Identification of *Saccharomyces cerevisiae* proteins that bind to nucleosomes at positions other than the N-terminal histone tails.

by

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“[This is the] puzzling limitation of our mind: our excessive confidence in what we believe we know, and our apparent inability to acknowledge the full extent of our ignorance and the uncertainty of the world we live in. We are prone to overestimate how much we understand about the world and to underestimate the role of chance in events. Overconfidence is fed by the illusory certainty of hindsight.” – Daniel Kahneman

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List of Abbreviations

- **ACF1** = ATP-dependent chromatin assembly factor large subunit
- **ARP** = Actin-related protein
- **ASF1** = Histone chaperone ASF1
- **ATP** = Adenosine triphosphate
- **ATRX** = α-Thalassemia X-linked retardation
- **BAH** = Bromo-adjacent homology (domain)
- **BDF1** = Bromodomain-containing factor 1
- **bp** = Base pairs
- **BR** = Broad range
- **BRD** = Bromodomain
- **BRG1** = Transcription activator BRG1
- **BRM** = Brahma
- **C18** = Octadecyl carbon chain
- **CAF-I** = Chromatin assembly factor
- **CAP** = Chromatin architectural protein
- **CBX5** = Chromobox protein homolog 5
- **CHD** = Chromodomain helicase DNA-binding (domain)
- **CIA** = Histone chaperone CIA (ASF1 homolog)
- **COFS** = Cerebro-oculo-facio-skeletal syndrome
- **CSB** = Cockayne syndrome B DNA repair-transcription-coupling factor
- **dCTP** = Deoxycytidine triphosphate
- **dsDNA** = Doubel strand DNA
- **ECT1** = Ethanolamine-phosphate cytidylyltransferase
- **EMANIC** = Electron microscopy assisted nucleosome capture
- **EPI** = Enhanced product ion (scan)
- **ESI** = Electrospray ionisation
- **GDP1** = Guanosine-diphosphatase
- **gH2A** = Globular domain of histone H2A
- **gH2B** = Globular domain of histone H2B
- **gH3** = Globular domain of histone H3
- **gH4** = Globular domain of histone H4
- **H2A** = Canonical histone H2A
- **H2B** = Canonical histone H2B
- **H3** = Canonical histone H3
- **H4** = Canonical histone H4
- **hCMV** = Human cytomegalovirus
- **HMG** = High mobility group box
- **HMGN** = High mobility group nucleosome-binding (protein)
- **HP1α** = Heterochromatin protein 1
- **HPLC** = High performance liquid chromatography
- **HSA** = Helicase-SANT-associated (domain)
- **IDA** = Information dependent acquisition
- **IE1** = Immediate early 1 protein
- **IL-33** = Interleukin 33

- **INO80** = Inositol requiring 80
- **IPTG** = Isopropyl β-D-1-thiogalactopyranoside
- **ISW** = ISWI subunit
- **ISWI** = Imitation switch remodelling complex
- **KSHV** = Kaposi's sarcoma-associated herpesvirus
- **LANA** = Latency-associated nuclear antigen
- **LC** = Liquid chromatography
- **LC-MS/MS** = Liquid chromatography-coupled tandem mass spectrometry
- **LRS** = Loss of rDNA silencing
- **MAD1** = Spindle assembly checkpoint component MAD1
- **MAK21** = Ribosome biogenesis protein MAK21
- **MALDI** = Matrix-assisted laser desorption ionisation
- **MCM22** = Minichromosome maintenance protein 22
- **MIi-2** = CHD family remodelers
- **MRT** = Malignant rhabdoid tumor
- **MS** = Mass spectrometry
- **MTA1-3** = Metastasis-associated proteins 1-3
- **MW** = Molecular weight
- **NAD** = Nicotinamide adenine dinucleotide
- **NCP** = Nucleosome core particle
- **NMR** = Nuclear magnetic resonance
- **NOC3** = Nucleolar complex-associated protein 3
- **NOP2** = Nucleolar complex protein 1
- **NuRD** = Nucleosome Remodeling Deacetylase
- **ORC** = Origin recognition complex
- **PDB** = Protein database
- **PDC1** = Pyruvate decarboxylase isozyme 1
- **PHD** = Plant homeodomain
- **POL5** = DNA polymerase V
- **PRP43** = Pre-mRNA-splicing factor ATP-dependent RNA helicase PRP43
- **PTM** = Posttranslational modification
- **PUF6** = Pumilio homology domain family member 6
- **RCC1** = Regulator of chromosome condensation 1
- **RPA12/ 49** = DNA-directed RNA polymerase I subunit RPA12/ 49
- **RPAB3** = DNA-directed RNA polymerases I, II, and III subunit RPABC3
- **RSC** = Chromatin structure remodelling complex
- **RVB1/ 2** = RuVB-like protein 1/ 2
- **SANT** = Swi3, Ada2, N-Cor, and TFIIIB (domain)
- **SGD1** = Suppressor of Glycerol Defect
- **SIR1/ 3** = Silent information regulator 1/ 3
- **SLIDE** = SANT-like (but with several insertions)
- **SWI/SNF** = SWItch/Sucrose Non-Fermentable
- **SWR1** = SWi2/snf2-Related
- **TAF1/ 9** = Transcription initiation factor TFIID subunit 1/ 9
- **TFIID** = Transcription factor II D

- **TOP2** = DNA topoisomerase 2
- **URB** = Nucleolar pre-ribosomal-associated protein
- **UTP9/ 10** = U3 small nucleolar RNA-associated protein 9/ 10
- **YHR127W** = Uncharacterized protein YHR127W
- **YNL108C** = Uncharacterized protein YNL108C
- **YRA1** = RNA annealing protein YRA1

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Introduction

The eukaryotic cell faces a dilemma: its DNA needs to be packed tightly to fit into the limited space available within the cellular nucleus, while still remaining accessible to the multitude of enzymes involved in maintaining biological function (Dutta *et al.* 2012). To this end the DNA is organised into chromatin. Chromatin consists of the 4 core histones, H2A, H2B, H3 and H4 assembled into octamers around which the DNA wraps (Figure 1.1). Around each octamer 145-147bp of DNA binds to form a nucleosome core particle (NCP). All of the NCPs will be linked via the linker DNA which is not associated with the histones. This resembles "beads-on-a-string" which can be further compacted into chromatin higher-order structures by association of linker histone H1. As the chromatin higher-order structure is the ultimate regulator of all DNA functions through regulation of DNA accessibility a whole section of the first chapter will be devoted to the structural dynamics thereof (Chen & Li 2010).

Essential to this regulation by chromatin are DNA methylation, histone posttranslational modifications, histone variants and non-histone, regulator proteins which bind the chromatin via the histone surfaces and N-terminal extensions (Figure 1.1) (Klose & Bird 2006)(Kouzarides 2007)(Biterge & Schneider 2014)(McBryant *et al.* 2006)(Clapier & Cairns 2009). Until recently it was maintained that DNA-dependent enzyme complexes had to simply "overcome" the steric hindrances imposed by chromatin in order to access the DNA (Luger *et al.* 2012). It is becoming clearer that these enzymes have not only become specialised in remodelling the chromatin structure for access but may also utilize the chromatin structure and the plethora of chromatin binding proteins associated with the DNA to properly and efficiently perform their functions (Eberharter *et al.* 2004)(Hou *et al.* 2005)(Narlikar *et al.* 2015).

As the accurate regulation of DNA processes is crucial to the maintenance of a functional biological state, the exploration of the underlying mechanisms of regulation is essential (Srivastava *et al.* 2010). Although originally believed to simply be a static substrate onto which the DNA is packaged, it has been established that chromatin is a dynamic entity that is actively involved in determining cellular fate (Luger *et al.* 2012). Fundamental to its regulation is the abundance of protein-protein interactions occurring in chromatin (Wolffe & Guschin 2000). Identifying all the possible interaction partners of chromatin is an important step in the elucidation of the overall process of regulation. To date a significant amount of work has been carried out on determining the distribution of post translational modifications in the intrinsically disordered N-terminal domains of the core histones as well as the proteins recruited by these modifications (Jenuwein & Allis 2001)(Hoffman & Vu 2004)(Strahl & Allis 2000). However the

nucleosomal surface formed by the globular domains of the core histones remains grossly neglected.

Instead of fulfilling the role as simple storage substrates for DNA, nucleosomes partner up with neighbouring nucleosomes as well as a host of other regulatory proteins to regulate DNA function by modulation of the large-scale chromatin organisation (Luger *et al.* 2012)(Clapier & Cairns 2009)(Chen & Li 2010). It is clear that factors effecting minute changes in the nucleosomal structure can generate dramatic effects in the higher order structure and subsequently in the execution of various DNA-related functions (Luger *et al.* 2012). These factors include post translational modification of histones, incorporation of histone variants, changes in the binding of the linker histone and the positioning of the nucleosomes along the DNA sequence (Rosa & Shaw 2013)(Luger *et al.* 2012). The different combinations of these factors create local chromatin states which in turn give rise to a variation in the population of non-histone proteins that is associated with a particular chromatin state (Rosa & Shaw 2013)(Clapier & Cairns 2009). These associated proteins include the chromatin remodelers, the architectural proteins, PTM “reader” and “writer” enzymes and transcription factors required for gene expression (Tan & Davey 2011). Dynamic interplay also exist between the associated proteins, adding even more to the complexity (Clapier & Cairns 2009).

This study focussed on the largely unexplored idea that regulatory proteins might interact with the globular domains of the nucleosomes. The first chapter is a study of the available literature which indicates the functional importance of these domains. It also looks at the importance of the regulatory proteins and literature which suggests or proves direct association with the nucleosome’s globular domains. Chapter 2 lays out the experimental part of the study, followed by the results discussed in chapter 3. Finally a short conclusion makes up chapter 4 with chapter 5 comprising the summary.

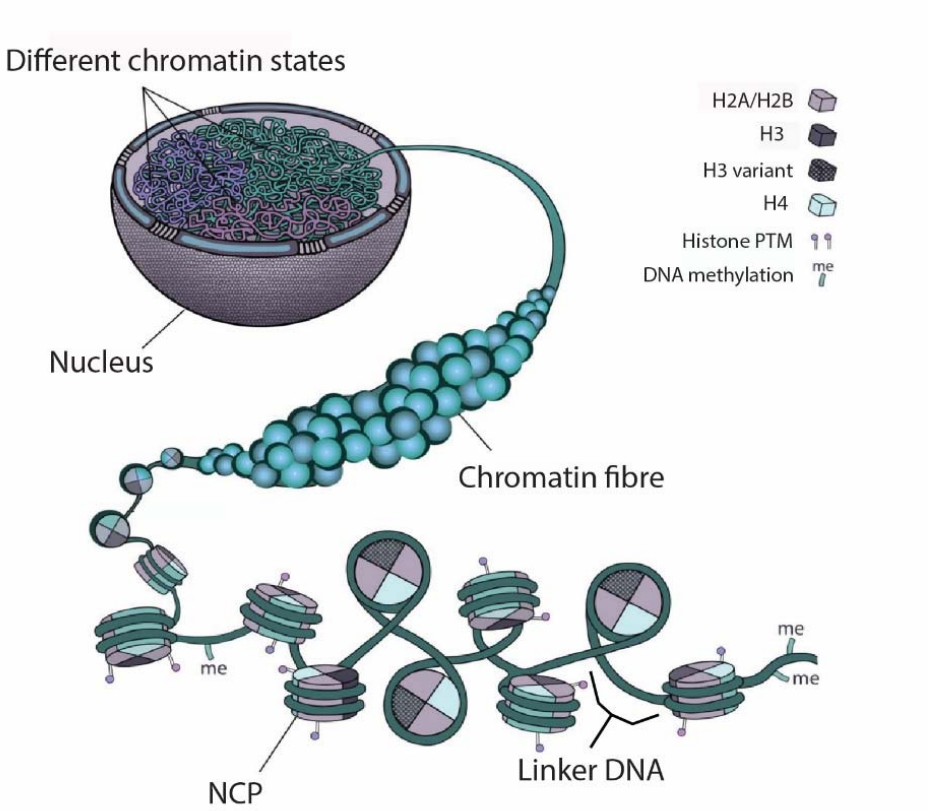

Figure 1.1: Chromatin compaction. (Rosa & Shaw 2013) *The simplified diagram adapted from Rosa & Shaw 2013 shows the various aspects of chromatin compaction and regulation.*

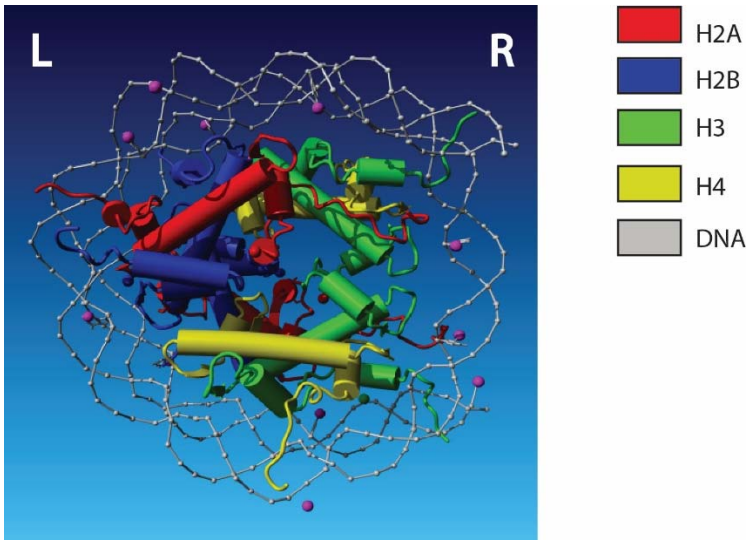

Figure 1.2: The Nucleosome core particle (PDB: 1ID3). The NCP consists of two copies of the four major type histones, H3, H4, H2A, H2B assembled into an octamer. Around this octamer ~145-147bp of DNA is wrapped in a tight, two-turn superhelix.(Luger, Rechsteiner, *et al.* 1997). *Histone H2A and H2B is clustered to one side of the NCP as two dimers (left in this orientation) while the H3-H4 tetramer is found on the opposite side (right in this orientation). This image was rendered with YASARA View (open source: www.yasara.org).*

Chapter 1

Literature Review

1.1. Chromatin structure and function

In order to understand the interactions with the regulatory proteins it is important to firstly understand the underlying conformational dynamics of the chromatin fiber (Luger *et al.* 2012). Most of our knowledge is derived from *in vitro* model system studies and much about the higher order chromatin fiber is still disputed (Li & Reinberg 2011). The nucleosome is the monomer unit of chromatin and comprises of ~145 – 147bp of DNA wrapped around a compact core of eight histones in a tight, two-turn superhelix (Luger, Mäder, *et al.* 1997). The canonical octamer consists of two copies of each of the four major type, unmodified histone proteins, H3, H4, H2A and H2B (Figure 1.2)(Luger, Rechsteiner, *et al.* 1997). Positively charged residues on the exposed histone surfaces interact with the negatively charged phosphate backbone of the DNA every ~10.4bp forming a relatively weak single interaction (Luger *et al.* 2012). Combined however, these 14 contacts provide positional stability and together with the protein-protein interactions between the histones, they stabilise the whole nucleosome (Luger, Mäder, *et al.* 1997)(Davey *et al.* 2002)(Rohs *et al.* 2009)(Luger *et al.* 2012). It has been observed through high-resolution crystal structures that the largest part of the octamer consists of structured domains found within the DNA superhelix while between 14 and 38 N-terminal residues of each histone extend outside of the complex (Luger, Mäder, *et al.* 1997)(Davey *et al.* 2002)(White *et al.* 2001). These tail-like structures play an important role in the assembly of higher order structures of chromatin as it has been proven extensively that arrays of which these tails have been removed by limited proteolysis do not form secondary or tertiary structures at all (Allan *et al.* 1982)(Fletcher & Hansen 1995)(Tse & Hansen 1997)(Schwarz *et al.* 1996)(Woodcock & Dimitrov 2001). Connected by linker DNA, nucleosomes form nucleosomal arrays which resemble beads on a string and can further compact into chromatin fibers (Figure 1.1)(Luger *et al.* 2012). The H1 or H5 linker histone is important to histone functionality (Thomas 1999). H1 stabilises the nucleosome by binding to the nucleosomal dyad and the linker DNA entering and exiting the NCP (Figure 1.3)(Carruthers *et al.* 1998)(Allan *et al.* 1980)(Goytisolo *et al.* 1996)(Bednar *et al.* 1998)(Zhou *et al.* 1998)(Widom 1998). The condensed chromosome is a result of the fiber-fiber interaction which follows this compaction (Tremethick 2007).

Histone variants. The nucleosomal arrays vary in amino acid sequence and post-translational modification status of the histones as well as in nucleosome position along the DNA sequence (Woodcock & Dimitrov 2001). This level of organisation is the primary structure of chromatin

which will define the higher order structures (Woodcock & Dimitrov 2001). Variation in the amino acid composition of the nucleosome can be attributed to histone variants being incorporated into the octamer (Biterge & Schneider 2014). These variants replace major type histones and are often found to be recruited to precise locations within the genome (Biterge & Schneider 2014). For example, H3.3 is enriched in regions of active transcription whereas H2A.Z is found to be significantly enriched at regions flanking active promotors (Henikoff *et al.* 2004)(Gu *et al.* 2015). Variants have been found to be highly conserved between species alluding to functional importance although much is still unknown about the specific functional roles they fulfil (Biterge & Schneider 2014)(Henikoff *et al.* 2004).

Post-translational modifications. Post-translational modifications (PTMs) are chemical modifications such as phosphorylation which are added after translation by specific modifying enzymes (Bannister & Kouzarides 2011). Histones exhibit an abundance of PTMs (Bannister & Kouzarides 2011)(Strahl & Allis 2000). Effectively all known protein modifications has been found on histones and new modifications are being discovered regulary (Tan *et al.* 2011). A vast number of studies focus on the PTMs found on the N-terminal extensions of the histones and the important roles they play in mediating DNA processes by recruiting and binding a large number of non-histone, chromatin associated proteins (Matsubara *et al.* 2007)(McBryant *et al.* 2006)(Clapier & Cairns 2009).

Nucleosome positioning. The relative position of the nucleosomes on the DNA sequence of the genome plays an integral part in the regulation of the DNA process (Jiang & Pugh 2009)(Struhl & Segal 2013). DNA that is densely populated with nucleosomes can become inaccessible to DNA-binding factors and thus *cis*-regulatory elements need to be in nucleosome-free regions or transiently “opened up” by specialised chromatin remodelers for transcription to proceed (Clapier & Cairns 2009). Technological advances in DNA sequencing have made it possible to perform genome-wide nucleosomal mapping and attempts have been made to predict nucleosome positioning based on DNA sequence alone (Kaplan *et al.* 2009)(Trifonov 2011). While it is still debatable whether the positioning can be determined by DNA sequence alone other nuclear factors like the chromatin architectural proteins and ATP-dependent remodelling factors definitely contribute to the final positioning and structure of nucleosomes *in vivo* (Zhang *et al.* 2009)(Segal *et al.* 2006)(Kaplan *et al.* 2010)(Zhang & Pugh 2015)(Zhang *et al.* 2011)(Radman-Livaja & Rando 2010)(Pugh 2010). It is important to understand the dynamic nature of the chromatin and that nucleosome spacing is not absolute but constantly changing to accommodate cellular functions (Luger *et al.* 2012).

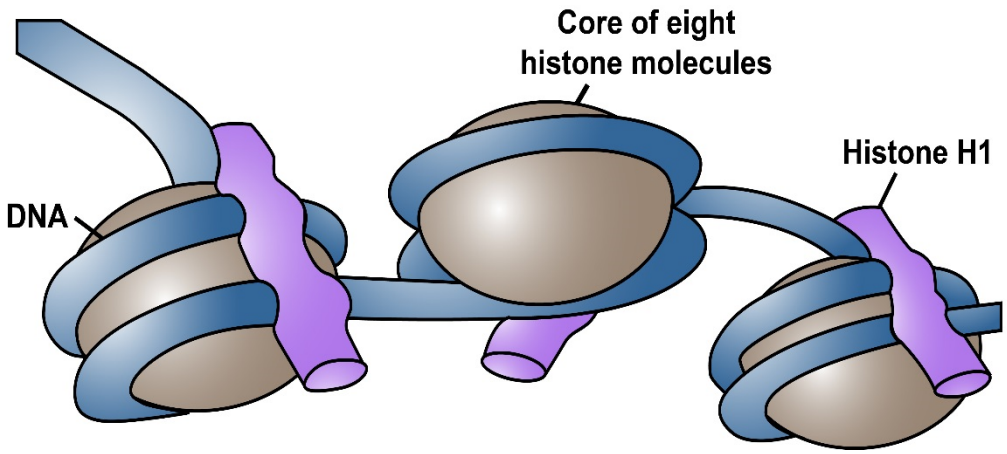

Figure 1.3: H1 stabilises the nucleosome by binding to the nucleosomal dyad and the linker DNA entering and exiting the NCP (Carruthers et al. 1998)(Allan et al. 1980)(Goytisolo et al. 1996)(Bednar et al. 1998)(Zhou et al. 1998)(Widom 1998).

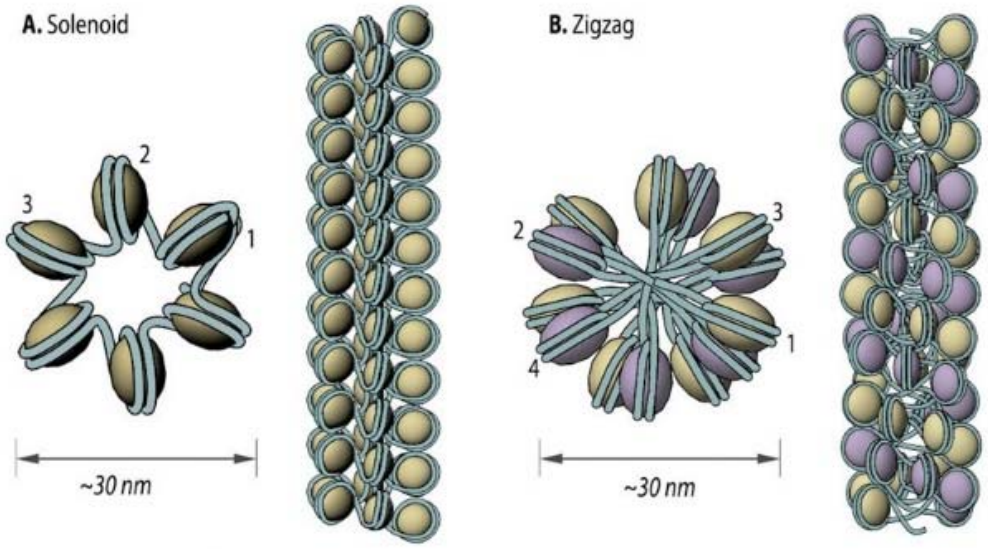

Figure 1.4: Proposed models for chromatin secondary structure. (A) The proposed "one-start" helix or solenoid model is defined by interactions between neighbouring nucleosomes. The sequential histones thus follow each other, bending the DNA to form a helical structure (Robinson & Rhodes 2006). (B) The zigzag model differs from the solenoid model in that alternate nucleosomes interact with each other and sequential nucleosomes (nucleosomes connected by linker DNA) are positioned opposite each other. This way the linker DNA can remain more rigid and a secondary structure resembling a "two start" helix is formed (Dorigo et al. 2004).

Higher order structure – 30nm fiber. To progress from the primary to the secondary structure nucleosomal arrays must compact into a defined structure such as the much disputed 30nm fiber (Fussner *et al.* 2011)(Grigoryev & Woodcock 2012). As chromatin higher order structures play a vital role in regulation of DNA processes, and thus ultimately all cellular processes, great effort has gone into attempting to solve the secondary structure of chromatin *in vivo* at interphase and the subsequent passage thereof into the highly condensed chromosomes observed at metaphase (Grigoryev & Woodcock 2012). To date, progress in this area has been slow and many results are unclear and/or controversial (Li & Reinberg 2011)(Fussner *et al.* 2011). Studies has shown that a defined array from purified DNA and histones will fold into structure resembling the 30nm fiber at physiological salt concentrations *in vitro* although the existence of the 30nm fiber *in vivo* remains disputed (Nishino *et al.* 2012)(Fussner *et al.* 2011)(Maeshima *et al.* 2010). The complete structure of the 30nm fibre also remains unsolved with two models proposed (Figure 1.4). For the one-start solenoid model it is suggested that neighbouring nucleosomes will interact with each other, bending the linker DNA to form a helix (Kruithof *et al.* 2009)(Li & Reinberg 2011). The other, two-start zig-zag structure proposes that two linear arrays of nucleosomes will interact and form a two-start helix, keeping the linker DNA relatively straight and zig-zagging between the stacks (Dorigo *et al.* 2004).

A study utilising electron microscopy-assisted nucleosome capture (EMANIC) recently found that chromatin assembled *in vitro* forms a heteromorphic fiber rather than adopting a single conformation (Grigoryev *et al.* 2009). It showed that the fiber predominantly adopted the two-start zig-zag structure but partially bent linker DNA typical of the solenoid model were scattered throughout the fiber. It was also confirmed that this heteromorphic structure was energetically more favourable than a uniform organization in the presence of linker histone and Mg^{2+} counter ions (conditions that promote compact folding) *in silico*. It is suggested that the length of linker DNA will be the determinant in fiber organization where nucleosome repeat lengths of between 173 and 209bp favoured compaction into the zig-zag structure while longer repeat lengths of 218 to 226bp will favour the solenoid structure. It is understandable that shorter repeat lengths may be less prone to bending of the linker DNA in the presence of linker histone whereas longer linker DNA stretches may have more freedom to bend (Grigoryev & Woodcock 2012)(Perisic *et al.* 2010)(Correll *et al.* 2012)(Kelbauskas *et al.* 2009). Linker histones also serve to stabilise the higher order structures by binding to nucleosomes with linker DNA (Caterino & Hayes 2011).

1.1.1. Functional importance of the nucleosomal surface

The core histones in the nucleosomes can be divided into two separate domains – an intrinsically unstructured N-terminal extension or “tail” and the structured globular domain

(Figure 1.5)(Luger *et al.* 2012). The majority of studies focus on the functional importance of the N-terminal extensions and their chemical modifications as they are targeted by many chromatin-associated factors (Matsubara *et al.* 2007)(Taverna *et al.* 2007)(Cosgrove *et al.* 2004). The globular domains are however equally well conserved between species and also contain functionally important features.(Matsubara *et al.* 2007) A well-known, functionally important feature of the globular domain is the acidic patch (Kalashnikova *et al.* 2013)(Chen & Li 2010)(Zhou *et al.* 2007).

The acidic patch is a grouping of acidic residues located in the H2A-H2B dimer (Figure 1.5) that has been shown to be central to nucleosome-nucleosome interaction and thus the folding of chromatin higher order structures (Zhou *et al.* 2007). Evidence suggests that the H4 N-terminal extension can interact with a neighbouring nucleosome's acidic patch to facilitate nucleosome-nucleosome interactions as well as with the acidic patch on its own nucleosome which might serve to stabilise the DNA wrapping at its entry and exit points on the nucleosome (Dorigo *et al.* 2004)(Kan *et al.* 2009)(Zhou *et al.* 2007). A growing list of proteins is also found to bind the acidic patch with potential effects on the chromatin structure (Luger *et al.* 2012). Of these, two are viral proteins (Karposi's sarcoma-associated herpesvirus protein latency-associated nuclear antigen (LANA) and human cytomegalovirus major immediate early 1 protein) that seem to mediate viral genome attachment to the chromosomes by docking to the acidic patch (Mucke *et al.* 2014)(Barbera *et al.* 2006). The group also include cytokine interleukin-33 (IL-33), regulator of chromosome condensation 1 (RCC1), silent information regulator 3 (SIR3) and high mobility group nucleosome-binding domain-containing protein 1 and 2 (HMGN1 and HMGN2) (Makde *et al.* 2010)(Armache *et al.* 2011)(Roussel *et al.* 2008)(Kato *et al.* 2011). The incorporation of H2A.Z with its expanded acidic patch was shown to promote the association of heterochromatin protein 1α (HP1α or CBX5) with condensed chromatin although direct interaction of HP1α with the acidic patch must still be verified (Luger *et al.* 2012)(Fan *et al.* 2004). These specific protein interactions will be discussed in greater detail in coming sections but it would seem that the acidic patch and subsequently the globular domains of histones play a greater role in mediating cellular functions through non-histone protein interaction than previously anticipated (Luger *et al.* 2012).

A 2007 study investigated the functional importance of the globular domains by mutating each of the major type histones' residues located on the nucleosome surface and analysing the effects of each separate mutation *in vivo* (Matsubara *et al.* 2007). The study analysed the effect of each mutation on growth rate, transcription, transcription elongation, DNA replication and

DNA repair by using phenotypic assays. It was found that out of the 320 mutants constructed only 8 mutations were lethal. Out of the viable 312 mutants in the extensive mutant library 10

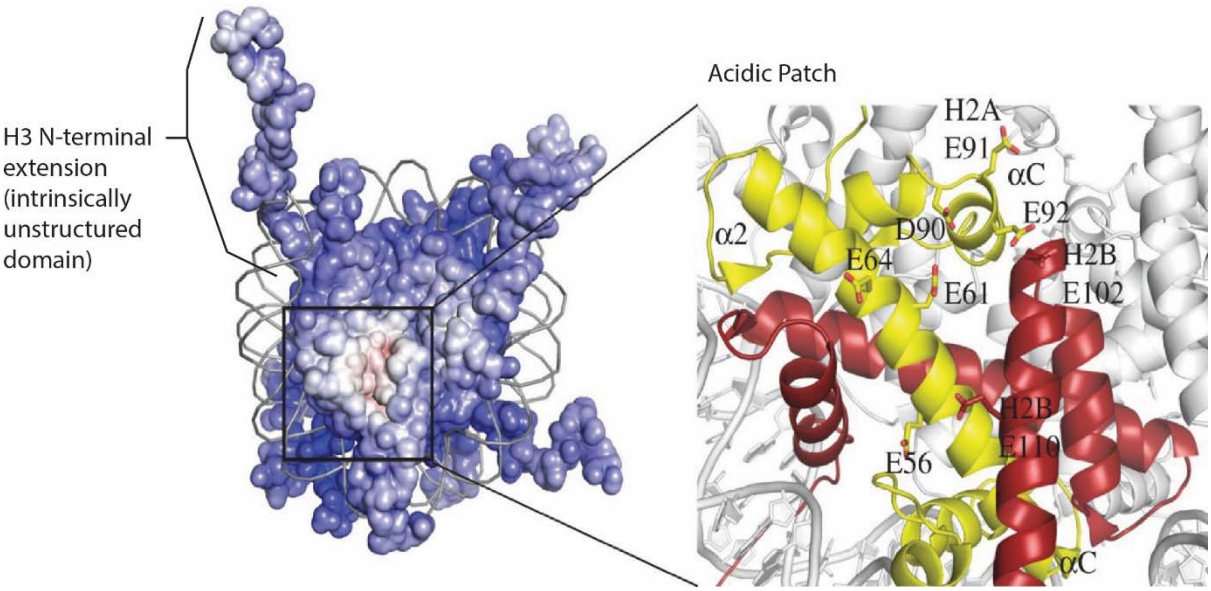

Figure 1.5: NCP and acidic patch. A figure adapted from a paper by Kalashnikova et al. 2013 showing the intrinsically unstructured domains protruding from the NCP core opposed to the globular domain around which the DNA is wrapped. The patch of acidic residues in the H2A-H2B dimer has been shown to play an essential role in chromatin compaction and has been implicated in interaction with an increasing number of proteins. (Kalashnikova *et al.* 2013)

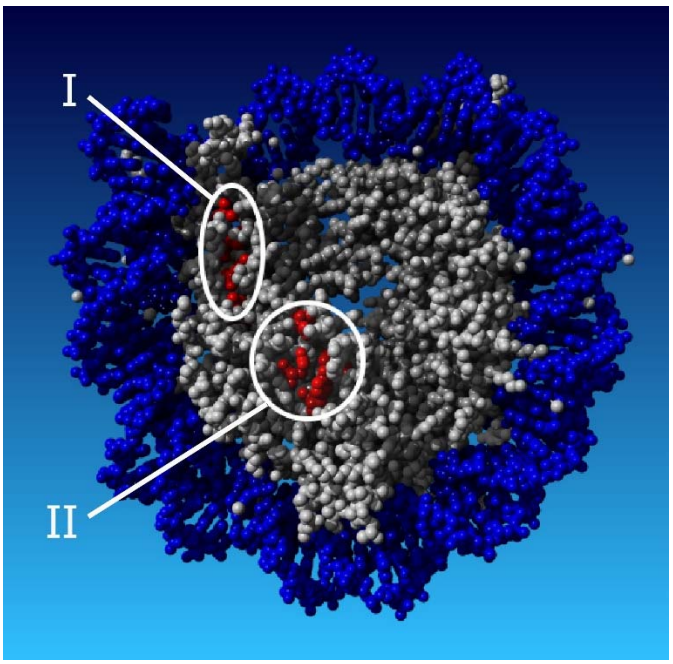

Figure 1.6: The two regions on the nucleosome face which caused lethality when mutated.(Matsubara *et al.* 2007)

displayed slow growth rates, 42 showed transcription inhibition, 8 blocked transcription elongation, 30 showed inhibition of DNA replication and 61 impaired DNA repair processes.

1.1.1.1. Lethal mutations. The residues which caused lethality when mutated were clustered into 2 different regions on the nucleosome surface (Figure 1.6)(Matsubara *et al.* 2007). The first cluster includes the 4 residues H3-L48, -I51, Q55 and H2A-R82. These residues all interact with the C-terminal extension of H2A as well as a region in the H4 histone fold (from helix α1 to loop L1). It was thus postulated that they play a central role in the development, maintenance and adaption of the nucleosome entry site. The second region (H2A-Y58, -E62, -D91 and H2B-L109) is located in the acidic patch.

1.1.1.2. Mutations affecting transcriptional initiation. Residues which were shown to influence transcriptional initiation were found to be mainly grouped into three different regions on the nucleosomal surface (Figure 1.7)(Matsubara *et al.* 2007). The first two regions map to regions previously shown to be DNA-interactive regions (White *et al.* 2001) The first region is located at the nucleosome entry site and contains 13 residues which are all accessible to the DNA and of which 6 are positively-charged (Matsubara *et al.* 2007). Residues H3-K56 and –L61 are also included in this region. These are known to recruit SNF5, a subunit of the SWI/SNF remodelling complex, to promoter regions and is known to hamper transcription when mutated (Duina & Winston 2004)(Xu *et al.* 2005). The other residues in this region (H4-R35, -R36, -G48, -L49 and –Y51) are distributed around the bromodomain factor 1 (BDF1) binding domain (Pamblanco *et al.* 2001). BDF1 is a transcription factor involved in the expression of a vast number of genes and is also known to play a role in yeast sporulation and DNA damage repair (Pamblanco *et al.* 2001)(Lygerou *et al.* 1994) (Chua & Roeder 1995)(Chang *et al.* 2002). Interestingly, BDF1 also blocks the propagation of SIR mediated silencing at telomeres and shields H4 from deacytelation (Ladurner *et al.* 2003)(Matangkasombut & Buratowski 2003).

The second region (H3-K115, -V117 and –Q120) falls within the domain that was shown to interact with human histone chaperone CCG1-interacting factor A (CIA) (Munakata *et al.* 2000). This protein has a counterpart in *Saccharomyces cerevisiae*, anti-silencing factor 1 (ASF1), which has been shown to have a functional and direct interaction with BDF1 (Chimura *et al.* 2002). It can thus be postulated that these regions play important roles in recruiting the appropriate proteins necessary to modify the nucleosome structure in such a way that it is conducive to active transcription (Matsubara *et al.* 2007).

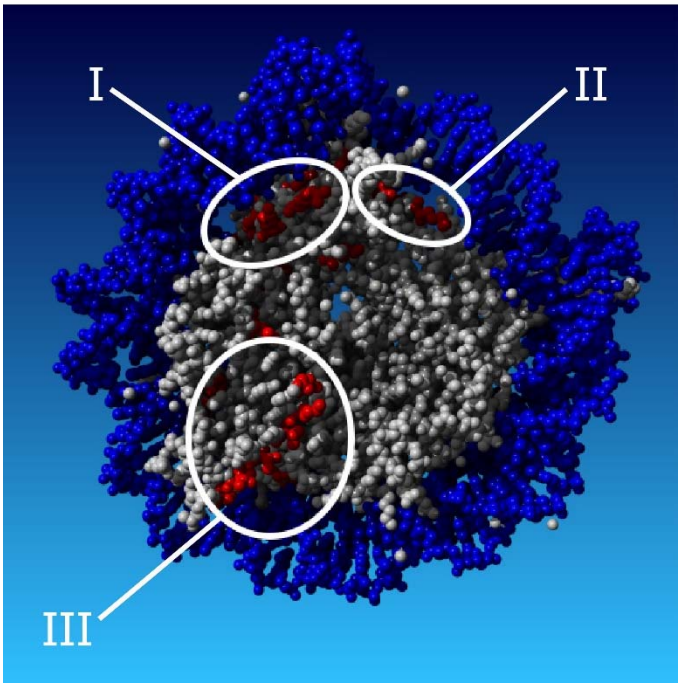

Figure 1.7: The three regions on the surface of the nucleosome which inhibits transcription initiation when mutated.(Matsubara *et al.* 2007)

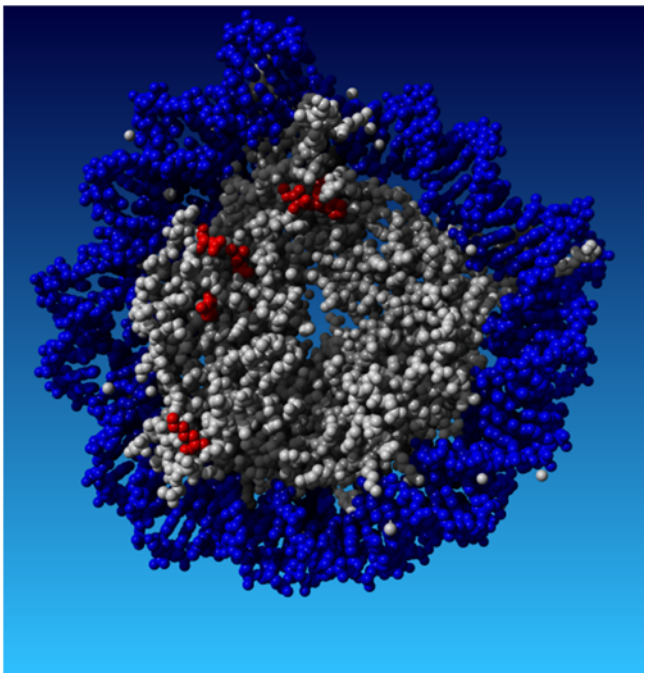

Figure 1.8: The residues on the face of the nucleosome which affects transcriptional elongation once mutated. They are not clustered into distinct regions but 7 of the 8 are residues of the H2A-H2B dimer.(Matsubara *et al.* 2007)

The third region (H2B-L105 to H2B-S125) cluster around and include H2B-E108 which has been shown by x-ray crystallography to interact with the neighbouring nucleosome (White *et al.* 2001). It is thus suggested that this region play a crucial role in regulation of chromatin structure through nucleosome-nucleosome interaction which distinguishes it from the previous regions in that it doesn't serve as binding domain for non-nucleosomal factors (Matsubara *et al.* 2007).

1.1.1.3. Mutations affecting transcriptional elongation. The 8 residues found to inhibit transcriptional elongation were all positioned on the one side of the nucleosome and 7 of them were residues of H2A or H2B (Figure 1.8)(Matsubara *et al.* 2007). Based on their positions, some of these residues were postulated to play a role in nucleosome-nucleosome interaction but it was also interesting to note that there were no overlapping residues with those that were shown to inhibit transcription initiation (Matsubara *et al.* 2007). It is thus conceivable that transcription initiation and transcription elongation are dependent on different areas of the nucleosomal surface which might suggest alternate protein interactions with the different regions of the nucleosomal surface (Matsubara *et al.* 2007).

1.1.1.4. Mutations affecting DNA replication. The 30 residues implicated in DNA replication were mapped to 3 regions on the nucleosome (Figure 1.9)(Matsubara *et al.* 2007). The first region overlaps the first region discussed for blocking transcription initiation (Figure 1.7) and the third region contains the residues responsible for the impairment of transcriptional elongation (Figure 1.8)(Matsubara *et al.* 2007). It makes sense if one considers that several chromatin factors associated with DNA replication such as ISW2 and CIA/Asf1 are also implicated in transcription(Deuring *et al.* 2000)(Chimura *et al.* 2002). Thus it comes as no surprise that regions known to interact with the ISW2 complex correspond with the overlapping regions for transcription initiation and DNA replication regulation on the nucleosome surface (Kagalwala *et al.* 2004). The first DNA replication region also has a known interaction with the replication-coupled chromatin assembly factor (CAF-I) and BDF1(Verreault *et al.* 1998). The second region falls in a domain essential for H3-H3 interaction and thus mutation of this domain might compromise nucleosome stability but it is not known how this affects DNA replication (Matsubara *et al.* 2007). The third region is found partly in the acidic patch, the H2A C-terminal area and H2BαC. This finding again alludes to functional importance of the acidic patch (Matsubara *et al.* 2007).

1.1.1.5. Mutations affecting DNA repair. The final 61 residues which were implicated in DNA repair were grouped into 3 regions on the nucleosome surface (Figure 1.10)(Matsubara *et al.* 2007). Complete overlap was observed with residues involved in DNA replication (Figure 1.9). It was suggested that this might be due to replication-coupled DNA repair by DNA replication

factors of which interaction with the nucleosome might be impaired by the mutations (Matsubara *et al.* 2007). Residues which fall outside of this overlap might be involved in alternative modes of DNA repair with proteins only concerned with DNA repair (Matsubara *et al.* 2007). The BDF1-interacting region on H4 and the CIA-interacting region on H3 are once again implicated (Matsubara *et al.* 2007)(Pamblanco *et al.* 2001). It was hypothesized that other proteins might interact with proteins such as BDF1 and CIA/Asf1 in order to achieve the desired DNA function (Matsubara *et al.* 2007). DNA repair also requires access to long stretches of the DNA sequence and thus chromatin remodelers might be needed to remove, slide or restructure the nucleosomes occupying the particular stretch of DNA (Clapier & Cairns 2009)(Matsubara *et al.* 2007). These remodelers might depend on the nucleosome surface for proper function (Clapier & Cairns 2009)(Eberharter *et al.* 2004).

As the complexity and dynamic nature of the chromatin structure comes to light it also becomes clear that the vast interplay of different chromatin factors is an important determinant of cell fate and function (Luger *et al.* 2012). It becomes easy to imagine the nucleosome as a docking station for a variety of proteins which in turn recruit and bind their own sets of partners to perform the needed DNA-mediated reactions. With the discussed global and functional analysis of the globular surfaces of the nucleosome, Matsubara and colleagues uncovered that the residues found to influence these DNA-mediated reactions were clustered to the one side of the nucleosome (left side in Figure 1.2) while silencing assays have shown that the opposite surface of the nucleosome (right side Figure 1.2) is involved in the regulation of transcriptional silencing (Park *et al.* 2002)(Thompson *et al.* 2003)(Hyland *et al.* 2005). Histones H2A and H2B, which are positioned mainly on the left side (Figure 1.2), seem to play important roles in both silencing and activating transcription by means of the acidic patch. It seems plausible that the nucleosome functions as a junction for a variety of functionally important proteins by means of its differently utilised regions.

1.2. Regulatory proteins

Non-histone proteins which regulate chromatin structure and function can be divided into the chromatin architectural proteins (CAPs), the histone chaperones, histone modifying enzymes and the chromatin remodelers (Luger *et al.* 2012). Histone chaperones, such as the replication-coupled chromatin assembly factor (CAF-I) mentioned above, are important proteins which are actively involved delivering the needed histones during nucleosome assembly (Burgess & Zhang 2013). CAF-I specifically is responsible for depositing canonical histone H3 onto the DNA during DNA-replication coupled assembly (Burgess & Zhang 2013). As these proteins mediate the deposition of new histones onto the chromatin, their extensive, and direct

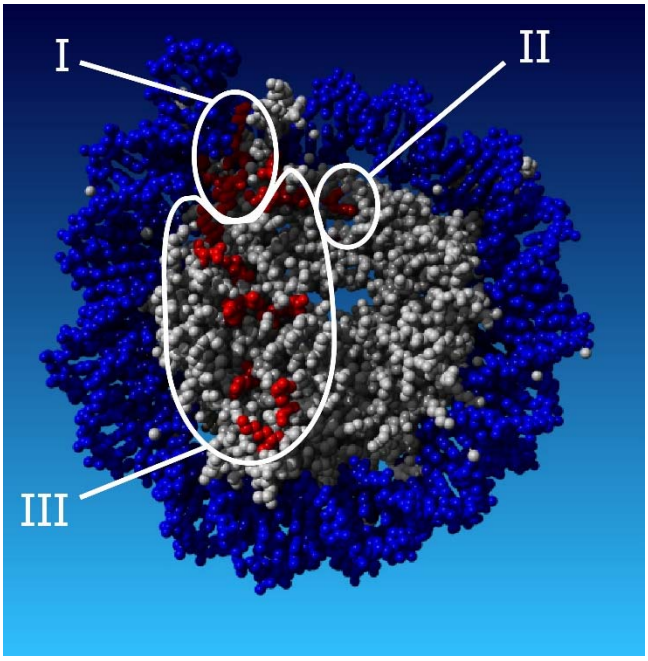

Figure 1.9: The residues affecting DNA replication when mutated can be grouped into three distinct regions (Matsubara *et al.* 2007).

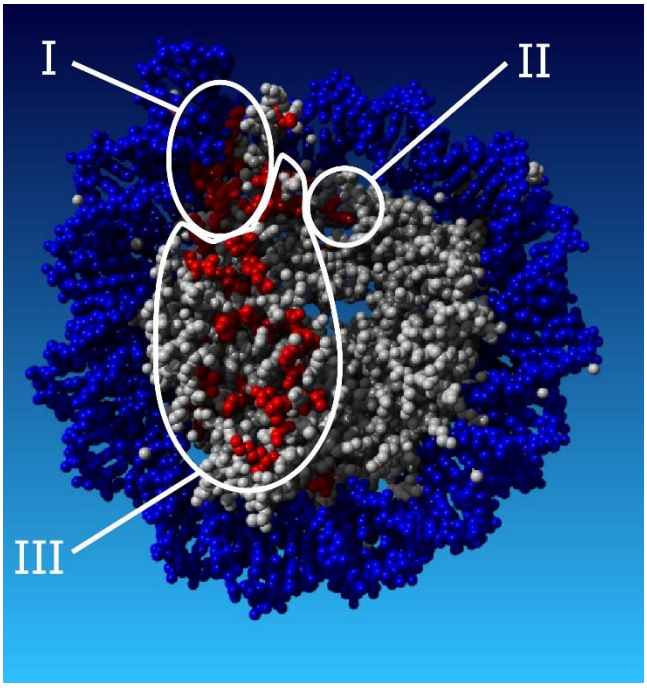

Figure 1.10: The three regions on the nucleosome surface that affects DNA repair when mutated (Matsubara *et al.* 2007).

interactions with the histones are to be expected. Likewise, the histone modification enzymes are directly associated with the N-terminal extensions they need to modify (Butler *et al.* 2012). What makes these protein groups interesting to this study, however, is that they form part of large protein complexes which works in synergy to modulate the chromatin structure (Clapier & Cairns 2009). These large protein complexes are known as chromatin remodelers and are of great interest when it comes to chromatin interaction studies as much of the underlying mechanisms of their activity remains unknown (Clapier & Cairns 2009). Also of importance are the CAPs, regulatory proteins with the ability to alter the chromatin's structural dynamics or architecture *in vitro* (McBryant *et al.* 2006). Many of these proteins are DNA binding proteins but many of them also interact directly with the histones, either through association of the N-terminal domains and their modifications, or by binding the globular domains (McBryant *et al.* 2006). For the purposes of this study we are interested in the possible interactions with the globular domains. What follows is a discussion of the nucleosome remodelling complexes and how they possibly interact directly with the nucleosome followed by a discussion of the CAPs with known interactions with the NCP globular domain.

1.2.1. Nucleosome remodelling complexes

In the highly dynamic chromatin organisation, remodelers use the energy of ATP hydrolysis to modulate chromatin structure (Tolkunov *et al.* 2011). In this way remodelers regulate the accessibility of the nucleosome surface areas and subsequently the DNA functions (Tolkunov *et al.* 2011)(Moshkin *et al.* 2012). Most remodeler complexes are built using one or two ATPases and a multitude of accessory proteins (Clapier & Cairns 2009). These complexes constitute multiple domains that are suspected to play role in nucleosome recognition but it is not clear whether they work cooperatively or as separate units (Bao & Shen 2007)(Marfella & Imbalzano 2007)(Wang 2003). Although important, the recruitment by histone modifications only constitutes a part of the overall remodelling process (Eberharter *et al.* 2004). To understand the mechanisms and dynamics underlying this process current evidence is pointing towards considering multiple domains and subunits working together and interacting with all the available surfaces of the nucleosome to direct, effect and regulate remodelling (Clapier & Cairns 2009).

At present there are four defined families of chromatin remodelers with distinctive domains conferring specialised functions for varying biological contexts on them (Clapier & Cairns 2009). All of the known remodelers have homologous DNA-dependent ATPase domains (Marfella & Imbalzano 2007)(Bao & Shen 2007)(Wang 2003)(Tsukiyama *et al.* 1995). They also share some other properties. Firstly they all show interaction with the nucleosome beyond DNA

interaction (Narlikar *et al.* 2015)(Clapier & Cairns 2009). They also possess domains that are able to recognise specific histone modifications and domains or protein subunits that regulate the ATPase activity and domains or subunits that interact with other non-histone chromatin and DNA associated proteins (Clapier & Cairns 2009). These remodelers make up specialised, multifaceted cell machinery using different domains that work in synergy to achieve nucleosome targeting, association and remodelling (Narlikar *et al.* 2015).

1.2.1.1. Functional domains associated with remodelling complexes

With the variety of functional domains associated with a single remodelling complex it makes sense that multiple domains might work in concert to recognise, bind and remodel specific areas of chromatin (Wang 2003)(Tsukiyama *et al.* 1995)(Marfella & Imbalzano 2007)(Bao & Shen 2007). Domains which recognise certain modification states of the nucleosome, such as the bromodomain (binds acetylated lysines), might work to target the remodeler to very specific nucleosomes, upon which the DNA binding domains (e.g. SANT and HMG domains) might bind DNA that needs to be translocated by the DNA-dependent ATPAses (Sanchez & Zhou 2009)(Clapier & Cairns 2009). Definitely the most studied part of the remodelling mechanism has been how covalent modifications are recognised by complexes and the derivation of a possible "histone code" (Jenuwein & Allis 2001)(Taverna *et al.* 2007)(Strahl & Allis 2000). Some domains that are abundantly distributed among remodelling complexes have however been indicated to be able to bind the nucleosome's globular surfaces (Eberharter *et al.* 2004)(Armache *et al.* 2011). This implies possible mechanisms for remodeler-nucleosome interaction and subsequent regulation of chromatin remodelling which involve more than one of the accessible nucleosome surface (Clapier & Cairns 2009).

Plant Homeodomain (PHD finger) as a module in multiple chromatin modifiers. A 2004 study found that the PHD fingers in ACF1, a subunit of the ISWI remodelling complex in *Drosophila*, interacted with recombinant histones minus their N-terminal extensions *in vitro* (Eberharter *et al.* 2004). Because they found that the PHD fingers binds to all the core histones it was suggested that the PHD fingers recognise a common structural moiety on the histone dimers which makes it possible for the remodeler to have multiple contacts with the nucleosome (Eberharter *et al.* 2004). Also prior to this study, it was found that inclusion of the ACF1 subunit leads to a striking increase in the efficiency of ISWI's remodelling reaction (Eberharter *et al.* 2001). They proposed a model in which the remodeler is anchored to the nucleosome by the PHD-histone contacts which allow more effective translocation of the DNA and sliding of the nucleosome (Figure 1.11)(Eberharter *et al.* 2004).

Bromo-adjacent homology (BAH) domain. The BAH domain is frequently found next to the bromodomains of a variety of proteins.(Yang & Xu 2013) It is found coupled to bromodomains in chromatin remodelers such as RSC1/2 but is also found on its own in other regulatory proteins such as SIR3 (Chambers *et al.* 2013)(Armache *et al.* 2011)(McBryant *et al.* 2006). In SIR3 it has been proven to bind the globular surfaces of the nucleosome directly as discussed below (Armache *et al.* 2011).

The fact that these domains have been shown to extensively associate with the surface of the nucleosome suggests a new, previously unexplored chromatin binding mechanism for regulatory proteins (Clapier & Cairns 2009). The extent to which proteins bind the nucleosomes independent of the chemically modified N-terminal extensions remain largely unknown (Clapier & Cairns 2009).

1.2.1.2. Nuclear actins and actin-related proteins (ARPs) as integral subunits of remodelers

Actin is a highly abundant protein known for a myriad of important functions in the cytoplasm of the cell (Kapoor & Shen 2014). Cytoplasmic actin polymerises to form microfilaments in a process regulated by ATP hydrolysis (Field & Lenart 2011). Critical for ATP hydrolysis is the central actin-fold which characterises actin (Dominguez & Holmes 2011). Actin-related proteins (ARPs) have a conserved actin fold and between 10% and 80% sequence identity with actin (Robinson *et al.* 2001)(Schafer & Schroer 1999). Both actin and ARPs have been established to be subunits of many chromatin remodelers (Boyer & Peterson 2000). It has however not been determined what their role as subunits are (Clapier & Cairns 2009). Other studies have postulated that actin and ARPs might regulate association of the remodelling complexes to the chromatin, assist in assembly and stabilisation of remodeler complex, enhance DNA-dependent ATPase activity, or play a role specifically in histone binding (Shen *et al.* 2003)(Olave *et al.* 2002)(Rando *et al.* 2002)(Boyer & Peterson 2000)(Szerlong *et al.* 2003). As none of these functions are general functions of all the ARPS, actin or even of the different remodelling complexes, it is plausible that different ARPs might play different roles in different biological contexts and might very well be found to also associate with the globular domains of the nucleosome (Visa & Percipalle 2010).

1.2.1.3. Chromatin remodeler families

SWI/SNF (switching defective/ sucrose nonfermenting) family. The SWI/SNF complex in *Saccharomyces cerevisiae* was the first remodelling complex to be described and comprises 8 to 14 subunits (Mohrmann & Verrijzer 2005). The motor unit in this family of complexes is

characterised by an ATPase domain that is divided into two parts and separated by a short insertion (Wang 2003). Flanking this ATPase domain is a helicase-SANT (HSA) domain and a C-terminal bromodomain (Wang 2003). The SANT domains are known to bind unmodified histone N-terminal extensions while bromodomains recognise acetylated lysines in proteins (Boyer *et al.* 2004)(Grune *et al.* 2003)(Boyer *et al.* 2002) (Sanchez & Zhou 2009).

It is important to note that none of SWI/SNF remodelers work as a single, defined complex, always effecting the same changes on the nucleosome (Wang 2003). By variation in subunit organisation functional differentiation is possible allowing, these remodelers to play a role in many distinct chromatin activities (Clapier & Cairns 2009). The distinct functions of each complex are determined by the structural domains found in the different subunits (Clapier & Cairns 2009). In addition to a number of unique DNA-binding motifs, SWI/SNF complexes also contain a host of possible histone binding domains (Wang 2003).

ISWI (imitation switch) family. The characteristic ATPase of this family contains a C-terminal SANT domain next to a SLIDE domain (Corona & Tamkun 2004). Together these two domains form a nucleosome recognition moiety which binds DNA and unmodified histone tails (Boyer *et al.* 2004). As with SWI/SNF, specialised accessory proteins adds other functional domains to the complex such as protein binding bromo- and plant homeodomains (PHDs)(Clapier & Cairns 2009). Because of the variation of subunit proteins, ISWI plays diverse roles in chromatin-based reactions (Clapier & Cairns 2009). The ISWI ATPase is an abundant protein which is crucial for cell survival in *Drosophila,* but not in yeast (Tsukiyama *et al.* 1995)(Deuring *et al.* 2000)(Tsukiyama *et al.* 1999). This highly conserved family of remodelling complexes play diverse and important roles in chromatin mediated cellular functions (Clapier & Cairns 2009).

CHD (chromodomain, helicase, DNA-binding) or Mi-2 family. This family of remodelers was first characterised in *Xenopus laevis* and is composed of 1 to 10 subunits associated with the characteristic catalytic subunit (Marfella & Imbalzano 2007). CHD proteins are characterised by N-terminally located, tandem chromodomains (Chromatin Organisation Modifier) and a central ATPase domain (Woodage *et al.* 1997)(Delmas *et al.* 1993). The chromodomain allows for chromatin interaction by binding DNA, RNA and methylated lysine 4 in the H3 N-terminal domain (Brehm *et al.* 2004)(Bouazoune *et al.* 2002)(A. Akhtar *et al.* 2000)(Fischle *et al.* 2003)(Flanagan *et al.* 2005)(Min *et al.* 2003)(Pray-Grant *et al.* 2005)(Sims *et al.* 2005)(Kim *et al.* 2006). The CHD family can be further subdivided into three subfamilies based on other domains associated with the complexes (Marfella & Imbalzano 2007).

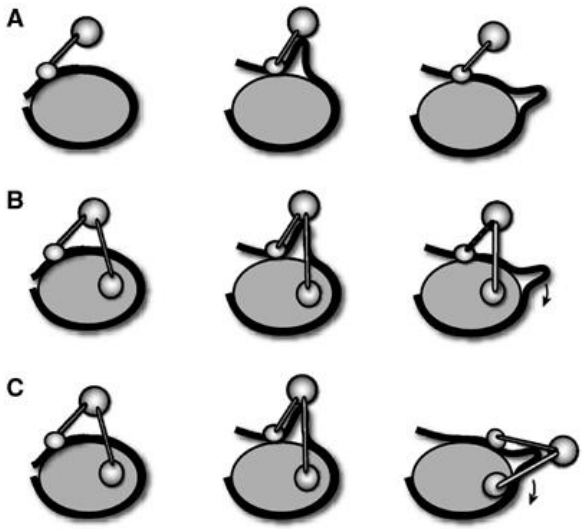

Figure 1.11: The possible models proposed to explain the increased sliding efficiency ISWI with ACF1. (A) On its own ISWI is known to interact with the linker DNA. (B &C) ACF1 provides additional interactions with the nucleosome surface, "anchoring" the remodelling complex to the NCP. This allows better translocation of the DNA during the nucleosome sliding process (Eberharter et al. 2004).

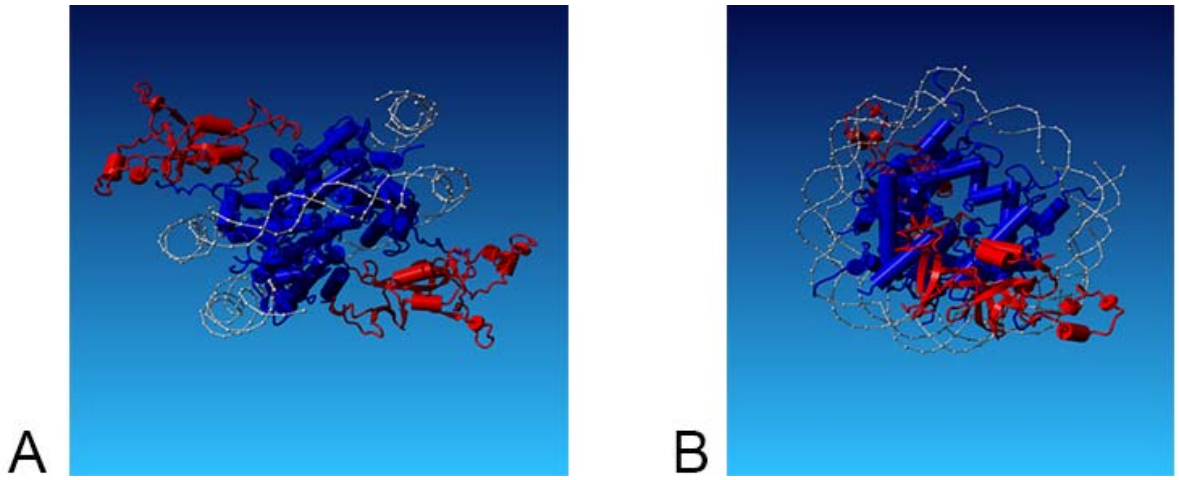

Figure 1.12: The SIR3 BAH domain (red) in association with the nucleosome core (blue). (A) The side view shows the pseudo two-fold symmetry. (B) The front view shows the BAH domain bound to the area in which the acidic patch is located. The crystal structure was obtained from PDB (ID: 3TU4) and rendered in YASARA (Armache et al. 2011).

Proteins of the CHD family have been implicated in a variety of cellular processes *in vitro* and *in vivo* (Marfella & Imbalzano 2007). Deletion strains for *Saccharomyces cerevisiae Chd1* (*ScChd1*) showed partial loss of chromatin assembly (Robinson & Schultz 2003). It has also been shown that ScCHD1 relocates nucleosomes to the centre of DNA fragments *in vitro* (Stockdale *et al.* 2006). In *Drosophila* dCHD1 could assemble chromatin without linker histone H1 but lost its chromatin assembly functionality in the presence of H1, giving it a putative function in assembly of transcriptionally active chromatin (Lusser *et al.* 2005). A number of studies also suggested that CHD1 is a possible transcription elongation factor by virtue of interacting with other proteins involved in transcriptional elongation (Woodage *et al.* 1997)(Kelley *et al.* 1999)(Krogan *et al.* 2002)(Krogan *et al.* 2003)(Srinivasan *et al.* 2005).

INO80 (inositol requiring 80) family. The INO80 family, containing the INO80 and SWR1 remodelling complexes, are very large complexes of more than 10 subunits and was first purified from *S.cerevisiae* (Bao & Shen 2007). The catalytic subunit of this family is highly related to that of the SWI/SNF family and contains a characteristic ATPase domain which is split in two by a 281bp spacer (Ebbert *et al.* 1999). The spacer contains binding motifs for an ARP protein and RVB1/2 (Walker *et al.* 1982). RVB1 and 2 are essential subunits of the complex that are conserved from yeast to mammals (Qiu *et al.* 1998)(Kanemaki *et al.* 1999)(Jonsson *et al.* 2004).

The SWR1 complex is highly related to the INO80 complex but has a unique function in replacing H2A-H2B dimers in canonical nucleosomes with variant H2A.Z-H2B dimers (Mizuguchi *et al.* 2004)(Krogan, Keogh, *et al.* 2003)(Kobor *et al.* 2004). BDF1 forms a part of this complex in *S.cerevisiae* and has been shown to bind to regions of H4 (Pamblanco *et al.* 2001)(Wu *et al.* 2009). Although the bromodomains in BDF1 would target this protein to acetylated lysine residues in the N-terminal extension of the histone, it has been shown to bind not only the 1-16 amino acid region, which can be acetylated, but also needs the first 59 residues of H4 for binding. It thus, in part, also binds the globular domain area adjacent to the N-terminal extension (Pamblanco *et al.* 2001).

1.2.1.4. The importance of remodelers in proper regulation of the human genome

The importance of chromatin remodelling complexes in the regulation of gene expression and genome maintenance is revealed by the number of diseased states linked to mutations affecting them (Nair & Kumar 2012)(Davis & Brackmann 2003)(Cho *et al.* 2004). Several syndromes as well cancerous states have been connected to chromatin remodelers (Cho *et al.* 2004). Cerebro-oculo-facio-skeletal syndrome (COFS), also known as Cockayne Syndrome, for

instance has been linked to mutations in the Cockayne Syndrome B DNA Repair-Transcription-Coupling Factor (CSB), a member of the SWI2/SNF2 remodelling family (Citterio *et al.* 2000)(Cho *et al.* 2013). CSB is thought to alter the nucleosome structure in such a way that DNA repair proteins can access DNA (Woudstra *et al.* 2002). COFS symptoms include, sensitivity to ultra-violet (UV), cataract growth, failure to thrive and severe neurological abnormalities (Citterio *et al.* 2000). Other syndromes include α-thalassemia X-linked mental retardation (ATRX-syndrome) and α-thalassemia myelodysplasia which are both caused by mutations in a SNF2-related ATPase (Xue *et al.* 2003). ATRX, Williams-Beuren syndrome is linked to the deletion of the transcription factor responsible for recruiting SNF2H to heterochromatic replication foci as well as CHARGE syndrome linked to low levels of CHD7 expression (Xue *et al.* 2003)(Baumann *et al.* 2008)(Vissers *et al.* 2004)(Poot *et al.* 2004).

Remodelling complexes' role in cancer is also widely studied (Wang *et al.* 2007)(Roberts & Orkin 2004). In the SWI/SNF complex SNF5 as well as the catalytic subunits have been linked to oncogenesis (Roberts & Orkin 2004). Mounting evidence suggests SNF5 is a tumour suppressive factor while both BRG1 and BRM has been shown to have properties of tumour suppressors (Roberts & Orkin 2004). The particularly aggressive human malignant rhabdoid tumors (MRTs) mostly coincides with bi-allelic loss of the SNF5 gene (Versteege *et al.* 1998). The probable tumour suppressive nature of SNF5 has been further established in studies of genetically targeted murine models (Guidi *et al.* 2001)(Klochendler-Yeivin *et al.* 2000)(Roberts *et al.* 2000)(Roberts *et al.* 2002). In approximately 30% of non-small-cell lung cancer and approximately 10% of primary non-small-cell lung cancers, coincidental loss of both the ATPase subunits of the SWI/SNF family, BRG1 and BRM, are detected (Reisman *et al.* 2003). The cancerous states linked to these concomitant losses have also been correlated with very poor prognosis (Reisman *et al.* 2003). Furthermore, bi-allelic loss of the *BRG1* gene has also been observed in pancreatic, lung, prostate and breast cancer cell lines, and BRG1 and BRM has been reported to interact with several known tumour suppressors *in vitro* (Roberts & Orkin 2004)(Bochar *et al.* 2000). However, the exact mechanism by which these subunits of SWI/SNF suppress oncogenesis remains unknown (Roberts & Orkin 2004).

Although much attention in the oncology field have been afforded to the SWI/SNF complexes and their involvement in oncogenesis, it is not the only complex which have been linked to cancer (Wang *et al.* 2007)(Clapier & Cairns 2009). Evidence suggests that members of the CHD family and the INO80 complex is also involved as INO80 family members in yeast are known to be recruited to DNA double-stranded breaks to mediate repair pathways (van Attikum *et al.* 2004)(Morrison *et al.* 2004)(Tsukuda *et al.* 2005)(Price & D'Andrea 2013). In the CHD

family overexpression of metastasis-associated protein 1 to 3 (MTA1-3), associated with the NuRD complex, has been connected to invasiveness in a multitude of cancers and is observed in more than 30% of primary carcinomas of the oesophagus, colon and stomach (Denslow & Wade 2007)(Bowen *et al.* 2004). The MTA proteins are also found to be overexpressed in breast tumours and has been confirmed to trigger tumourigenesis (Kumar *et al.* 2002)(Fujita *et al.* 2003).

The remaining challenge is to establish the direct way in which these remodeler mutations contribute to development of disease states (Cho *et al.* 2004). Upon elucidation of the pathologies underlying the various diseases, therapies can then be designed to directly target and overcome the aberrations (Clapier & Cairns 2009).

1.2.2. Chromatin Architectural Proteins (CAPs) with known interactions with the nucleosome globular domains

1.2.2.1. Silent information regulator 3 (SIR3)

As eukaryotic cells contain a complete set of genes needed for every possible cell type, gene silencing is a vital part of modulating cellular fate in eukaryotes (Loo & Rine 1995). The underlying mechanism involved in silencing seem to be conserved between *Saccharomyces cerevisiae* and higher eukaryotes, even though several diverse protein families are implicated across species (Rusche *et al.* 2003). Thus *Saccharomyces cerevisiae* modulation of mating type loci can be regarded as a prototype of silencing in eukaryotes (Rusche *et al.* 2003). In the determination of mating type of the yeast cells, the SIR proteins are crucial for silencing at the *HMLα* and *HMRa,* as well as ribosomal DNA (rDNA) loci and the telomeres (Rusche *et al.* 2003)(Loo & Rine 1995)(Ivy *et al.* 1986). It has long been postulated that these SIR proteins work in *trans* to establish domains of silent chromatin by association with the nucleosome to create repressive chromatin architectures (Rusche *et al.* 2003). The observation that the SIR proteins, and even SIR3 independently, can assemble compact nucleosomal arrays *in vitro,* supports this theory (McBryant *et al.* 2008)(Martino *et al.* 2009)(Johnson *et al.* 2009). Generally it is believed that SIR1 targets the silencing proteins to the specific chromatin areas by interacting with the origin recognition complex (ORC) component ORC1 (Hsu *et al.* 2005)(Hou *et al.* 2005). A stable SIR3/SIR4 complex is then in turn recruited to SIR1 through direct association of SIR1 and SIR4 (Bose *et al.* 2004). The SIR2 protein is a NAD-dependent histone deacetylase and association of the SIR1/2/4 complex with the chromatin results in hypoacetylated histones, to which the SIR3 protein is finally recruited (Marmorstein 2004)(Chang *et al.* 2003). Binding of SIR3 to the nucleosomes lead to propagation of silenced

chromatin *in vivo* (Kristjuhan *et al.* 2003). This important interaction between SIR3 and the nucleosome has been solved in a recent x-ray crystallography study (Armache *et al.* 2011).

The aforementioned study solved the crystal structure of the nucleosome core particle of *Xenopus laevis* in complex with the SIR3 bromo-adjacent homology domain (BAH_{SIR3}), containing mutation D205N known to enhance silencing. Strikingly, of the histone residues implicated in the BAH_{SIR3} interaction, only one (H4V21) differed between *Xenopus laevis* and *Saccharomyces cerevisiae* (Armache *et al.* 2011). This alludes to a conserved mechanism of silencing (Armache *et al.* 2011). Similar to the RCC1-nucleosome complex solved earlier, the BAH_{SIR3} interacted with the two faces of the nucleosome in a comparable manner forming a pseudo-two-fold symmetry (Figure 1.12)(Makde *et al.* 2010)(Armache *et al.* 2011). BAH_{SIR3} was shown to interact with the intrinsically disordered H4 tail, which adopted an ordered structure upon binding (Armache *et al.* 2011). Further interaction was established with regions in the globular part of the nucleosome (Armache *et al.* 2011). These regions included the loss of rDNA silencing (LRS) domain formed by H3 and H4 as well as a region of H2B neighbouring the LRS and H2A/H2B acidic patch surfaces (Armache *et al.* 2011). The protein-protein interactions are fixed by flexible regions in the proteins which become ordered upon interaction (Armache *et al.* 2011). Further stabilisation of the BAH_{SIR3} interaction with the globular nucleosome domain surfaces is mediated by acetylation of the N-terminus of the SIR3 protein (Arnaudo *et al.* 2013).

1.2.2.2. Cytokine interleukin-33 (IL-33) and viral proteins

IL-33 is a protein with dual-function as a cytokine and an intracellular nuclear factor found to tether to chromatin by binding to the H2A-H2B acidic patch (Roussel *et al.* 2008). Curiously this is done by the same mechanism Karposi sarcoma herpesvirus (KSHV) and more recently human cytomegalovirus (hCMV) has been shown to utilise for the attachment of their genomes to host chromatin (Mucke *et al.* 2014)(Barbera *et al.* 2006). KSHV achieves this by docking of the latency-associated nuclear antigen (LANA) peptide to the H2A-H2B acidic patch while hCMV docks its major immediate early 1 (IE1) protein in the same fashion (Mucke *et al.* 2014)(Barbera *et al.* 2006). As the acidic patch is important for regulation of chromatin compaction it came as no surprise that the IL-33 chromatin-binding motif was found to alter chromatin compaction *in vitro* by enhancing condensation via self-association of the nucleosomal arrays (Chodaparambil *et al.* 2007)(Zhou *et al.* 2007) (Roussel *et al.* 2008). This increased condensation is also in line with the transcriptional repressive function of IL-33 and the same effects on the chromatin has been observed in the presence of KSHV LANA (Carriere *et al.* 2007). *In vivo* IL-33 alters nuclear organisation in the same way as KSHV LANA

(Chodaparambil *et al.* 2007). It is however still unknown what the specific transcriptional targets and effects of IL-33 are (Miller 2011).

Naturally the interaction between viral proteins and host chromatin is of great interest as further characterisation of such interactions can lead to identification of new therapeutic targets for disease treatment (Barbera *et al.* 2006)(Mucke *et al.* 2014). KSHV LANA has been implicated in viral genome tethering and maintenance (Barbera *et al.* 2006). By linking the viral DNA to the host genome the viral genome is retained in the nucleus and maintained by the host's cellular machinery (Barbera *et al.* 2006)(Ohsaki & Ueda 2012). This bridging also allows the viral genome to be "passed on" to daughter nuclei during cell division, maintaining latent infection (Ohsaki & Ueda 2012). It would be interesting to see if these functions are conserved in other DNA-viruses and what the possible therapeutic implications are.

1.2.2.3. Regulator of chromosome condensation 1 (RCC1)

RCC1 is a β-propeller protein known to bind the nucleosomes directly to recruit the RAN enzyme to the chromatin (Renault *et al.* 1998). It also activates the enzyme's nucleotide exchange activity through direct interactions with the enzyme as well as the nucleosome (Hadjebi *et al.* 2008). The concentration gradient of the GTPase Ran enzyme surrounding the chromosomes regulates cellular functions that are vital for eukaryotic cell survival (Renault *et al.* 2001)(Hadjebi *et al.* 2008). These include macromolecule transport between the nucleus and cytoplasm and development of the nuclear envelope and mitotic spindles (Makde *et al.* 2010)(Carazo-Salas *et al.* 1999)(Clarke & Zhang 2008)(Kalab & Heald 2008). A 2010 crystallography study revealed that the RCC1-nucleosome complex forms a pseudo-two-fold symmetry much like the SIR3-nucleosome complex (Figure 1.13)(Makde *et al.* 2010).

1.2.2.4. High mobility group nucleosome-binding domain-containing protein 1 and 2 (HMGN1 and HMGN2)

These two structurally related proteins are part of a larger, highly abundant high mobility group nucleosomal (HMGN) protein family found in all higher eukaryotes (Bustin & Reeves 1996)(Bustin *et al.* 1995). This family of proteins modulates a variety of chromatin functions, including but not restricted to transcription (Kato *et al.* 2011). HMGN1 and 2 bind to the core histones, independent of the DNA sequence or modification status of residues in the N-terminal extensions (Shirakawa *et al.* 2000)(Shimahara *et al.* 2013). It is suggested that binding of HMGN proteins to the chromatin causes compacted chromatin to decondense which subsequently upregulates DNA-dependent activities (Crippa *et al.* 1993)(Tremethick & Hyman 1996)(Trieschmann *et al.* 1995)(Vestner *et al.* 1998).

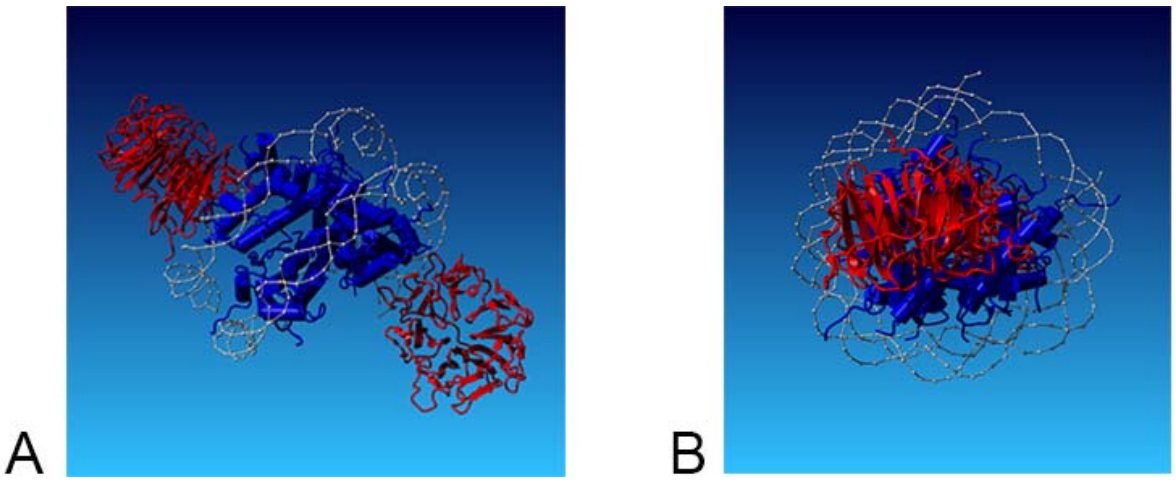

Figure 1.13: The RCC1-nucleosome core particle complex as obtained from PDB (ID: 3MVD) and rendered in YASARA. (A) The same pseudo two-fold symmetry as observed for the SIR3 BAH domain-NCP complex is observed as the two RCC1 molecules (red) form identical interactions on the opposite sides of the NCP (blue). (B) A front view (looking down the DNA superhelical axis)(Makde *et al.* 2010).

The basis of interaction between the HMGN proteins and the nucleosomes is largely unknown (Kato *et al.* 2011). A recent study revealed the mode of interaction between HMGN2 and the nucleosome by virtue of a combination of methyl-transverse relaxation optimized nuclear magnetic resonance spectroscopy (NMR)(Kato *et al.* 2011). They demonstrated that HMGN2 binds to the H2A-H2B acidic patch as well as the DNA near the entry/exit point, thus effectively fixing the DNA to the nucleosome and interfering with the binding of the linker histone H1 (Kato *et al.* 2011). The chromatin decondensation effect of the HMGN binding might be attributed to this inhibition of the binding of H1 (Kato *et al.* 2011). The association with both the nucleosome globular surface and the DNA will stabilise the nucleosome and may work in direct opposition with the ATP-dependent chromatin remodelers by either sterically hindering the remodelling complexes from association with the nucleosome or by limiting DNA movement (Kato *et al.* 2011). The long C-terminal tail of the HMGN2 protein effectively prohibits the linker histone from binding to the dyad axis and linker DNA (Thomas 1999)(Kato *et al.* 2011). Phosphorylation of Ser24 and Ser28 during mitosis has been shown to cause the dissociation of the HMGN proteins from the chromatin (Prymakowska-Bosak *et al.* 2001). Structurally this made sense as these two residues are located in close proximity to the acidic patch and the negative charges attributed to the phosphor groups will produce repulsive electrostatic interactions with the acidic patch, destabilising the complex (Kato *et al.* 2011). This is a good illustration of how the interplay between different non-histone chromatin proteins and chemical modifications dictates cellular function. The HMGN family of proteins is a large constituent of the nuclear make-up and plays a key role in the regulation of chromatin structure and function and thus determination of cell fate (Bianchi & Agresti 2005).

1.3. Aim of this study

It seems possible that chromatin remodelling factors and architectural proteins play a key role in regulating DNA functions by determining accessibility of the different regions of the nucleosomal surface. To clarify the underlying mechanisms of regulation it is important to understand the different protein-protein interactions within the context of the dynamic chromatin structure. The importance of these regulatory proteins and their proper interaction with chromatin is clearly demonstrated by the various disease states which arises upon their mutation (Wang *et al.* 2007)(Hadjebi *et al.* 2008)(Miller 2011)(Burgess & Zhang 2013)(Cho *et al.* 2004). In our pursuit of new therapeutic approaches understanding the underlying mechanisms of chromatin regulation is crucial. Up until now the focus has mainly been on the modification status of histones and the interaction of the modified residues with regulatory proteins (Strahl & Allis 2000). An example of this is the selective inhibition of bromodomains in

oncoprotein BRD4 by cell-permeable small molecule JQ1 (Filippakopoulos *et al.* 2010). BRD4 has been implicated as a partner in the fusion oncogene in an aggressive, incurable squamous carcinoma caused by recurrent chromosomal translocation (French *et al.* 2001)(French *et al.* 2003). JQ1 displaced the fusion oncogene from the chromatin by binding the bromodomains in BRD4, disrupting their association with the histones' acetylated lysines (Filippakopoulos *et al.* 2010). This has been shown to stop proliferation of the BRD4-dependent cancer cell lines (Filippakopoulos *et al.* 2010). This demonstrated a proof-of-concept for specific targeting of protein-nucleosome interactions in the treatment of cancer. As our knowledge of such interactions improves, new possible avenues for therapeutic advances become available. A significant amount of research focusses on the identification of all chromatin readers as well as small molecules that interfere with the binding of protein domains known to bind the specific epigenetic patterns of the histone N-terminal extensions (Hoelder *et al.* 2012).

Although it is known that the lateral and distal surfaces of the nucleosome is important for the binding of chromatin remodelers as well as other regulatory proteins, no systematic study has been carried out to identify all proteins that bind to these surfaces. As these proteins are so important for proper genome regulation these surfaces are also probable targets for therapeutic approaches in diseases of transcriptional mis-regulation such as cancer. Mounting evidence also suggests that interaction with the nucleosome is crucial for certain DNA viruses' episome persistence and thus one of the main determinants of persistent latent infections of viruses such as the Herpesvirus family (Barbera *et al.* 2006)(Mucke *et al.* 2014). Interfering with these interactions might provide ways in which to oust these latent viral infections from the host. Thus, in an attempt to better understand the regulation of DNA function, we aim with this project to isolate and identify proteins that bind the non-tail parts of the nucleosome.

Chapter 2

Material and Methods

2.1. Introduction

This study utilised affinity purification in conjunction with mass spectrometry to identify proteins bound to specific domains of the NCPs. Central to the feasibility of this project is the ability to over-express *Xenopus laevis* histone proteins and combine them with a defined sequence DNA fragment to reconstitute nucleosome core particles (NCPs). The comprehensive protocol for this approach as developed and published by Luger *et al.* was adapted and used to complete the first part of the study (Luger *et al.* 1999). Before the publication of this protocol *in vitro* studies concerned with nucleosomes were limited to the use of histones and nucleosomes isolated from chromatin extracted from nuclei. Luger's approach has several advantages over this approach (Luger *et al.* 1999). It achieves higher yields of histone proteins which are not degraded by contaminating proteases (Mellado *et al.* 1983). It also does not rely on the availability of fresh blood or tissue. With this approach it is possible to produce homogenous samples where methods relying on natural sources yield varying levels of heterogeneity due to different histone isotypes and posttranslational modifications (Luger *et al.* 1999)(Rill *et al.* 1975). This heterogeneity depends on the type of tissue used as well as the developmental stage at which the tissue was harvested (Luger *et al.* 1999)(Rill *et al.* 1975). Histones of which the *N*-terminal extensions have been removed would be lethal *in vivo*. Thus this *in vitro* approach was the best way to study these specific protein interactions.

The four core histone proteins from *Xenopus laevis*, H2A, H2B, H3 and H4, was expressed both as full length canonical proteins and *N*-terminally truncated, globular domains. Two plasmids (obtained from Karolin Lüger at Colorado State University), pXLHW7 and pXLHW8, containing the *Xenopus laevis* histone gene cluster were utilised as the source of the coding regions for core histones H2A, H2B, H3 and H4 full-length proteins as well as for the globular domains (Old *et al.* 1982). The plasmid containing the defined sequence fragment was obtained from Bing Li's lab at Texas A&M. The plasmid was constructed as described by Richmond and Searles in 1988 to contain 16 repeats of a 146mer derived from human α-satellite DNA in a pUC-based vector (Luger, Mäder, *et al.* 1997)(Richmond *et al.* 1988). The isolated histones were reconstituted onto the defined sequence DNA fragment into which a biotinylated dCTP was incorporated. This allowed the affinity purification or "pull-down" of the NCP with streptavidin-coated beads. Before the pull-down, the reconstituted NCPs were incubated with proteins extracted from *Saccharomyces cerevisiae*. This way all the proteins which bound the

NCPs were pulled down upon incubation with the streptavidin-coated beads. To identify the binding partners the proteins were trypsinized and analysed on an in-house AB SCIEX 4000 QTRAP mass spectrometer. What follows is a brief introduction to the major techniques used in this study.

2.1.1. Gel-filtration. Gel filtration is the chromatography technique used to purify the individual histone proteins as well as the refolded nucleosomes. Gel filtration chromatography, also known as size exclusion, separates biomolecules according to their size (Malawer & Senak 2003). Like all chromatography techniques the sample was dissolved in the mobile phase (in this case the protein buffer) which then carries it through the stationary phase. The stationary phase is the phase which will remain fixed in place while the mobile phase moves through it, carrying the analytes (Malawer & Senak 2003). In gel-filtration, unlike other liquid chromatography techniques, the proteins will not bind to the stationary phase (Malawer & Senak 2003). The stationary phase consists of inert, porous beads into which small proteins will enter (Malawer & Senak 2003). In this way smaller proteins will be retained longer on the column than larger proteins which will pass through the gaps between the beads (Malawer & Senak 2003). Different commercially available mediums have different pore sizes which will determine their different fractionation ranges (Twyman 2004). Sephacryl S-200 HR was chosen as a gel-filtration medium for the purification of the individual histones as well as the refolded octamers because of its broader fractionation range (5kDa – 250kDa), chemical stability and tolerance of high flow rates (10 – 35 cm/h)(Amersham Biosciences 2002). This is an example of preparative chromatography which is a purification technique used to separate molecules at higher concentrations to be used for downstream experiments.

2.1.2. Affinity purification. To be able to screen for previously unknown proteins interactions with the globular domain of the NCP this study employed affinity purification partnered with mass spectrometry. Affinity purification is a biochemical purification technique which exploits a highly specific interaction such as interactions between proteins and ligands, antibodies and antigens or enzymes and their substrates (Dunham *et al.* 2012). One of the interaction partners is fixed onto the molecule of interest while the other partner is bound to a stationary phase (Dunham *et al.* 2012). When the interaction takes place the molecule of interest can be retained. In this study biotin was fixed onto a “bait” protein complex (the NCP) which was then used to “fish” for its interaction partners.

Streptavidin is a protein with an affinity for biotin as a ligand. The interaction between streptavidin and biotin is the strongest known non-covalent interaction ($K_d = 10^{-15}$M)(Chodosh 2001). Once formed this interaction is very stable even in the presence of organic solvents,

chaotropic agents, detergents, proteases, and extreme temperatures and pH ranges (Chodosh 2001). These characteristics are useful for affinity purification and biotin-streptavidin purification has been widely adopted in the molecular biology field. This study used magnetic beads covered with streptavidin and biotinylated dCTP incorporated into the DNA fragment by means of a restriction digest followed by a Klenow reaction. This could then be bound to the streptavidin-coated magnetic beads which could be pulled down with a magnet.

2.1.3. High performance liquid chromatography (HPLC). High performance liquid chromatography (HPLC) uses a pump to push a pressurised solvent (mobile phase) through a column packed with the stationary phase (Cunico *et al.* 1998a). The stationary phase used here was octadecyl carbon chain (C18)-bonded silica. This is a reversed phase HPLC technique for peptide separation based on analyte hydrophobicity (Cunico *et al.* 1998b). The C18 bound to the silica creates a hydrophobic stationary phase with a high affinity for the hydrophobic residues in the peptides (Cunico *et al.* 1998b). Hydrophobic peptides will bind to the column and can then be eluted by decreasing the polarity of the mobile phase by addition of a non-polar organic solvent at a gradient (Cunico *et al.* 1998b). The more hydrophobic peptides will elute last when the polarity of the mobile phase is at its lowest while the more hydrophilic peptides will elute first (Cunico *et al.* 1998b). This is an example of analytical chromatography where smaller amounts of sample is processed for direct analysis by a detector. In this study the mass spectrometer was applied as a detector.

2.1.4 Electrospray ionisation (ESI). To ionise the analytes we used electrospray ionisation (ESI), the most widely used ionisation technique. Briefly, ions are produced by applying a high voltage to a liquid to generate an aerosol of charged droplets from which the liquid is evaporated to yield gas phase ions (Wilm 2011). It is used for both chemical and biochemical analysis due to its low chemical specificity (Wilm 2011). As it ionises the analytes directly from the liquid phase it is easily combined with a high performance liquid chromatograph (HPLC) for molecular fractionation prior to analysis by the mass spectrometer (Ho *et al.* 2003). Electrospray generated ions are very stable unlike the ions released by matrix-assisted laser desorption ionisation (MALDI) that are often in the excited state and will decay rapidly (Ho *et al.* 2003). Because ionisation is not limited by molecular mass and the high ionisation efficiency of this technique it is ideal for the biomolecular sciences (Ho *et al.* 2003).

2.1.5. Mass spectrometry (MS). For the mass spectrometric analysis we used an AB SCIEX 4000 QTrap hybrid triple quadrupole linear ion trap mass spectrometer. A typical protein identification method on this instrument consists of 4 steps. (1) A survey scan over a defined mass range is performed to identify the precursor peptides that are being introduced into the

MS. This mass range ideally needs to be optimised for the particular experiment and analytes. Broader mass ranges require longer scan times which increases the cycle time. Shorter cycle times are beneficial as the instrument is essentially blind to ions entering while it is busy with a scan. Because this study analysed an unknown protein sample, containing unknown peptide masses a broader mass range of 400-1200 Da was used. (2) Information gathered in the survey scan is compared to the information dependent acquisition (IDA) parameters defined by the user in the second step. The IDA parameters determine the number of most intense peaks the software will pick for tandem MS analysis. (3) The ions picked by the software will then be subjected to an enhanced resolution scan to determine their charge states. Because the AB SCIEX 4000 QTrap is a lower resolution instrument with a nano-electrospray source only doubly and triply charged ions are considered in an effort to eliminate background noise from singly charged contaminants. (4) The final step is the enhanced product ion (EPI) scan. For this step the ions selected during the enhanced resolution scan is fragmented in the collision cell before being collected in the ion-trap to increase the concentration that can be scanned and recorded by the detector. The EPI scan yields the final data which is converted to a peak list and searched against the Swiss-Prot database by the Mascot software (Matrix Science) package. This enables the identification of peptides which can be used for protein assignment.

2.1.6. Data analysis. Although a number of popular databases exist online Swiss-Prot is a good database to use for a sample containing unknown proteins as it is a high quality, curated database with well annotated entries. As it is a non-redundant database it is small and it is easier to obtain statistically significant identifications. Our study also seek to identify proteins from *Saccharomyces cerevisiae*, a well-characterised organism which is well-represented in the Swiss-Prot database.

The following section is a detailed breakdown of the experimental procedures that were followed.

2.2. General materials used

2.2.1 Strains and plasmids used in this study

Table 2.1: Plasmids

Plasmid	Backbone vector	Promoter	Cloned Gene	Gene Origin	Terminator	Selective marker
H2A-expression-vector	pET3a	T7	*Xenopus laevis* histone H2A gene	pXLHW8	T7	Ampicillin
H2B-expression-vector	pET3a	T7	*Xenopus laevis* histone H2B gene	pXLHW7	T7	Ampicillin
H3-expression-vector	pET3d	T7	*Xenopus laevis* histone H3 gene	pXLHW7	T7	Ampicillin
H4-expression-vector	pET3a	T7	*Xenopus laevis* histone H4 gene	Synthesized	T7	Ampicillin
gH2A-expression-vector	pET3a	T7	*Xenopus laevis* histone H2A gene	pXLHW8	T7	Ampicillin
gH2B-expression-vector	pET3a	T7	*Xenopus laevis* histone H2B gene	pXLHW7	T7	Ampicillin

gH3-expression-vector	pET3d	T7	*Xenopus laevis* histone H3 gene	pXLHW7	T7	Ampicillin
gH4-expression-vector	pET3a	T7	*Xenopus laevis* histone H4 gene	Synthesized	T7	Ampicillin
pBL634-196-16x	pBluescript	n/a	Part of the 5 S RNA gene	Synthesized	n/a	Ampicillin
pLysS			T7 Lysozyme			Chloramphenicol

Table 2.2: Strains

Strain	Genotype	Source
Escherichia coli **Top10 competent cells**	*F- mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 nupG recA1 araD139 Δ(ara-leu)7697 galE15 galK16 rpsL(Str^R) endA1 λ$^-$*	Invitrogen
Escherichia coli **BL21-Gold(DE3) competent cells**	*F$^-$ ompT gal dcm lon hsdS$_B$(r$_B^-$ m$_B^-$) λ(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5])*	Agilent
Escherichia coli **Rosetta^{TM}(DE3)pLysS competent cells**	*F$^-$ ompT hsdS$_B$(r$_B^-$ m$_B^-$) gal dcm (DE3) pLysSRARE (Cam^R)*	Merck Millipore
Saccharomyces cerevisiae **BY4742**	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	UFS laboratory stocks

2.2.2 Cultivation media and growth conditions

For this study the following cultivation media was used:

Luria-Bertani (LB) broth*: 1% (w/v) Tryptone, 0.5% (w/v) Yeast extract, 1% (w/v) NaCl, pH 7.0

Psi broth: 2% (w/v) Tryptone, 0.5% (w/v) Yeast extract, 0.5% (w/v) $MgSO_4.7H_2O$, pH 6.0

2xTY broth: 1.6% (w/v) Bacto-tryptone, 1% (w/v) Yeast extract, 0.5% (w/v) NaCl, 0.1% (w/v) Glucose

YPD broth*: 1% (w/v) Yeast extract, 2% (w/v) Peptone, 2% (w/v) Glucose

SOC broth: 0.5% (w/v) Yeast extract, 2% (w/v) Tryptone, 10mM NaCl, 2.5mM KCl, 10mM $MgCl_2$, 10mM $MgSO_4$, 20mM Glucose

*LB as well as YPD plates were also used. For agar plates 1.5% agar was added to broth recipes.

2.2.3 Kits, enzymes and other consumables used

Dialysis

Snakeskin dialysis tubing (MW cut-off of 7kDa) obtained from Thermo Scientific was used to dialyse the individually purified histones (2.4) as well as to refold the octamer (2.5).

For concentrating the octamer (2.5) Amicon® membranes with a molecular weight (MW) cut-off of 15kDa was used.

For reconstitution of the NCP Single Use Micro DispoDialyzers from Harvard Apparatus with a MW cut-off of 5kDa were used.

Enzymes

EcoRV, AvaII and Klenow Fragment which was used in the preparation of the defined sequence DNA fragment were all obtained from Thermo Scientific. Trypsin was from Promega.

Molecular weight markers.

DNA Molecular Weight Marker III from Roche and the 100bp DNA ladder from Invitrogen were used as DNA ladders.

Unstained molecular marker from Thermo Scientific was used as a protein ladder.

Plasmid purification kits

Plasmids were purified using a Promega PureYield™ Maxiprep System.

Concentration determination

For all protein and DNA samples concentration was determined with the aid of an Invitrogen Qubit® fluorometer. For protein concentrations the accompanying Qubit® Protein Assay Kit was used while DNA samples were analysed using the Qubit® dsDNA BR Assay Kit.

2.3. Methods used

2.3.1 Reconstitution of Nucleosome Core Particles (NCP) from *Xenopus laevis* histones and defined-sequence DNA fragments

2.3.1.1. Transformation of *BL21(DE3)Gold* for expression

Competent *BL21(DE3)Gold* as well as *TOP10* cells were prepared by means of an adapted version of the $RbCl_2$ method as described by Hanahan (Hanahan 1983). For this, 2 separate tubes of 5mL LB broth was inoculated with *BL21(DE3)Gold* and *TOP10* cells from glycerol stocks stored at -80˚C and incubated overnight at 37˚C with aeration. Psi broth (100mL) was inoculated with 1mL of this pre-inoculum. The cultures were grown to OD_{600} of 0.4-0.6. The cultures were cooled down on ice for 15 min and cells were harvested by centrifugation at 4˚C. Pellets were resuspended in 40mL ice cold TFB1 medium and incubated on ice for 15 min where after cells were spun down again. Pellets were resuspended in 4mL TFB2 and incubated on ice for 15-60 min. Aliquots of 50µL were snap frozen in liquid nitrogen and stored at -80˚C.

Aliquots (50µL) of the competent cells were simultaneously transformed with a pET-histone expression plasmid and pLysS by adding 100ng of each plasmid to the cells and incubating the mixture on ice for 1 hour. The cells were subjected to heat shock at 42˚C for 42 seconds and subsequently incubated on ice for 2 min. One millilitre of SOC medium was added to the cells and cells were resuscitated at 37˚C with gentle agitation for 60 min. The cells were spun down for approximately 30 seconds and most of the supernatant was discarded leaving about 300µl in which the cells were resuspended. The resuspended *BL21(DE3)Gold* cells were plated on LB agar plates containing 100 µg/mL ampicillin and 34 µg/mL chloramphenicol and incubated at 37°C overnight.

2. 3.1.2 Test expressions

Methods for test expressions as well as large-scale expressions and histone purification were adapted from protocols by Luger *et al.*(Luger, Thomas J Rechsteiner, *et al.* 1999). Four tubes with 5mL 2X TY plus ampicillin (100µg/mL) and chloramphenicol (34µg/mL) were inoculated with a single colony each. This was incubated with agitation at 37°C to OD_{600} between 0.3 and 0.6. Glycerol stocks were prepared of each culture by adding 0.5mL thereof to 0.5mL sterile glycerol (30%). In all but one of the remainder of the cultures expression was induced by addition of Isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 0.2mM. The uninduced sample served as a negative control. Samples were incubated for a further 2 hours at 37°C. Finally the cells were harvested by centrifugation and boiled in 100µl protein gel sample buffer (5% SDS, 20% glycerol, 250mM DTT, 65mM pH 6.8 Tris, 0.2% bromophenol blue). The samples were analysed on a 15% SDS-PAGE gel to determine expression levels.

2.3.1.3 Large-scale expressions

Based on the test expression results colonies with high expression levels were chosen for the large-scale expression. The glycerol stocks for these cultures were used to inoculate 3 aliquots of 5mL 2X TY broth supplemented with ampicillin and chloramphenicol. These were incubated at 37˚C overnight but no longer than 12 hours. The overnight cultures were used to inoculate three 500mL Erlenmeyer flasks containing 100mL TY broth which was then incubated with shaking at 37˚C until slightly turbid. Finally three 5L Erlenmeyer flask with 2L 2X TY broth were each inoculated with 100mL pre-culture. The 2L cultures were incubated at 37˚C whilst shaking at 180rpm until an OD_{600} of approximately 0.6 has been reached. Expression was induced by adding IPTG to a final concentration of 0.2mM. Cultures were incubated at 37˚C for a further 2 hours for the expression of histones (3 hours for both globular and canonical H2B). All cells were harvested by centrifugation, resuspended in 50mL wash buffer (50mM Tris-HCl, pH7.5, 100mM NaCl, 1mM Na-EDTA, 1mM Benzamidine, 5mM β-mercaptoethanol) and flash frozen in liquid nitrogen.

2.3.1.4 Purification of histones

2.3.1.4.1. Inclusion body preparation. The frozen cell pellets were thawed at 37˚C to initiate lysis. The thawed samples were passed through a One Shot Model cell disrupter (Constant Systems) at 28kPSI. Inclusion bodies were harvested by centrifugation at 12 000*xg* for 20 min and washed once with wash buffer, 1% Triton X-100 (50mM Tris-HCl, pH 7.5, 100mM NaCl,

1mM benzamidine, 1mM β-mercaptoethanol). Dimethyl sulfoxide (DMSO) was added to the drained pellet to solubilise the inclusion bodies before the pellet was resuspended in unfolding buffer (7M Guanidium hydrochloride, 20mM Tris-HCl, pH 7.5, 10mM DTT). Residual insoluble debris was removed by centrifugation.

2.3.1.4.2. Purification by gel filtration. The supernatant containing the unfolded histone proteins was loaded onto an equilibrated Sephacryl S-200 column (2.5x75cm; V_0 ~ 120mL; V_i ~ 370mL). Proteins were eluted at a flow rate of 2mL/min and the elution profile was recorded at an ultra-violet wavelength of 280nm. Peaks were collected in 5mL fractions. Fractions were analysed with 15% SDS-PAGE and those containing the histone proteins were pooled. The pooled sample was dialysed at 4˚C against three changes of 2mM β-mercaptoethanol. After dialysis the proteins were lyophilised. Lyophilised proteins were dissolved in pure, sterile water and concentrations thereof were determined. Aliquots of 2mL were snap-frozen in liquid nitrogen and stored at -80˚C until needed.

2.3.1.5 Refolding and purification of the histone octamers

2.3.1.5.1. Refolding. Aliquots making up approximately 8mg of each of the four core histones were thawed on ice. These were then dissolved to approximately 2mg/mL in freshly prepared unfolding buffer and allowed to unfold for 1 hour. The concentrations of the unfolded proteins were determined and the four histones were mixed in equimolar ratios. The mixture was dissolved in unfolding buffer to a final concentration of 1mg/mL. This was dialysed at 4˚C against 3 changes of refolding buffer (2M NaCl, 10mM Tris-HCl, pH 7.5, 1mM Na-EDTA, 5mM β-Mercaptoethanol). This refolding process was completed for both the canonical as well as the globular histones.

2.3.1.5.2. Purification by gel filtration. The dialysed sample was loaded onto the Sephacryl S-200 column previously used to purify histones after the column was washed and equilibrated with refolding buffer. Proteins were eluted at a flow rate of 2mL/min at 4˚C and peaks were collected in 5mL fractions. Fractions were analysed on a 15% SDS-PAGE gel and the fractions containing the octamer were pooled. The pooled fractions were concentrated in an Amicon® stirred cell to a final concentration of approximately 1mg/mL. Aliquots of 500µL were snap-frozen in liquid nitrogen and stored at -80˚C.

2.3.1.6 Preparation and purification of the defined-sequence DNA fragment

As previously noted a plasmid containing 16 repeats of the desired DNA fragment was obtained from Bing Li's laboratory. The cloning strategy for this plasmid was outlined by Dyer *et al.* (Dyer *et al.* 2004).

2.3.1.6.1. Preparation of 202bp blunt fragment. The plasmid was propagated in *Escherichia coli* Top10 cells. Plasmid DNA was purified from the cells with a Promega PureYield™ Plasmid Maxiprep system as per manufacturer specifications. The plasmid was digested with 30 units EcoRV per nanomole cutting site at a plasmid concentration of 1 mg/mL. For complete large-scale digestion the digest reaction was incubated at 37°C overnight. The digested plasmid DNA was run on a 1% agarose gel to check for completion.

The 202bp EcoRV fragment was separated from the plasmid backbone by adding 0.192 volumes of 4M NaCl and 0.346 volumes of 40% PEG 6000 to the reaction and then incubating on ice for at least 1 hour. Vector DNA was spun down at 27 000 *xg* and 4°C for 20 min. The supernatant was decanted into a clean tube and both the supernatant and the pellet (resuspended in 1mL distilled H_2O) was checked on a 1% agarose gel for cross contamination. For precipitation of the 202bp fragment 2.5 volumes of 100% cold ethanol was added and the DNA was spun down as before. After the supernatant was decanted and the remaining DNA was air dried briefly, the fragment was resuspended in 5 mL TE buffer.

2.3.1.7 Reconstitution of NCP and high resolution gel shift assay

To correct for possible errors in concentration determinations titrations were performed by varying the molar ratio of DNA fragment to histone complexes. For the first experiment ratios of 1.0:1.0, 1.1:1.0, 1.2:1.0, 1.3:1.0 and 1.4:1.0 DNA to octamer ratios were used. To achieve this, the DNA concentration was kept at 0.02nM while the octamer concentrations were varied. Separate Single Use Micro DispoDialyzers (Harvard Apparatus) were set up with the different ratios of DNA to octamer. NaCl concentration was adjusted to 2 M before adding octamer and the final volume was adjusted to 100µl. Stepwise dialysis at 4˚C against 300 mL each of TCS buffer (20mM Tris-HCl, pH7.5, 1mM EDTA, 1mM DTT) containing 2 M, 0.85 M, 0.65 M and 0.2 M NaCl was performed for 90 min per step. The content was removed from the DispoDialyzers by brief centrifugation and analysed using the high-resolution gel-shift assay described below. As discussed in the results (chapter 3), a lot of unbound DNA was observed and subsequently the ratios were taken up to 1.0:2.0, 1.0:2.5, 1.0:3.0, 1.0:6.0 DNA to octamer ratios. The experiment was repeated again with ratios of 3.0:1.0, 3.5:1.0, 4.0:1.0, 4.5:1.0, 5.0:1.0, and 5.5:1.0.

Samples were analysed on a 6% polyacrylamide running gel with a 4% polyacrylamide stacking gel under non-denaturing conditions. The gel was pre-run in 1 x TAE buffer (40mM Tris-acetate, 1mM EDTA) for 1 hour at 4˚C and 100V before running the samples. The gel was stained with ethidium bromide. In this way the amount of DNA which shifted on the gel as it was bound by octamer complexes versus the unbound DNA which would run lower could be observed.

2.3.2. Preparation of labelled DNA fragment, affinity purifications and mass spectrometric analysis of protein complexes

2.3.2.1 Preparation of biotinylated DNA fragment

The pBL634-196-16x plasmid was propagated and purified as discussed. It was then digested with AvaII overnight to yield a 169bp fragment with a -CWG 5' overhang. The 169bp fragment was purified by means of preparative electrophoresis as described previously (Hediger 1986). The Bio-Rad Mini Prep Cell was used to separate the restriction fragments. A 3% polyacrylamide gel solution (2,85% (w/v) acrylamide, 0.15% (w/v) methylenebisacrylamide, 0.115% (w/v) ammonium persulphate, 0.034% (w/v) Temed) was prepared and allowed to polymerise in the gel tube of the mini prep cell. TBE (100mM Tris, 100mM boric acid, 2mM EDTA pH 8.5) was used as an electrode and elution buffer. The electrophoresis was performed at 100V and elution buffer was pumped through the elution manifold with a peristaltic pump at 10mL/h. Fractions (1mL) were collected with a fraction collector and were analysed on a 1% (w/v) agarose gel. Fractions containing the desired fragment were pooled and ethanol precipitated.

Precipitated DNA was resuspended in pure water and the overhang was blunted with a Klenow reaction in which the standard dCTP was substituted by a biotinylated dCTP to yield a labelled fragment for the affinity purification. The DNA was precipitated with cold ethanol to remove any unincorporated dNTPs.

2.3.2.2 Nuclear extract preparation

2.3.2.2.1. Extraction of Saccharomyces cerevisiae nuclei. Two pre-cultures were prepared by inoculating 10mL YPD with *Saccharomyces cerevisiae* BY4742 and incubating them at 30˚C overnight. After the pre-cultures were checked under a microscope for contamination, they were used to inoculate 2X 1l YPD. This was incubated at 30˚C until an OD_{600} of 0.6 was reached. The cells were harvested by centrifugation and washed with sterile water. The washed pellet was resuspended in DTT/Tris Buffer (0.1M Tris-HCl pH 9.4, 10mM DTT) and incubated at 30˚C with shaking for 15 min after which it was pelleted by centrifugation at 3000*xg* for 5 min at 4˚C.

The pellet was washed once with buffer 2 (1.2M Sorbitol, 20mM Hepes pH 7.4, 1mM PMSF, 0.5µg/mL Leupeptin, 0.7µg/mL Pepstatin) and resusupended again in buffer 2. Zymolase (20mg) was added to the resuspended cells and cells were incubated at 30˚C for 1 hour. Ice cold buffer 3 (1.2M Sorbitol, 20mM Pipes pH 6.8, 1mM $MgCl_2$, 1mM PMSF, 0.5µg/mL Leupeptin, 0.7µg/mL Pepstatin) was added and ghosted cells were centrifuged at 3500*xg* for 5 min at 4˚C. The pellet was washed 3 times with nuclei isolation buffer (0.25M Sucrose, 60mM KCl, 14mM NaCl, 5mM $MgCl_2$, 1mM $CaCl_2$, 15mM MES pH 6.6, 0.8% Triton X-100, 1mM PMSF, 0.5µg/mL Leupeptin, 0.7µg/mL Pepstatin) to yield nuclei.

2.3.2.2.2. Ammonium sulphate precipitation of nucleic proteins. Nuclei were lysed by slowly adding 3M $(NH_4)_2SO_4$ (pH 7.6) to the slowly stirring nuclei solution until a final $(NH_4)_2SO_4$ concentration of 0.5M was reached. The solution was left to stir at 4˚C for 30 min. The insoluble mass was spun down at 28 000 g for 75 min and stored for analysis to make sure that no proteins of interest were pulled down with the debris and subsequently lost. The volume of the supernatant was measured and 0.35g of $(NH_4)_2SO_4$ was added per mL while slowly stirring at 4˚C for 30 min. The precipitated proteins were spun down at 20K *xg* for 30 min. The supernatant was removed and stored to be analysed. The pellet was briefly centrifuged again and any residual supernatant was removed before resuspension in 1 mL buffer C (20mM Hepes pH 7.6, 10mM $MgSO_4$, 10mM EGTA, 20% glycerol, 5mM DTT, 1mM PMSF, 0.5µg/mL Leupeptin, 0.7µg/mL Pepstatin) and dialysed against 2 changes of 500mL buffer C. Of the dialysed protein mixture 10µl was also analysed with the other fractions. The remainder of the protein solution was divided into 200µl aliquots and stored at -80˚C.

2.3.2.2.3. SDS-PAGE and tryptic digestion of fractions. The protein concentration of the different fraction were determined and resuspended in 10µl pure water and analysed on a 15% SDS-PAGE gel. Each gel lane were cut out and divided into 10 fractions. Each fraction was cut into roughly $1mm^3$ cubes and washed with water followed by 50% acetonitrile. This was repeated twice. The pieces were then shrunk with 100% acetonitrile. The shrunken, dried pieces were swollen in 10mM DTT in 0.1M NH_4HCO_3 and incubated at 56˚C for 45 minutes to reduce the proteins. The proteins were then alkylated by adding 55mM iodoacetamide in 0.1M NH_4HCO_3 to the pieces and incubating this for 30 minutes in the dark at room temperature. The water/acetonitrile washing steps were repeated and the pieces were shrunk again with 100% acetonitrile and dried. The digestion solution (10ng/µl Trypsin, 0.1M NH_4HCO_3) was added to provide a roughly 1:20 (w/w) ratio of protein to protease. This was incubated at 37˚C overnight. To wash out the digested peptides 5% formic acid was added to the gel pieces which were then incubated at room temperature for 15 minutes. After incubation the pieces were spun down

briefly at maximum rpm in the microfuge and the supernatant was transferred to a clean sample tube. This was repeated with 100% acetonitrile and then both washing steps were repeated again to wash out any residual peptides. The peptides were dried in a Thermo Scientific Savant^{TM} Speedvac^{TM} concentrator and analysed by mass spectrometer.

2.3.2.2.4. Mass spectrometric analysis. When analysing the sample by LC/MS/MS 8µl of each digest was injected by autosampler onto a C18 reverse phase trapping column [Agilent Zorbax 300SB, 5uM, 5.0 x 0.3mm] for sample desalting using an Agilent 1200 series capillary LC pump at 5uL/min. This was followed by gradient elution onto a custom packed C18 reverse phase column (150mm x 75uM) for peptide separation using an Agilent 1200 series nano LC pump at 0.600 uL/min. The elution gradient consisted of a 60 minute ramp from 10 to 25% Eluent B (100% Actetonitrile/0.1% formic acid) for a total run time including re-equilibration of 120 minutes. The eluting peptides were analyzed as previously described.

2.3.2.3 SIR3 BAH domain overexpression and purification for a positive control

A his-tagged recombinant protein consisting of the first 380 amino acids of the SIR3 protein was expressed using the plasmid and protocol published by Connelly *et al.* (Connelly *et al.* 2006). The pJC65 plasmid was obtained from the corresponding author of the publication and transfected into Rosetta^{TM}(DE3)pLysS competent cells.

2.3.2.3.1. Test expressions Test expressions were done as in 3.2. Four tubes with 5ml LB plus kanamycin (50µg/ml) were inoculated with a single colony each. This was incubated with agitation at 37°C to OD_{600} between 0.3 and 0.6. Glycerol stocks were prepared of each culture by adding 0.5ml thereof to 0.5ml sterile glycerol (30%). In all but one of the remainder of the cultures expression was induced by addition of IPTG to a final concentration of 1mM. The uninduced sample served as a negative control. Samples were incubated for a further 2 hours at 37°C. Finally the cells were harvested by centrifugation and boiled in 100µl protein gel sample buffer (5% SDS, 20% glycerol, 250mM DTT, 65mM pH 6.8 Tris, 0.2% bromophenol blue). The samples were analysed on a 15% SDS-PAGE gel to determine expression levels.

2.3.2.3.2. Large scale expressions Expression was scaled up to 6l culture as for the histones in chapter 2. One of the glycerol stocks prepared was used to inoculate 3 aliquots of 5mL LB broth supplemented with kanamycin (50µg/ml). These were incubated at 37˚C overnight but no longer than 12 hours. The overnight cultures were used to inoculate 3X 100mL LB broth which was then incubated in the shaker at 37˚C until slightly turbid. Finally 3X 2L LB broth were each inoculated with 100mL pre-culture. The 2L cultures were incubated at 37˚C whilst shaking at 180rpm until an OD_{600} of approximately 0.6 has been reached. Expression was induced by

adding IPTG to a final concentration of 1mM and 3% ethanol. Cultures were incubated at 37˚C for a further 6 hours. All cells were harvested by centrifugation, resuspended in 10ml HisTrap binding buffer (20mM sodium phosphate, 0.5M NaCl, 40mM imidazole pH7.4) and flash frozen in liquid nitrogen.

2.3.2.3.3. Lysis and purification of his-tagged protein. The frozen cell pellets were thawed at 37˚C to initiate lysis. The thawed samples were passed through a Constant Systems One Shot Model cell disrupter at 28kPSI. The lysate was centrifuged at 12 000g for 20 minutes to pellet any debris and insoluble matter. The supernatant were loaded onto a prepacked and precharged 1ml HisTrap™ FF column which was equilibrated with HisTrap binding buffer. The column was washed with binding buffer until a stable baseline was observed at 280nm. His-trapped proteins were released by gradient elution using elution buffer (20mM sodium phosphate, 500mM NaCl, 1M imidazole pH7.4). The protein was collected by an online fraction collector as it eluted and aliquoted to be used in positive control experiments.

2.3.2.4 Affinity purifications

For the affinity purification, canonical as well as globular NCPs were reconstituted with the labelled DNA using the same protocol as for the gelshift assay discussed in the previous chapter. Five biological replicates were performed for each of the experiments listed below. Replicates were pooled before MS analysis in an effort to overcome low concentrations.

2.3.2.4.1. Positive control. To validate the protocol a positive control was set-up using the BAH-domain of the Sir 3 protein which was overexpressed and purified as discussed in the previous section. The reconstituted NCP were incubated with the recombinant Sir3 domain for 2 hours with rotation in binding buffer at 4˚C. The protein complexes were immobilised on the beads by adding the mixture to 1.5mg washed Streptavidin-coupled Dynabeads® (Life Technologies) and incubating for a further hour at 4˚C. The beads and any proteins bound to them were pulled down with the DynaMag™-2 magnet and washed twice with binding buffer.

2.3.2.4.2. Affinity purification first set. The pull-down experiments were performed in two sets. For the first group of pull-downs 5 replicate reconstitutions were done for the canonical as well as the globular NCP. For each of the replicates 0.1nM DNA was used. Average concentrations of the octamers were used (discussed in chapter 3) and 0.4nM were added to the DNA. The reconstituted NCP were incubated at 4˚C for 1 hour with 1.5mg washed Streptavidin-coupled Dynabeads®. The unbound NCP was washed away by pulling down the beads with the DynaMag™-2 magnet and removing the supernatant. The beads were washed twice with binding buffer (20% glycerol, 20mM Tris-HCl pH 7.5, 1mM DTT, 1mM EDTA, 5mM $MgCl_2$,

0.2mM PMSF, 100mM NaCl). The immobilised NCP were incubated with 150µg of the nuclear extract in binding buffer, with rotation at 4˚C for 2 hours. The beads were pulled down and washed twice with binding buffer.

2.3.2.4.3. Affinity purification second set. For this group of pull-downs the reconstituted NCP were first incubated with the nuclear proteins for 2 hours in binding buffer at 4˚C before immobilising them on the beads. The protein mixture was added to 1.5mg washed Streptavidin-coupled Dynabeads® and incubated for a further hour at 4˚C. The beads and any proteins bound to them were pulled down and washed twice with binding buffer.

2.3.2.4.4. Streptavidin-coupled Dynabeads® non-specific binding control. To correct for proteins which bind to the magnetic beads non-specifically rather than to the NCP 1.5mg of beads were incubated with 150µg of the nuclear extract under the same conditions as for the pull-down experiments. The beads were pulled down and washed twice with binding buffer.

2.3.2.4.5. Unbound DNA control. A control was also incorporated to see which proteins would bind to the DNA if it is not bound to the histone octamers to correct for any free, labelled DNA in the samples which might pull down proteins. The labelled DNA (0.1nM) was treated in the same way as the reconstituted NCP in the pull-down experiments.

2.3.2.4.6. Tryptic digestion. The immobilised complexes were digested in solution and on-bead. The protocol for this was adapted from an Agilent Technologies protocol. (Meza *et al.* 2014) Each sample of beads and immobilised proteins were resuspended in denaturing buffer (25mM NH_4HCO_3, 10mM DTT, 50% 2,2,2-Triflurouethanol) and incubated at 60˚C for 45 min). This was diluted ten-fold with 25mM NH_4HCO_3 before trypsin was added at a ratio of roughly 1:20 (w/w) of protein to protease. Digestion was allowed to proceed overnight at 37˚C. Beads were pulled down magnetically as before and the supernatant was removed and added to a clean sample tube. To make sure that all peptides were eluted the beads were washed twice with 25mM NH_4HCO_3. This was added to the first supernatant before drying peptides in a Thermo Scientific Savant™ Speedvac™ concentrator.

2.3.2.4.7. Mass spectrometric analysis. The mass spectrometric analysis were performed as discussed in 3.2.2.4.

2.3.2.4.8. Data analysis. All data generated by the MS was acquired using Analyst 1.5.2 (AB Sciex). Raw LC-MS/MS data files were processed into peak lists and searched using MASCOT 2.3 (Matrix Science, Boston, MA) against a target Saccharomyces cerevisiae database (SwissProt, Oct 2014). Oxidation (M) was set as a variable modification and a maximum of 2

missed tryptic cleavages were included. Data searches were repeated with semitrypsin as enzyme. The precursor ion search tolerance were 1.2 Da and the MS/MS tolerance was set to 0.6 Da. Data filtering was done by importing the Mascot data files from the different biological and technical replicates, (including the semitryptic searches) into ProteoIQ (Premierbiosoft) and combining the results into one biological sample. Proteins with a p>0.9 were used. See each table caption for further filtering parameters.

Chapter 3

Results and Discussion

3.1. Results

3.1.1. Reconstitution of NCPs from *Xenopus laevis* histones and defined-sequence DNA fragments

3.1.1.1. Histones test expressions

All the histones showed good levels of expression on SDS-PAGE (figures 3.1 and 3.2) with the globular domain of histone H2B (gH2B) having a visibly lower expression level. Upon investigation of the H2B DNA sequence it was observed that the insert contained rare codons which might be the cause of the poor expression. The plasmid for the canonical H2B was subsequently transfected into Rosetta^{TM}(DE3)pLysS competent cells and a small scale test expression showed markedly better results than the test expression originally performed for gH2B. Rosetta^{TM}(DE3)pLysS cells are BL21 derivatives adapted for expression of proteins which contain codons that are rarely used by *E.coli*. The molecular weight of the domains were computed by the ExPASy online compute PI/Mw tool (Gasteiger *et al.* 2005).

3.1.1.2. Large scale expression and purification of histones

All the histones were expressed in 6L scaled up experiments and purified on a preparative Sephacryl S-200 HR column. The peaks (figures 3.5 and 3.6) were collected as they eluted and analysed on an SDS-PAGE gel (figures 3.3 and 3.4). The histone proteins eluted in peak B. Peak A in every case consisted of larger, contaminating proteins that were not washed from the inclusion body pellet and peak C was confirmed to be a salt peak. Since high levels of salt in the running buffer influenced the protein concentration determination 5µl was loaded from each fraction.

3.1.1.3. Purification of refolded octamer complexes

Even though resolution for the purification of the refolded octamer was low (figure 3.7) sufficient amounts of octamer was obtained by pooling fractions containing equimolar levels of the four different histones. The refolded octamer eluted in peak B in both cases. Peak C of both chromatograms did not yield any result when tested with SDS-PAGE. Peak A from the chromatogram for the globular and canonical octamer, was not sampled due to its low intensity and did not yield any bands on SDS-PAGE gels.

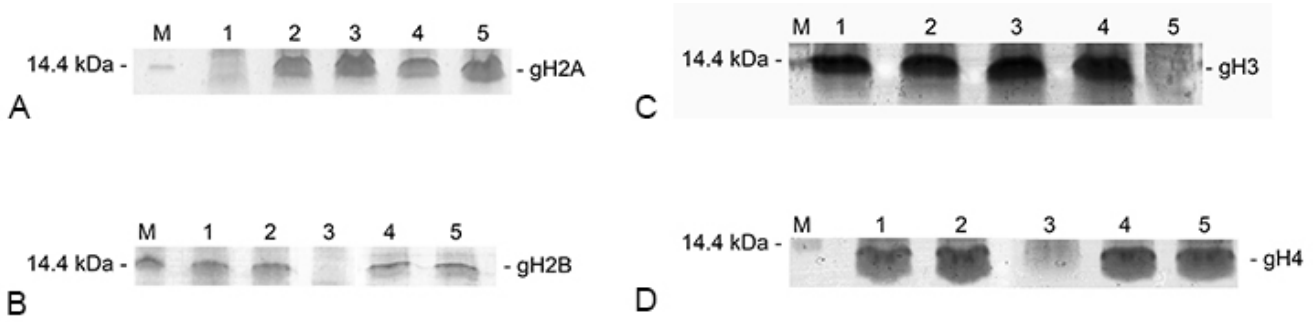

Figure 3.1: The SDS-PAGE results of the test expressions performed for the globular domains of the core histones. (A)The globular domain of histone H2A has an average molecular weight of 11,73 kDa. Lane 1 is the uninduced control and lanes 2 to 5 are the experiments where expression of gH2A was induced by the addition of IPTG. (B) The globular domain of histone H2B has an average molecular weight of 11,29 kDa. Lane 3 is the uninduced control and lanes 1, 2, 4 and 5 are the experiments where expression of gH2B was induced by the addition of IPTG. (C) The globular domain of histone H3 has an average molecular weight of 12,58 kDa. Lane 5 is the uninduced control and lanes 1 to 4 are the experiments where expression of gH3 was induced by the addition of IPTG. (D) The globular domain of histone H4 has an average molecular weight of 9,52 kDa. Lane 3 is the uninduced control and lanes 1, 2, 4 and 5 are the experiments where expression of gH4 was induced by the addition of IPTG.

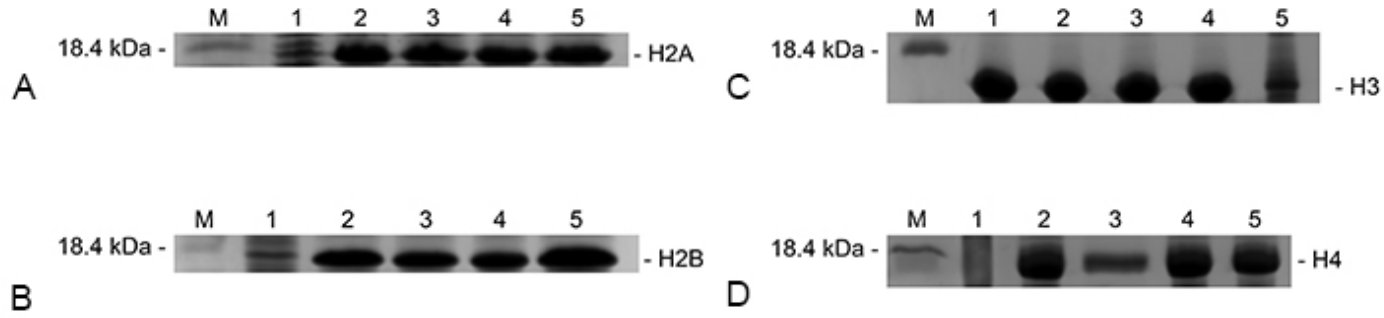

Figure 3.2: The SDS-PAGE results of the test expressions performed for the canonical (full length) core histones. (A) Histone H2A has an average molecular weight of 14.08 kDa. Lane 1 is the uninduced control and lanes 2 to 5 are the experiments where expression of H2A was induced by the addition of IPTG. (B) Histone H2B has an average molecular weight of 13.62 kDa. Lane 1 is the uninduced control and lanes 2 to 5 are the experiments where expression of H2B was induced by the addition of IPTG. (C) Histone H3 has an average molecular weight of 15.40 kDa. Lane 5 is the uninduced control and lanes 1 to 4 are the experiments where expression of H3 was induced by the addition of IPTG. (D) Histone H4 has an average molecular weight of 11.37 kDa. Lane 1 is the uninduced control and lanes 2 to 5 are the experiments where expression of H4 was induced by the addition of IPTG.

3.1.1.4. Preparation and purification of the defined-sequence DNA fragment

The plasmid (figure 3.10) containing the defined sequence (also referred to as the positioning sequence) was digested by EcoRV (figure 3.8) and the resulting 202bp sequence was precipitated with polyethylene glycol (figure 3.9). This DNA fragment was blunt and unlabelled and was only used for the gelshift assays.

3.1.1.5. Gel-shift assays

A high resolution gelshift assay was used to estimate the optimal ratio of octamer to DNA concentration that would yield the least amount of unbound DNA. If the DNA binds the refolded octamer the resulting complex will shift up on the native PAGE gel to the reconstituted NCP position. This was the only way to determine the optimal, relative concentration as it proved difficult to accurately determine the concentration of the octamers through traditional methods as they precipitated readily in the sample buffers used for quantitation. In all gelshift experiments the DNA concentration was kept constant at 0.02nM while varying the octamer concentration. The experiment was repeated with varying DNA : Octamer ratios to get a clearer idea of where the maximum amount of DNA binds the octamers with minimum aggregation. DNA: Octamer ratios of 1.1 to 0.7 (figure 3.11), 0.5 to 0.17 (figure 3.12) and 0.3 to 0.17 (figure 3.13) were tested. From this results it was decided to use a ratio of 1.0:4.0. An unbound DNA control was added to correct for any proteins pulled down with the unbound DNA still in the reaction mixture.

3.1.2. Preparation of labelled DNA fragment, affinity purifications and mass spectrometric analysis of protein complexes.

3.1.2.1. Preparation of biotinylated DNA fragment

Preparative gel electrophoresis was performed to produce a large amount of DNA for the octamer binding. The plasmid (figure 3.14) containing the fragments was digested with AvaII and preparative electrophoresis was used to purify the 169bp AvaII digested fragments. The 169bp fragment has a 5' overhang to be filled in with Klenow. As discussed, a biotinylated dCTP was incorporated by this reaction. This made the pull-down with Streptavidin-coupled beads possible. Figure 3.15 shows the fractions sampled and analysed on a 1% (w/v) agarose gel. As the preparative gel electrophoresis has a high resolution the 169bp fragments could be separated from the 222bp fragment from the plasmid backbone generated by AvaII digest. The purified 169bp fractions were pooled, ethanol precipitated and biotinylated.

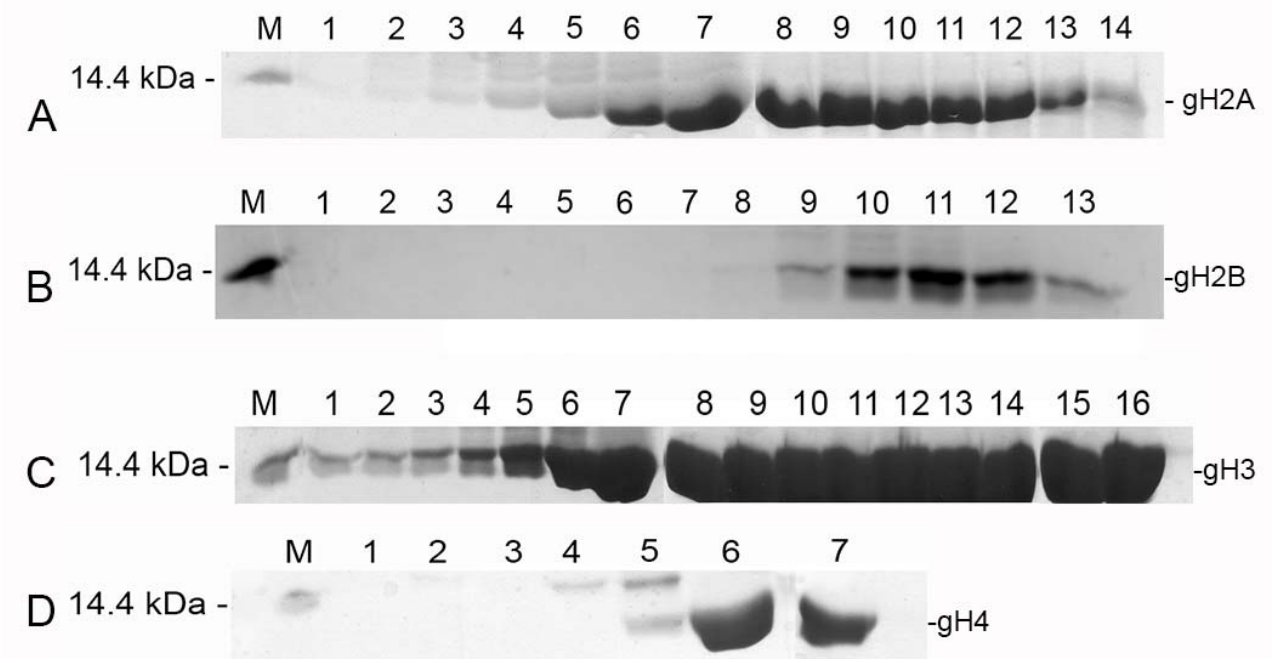

Figure 3.3: SDS-PAGE results of FPLC fractions of the globular histone purifications. (A) FPLC fractions of globular H2A. (B) FPLC fractions of globular H2B. (C) FPLC fractions of globular H3. (D) FPLC fractions of globular H4.

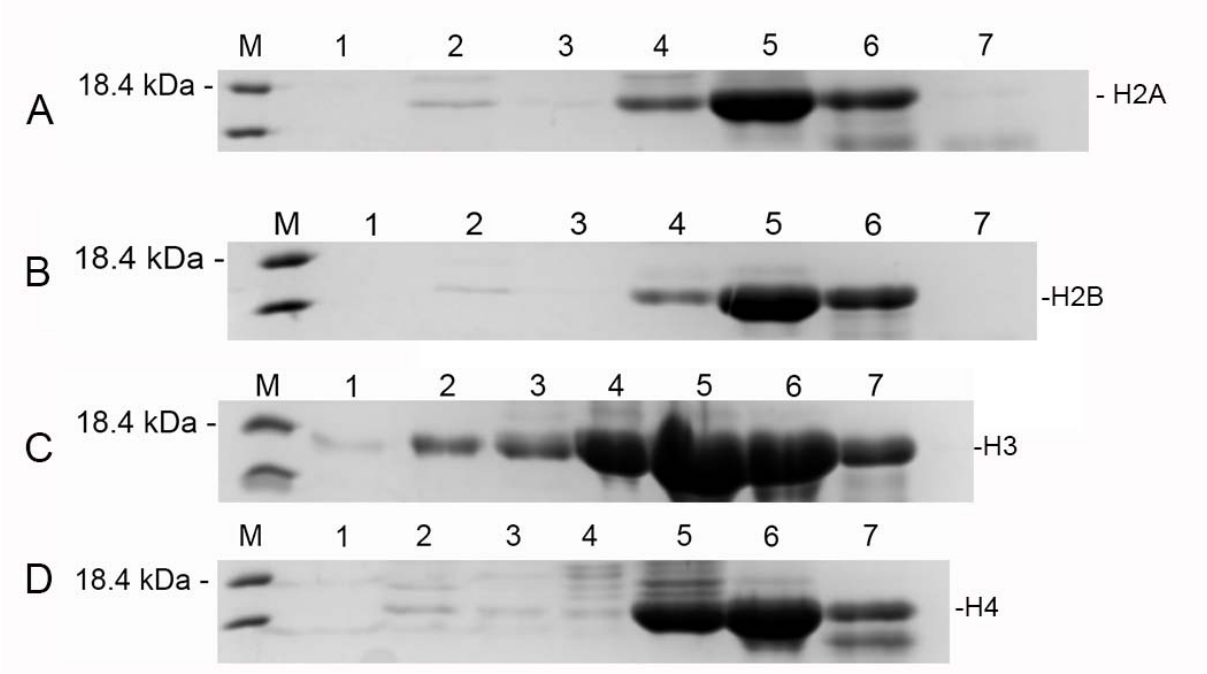

Figure 3.4: SDS-PAGE results of FPLC fractions of the canonical histone purifications. A) FPLC fractions of H2A. (B) FPLC fractions of H2B. (C) FPLC fractions of H3. (D) FPLC fractions of H4.

3.1.2.2. *Saccharomyces cerevisiae* nuclear extract preparation

A nuclear extract from *S.cerevisiae* was prepared as source of the proteins interacting with the NCP. Figure 3.16 shows the proteins on SDS-PAGE for the different fractions of the nuclear extract preparation. The pellet containing the cell debris as well as proteins precipitated together with the insoluble mass (lane 1) was identified by LCMSMS to see if any proteins of interest would be lost to downstream experiments. The proteins successfully precipitated from the supernatant (lane 3) to be used in the affinity purifications was also identified (Table 3.1.1).

3.1.2.3. SIR3 BAH domain expression and positive control for affinity purifications

Following test expression of $SIR3^{1-380}$ (figure 3.17) the expression was scaled up and purified by a His-trap column. Due to non-specific background binding (figure 3.18) fractions containing lower levels of these contaminating proteins (lanes 6 and 7) were combined (figure 3.19) for subsequent experiments. Since $SIR3^{1-380}$ was only to be used as a positive control for the affinity purifications it was needed in detectable amounts, the contaminating proteins would not be an issue.

This control pull down experiment showed that $SIR3^{1-380}$ bind both the globular as well as the canonical NCP (tables 3.2.1. and 3.2.2.). A non-specific binding control showed that $SIR3^{1-380}$ also binds the Streptavidin-coupled Dynabeads® with protein identifications at lower confidence as well as with a lower sequence coverage suggesting that the NCP pull-down did in fact enrich for $SIR3^{1-380}$. A control experiment using unbound beads was incorporated to correct for any non-specific binding to the beads.

3.1.2.4. Affinity purifications (pull-downs)

Comparing the proteins (probability ≥0.9) pulled down in the globular NCP pull down experiment and using them as reference to the occurrence of these proteins in the other experiments, the results can be represented in a heat map (Table 3.3.1). From these listed proteins two filtered groups were created by ProteoIQ: group one (table 3.3.2) was the identifications that were shared between the canonical NCP and the globular domain pull-downs after any identifications observed in the DNA and bead control experiments were removed. Group two (table 3.3.3) was identifications which were unique to the globular domain pull-down and not pulled down with the canonical NCP.

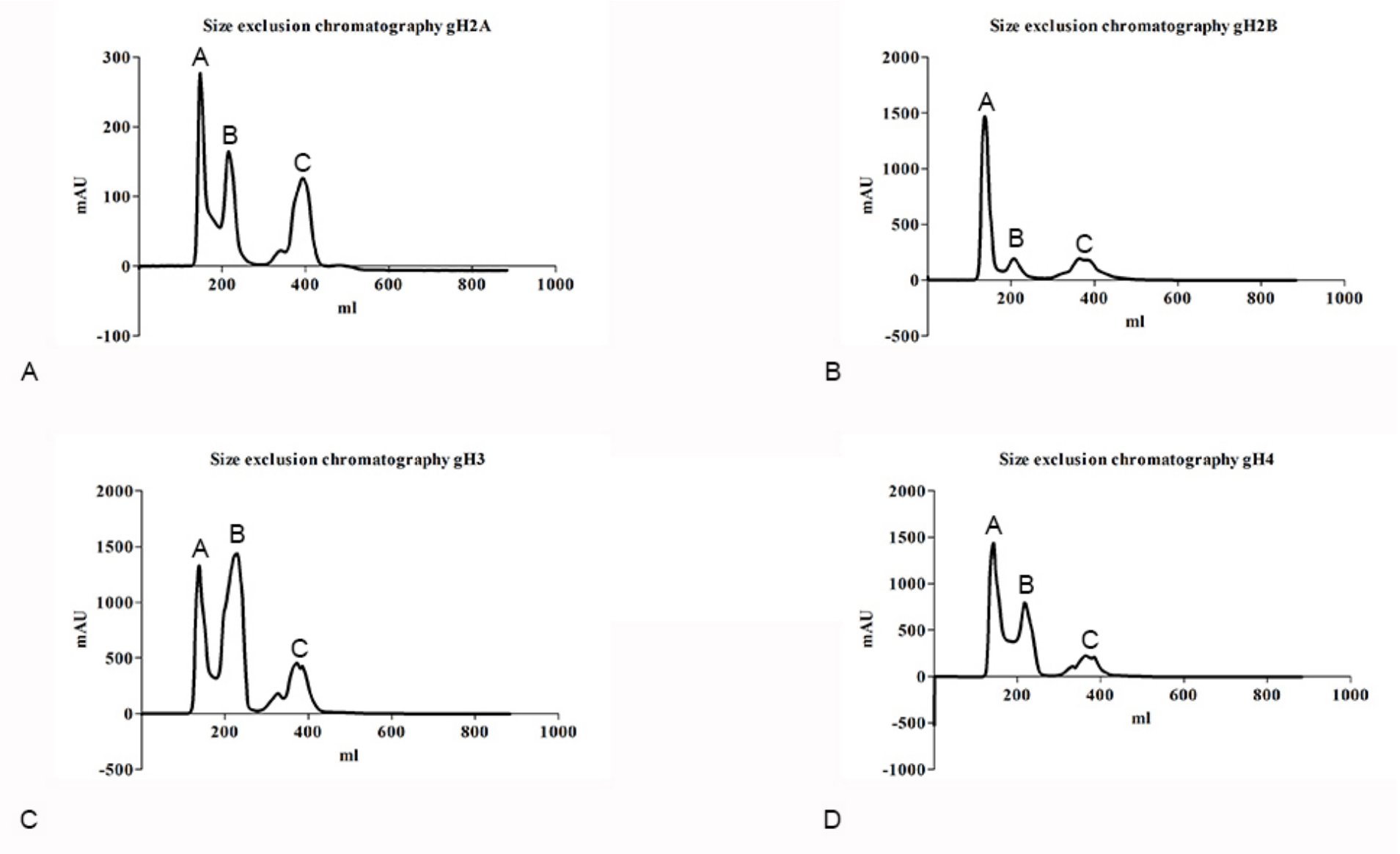

Figure 3.5: Chromatograms of globular histones. (A) Elution profile of globular H2A. (B) Elution profile of globular H2B. (C) Elution profile of globular H3. (D) Elution profile of globular H4.

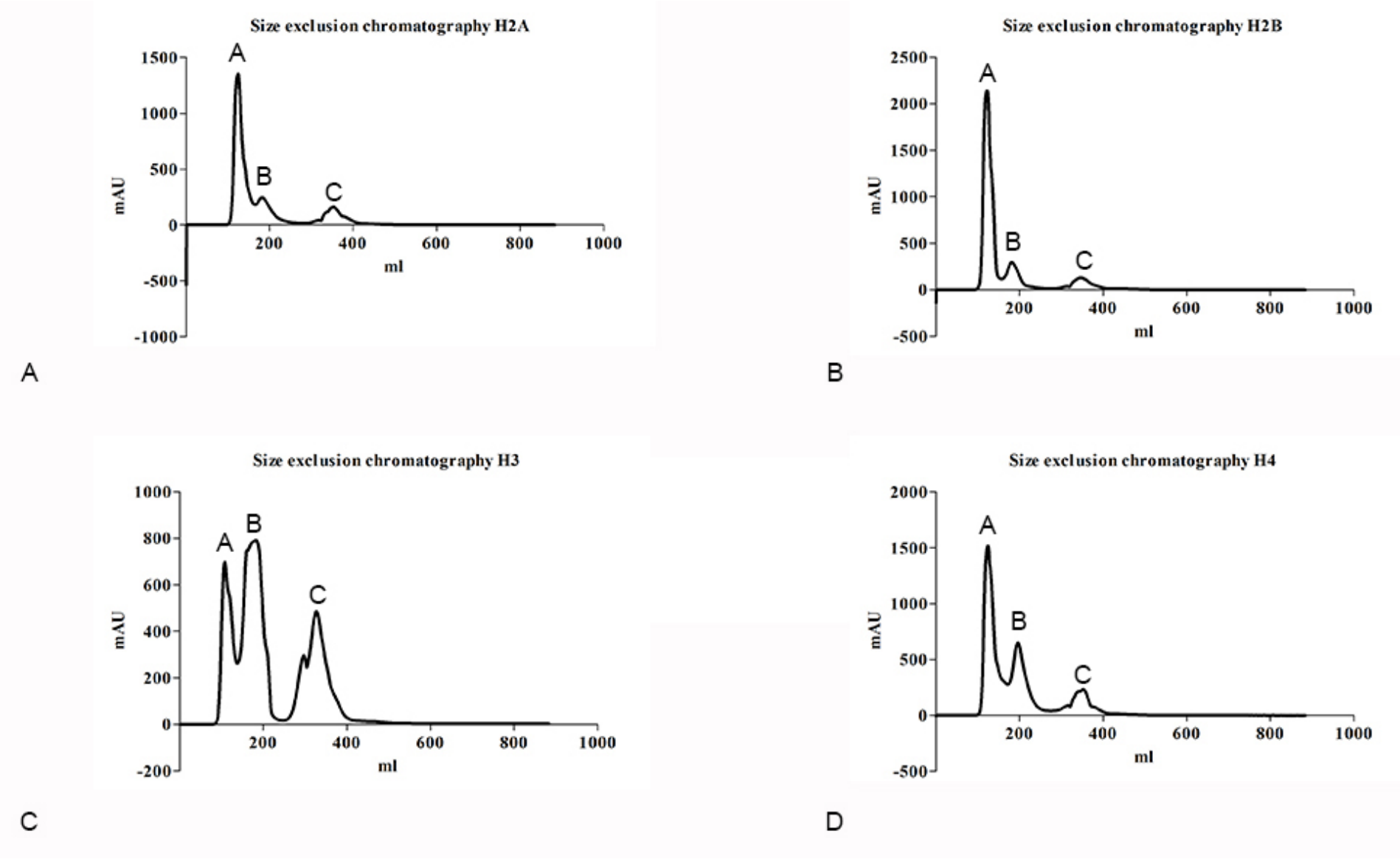

Figure 3.6: Chromatograms of the canonical histones. (A) Elution profile of canonical H2A. (B) Elution profile of canonical H2B. (C) Elution profile of canonical H3. (D) Elution profile of canonical H4.

The proteins in both these tables were searched on UniProt (www.uniprot.org) to determine their cellular localization and function. The IntAct database (http://www.ebi.ac.uk/intact/) was used to determine possible protein interactions. Subsequently all proteins which were known to not be localized in the nucleus were removed and the remaining proteins were grouped according to function (table 3.3.4) and will be discussed below.

3.2. Discussion

When SIR3 was not identified in the final results, the protein identification results for the nuclear fractions (table 3.1.) were inspected to see if SIR3 was present at all in the soluble fraction. SIR3 is known to bind the globular domains of the NCP (chapter 1) and was thus expected to be pulled-down. Surprisingly it was not observed in any of the fractions. This was most likely due to too low concentration. One of the drawbacks of analysing a complex sample such as the nuclear extract on a mass spectrometer is that the higher concentration peptides will be sampled more readily by the mass spectrometer and will in effect overshadow the lower concentration peptides. Thus, if proteins of interest are only present in low concentrations they might be concealed by the higher concentration proteins. As the affinity purification would enrich for proteins bound to the NCP the hope was that SIR3 and other low concentration proteins of interest would be observed after affinity purification.

The unexpected high level of nonspecific binding to the Streptavidin-coupled Dynabeads® is troublesome. It is plausible that a lot of important proteins bind the beads non-specifically, causing them to be filtered out automatically during data processing. Proteins bound non-specifically to the beads will also be analysed by the MS contributing to the concealment of the lower concentration NCP bound proteins. A better matrix to which the Streptavidin can be coupled is thus needed (e.g. Streptavidin-coupled sepharose).

None of the proteins originally expected in the results were observed. For potential explanations, let's first explore the proteins used in the pull down experiments. An *Escherichia coli* expression system was used to produce histones free from PTMs. The aim was to investigate binding proteins independent of PTMs. Although this served the purpose, the question can now be asked whether PTMs are indeed needed to form the interaction with many proteins. These unexpected results might indicate that they do in fact need specific PTMs to bind. Furthermore, the stability of the interactions formed between the NCP and the protein being pulled down can be less than what is needed for the protein to make it all the way into the final experimental step. One way to help retain less stable interactions is to perform a cross-linking reaction. Cross-linking reagents such as formaldehyde is used to fix peptides in close

proximity to each other. The data subsequently obtained from the MS needs to be treated differently as the cross-linked peptides will remain cross-linked after digestion, yielding different masses. This allows the inference of structural information as the cross-linked partners will reveal which parts of the proteins are close proximity with each other. This way it will also be possible to separate the indirect associations from the proteins bound directly to the NCPs. Another issue was the high levels of cytoplasmic proteins contaminating the nuclear extract even after washing the nuclei several times. This seemed to be a problem inherent to yeast nuclear preparations. The final factor to consider is the concentration of the proteins being pulled down. To solve this the higher concentration proteins which will conceal the lower concentration ones will need to be excluded or the sample will have to be enriched for specific low concentration proteins. This then becomes a more directed approach where very specific interactions are tested. This study aimed to take a broader approach. The hope was that the affinity purification technique will enrich for the proteins but only the higher concentration proteins seen in the MS analysis of the nuclear extract was identified. It is important to keep in mind that some proteins do get lost in the preparation of the nuclear extract by precipitating with the insoluble debris. This might contribute to the concentration of some proteins being low.

All results obtained for the affinity purifications were subsequently divided based on cellular localisation and only proteins localised to the nucleus would be considered. Some of the contaminating non-nuclear proteins did bind the NCPs as well as the beads. This might have interfered with binding of other nuclear proteins of interest and again would have contributed to the concealment of lower concentration peptides.

The proteins that were identified to bind the globular domains of the NCP are listed in tables 3.3.2. and 3.3.3. and a summary of the final results are found in table 3.3.4. These are discussed in detail below.

The majority of the final results were proteins involved in ribosome biogenesis and the transport of ribosomal subunits between the nucleus and the cytoplasm (figure 3.20). The high occurrence of the ribosomal proteins might be because histones, like ribosomal subunits, are small, basic proteins. Thus the interactions might be non-specific interactions which will never occur in the tightly controlled *in vivo* chromatin. Some of the cytoplasmic proteins filtered out were the 40s and 60s ribosomal subunits. It might be that these subunits formed interactions with the histones they would normally be separated from. The proteins involved in their transport and biogenesis could then interact with them but will still be pulled down with the complex. It is also difficult to say whether the proteins identified interacted with the NCPs directly. Some of the proteins identified have many interaction partners of which some are also among the results.

MAK21 (MAK21_YEAST, table 3.3.4.) for instance shows interactions with 86 other proteins according to the IntAct database. Among these interaction partners are NOP2, NOG1, NOC2 and URB1 which are all observed in the results (table 3.3.4) as well (Krogan *et al.* 2006). POL5 (DPO5_YEAST, table 3.3.4.), functions in the synthesis of rRNA and has been shown previously to bind histone H4 but it also binds a number of the other proteins identified by this study, including PUF6, TAF9, UTP10 and UTP9 (Shimizu *et al.* 2002) (Lambert *et al.* 2010) (Krogan *et al.* 2006)(Gavin *et al.* 2006)(Lee *et al.* 2011). Any one of these proteins could have interacted with the NCP and pulled the others down with it. Another important fact to keep in mind is that the *in vitro* conditions of this experiment are very far removed from the *in vivo* reality and all results should be regarded accordingly and be investigated further for confirmation. This can either be done through *in silico* simulations or through more direct approaches where specific interactions are studied *in vivo*.

One of the proteins of the ribosome biogenesis group that have previously been implicated in chromatin association was NOC3 (NOC3_YEAST, table 3.3.4.). This protein is not only important for ribosomal subunit synthesis and transport but is also crucial to the initiation of DNA replication (Zhang *et al.* 2002). As the detailed underlying mechanism of DNA replication is still unclear and the globular domains have been shown to be important to this cellular function this might be an interesting interaction to pursue further (Matsubara *et al.* 2007). NOC3 is known to interact with the origin recognition complex (ORC) as well as the mini chromosome maintenance (MCM) protein complex and has been shown to associate with the chromatin and replicator proteins through the whole cell cycle. The ORC1 subunit of the ORC protein complex is known to contain a BAH domain which, as discussed in chapter 1, might implicate it in the binding of the globular domains of the NCP (Kuo *et al.* 2012). This study, however, failed to identify ORC1 as a binding partner to the NCP. The ORC complex was also missing from the identification results obtained for the total nuclear fractions which indicates that this might be due to too low concentration. It has been shown previously that NOC3 is needed for the association of CDC and MCM2, both essential proteins for DNA replication, with chromatin (Zhang *et al.* 2002). It was however found that ORC is needed to ensure a stable interaction between NOC3 and the chromatin (Zhang *et al.* 2002). It is thus postulated that ORC binds the NCP directly and that NOC3 binds ORC and links it to the other initiator proteins (Zhang *et al.* 2002). The fact that NOC3 was pulled-down by the globular domains but not the ORC complex might suggest an alternate mode of interaction.

The other large group of proteins identified were involved in transcription. Under these proteins are the high mobility group protein 1 (HMO1_YEAST, table 3.3.4.). High mobility group protein

1 and 2 (HMGN1 and HMGN2) have previously been shown to bind the globular domains of the NCP directly (Chapter 1)(Kato *et al.* 2011). Most of the transcription factors identified here are known DNA-binding proteins and it is interesting that they were identified in experiments where the DNA was bound to the histone octamer rather than in the free DNA control experiment. This might indicate a novel way of interacting with the DNA, mediated by the NCP. As most of them form subunits of large multi-protein transcription factors, they show interaction with each other and again it becomes quite difficult to know the exact hierarchy of the interaction. RPAB3 (RPAB3_YEAST, table 3.3.4.) for example has known interactions with RPA49 (RPA49_YEAST, table 3.3.4.) and RPA12 (RPA12_YEAST, table 3.3.4.) and TAF1 (TAF1_YEAST, table 3.3.4.), TAF6 (TAF6_YEAST, table 3.3.4.) and TAF9 (TAF9_YEAST, table 3.3.4.) together form a part of the TFIID transcription factor complex (Krogan *et al.* 2006).

Three of the other identified proteins, MCM22 (MCM22_YEAST, table 3.3.4.), MAD1 (MAD1_YEAST, table 3.3.4.) and TOP2 (TOP2_YEAST, table 3.3.4.) have functional implications in the segregation of chromosomes during cell division. MCM22 ensures proper chromosome segregation during cell division by facilitating binding of the centromere to the mitotic spindle.(Poddar *et al.* 1999) MAD1 plays a role the spindle checkpoint activation to ensure proper spindle-chromosome attachment and subsequently ensure proper chromosome segregation.(Kastenmayer *et al.* 2005) Attachment to the globular domains of the NCP makes sense in this context and these might be interesting interactions to investigate further. TOP2 is a DNA topoisomerase which makes and rejoins double-stranded breaks in the DNA and is important for proper segregation of daughter chromosomes during cell division (Schmidt *et al.* 2012).

Proteins PUF6 (PUF6_YEAST, table 3.3.4.) and YRA1 (YRA1_YEAST, table 3.3.4.) are both known RNA-binding proteins implicated in posttranscriptional regulation of mRNA (Gu *et al.* 2004)(Kashyap *et al.* 2005). PRP43 (PRP43_YEAST, table 3.3.4.) is also involved in pre-mRNA processing by dissembling the spliceosomes after mRNA has been spliced and mature mRNA has been released (Boon *et al.* 2006)(Arenas & Abelson 1997). There does not seem to be a logical explanation as to why they would associate with the NCP. PDC1 (PDC1_YEAST, table 3.3.4.) is a pyruvate decarboxylase enzyme which converts pyruvate to acetaldehyde and carbon dioxide during anaerobic alcohol fermentation.(Dickinson *et al.* 2003) The enzyme plays an important role in amino acid catabolism by catalysing the decarboxylation of the amino acids.(Dickinson *et al.* 1998)(Dickinson *et al.* 2000) It has a lot of interaction partners according to the IntAct database and it is likely that it just has a high specificity for certain amino acid sidechains.

SGD1 (SGD1_YEAST, table 3.3.4.) regulates the expression of GDP1 through an unknown mechanism (N. Akhtar *et al.* 2000)(Lin *et al.* 2002). An earlier study has established an interaction with histone H4 and another linked it to the ISWI remodeler complex's ATPase ISW1 (Krogan *et al.* 2006)(Lambert *et al.* 2010). If linked to ISWI it might play a role in the function of the remodeler which might be the way in which it regulates the expression of GDP1. This might warrant some further investigation.

ECT1 (ECT1_YEAST, table 3.3.4.) is a protein involved in plasma membrane maintenance by playing a role in phosphatidylethanolamine biosynthesis.(Choi & Carman 2007)(Deng *et al.* 2008) It is also essential for sporulation (Deng *et al.* 2008). There was also two uncharacterised proteins in the results; YNL108C (YNK8_YEAST, table 3.3.4.) and YHR127W (YHS7_YEAST, table 3.3.4.). Although their functions and interaction networks remain largely unknown, YHR127W has been implicated in the process of mitotic spindle elongation by a mutation study and YNL108C has been shown to have phosphatase activity.(Schoner *et al.* 2008)(Taylor *et al.* 2013) YHR127W has also been linked to one of the RSC remodeler's subunits, RSC58, and has been shown to interact with histone H2A.2 in a previous study (Lambert *et al.* 2010).

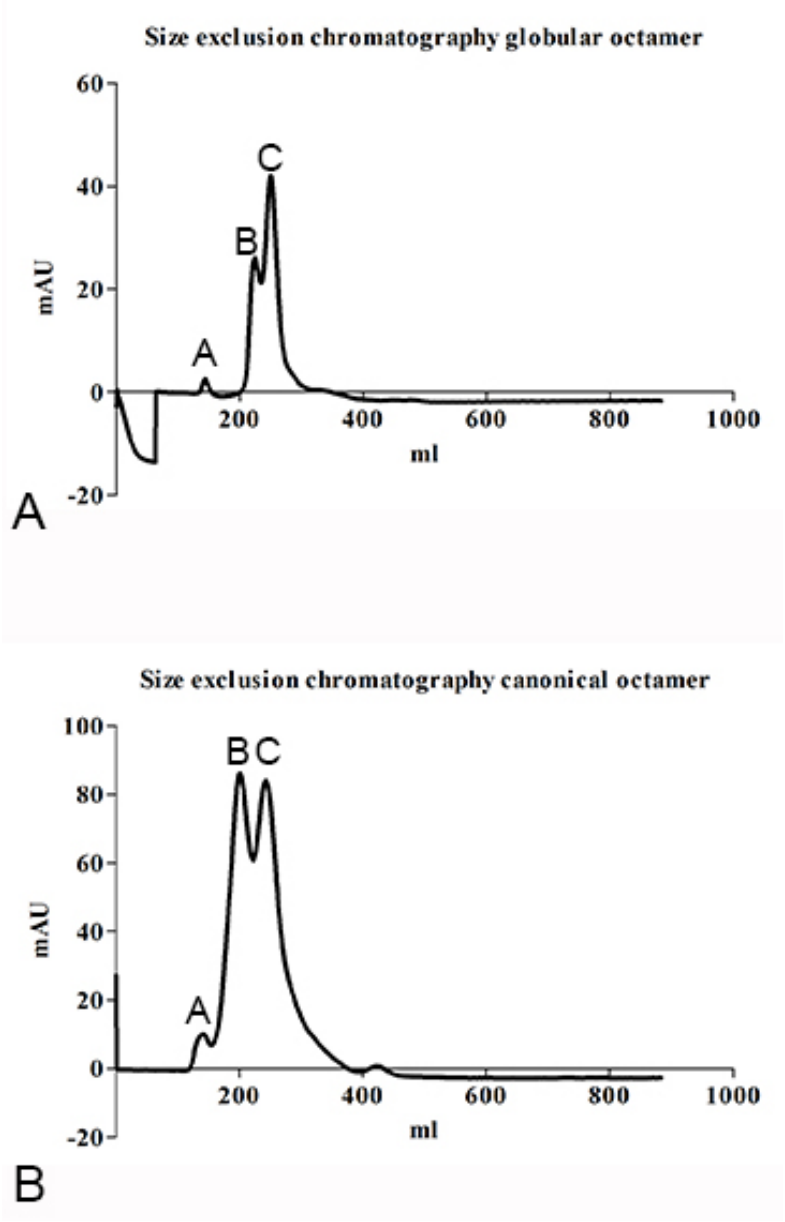

Figure 3.7: (A) Chromatogram of refolded, globular octamer. (B) Chromatogram of refolded, canonical octamer.

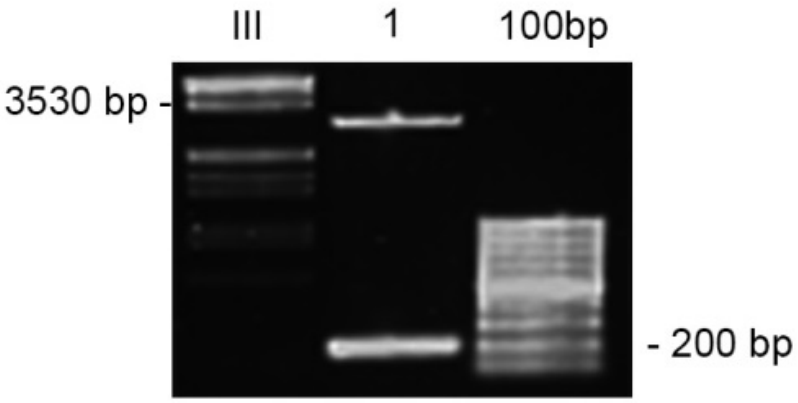

Figure 3.8: EcoRV digestion of pBL634-196-16x. Lane 1 is the digest reaction. The lane marked 100bp is the 100bp DNA ladder (Invitrogen).

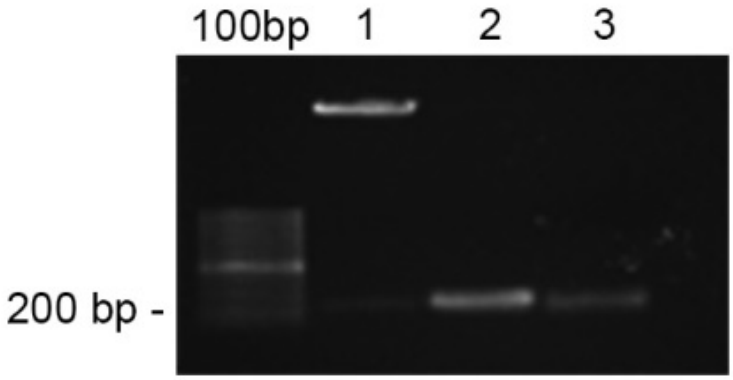

Figure 3.9: PEG precipitation of 202bp insert. Lane 1 is the pellet obtained from the PEG precipitation reaction containing the linearised plasmid. Lane 2 is the supernatant containing the 202bp fragment and lane 3 is the 202bp fragment precipitated with cold ethanol to get rid of the PEG and salts.

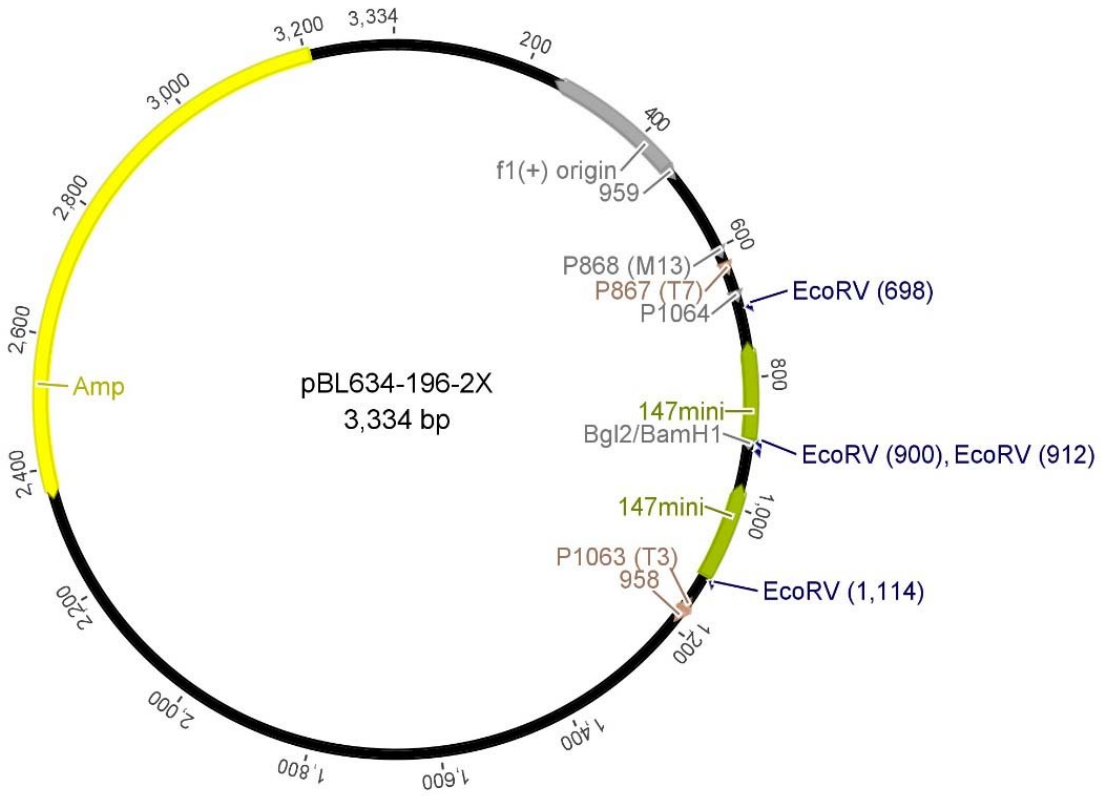

Figure 3.10: Plasmid map of pBL634-196-2X with EcoRV digest points and 2 of the defined sequence inserts (green) indicated. The real plasmid contained 16 of these inserts.

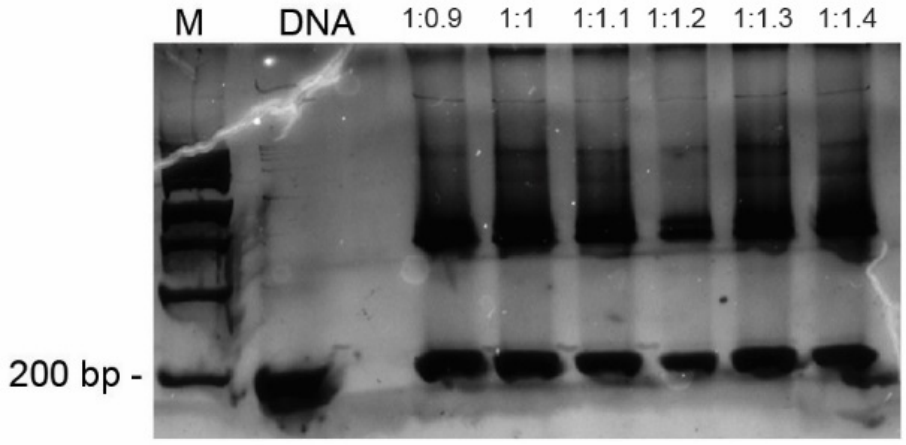

Figure 3.11: Silver stained native PAGE showing the gelshift assay for globular octamer for DNA : Octamer ratios of 1.0:0.9 – 1.0:1.4. DNA concentrations were kept at 0.02nM while octamer concentrations were varied to yield DNA : Octamer ratios of 1.0:0.9 to 1.0:1.4 as indicated by the lanes' labels. The lane labelled DNA contains free DNA as a reference.

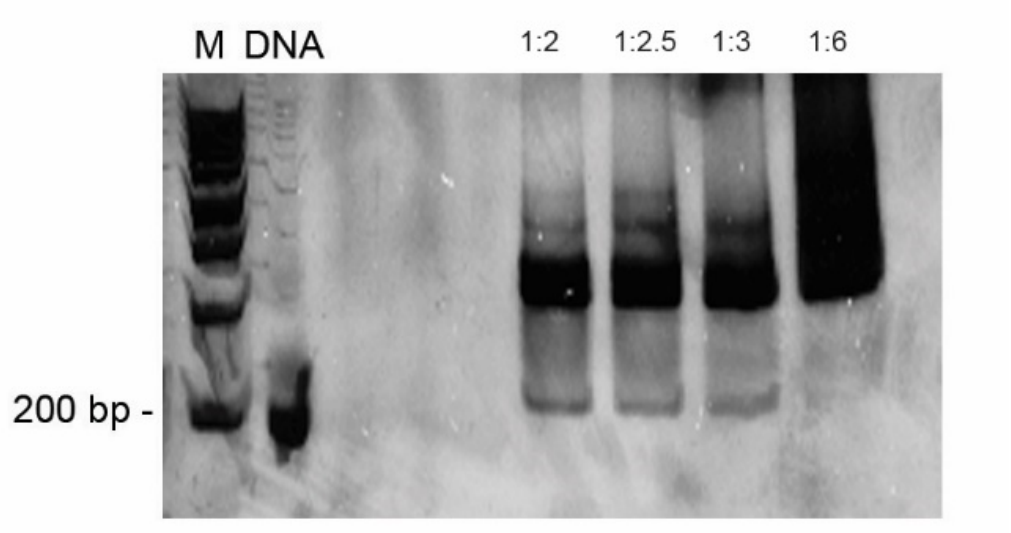

Figure 3.12: Gelshift assay for globular octamer for DNA : Octamer ratios of 1.0:2.0 – 1.0:6.0

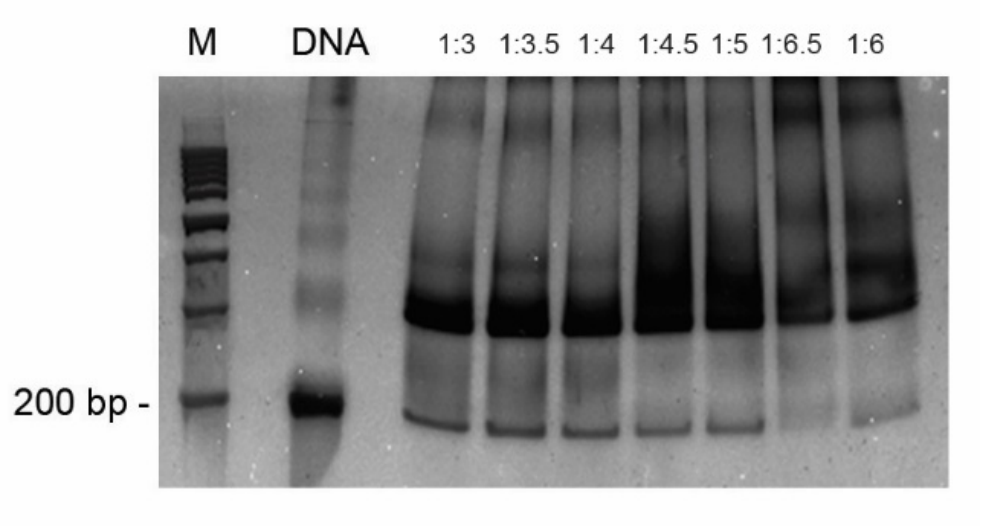

Figure 3.13: Gelshift assay for globular octamer for DNA : Octamer ratios of 1.0:3.0 – 1.0:6.0

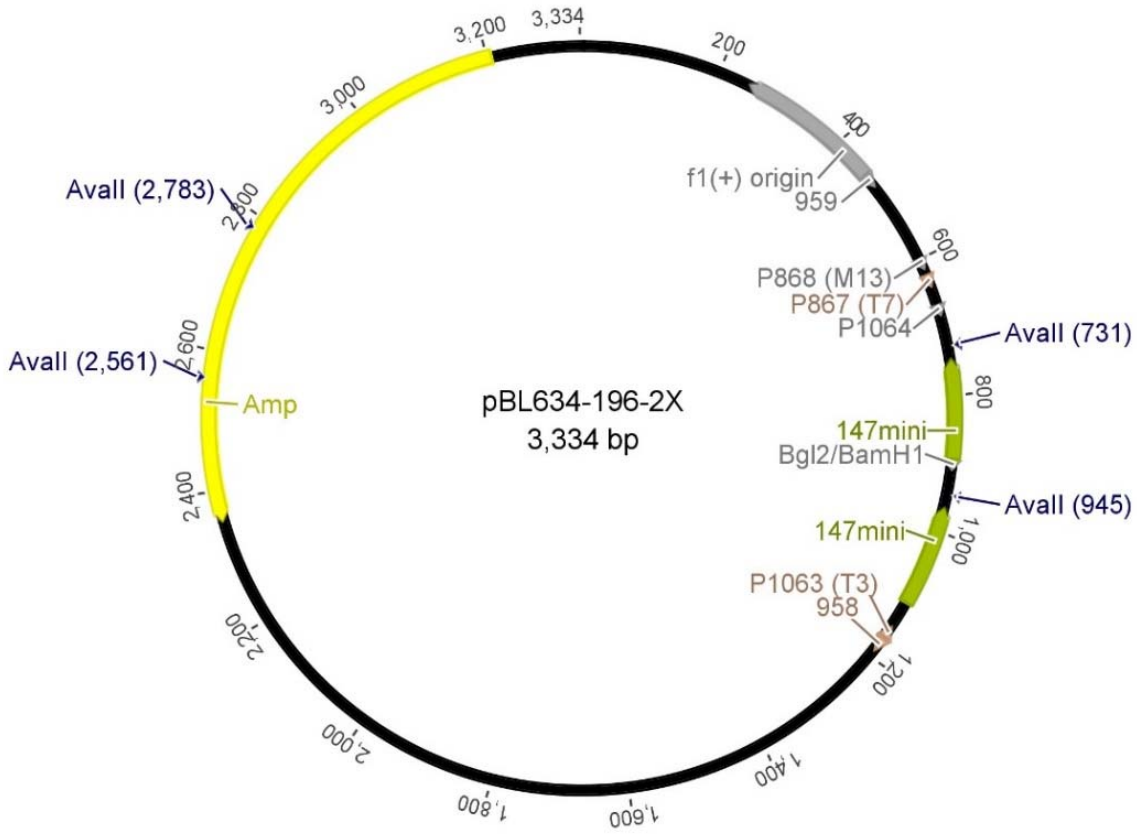

Figure 3.14: Plasmid map of pBL634-196-2X with AvaII and EcoRV digest points indicated.

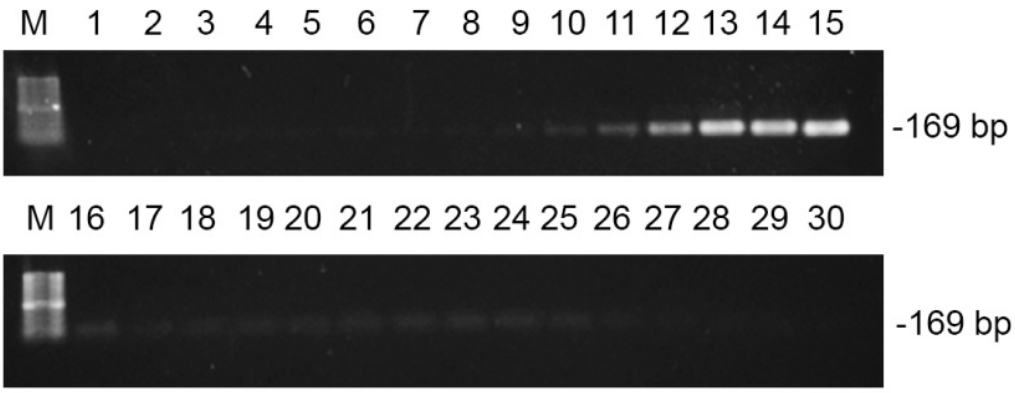

Figure 3.15: Fractions eluted during preparative gel electrophoresis of AvaII digested pBL634-196-16x.

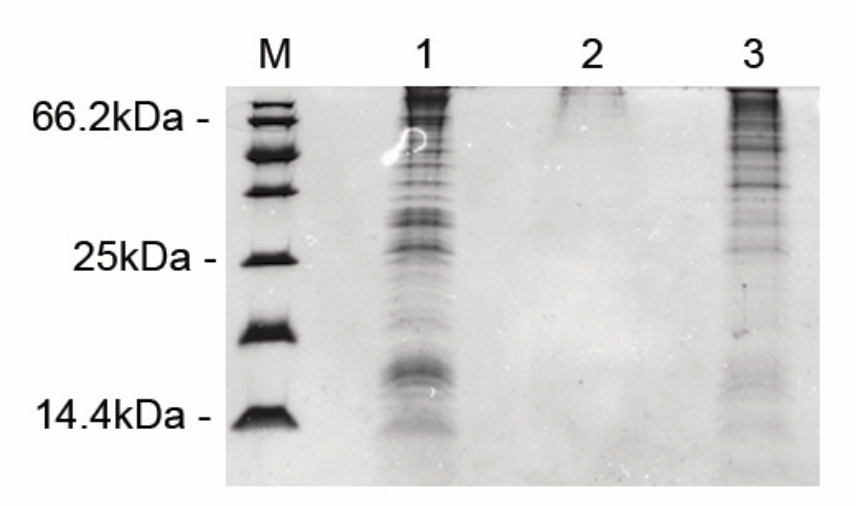

Figure 3.16: Fractions of nuclear extraction analysed on a 15% SDS-PAGE. Lane 1 shows the pellet containing any cell debris as well as proteins precipitated together with the insoluble mass. . Lane 2 shows the proteins left behind after the ammonium sulphate precipitation. Lane 3 shows the group of proteins successfully precipitated to be used in the affinity purifications.

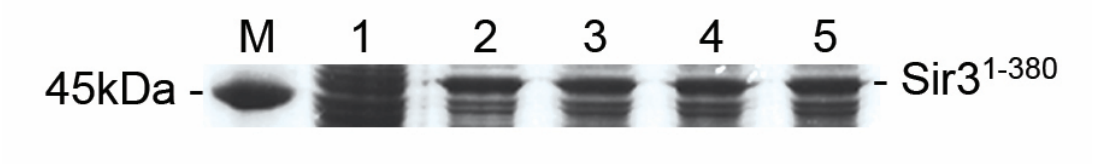

Figure 3.17: Test expressions for Sir3$^{1-380}$ analysed on a 15% SDS-PAGE gel. Lane 1 is the uninduced control and lanes 1-5 are the lanes were protein expression was induced by IPTG.

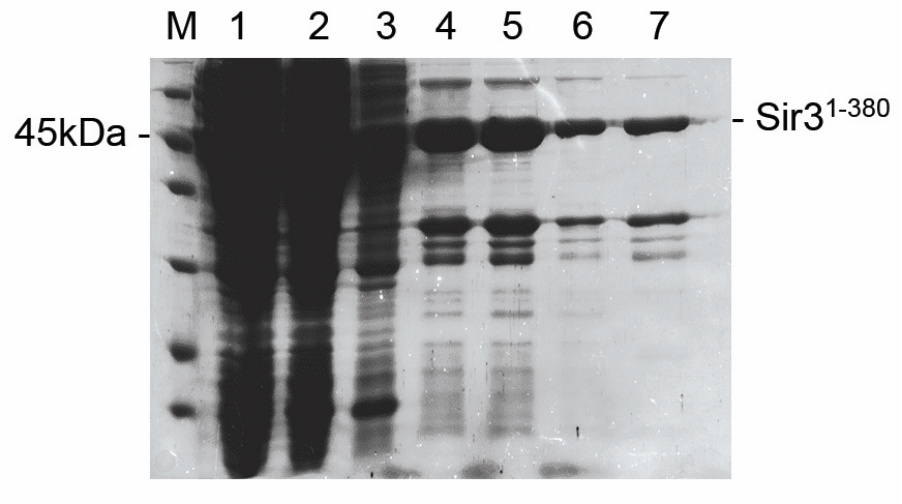

Figure 3.18: His-trap fractions of Sir3$^{1-380}$. Lane M is the molecular weight marker used and lanes 1-7 are the fractions collected as they eluted of the his-trap.

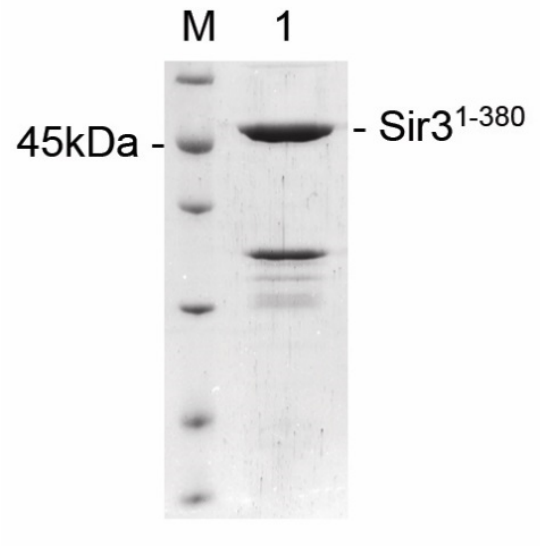

Figure 3.19: Pooled His-trap fractions of Sir3 1-380.

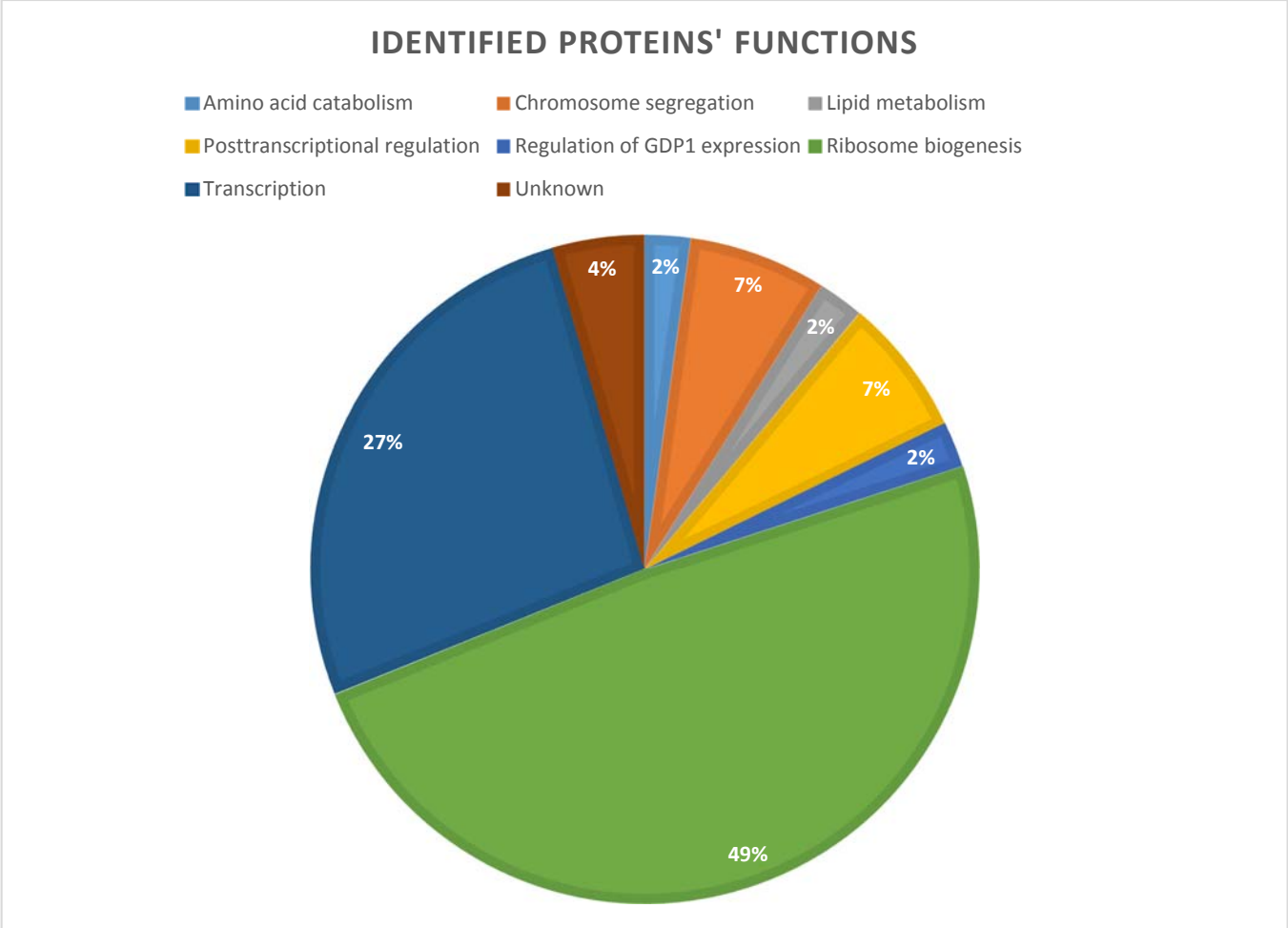

Figure 3.20: Distribution of final results' functions. The data is listed in table 3.3.4.

Table 3.1. Proteins identified for the different nuclear fractions (p≥0.9). Fraction P is the insoluble fraction (pellet) and Fraction S is the soluble fraction. The proteins which were only identified in the insoluble fraction and thus would be lost to downstream experiments are highlighted.

Sequence ID	Sequence Name	Fraction
sp|P02400|RLA4_YEAST	60S acidic ribosomal protein P2-beta	P
sp|P02407|RS17A_YEAST	40S ribosomal protein S17-A	P
sp|P04819|DNLI1_YEAST	DNA ligase 1	P
sp|P05739|RL6B_YEAST	60S ribosomal protein L6-B	P
sp|P05748|RL15A_YEAST	60S ribosomal protein L15-A	P
sp|P06704|CDC31_YEAST	Cell division control protein 31	P
sp|P0CX35|RS4A_YEAST	40S ribosomal protein S4-A	P
sp|P0CX36|RS4B_YEAST	40S ribosomal protein S4-B	P
sp|P0CX37|RS6A_YEAST	40S ribosomal protein S6-A	P
sp|P0CX38|RS6B_YEAST	40S ribosomal protein S6-B	P
sp|P0CX43|RL1A_YEAST	60S ribosomal protein L1-A	P
sp|P0CX44|RL1B_YEAST	60S ribosomal protein L1-B	P
sp|P0CX53|RL12A_YEAST	60S ribosomal protein L12-A	P
sp|P0CX54|RL12B_YEAST	60S ribosomal protein L12-B	P
sp|P0CX55|RS18A_YEAST	40S ribosomal protein S18-A	P
sp|P0CX56|RS18B_YEAST	40S ribosomal protein S18-B	P
sp|P0CX82|RL19A_YEAST	60S ribosomal protein L19-A	P
sp|P0CX83|RL19B_YEAST	60S ribosomal protein L19-B	P
sp|P10664|RL4A_YEAST	60S ribosomal protein L4-A	P
sp|P10962|MAK16_YEAST	Protein MAK16	P
sp|P12385|ERF1_YEAST	Eukaryotic peptide chain release factor subunit 1	P
sp|P14126|RL3_YEAST	60S ribosomal protein L3	P
sp|P14127|RS17B_YEAST	40S ribosomal protein S17-B	P
sp|P23585|HXT2_YEAST	High-affinity glucose transporter HXT2	P
sp|P25367|RNQ1_YEAST	[PIN+] prion protein RNQ1	P
sp|P26786|RS7A_YEAST	40S ribosomal protein S7-A	P
sp|P29496|MCM5_YEAST	Minichromosome maintenance protein 5	P
sp|P32366|VA0D_YEAST	V-type proton ATPase subunit d	P
sp|P32466|HXT3_YEAST	Low-affinity glucose transporter HXT3	P
sp|P32495|NHP2_YEAST	H/ACA ribonucleoprotein complex subunit 2	P
sp|P38011|GBLP_YEAST	Guanine nucleotide-binding protein subunit beta-like protein	P
sp|P38779|CIC1_YEAST	Proteasome-interacting protein CIC1	P
sp|P39926|SSO2_YEAST	Protein SSO2	P
sp|P40212|RL13B_YEAST	60S ribosomal protein L13-B	P
sp|P40693|RLP7_YEAST	Ribosome biogenesis protein RLP7	P
sp|P53163|MNP1_YEAST	54S ribosomal protein L12, mitochondrial	P
sp|P53276|UTP8_YEAST	U3 small nucleolar RNA-associated protein 8	P
sp|Q02326|RL6A_YEAST	60S ribosomal protein L6-A	P
sp|Q02892|NOG1_YEAST	Nucleolar GTP-binding protein 1	P
sp|Q02931|UTP17_YEAST	NET1-associated nuclear protein 1	P
sp|Q03532|HAS1_YEAST	ATP-dependent RNA helicase HAS1	P
sp|Q05946|UTP13_YEAST	U3 small nucleolar RNA-associated protein 13	P
sp|Q06679|UTP4_YEAST	U3 small nucleolar RNA-associated protein 4	P
sp|Q06689|YL413_YEAST	Cell membrane protein YLR413W	P

sp|Q08746|RRS1_YEAST	Regulator of ribosome biosynthesis	P
sp|Q12207|NCE2_YEAST	Non-classical export protein 2	P
sp|Q12213|RL7B_YEAST	60S ribosomal protein L7-B	P
sp|Q12464|RUVB2_YEAST	RuvB-like protein 2	P
sp|Q12522|IF6_YEAST	Eukaryotic translation initiation factor 6	P
sp|Q12690|RL13A_YEAST	60S ribosomal protein L13-A	P
sp|Q3E757|RL11B_YEAST	60S ribosomal protein L11-B	P
sp|Q6FN88|FBRL_CANGA	rRNA 2'-O-methyltransferase fibrillarin	P
sp|Q6FTJ2|RL3_CANGA	60S ribosomal protein L3	P
sp|A7TNP9|RS3A_VANPO	40S ribosomal protein S1	S
sp|O14467|MBF1_YEAST	Multiprotein-bridging factor 1	S
sp|P00549|KPYK1_YEAST	Pyruvate kinase 1	S
sp|P00950|PMG1_YEAST	Phosphoglycerate mutase 1	S
sp|P02294|H2B2_YEAST	Histone H2B.2	S
sp|P04456|RL25_YEAST	60S ribosomal protein L25	S
sp|P07253|CBP6_YEAST	Cytochrome B pre-mRNA-processing protein 6	S
sp|P07260|IF4E_YEAST	Eukaryotic translation initiation factor 4E	S
sp|P07342|ILVB_YEAST	Acetolactate synthase catalytic subunit, mitochondrial	S
sp|P08964|MYO1_YEAST	Myosin-1	S
sp|P09435|HSP73_YEAST	Heat shock protein SSA3	S
sp|P09950|HEM1_YEAST	5-aminolevulinate synthase, mitochondrial	S
sp|P10591|HSP71_YEAST	Heat shock protein SSA1	S
sp|P10592|HSP72_YEAST	Heat shock protein SSA2	S
sp|P10964|RPA1_YEAST	DNA-directed RNA polymerase I subunit RPA190	S
sp|P12383|PDR1_YEAST	Transcription factor PDR1	S
sp|P12683|HMDH1_YEAST	3-hydroxy-3-methylglutaryl-coenzyme A reductase 1	S
sp|P14737|RAD9_YEAST	DNA repair protein RAD9	S
sp|P15454|KGUA_YEAST	Guanylate kinase	S
sp|P15705|STI1_YEAST	Heat shock protein STI1	S
sp|P16387|ODPA_YEAST	Pyruvate dehydrogenase E1 component subunit alpha, mitochondrial	S
sp|P16965|STF2_YEAST	ATPase-stabilizing factor 15 kDa protein	S
sp|P18962|DAP2_YEAST	Dipeptidyl aminopeptidase B	S
sp|P20134|SFL1_YEAST	Flocculation suppression protein	S
sp|P20436|RPAB3_YEAST	DNA-directed RNA polymerases I, II, and III subunit RPABC3	S
sp|P21576|VPS1_YEAST	Vacuolar protein sorting-associated protein 1	S
sp|P22133|MDHC_YEAST	Malate dehydrogenase, cytoplasmic	S
sp|P22216|RAD53_YEAST	Serine/threonine-protein kinase RAD53	S
sp|P22696|ESS1_YEAST	Peptidyl-prolyl cis-trans isomerase ESS1	S
sp|P25294|SIS1_YEAST	Protein SIS1	S
sp|P25342|CDC10_YEAST	Cell division control protein 10	S
sp|P25349|YCP4_YEAST	Flavoprotein-like protein YCP4	S
sp|P25571|YCE1_YEAST	Putative uncharacterized protein YCL041C	S
sp|P25605|ILV6_YEAST	Acetolactate synthase small subunit, mitochondrial	S
sp|P25846|MSH1_YEAST	DNA mismatch repair protein MSH1, mitochondrial	S
sp|P27882|ERV1_YEAST	Mitochondrial FAD-linked sulfhydryl oxidase ERV1	S
sp|P28007|GAR1_YEAST	H/ACA ribonucleoprotein complex subunit 1	S
sp|P28273|OPLA_YEAST	5-oxoprolinase	S
sp|P32074|COPG_YEAST	Coatomer subunit gamma	S
sp|P32324|EF2_YEAST	Elongation factor 2	S
sp|P32386|YBT1_YEAST	ATP-dependent bile acid permease	S

sp|P32573|SPS19_YEAST	Peroxisomal 2,4-dienoyl-CoA reductase SPS19	S
sp|P32588|PUB1_YEAST	Nuclear and cytoplasmic polyadenylated RNA-binding protein PUB1	S
sp|P32589|HSP7F_YEAST	Heat shock protein homolog SSE1	S
sp|P32606|PT127_YEAST	Putative mitochondrial translation system component PET127	S
sp|P32639|BRR2_YEAST	Pre-mRNA-splicing helicase BRR2	S
sp|P32787|MG101_YEAST	Mitochondrial genome maintenance protein MGM101	S
sp|P32861|UGPA1_YEAST	UTP--glucose-1-phosphate uridylyltransferase	S
sp|P33322|CBF5_YEAST	H/ACA ribonucleoprotein complex subunit 4	S
sp|P34756|FAB1_YEAST	1-phosphatidylinositol 3-phosphate 5-kinase FAB1	S
sp|P35191|MDJ1_YEAST	DnaJ homolog 1, mitochondrial	S
sp|P35209|SPT21_YEAST	Protein SPT21	S
sp|P36000|AP1B1_YEAST	AP-1 complex subunit beta-1	S
sp|P36010|NDK_YEAST	Nucleoside diphosphate kinase	S
sp|P36037|DOA1_YEAST	Protein DOA1	S
sp|P36093|PHD1_YEAST	Putative transcription factor PHD1	S
sp|P36160|RPF2_YEAST	Ribosome biogenesis protein RPF2	S
sp|P36161|NU133_YEAST	Nucleoporin NUP133	S
sp|P37838|NOP4_YEAST	Nucleolar protein 4	S
sp|P38009|PUR92_YEAST	Bifunctional purine biosynthesis protein ADE17	S
sp|P38074|HMT1_YEAST	HNRNP arginine N-methyltransferase	S
sp|P38129|TAF5_YEAST	Transcription initiation factor TFIID subunit 5	S
sp|P38132|MCM7_YEAST	DNA replication licensing factor MCM7	S
sp|P38207|APN2_YEAST	DNA-(apurinic or apyrimidinic site) lyase 2	S
sp|P38251|RFC5_YEAST	Replication factor C subunit 5	S
sp|P38427|TSL1_YEAST	Trehalose synthase complex regulatory subunit TSL1	S
sp|P38631|FKS1_YEAST	1,3-beta-glucan synthase component FKS1	S
sp|P38713|OSH3_YEAST	Oxysterol-binding protein homolog 3	S
sp|P38737|ECM29_YEAST	Proteasome component ECM29	S
sp|P38775|YHK5_YEAST	Putative uncharacterized protein YHR045W	S
sp|P38783|FYV4_YEAST	Protein FYV4, mitochondrial	S
sp|P38788|SSZ1_YEAST	Ribosome-associated complex subunit SSZ1	S
sp|P38811|TRA1_YEAST	Transcription-associated protein 1	S
sp|P38920|MLH1_YEAST	DNA mismatch repair protein MLH1	S
sp|P38934|BFR1_YEAST	Nuclear segregation protein BFR1	S
sp|P39730|IF2P_YEAST	Eukaryotic translation initiation factor 5B	S
sp|P39744|NOC2_YEAST	Nucleolar complex protein 2	S
sp|P39928|SLN1_YEAST	Osmosensing histidine protein kinase SLN1	S
sp|P40012|PPOX_YEAST	Protoporphyrinogen oxidase	S
sp|P40095|YEY8_YEAST	Uncharacterized protein YER158C	S
sp|P40157|VID27_YEAST	Vacuolar import and degradation protein 27	S
sp|P40395|RIC1_YEAST	Protein RIC1	S
sp|P40508|YIH7_YEAST	Uncharacterized protein YIL077C	S
sp|P40825|SYA_YEAST	Alanine--tRNA ligase, mitochondrial	S
sp|P40990|MSS2_YEAST	Protein MSS2, mitochondrial	S
sp|P40991|NOP2_YEAST	Putative ribosomal RNA methyltransferase Nop2	S
sp|P41745|EF1A_BLAAD	Elongation factor 1-alpha	S
sp|P42846|KRI1_YEAST	Protein KRI1	S
sp|P42951|LSB6_YEAST	Phosphatidylinositol 4-kinase LSB6	S
sp|P47079|TCPQ_YEAST	T-complex protein 1 subunit theta	S
sp|P50109|PSP2_YEAST	Protein PSP2	S

sp|P50111|ZDS1_YEAST	Protein ZDS1	S
sp|P53086|KIP3_YEAST	Kinesin-like protein KIP3	S
sp|P53120|YGO0_YEAST	Uncharacterized membrane protein YGL140C	S
sp|P53128|MTHR2_YEAST	Methylenetetrahydrofolate reductase 2	S
sp|P53150|LIF1_YEAST	Ligase-interacting factor 1	S
sp|P53164|NPY1_YEAST	NADH pyrophosphatase	S
sp|P53206|CYSK_YEAST	Putative cysteine synthase	S
sp|P53232|YG1Y_YEAST	Putative uncharacterized protein YGR051C	S
sp|P53236|RSC1_YEAST	Chromatin structure-remodeling complex subunit RSC1	S
sp|P53280|CF130_YEAST	Protein CAF130	S
sp|P53307|YG4O_YEAST	Uncharacterized protein YGR219W	S
sp|P53338|MAL13_YEAST	Maltose fermentation regulatory protein MAL13	S
sp|P53584|YTP1_YEAST	Protein YTP1	S
sp|P53751|YN94_YEAST	Uncharacterized membrane glycoprotein YNR065C	S
sp|P54005|DIA1_YEAST	Protein DIA1	S
sp|P54838|DAK1_YEAST	Dihydroxyacetone kinase 1	S
sp|Q00402|NUM1_YEAST	Nuclear migration protein NUM1	S
sp|Q01662|AMPM1_YEAST	Methionine aminopeptidase 1	S
sp|Q01939|PRS8_YEAST	26S protease regulatory subunit 8 homolog	S
sp|Q02486|ABF2_YEAST	ARS-binding factor 2, mitochondrial	S
sp|Q02554|CUS1_YEAST	Cold sensitive U2 snRNA suppressor 1	S
sp|Q02725|VTC3_YEAST	Vacuolar transporter chaperone 3	S
sp|Q03388|VPS72_YEAST	Vacuolar protein sorting-associated protein 72	S
sp|Q03786|GNTK_YEAST	Probable gluconokinase	S
sp|Q03787|YD249_YEAST	Uncharacterized protein YDR249C	S
sp|Q04007|YDR86_YEAST	Uncharacterized protein YDR186C	S
sp|Q04311|VMS1_YEAST	Protein VMS1	S
sp|Q04373|PUF6_YEAST	Pumilio homology domain family member 6	S
sp|Q05022|RRP5_YEAST	rRNA biogenesis protein RRP5	S
sp|Q05854|YL278_YEAST	Uncharacterized transcriptional regulatory protein YLR278C	S
sp|Q05924|DCR2_YEAST	Phosphatase DCR2	S
sp|Q06164|MMS22_YEAST	Methyl methanesulfonate-sensitivity protein 22	S
sp|Q06205|FKBP4_YEAST	FK506-binding protein 4	S
sp|Q06680|CND3_YEAST	Condensin complex subunit 3	S
sp|Q07623|NOP6_YEAST	Nucleolar protein 6	S
sp|Q07829|RRT7_YEAST	Putative regulator of rDNA transcription protein 7	S
sp|Q07878|VPS13_YEAST	Vacuolar protein sorting-associated protein 13	S
sp|Q07881|YL047_YEAST	Putative uncharacterized protein YLL047W	S
sp|Q07959|IZH3_YEAST	ADIPOR-like receptor IZH3	S
sp|Q08096|RCL1_YEAST	RNA 3'-terminal phosphate cyclase-like protein	S
sp|Q08109|HRD1_YEAST	ERAD-associated E3 ubiquitin-protein ligase HRD1	S
sp|Q08220|GSHB_YEAST	Glutathione synthetase	S
sp|Q08952|OXR1_YEAST	Oxidation resistance protein 1	S
sp|Q08958|YP205_YEAST	Putative uncharacterized protein YPL205C	S
sp|Q12059|ULA1_YEAST	NEDD8-activating enzyme E1 regulatory subunit	S
sp|Q12152|YP150_YEAST	Putative serine/threonine-protein kinase YPL150W	S
sp|Q12159|YRA1_YEAST	RNA annealing protein YRA1	S
sp|Q12177|YL056_YEAST	Uncharacterized protein YLL056C	S
sp|Q12263|GIN4_YEAST	Serine/threonine-protein kinase GIN4	S
sp|Q12275|YO093_YEAST	Uncharacterized protein YOR093C	S
sp|Q12325|SUL2_YEAST	Sulfate permease 2	S

sp|Q12326|PMG3_YEAST	Phosphoglycerate mutase 3	S
sp|Q12358|JLP1_YEAST	Alpha-ketoglutarate-dependent sulfonate dioxygenase	S
sp|Q12377|RPN6_YEAST	26S proteasome regulatory subunit RPN6	S
sp|Q12400|TRM10_YEAST	tRNA (guanine(9)-N1)-methyltransferase	S
sp|Q12513|TMA17_YEAST	Translation machinery-associated protein 17	S
sp|Q12745|SEC39_YEAST	Protein transport protein SEC39	S
sp|Q6Q560|ISD11_YEAST	Protein ISD11	S
sp|Q99186|AP2M_YEAST	AP-2 complex subunit mu	S
sp|A6ZPE5|NOP58_YEAS7	Nucleolar protein 58	S, P
sp|P02406|RL28_YEAST	60S ribosomal protein L28	S, P
sp|P02994|EF1A_YEAST	Elongation factor 1-alpha	S, P
sp|P04147|PABP_YEAST	Polyadenylate-binding protein, cytoplasmic and nuclear	S, P
sp|P05030|PMA1_YEAST	Plasma membrane ATPase 1	S, P
sp|P05317|RLA0_YEAST	60S acidic ribosomal protein P0	S, P
sp|P05737|RL7A_YEAST	60S ribosomal protein L7-A	S, P
sp|P05740|RL17A_YEAST	60S ribosomal protein L17-A	S, P
sp|P05750|RS3_YEAST	40S ribosomal protein S3	S, P
sp|P05756|RS13_YEAST	40S ribosomal protein S13	S, P
sp|P06105|SC160_YEAST	Protein SCP160	S, P
sp|P06169|PDC1_YEAST	Pyruvate decarboxylase isozyme 1	S, P
sp|P07283|PMM_YEAST	Phosphomannomutase	S, P
sp|P11484|HSP75_YEAST	Heat shock protein SSB1	S, P
sp|P12695|ODP2_YEAST	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial	S, P
sp|P15646|FBRL_YEAST	rRNA 2'-O-methyltransferase fibrillarin	S, P
sp|P17076|RL8A_YEAST	60S ribosomal protein L8-A	S, P
sp|P17255|VATA_YEAST	V-type proton ATPase catalytic subunit A	S, P
sp|P19657|PMA2_YEAST	Plasma membrane ATPase 2	S, P
sp|P24545|PMA1_ZYGRO	Plasma membrane ATPase	S, P
sp|P25443|RS2_YEAST	40S ribosomal protein S2	S, P
sp|P26321|RL5_YEAST	60S ribosomal protein L5	S, P
sp|P26783|RS5_YEAST	40S ribosomal protein S5	S, P
sp|P26784|RL16A_YEAST	60S ribosomal protein L16-A	S, P
sp|P27476|NSR1_YEAST	Nuclear localization sequence-binding protein	S, P
sp|P29453|RL8B_YEAST	60S ribosomal protein L8-B	S, P
sp|P32445|RIM1_YEAST	Single-stranded DNA-binding protein RIM1, mitochondrial	S, P
sp|P32473|ODPB_YEAST	Pyruvate dehydrogenase E1 component subunit beta, mitochondrial	S, P
sp|P33201|MRT4_YEAST	mRNA turnover protein 4	S, P
sp|P33302|PDR5_YEAST	Pleiotropic ABC efflux transporter of multiple drugs	S, P
sp|P36049|EBP2_YEAST	rRNA-processing protein EBP2	S, P
sp|P38701|RS20_YEAST	40S ribosomal protein S20	S, P
sp|P38911|FKBP3_YEAST	FK506-binding nuclear protein	S, P
sp|P39990|SNU13_YEAST	13 kDa ribonucleoprotein-associated protein	S, P
sp|P40150|HSP76_YEAST	Heat shock protein SSB2	S, P
sp|P41805|RL10_YEAST	60S ribosomal protein L10	S, P
sp|P48589|RS12_YEAST	40S ribosomal protein S12	S, P
sp|P49626|RL4B_YEAST	60S ribosomal protein L4-B	S, P
sp|P53252|PIL1_YEAST	Sphingolipid long chain base-responsive protein PIL1	S, P
sp|Q01855|RS15_YEAST	40S ribosomal protein S15	S, P

sp|Q12117|MRH1_YEAST	Protein MRH1	S, P
sp|Q12230|LSP1_YEAST	Sphingolipid long chain base-responsive protein LSP1	S, P
sp|Q12335|PST2_YEAST	Protoplast secreted protein 2	S, P
sp|Q12460|NOP56_YEAST	Nucleolar protein 56	S, P
sp|Q12499|NOP58_YEAST	Nucleolar protein 58	S, P
sp|P16521|EF3A_YEAST	Elongation factor 3A	S, P

3.2.1. Positive control for globular domain pull-down experiment. *SIR3 is highlighted in blue where it was identified.*

Replicate 1 Accession number	**Protein Description**	**Protein Score**	**Peptides matched**	**% Coverage**
SIR3_YEAST	Regulatory protein SIR3	56	9	7.2
H2B1_YEAST	Histone H2B.1	44	2	6.9
RT51_YEAST	37S ribosomal protein MRP51, mitochondrial	34	2	5.5
Replicate 2 Accession number	**Protein Description**	**Protein Score**	**Peptides matched**	**% Coverage**
H2B1_YEAST	Histone H2B.1	61	3	6.9
SIR3_YEAST	Regulatory protein SIR3	53	11	7.9
H2AZ_YEAST	Histone H2A.Z	45	7	19.4
Replicate 3 Accession number	**Protein Description**	**Protein Score**	**Peptides matched**	**% Coverage**
SIR3_YEAST	Regulatory protein SIR3	71	8	9.6
H2B1_YEAST	Histone H2B.1	60	7	24.4
H2AZ_YEAST	Histone H2A.Z	52	9	27.6
Replicate 4 Accession number	**Protein Description**	**Protein Score**	**Peptides matched**	**% Coverage**
SIR3_YEAST	Regulatory protein SIR3	78	13	12.9
H2B1_YEAST	Histone H2B.1	49	3	16.8
H2AZ_YEAST	Histone H2A.Z	43	9	23.9

3.2.2 Positive control for canonical octamer pull-down experiment. *SIR3 is highlighted in blue where it was identified.*

Replicate 1 Accession number	**Protein Description**	**Protein Score**	**Peptides matched**	**% Coverage**
SIR3_YEAST	Regulatory protein SIR3	69	12	9.6
H4_YEAST	Histone H4	51	10	28.2
H3_YEAST	Histone H3	41	10	11.8
H2B1_YEAST	Histone H2B.1	39	1	6.9
CYPH_YEAST	Peptidyl-prolyl cis-trans isomerase	33	1	4.3
Replicate 2 Accession number	**Protein Description**	**Protein Score**	**Peptides matched**	**% Coverage**
SIR3_YEAST	Regulatory protein SIR3	75	6	6.9
H3_YEAST	Histone H3	57	10	12.5
Replicate 3 Accession number	**Protein Description**	**Protein Score**	**Peptides matched**	**% Coverage**
SIR3_YEAST	Regulatory protein SIR3	74	10	6.6
H3_YEAST	Histone H3	46	6	9.6
H2B1_YEAST	Histone H2B.1	35	1	6.9
Replicate 4 Accession number	**Protein Description**	**Protein Score**	**Peptides matched**	**% Coverage**
H4_YEAST	Histone H4	72	16	22.3
SIR3_YEAST	Regulatory protein SIR3	62	17	9.7
H3_YEAST	Histone H3	62	6	5.1
H2AZ_YEAST	Histone H2A.Z	35	9	19.4

3.2.3 Non-specific binding of SIR$^{1-380}$ to Streptavidin-coupled Dynabeads®. *SIR3 is highlighted in blue where it was identified.*

Replicate 1 Accession number	**Protein Description**	**Protein Score**	**Peptides matched**	**% Coverage**
SIR3_YEAST	Regulatory protein SIR3	60	7	4.8
Replicate 2 Accession number	**Protein Description**	**Protein Score**	**Peptides matched**	**% Coverage**
SIR3_YEAST	Regulatory protein SIR3	34	2	1.1

Table 3.3.1. Heat map for all confident identifications. *Green indicates the proteins identified with a probability ≥ 0.9. Red indicates absence from the sample (probability = 0) and orange indicates the possible presence (probability > 0 but < 0.9) of these in the sample.*

Sequence Id	**Sequence Name**	**Beads Protein Probability**	**DNA Protein Probability**	**Canonical Protein Probability**	**Globular Protein Probability**
sp|P10964|RPA1_YEAST	DNA-directed RNA polymerase I subunit RPA190	1	1	1	1
sp|Q12460|NOP56_YEAST	Nucleolar protein 56	1	1	1	1
sp|P22138|RPA2_YEAST	DNA-directed RNA polymerase I subunit RPA135	1	1	1	1
sp|P05030|PMA1_YEAST	Plasma membrane ATPase 1	1	1	1	1
sp|Q05022|RRP5_YEAST	rRNA biogenesis protein RRP5	1	1	1	1
sp|P33322|CBF5_YEAST	H/ACA ribonucleoprotein complex subunit 4	1	1	1	1
sp|P53252|PIL1_YEAST	Sphingolipid long chain base-responsive protein PIL1	1	1	1	1
sp|Q12230|LSP1_YEAST	Sphingolipid long chain base-responsive protein LSP1	1	1	1	1
sp|Q12117|MRH1_YEAST	Protein MRH1	1	1	1	1
sp|Q07791|YD23B_YEAST	Transposon Ty2-DR3 Gag-Pol polyprotein	1	1	1	0.99
sp|P04801|SYTC_YEAST	Threonine--tRNA ligase, cytoplasmic	1	0.99	1	1

sp|P0C2H7|RL27B_YEAST	60S ribosomal protein L27-B	1	0.98	1	1
sp|P28007|GAR1_YEAST	H/ACA ribonucleoprotein complex subunit 1	1	0.48	1	1
sp|P05756|RS13_YEAST	40S ribosomal protein S13	1	0.39	1	0.99
sp|P32495|NHP2_YEAST	H/ACA ribonucleoprotein complex subunit 2	1	0.27	1	1
sp|Q06205|FKBP4_YEAST	FK506-binding protein 4	1	0	0.99	1
sp|P19657|PMA2_YEAST	Plasma membrane ATPase 2	1	0	0.99	0.99
sp|P37838|NOP4_YEAST	Nucleolar protein 4	0.99	0	1	1
sp|Q08208|NOP12_YEAST	Nucleolar protein 12	0.99	0.99	0.99	0.99
sp|A6ZPE5|NOP58_YEAS7	Nucleolar protein 58	0.98	0.78	1	1
sp|Q12499|NOP58_YEAST	Nucleolar protein 58	0.98	0.78	1	1
sp|P39990|SNU13_YEAST	13 kDa ribonucleoprotein-associated protein	0.98	0	1	1
sp|P53914|KRE33_YEAST	UPF0202 protein KRE33	0.97	0	1	1
sp|P07703|RPAC1_YEAST	DNA-directed RNA polymerases I and III subunit RPAC1	0.96	0.99	1	1
sp|P46669|RPA43_YEAST	DNA-directed RNA polymerase I subunit RPA43	0.95	0.88	1	1
sp|P15646|FBRL_YEAST	rRNA 2'-O-methyltransferase fibrillarin	0.95	0.8	1	1
sp|P24545|PMA1_ZYGRO	Plasma membrane ATPase	0.95	0	0.9	0.92
sp|P38911|FKBP3_YEAST	FK506-binding nuclear protein	0.94	1	1	1
sp|P26321|RL5_YEAST	60S ribosomal protein L5	0.94	0	0.96	0.96
sp|P38144|ISW1_YEAST	ISWI chromatin-remodeling complex ATPase ISW1	0.91	0	1	0.99
sp|P47167|nop14_YEAST	Central kinetochore subunit MCM22	0.88	0.24	0.51	0.95
sp|P02309|H4_YEAST	Histone H4	0.87	0.85	0.97	0.98
sp|Q8NIG3|H4_CANGA	Histone H4	0.87	0.85	0.96	0.98
sp|P39744|NOC2_YEAST	Nucleolar complex protein 2	0.83	0.83	0.97	1
sp|Q3E757|RL11B_YEAST	60S ribosomal protein L11-B	0.81	0.57	0.74	0.96
sp|P26785|RL16B_YEAST	60S ribosomal protein L16-B	0.8	0	0.99	0.98
sp|Q08235|BRX1_YEAST	Ribosome biogenesis protein BRX1	0.77	1	0.99	1
sp|P31539|HS104_YEAST	Heat shock protein 104	0.73	0.75	0.57	0.92
sp|P06786|TOP2_YEAST	DNA topoisomerase 2	0.69	0	1	1
sp|P05317|RLA0_YEAST	60S acidic ribosomal protein P0	0.67	0	0.5	0.98
sp|P02994|EF1A_YEAST	Elongation factor 1-alpha	0.64	0	0.99	1
sp|A6ZPU3|DBP4_YEAS7	ATP-dependent RNA helicase DBP4	0.55	0	0.89	0.93

sp|P20448|DBP4_YEAST	ATP-dependent RNA helicase HCA4	0.55	0	0.89	0.93
sp|P05737|RL7A_YEAST	60S ribosomal protein L7-A	0.5	0.5	1	0.99
sp|Q12213|RL7B_YEAST	60S ribosomal protein L7-B	0.5	0.5	1	0.99
sp|P0CX49|RL18A_YEAST	60S ribosomal protein L18-A	0.47	0	0.66	0.92
sp|P0CX50|RL18B_YEAST	60S ribosomal protein L18-B	0.47	0	0.66	0.92
sp|P12695|ODP2_YEAST	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial	0.47	1	0.51	1
sp|P37292|GLYM_YEAST	Serine hydroxymethyltransferase, mitochondrial	0.42	0	0.4	1
sp|P35194|UTP20_YEAST	U3 small nucleolar RNA-associated protein 20	0.35	0.35	1	1
sp|P20436|RPAB3_YEAST	DNA-directed RNA polymerases I, II, and III subunit RPABC3	0.35	0.22	1	1
sp|Q12176|MAK21_YEAST	Ribosome biogenesis protein MAK21	0.33	0.45	1	1
sp|P40558|CFD1_YEAST	Cytosolic Fe-S cluster assembly factor CFD1	0.24	0.28	0	0.91
sp|P22147|XRN1_YEAST	5'-3' exoribonuclease 1	0	0.98	1	1
sp|P53254|UTP22_YEAST	U3 small nucleolar RNA-associated protein 22	0	0.73	1	0.94
sp|P39985|DPO5_YEAST	DNA polymerase V	0	0.6	1	1
sp|P20967|ODO1_YEAST	2-oxoglutarate dehydrogenase, mitochondrial	0	0	1	1
sp|Q03532|HAS1_YEAST	ATP-dependent RNA helicase HAS1	0	0	1	1
sp|Q08965|BMS1_YEAST	Ribosome biogenesis protein BMS1	0	0	1	1
sp|P42945|UTP10_YEAST	U3 small nucleolar RNA-associated protein 10	0	0	1	1
sp|Q01080|RPA49_YEAST	DNA-directed RNA polymerase I subunit RPA49	0	0	1	1
sp|P47006|RPA34_YEAST	DNA-directed RNA polymerase I subunit RPA34	0	0	1	1
sp|P19262|ODO2_YEAST	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	0	0	1	1
sp|P09624|DLDH_YEAST	Dihydrolipoyl dehydrogenase, mitochondrial	0	0	1	1
sp|Q00955|ACAC_YEAST	Acetyl-CoA carboxylase	0	0	1	1
sp|Q99207|NOP14_YEAST	Nucleolar complex protein 14	0	0	1	1
sp|Q03973|HMO1_YEAST	High mobility group protein 1	0	0	1	1
sp|Q12159|YRA1_YEAST	RNA annealing protein YRA1	0	0	1	1
sp|Q06287|NEP1_YEAST	Ribosomal RNA small subunit methyltransferase NEP1	0	0	1	1

sp|P06169|PDC1_YEAST	Pyruvate decarboxylase isozyme 1	0	0	1	1
sp|P29453|RL8B_YEAST	60S ribosomal protein L8-B	0	0	1	1
sp|Q12035|FCF2_YEAST	rRNA-processing protein FCF2	0	0	1	1
sp|Q07896|NOC3_YEAST	Nucleolar complex-associated protein 3	0	0	1	1
sp|P20434|RPAB1_YEAST	DNA-directed RNA polymerases I, II, and III subunit RPABC1	0	0	1	1
sp|P36049|EBP2_YEAST	rRNA-processing protein EBP2	0	0	1	1
sp|P25270|MRM1_YEAST	rRNA methyltransferase, mitochondrial	0	0	0.99	1
sp|P28000|RPAC2_YEAST	DNA-directed RNA polymerases I and III subunit RPAC2	0	0	0.99	0.99
sp|P04456|RL25_YEAST	60S ribosomal protein L25	0	0	0.99	0.9
sp|P38811|TRA1_YEAST	Transcription-associated protein 1	0	0	0.98	1
sp|P47068|BBC1_YEAST	Myosin tail region-interacting protein MTI1	0	0	0.98	1
sp|P38333|ENP1_YEAST	Essential nuclear protein 1	0	0	0.98	1
sp|P38833|YHS7_YEAST	Uncharacterized protein YHR127W	0	0	0.98	1
sp|P40509|COPE_YEAST	Coatomer subunit epsilon	0	0	0.98	0.99
sp|P32529|RPA12_YEAST	DNA-directed RNA polymerase I subunit RPA12	0	0	0.98	0.98
sp|P40010|NUG1_YEAST	Nuclear GTP-binding protein NUG1	0	0.22	0.97	1
sp|P46655|SYEC_YEAST	Glutamate--tRNA ligase, cytoplasmic	0	0	0.96	1
sp|P46677|TAF1_YEAST	Transcription initiation factor TFIID subunit 1	0	0	0.96	0.99
sp|A7A1G0|DBP9_YEAS7	ATP-dependent RNA helicase DBP9	0	0	0.95	0.99
sp|Q06218|DBP9_YEAST	ATP-dependent RNA helicase DBP9	0	0	0.95	0.99
sp|P34241|URB1_YEAST	Nucleolar pre-ribosomal-associated protein 1	0	0	0.93	0.99
sp|A7A0P8|DRS1_YEAS7	ATP-dependent RNA helicase DRS1	0	0	0.89	0.96
sp|P32892|DRS1_YEAST	ATP-dependent RNA helicase DRS1	0	0	0.89	0.96
sp|Q02892|NOG1_YEAST	Nucleolar GTP-binding protein 1	0	0	0.88	0.93
sp|P20435|RPAB2_YEAST	DNA-directed RNA polymerases I, II, and III subunit RPABC2	0	0	0.8	1
sp|P14126|RL3_YEAST	60S ribosomal protein L3	0	0	0.74	1
sp|P53131|PRP43_YEAST	Pre-mRNA-splicing factor ATP-dependent RNA helicase PRP43	0	0	0.47	0.93
sp|P32333|MOT1_YEAST	TATA-binding protein-associated factor MOT1	0	0	0.42	0.95

sp|P53040|TAF6_YEAST	Transcription initiation factor TFIID subunit 6	0	0	0.41	0.99
sp|P09440|C1TM_YEAST	C-1-tetrahydrofolate synthase, mitochondrial	0	0.99	0	0.99
sp|P38198|STU1_YEAST	Protein STU1	0	0.33	0	0.9
sp|P37012|PGM2_YEAST	Phosphoglucomutase-2	0	0.32	0	0.92
sp|P47108|URB2_YEAST	Nucleolar pre-ribosomal-associated protein 2	0	0.25	0	1
sp|P42846|KRI1_YEAST	Protein KRI1	0	0	0	1
sp|P53622|COPA_YEAST	Coatomer subunit alpha	0	0	0	1
sp|P40991|NOP2_YEAST	Putative ribosomal RNA methyltransferase Nop2	0	0	0	1
sp|P32481|IF2G_YEAST	Eukaryotic translation initiation factor 2 subunit gamma	0	0	0	1
sp|P40957|MAD1_YEAST	Spindle assembly checkpoint component MAD1	0	0	0	0.99
sp|P32844|SEC6_YEAST	Exocyst complex component SEC6	0	0	0	0.98
sp|P32341|VPH2_YEAST	Vacuolar ATPase assembly integral membrane protein VPH2	0	0	0	0.97
sp|P53742|NOG2_YEAST	Nucleolar GTP-binding protein 2	0	0	0	0.97
sp|P53929|YNK8_YEAST	Uncharacterized protein YNL108C	0	0	0	0.97
sp|Q05027|TAF9_YEAST	Transcription initiation factor TFIID subunit 9	0	0	0	0.96
sp|P33412|ECT1_YEAST	Ethanolamine-phosphate cytidylyltransferase	0	0	0	0.96
sp|P26448|BUB2_YEAST	Mitotic check point protein BUB2	0	0	0	0.96
sp|P38882|UTP9_YEAST	U3 small nucleolar RNA-associated protein 9	0	0	0	0.95
sp|Q02354|UTP6_YEAST	U3 small nucleolar RNA-associated protein 6	0	0	0	0.94
sp|Q04373|PUF6_YEAST	Pumilio homology domain family member 6	0	0	0	0.93
sp|P06779|RAD7_YEAST	DNA repair protein RAD7	0	0	0	0.92
sp|Q07622|ACK1_YEAST	Activator of C kinase protein 1	0	0	0	0.92
sp|Q02207|FOX2_YEAST	Peroxisomal hydratase-dehydrogenase-epimerase	0	0	0	0.91
sp|Q06132|SGD1_YEAST	Suppressor of glycerol defect protein 1	0	0	0	0.91
sp|P40422|RPAB4_YEAST	DNA-directed RNA polymerases I, II, and III subunit RPABC4	0	0	0	0.91

Table 3.3.2. Identifications shared by the globular domain as well as the canonical octamer pull-down

Sequence Id	Sequence Name	Canonical Protein Probability	Globular Protein Probability	Localization
sp|Q12176|MAK21_YEAST	Ribosome biogenesis protein MAK21	1	1	Nucleus
sp|Q03532|HAS1_YEAST	ATP-dependent RNA helicase HAS1	1	1	Nucleus
sp|Q08965|BMS1_YEAST	Ribosome biogenesis protein BMS1	1	1	Nucleus, Cytoplasm
sp|Q01080|RPA49_YEAST	DNA-directed RNA polymerase I subunit RPA49	1	1	Nucleus
sp|P35194|UTP20_YEAST	U3 small nucleolar RNA-associated protein 20	1	1	Nucleus, Cytoplasm
sp|P47006|RPA34_YEAST	DNA-directed RNA polymerase I subunit RPA34	1	1	Nucleus
sp|Q03973|HMO1_YEAST	High mobility group protein 1	1	1	Nucleus
sp|Q99207|NOP14_YEAST	Nucleolar complex protein 14	1	0.98	Nucleus
sp|P38811|TRA1_YEAST	Transcription-associated protein 1	0.98	1	Nucleus
sp|Q06287|NEP1_YEAST	Ribosomal RNA small subunit methyltransferase NEP1	1	1	Nucleus
sp|P40010|NUG1_YEAST	Nuclear GTP-binding protein NUG1	0.97	1	Nucleus
sp|P39985|DPO5_YEAST	DNA polymerase V	1	1	Nucleus
sp|P20436|RPAB3_YEAST	DNA-directed RNA polymerases I, II, and III subunit RPABC3	1	1	Nucleus
sp|P06786|TOP2_YEAST	DNA topoisomerase 2	0.91	1	Nucleus
sp|P06169|PDC1_YEAST	Pyruvate decarboxylase isozyme 1	1	1	Nucleus, Cytoplasm
sp|P46677|TAF1_YEAST	Transcription initiation factor TFIID subunit 1	0.92	0.99	Nucleus
sp|Q12035|FCF2_YEAST	rRNA-processing protein FCF2	1	1	Nucleus
sp|P42945|UTP10_YEAST	U3 small nucleolar RNA-associated protein 10	0.93	0.98	Nucleus, Mitochondrion
sp|P36049|EBP2_YEAST	rRNA-processing protein EBP2	1	1	Nucleus
sp|Q12159|YRA1_YEAST	RNA annealing protein YRA1	0.99	0.99	Nucleus
sp|P38333|ENP1_YEAST	Essential nuclear protein 1	0.98	1	Nucleus, Cytoplasm
sp|Q07896|NOC3_YEAST	Nucleolar complex-associated protein 3	0.98	0.99	Nucleus
sp|P38833|YHS7_YEAST	Uncharacterized protein YHR127W	0.98	1	Nucleus
sp|P28000|RPAC2_YEAST	DNA-directed RNA polymerases I and III subunit RPAC2	0.99	0.99	Nucleus
sp|P32529|RPA12_YEAST	DNA-directed RNA polymerase I subunit RPA12	0.98	0.98	Nucleus

Table 3.3.3. Identifications unique to the globular domain pull-down

Sequence Id	Sequence Name	Globular Protein Probability	Localization
sp|P47167|MCM22_YEAST	Central kinetochore subunit MCM22	0.95	Nucleus
sp|P20435|RPAB2_YEAST	DNA-directed RNA polymerases I, II, and III subunit RPABC2	1	Nucleus
sp|P40422|RPAB4_YEAST	DNA-directed RNA polymerases I, II, and III subunit RPABC4	0.91	Nucleus
sp|P33412|ECT1_YEAST	Ethanolamine-phosphate cytidylyltransferase	0.96	Nucleus, Cytoplasm
sp|P39744|NOC2_YEAST	Nucleolar complex protein 2	1	Nucleus
sp|Q02892|NOG1_YEAST	Nucleolar GTP-binding protein 1	0.93	Nucleus
sp|P53742|NOG2_YEAST	Nucleolar GTP-binding protein 2	0.97	Nucleus
sp|P34241|URB1_YEAST	Nucleolar pre-ribosomal-associated protein 1	0.99	Nucleus
sp|P47108|URB2_YEAST	Nucleolar pre-ribosomal-associated protein 2	1	Nucleus
sp|P53131|PRP43_YEAST	Pre-mRNA-splicing factor ATP-dependent RNA helicase PRP43	0.9	Nucleus
sp|P42846|KRI1_YEAST	Protein KRI1	1	Nucleus
sp|Q04373|PUF6_YEAST	Pumilio homology domain family member 6	0.91	Nucleus
sp|P40991|NOP2_YEAST	Putative ribosomal RNA methyltransferase Nop2	1	Nucleus
sp|P40957|MAD1_YEAST	Spindle assembly checkpoint component MAD1	0.99	Nucleus
sp|Q06132|SGD1_YEAST	Suppressor of glycerol defect protein 1	0.91	Nucleus
sp|P53040|TAF6_YEAST	Transcription initiation factor TFIID subunit 6	0.96	Nucleus
sp|Q05027|TAF9_YEAST	Transcription initiation factor TFIID subunit 9	0.96	Nucleus
sp|Q02354|UTP6_YEAST	U3 small nucleolar RNA-associated protein 6	0.93	Nucleus
sp|P38882|UTP9_YEAST	U3 small nucleolar RNA-associated protein 9	0.95	Nucleus
sp|P53929|YNK8_YEAST	Uncharacterized protein YNL108C	0.97	Unknown

Table 3.3.4. Final results (canonical and globular pull down combined) grouped on function

Sequence Id	Sequence Name	Function
sp|P06169|PDC1_YEAST	Pyruvate decarboxylase isozyme 1	Amino acid catabolism
sp|P06786|TOP2_YEAST	DNA topoisomerase 2	Chromosome segregation
sp|P47167|MCM22_YEAST	Central kinetochore subunit MCM22	Chromosome segregation
sp|P40957|MAD1_YEAST	Spindle assembly checkpoint component MAD1	Chromosome segregation
sp|P33412|ECT1_YEAST	Ethanolamine-phosphate cytidylyltransferase	Lipid metabolism
sp|Q12159|YRA1_YEAST	RNA annealing protein YRA1	Posttranscriptional regulation
sp|P53131|PRP43_YEAST	Pre-mRNA-splicing factor ATP-dependent RNA helicase PRP43	Posttranscriptional regulation
sp|Q04373|PUF6_YEAST	Pumilio homology domain family member 6	Posttranscriptional regulation
sp|Q06132|SGD1_YEAST	Suppressor of glycerol defect protein 1	Regulation of GDP1 expression
sp|Q12176|MAK21_YEAST	Ribosome biogenesis protein MAK21	Ribosome biogenesis
sp|Q03532|HAS1_YEAST	ATP-dependent RNA helicase HAS1	Ribosome biogenesis
sp|Q08965|BMS1_YEAST	Ribosome biogenesis protein BMS1	Ribosome biogenesis
sp|P35194|UTP20_YEAST	U3 small nucleolar RNA-associated protein 20	Ribosome biogenesis
sp|Q99207|NOP14_YEAST	Nucleolar complex protein 14	Ribosome biogenesis
sp|Q06287|NEP1_YEAST	Ribosomal RNA small subunit methyltransferase NEP1	Ribosome biogenesis
sp|P40010|NUG1_YEAST	Nuclear GTP-binding protein NUG1	Ribosome biogenesis
sp|P39985|DPO5_YEAST	DNA polymerase V	Ribosome biogenesis
sp|Q12035|FCF2_YEAST	rRNA-processing protein FCF2	Ribosome biogenesis
sp|P42945|UTP10_YEAST	U3 small nucleolar RNA-associated protein 10	Ribosome biogenesis
sp|P36049|EBP2_YEAST	rRNA-processing protein EBP2	Ribosome biogenesis
sp|P38333|ENP1_YEAST	Essential nuclear protein 1	Ribosome biogenesis
sp|Q07896|NOC3_YEAST	Nucleolar complex-associated protein 3	Ribosome biogenesis and DNA replication
sp|P39744|NOC2_YEAST	Nucleolar complex protein 2	Ribosome biogenesis
sp|Q02892|NOG1_YEAST	Nucleolar GTP-binding protein 1	Ribosome biogenesis
sp|P53742|NOG2_YEAST	Nucleolar GTP-binding protein 2	Ribosome biogenesis
sp|P34241|URB1_YEAST	Nucleolar pre-ribosomal-associated protein 1	Ribosome biogenesis
sp|P47108|URB2_YEAST	Nucleolar pre-ribosomal-associated protein 2	Ribosome biogenesis

sp|P42846|KRI1_YEAST	Protein KRI1	Ribosome biogenesis
sp|P40991|NOP2_YEAST	Putative ribosomal RNA methyltransferase Nop2	Ribosome biogenesis
sp|Q02354|UTP6_YEAST	U3 small nucleolar RNA-associated protein 6	Ribosome biogenesis
sp|P38882|UTP9_YEAST	U3 small nucleolar RNA-associated protein 9	Ribosome biogenesis
sp|Q01080|RPA49_YEAST	DNA-directed RNA polymerase I subunit RPA49	Transcription
sp|P47006|RPA34_YEAST	DNA-directed RNA polymerase I subunit RPA34	Transcription
sp|Q03973|HMO1_YEAST	High mobility group protein 1	Transcription
sp|P38811|TRA1_YEAST	Transcription-associated protein 1	Transcription
sp|P20436|RPAB3_YEAST	DNA-directed RNA polymerases I, II, and III subunit RPABC3	Transcription
sp|P46677|TAF1_YEAST	Transcription initiation factor TFIID subunit 1	Transcription
sp|P28000|RPAC2_YEAST	DNA-directed RNA polymerases I and III subunit RPAC2	Transcription
sp|P32529|RPA12_YEAST	DNA-directed RNA polymerase I subunit RPA12	Transcription
sp|P20435|RPAB2_YEAST	DNA-directed RNA polymerases I, II, and III subunit RPABC2	Transcription
sp|P40422|RPAB4_YEAST	DNA-directed RNA polymerases I, II, and III subunit RPABC4	Transcription
sp|P53040|TAF6_YEAST	Transcription initiation factor TFIID subunit 6	Transcription
sp|Q05027|TAF9_YEAST	Transcription initiation factor TFIID subunit 9	Transcription
sp|P38833|YHS7_YEAST	Uncharacterized protein YHR127W	Unknown
sp|P53929|YNK8_YEAST	Uncharacterized protein YNL108C	Unknown

Chapter 4

Conclusions

The idea that regulatory proteins might interact with the globular domains of the nucleosomes remain a new and largely unexplored one. In the literature overview it was highlighted that these domains play a vital role in the DNA functions which are ultimately mediated by these proteins. It thus seems highly plausible that the regulatory proteins will have some degree of interaction with the globular domains. The importance of regulatory proteins such as the chromatin remodelers and chromatin architectural proteins and their proper interaction with chromatin have been discussed at length in the first chapter. In an attempt to better understand this level of regulation, this study aimed to isolate and identify proteins that bind the non-tail parts of the nucleosome.

To isolate these proteins the globular domains of the four core histones were expressed in *Escherichia coli*. These were then reconstituted with labelled DNA to form biotinylated octamers to be used in a pull-down experiment. This work did find novel interactions which will now be explored through computer simulations to firstly confirm the interactions but also to investigate the manner in which these proteins bind the NCP.

One of these proteins was NOC3. This protein is not only important for ribosomal subunit synthesis and transport but is also crucial to the initiation of DNA replication. Then there is the high mobility group protein 1 which are known to bind the nucleosome. The transcription factors identified here are known DNA-binding proteins, although they were identified in experiments where the DNA was bound to the histone octamer rather than in the free DNA control experiment, might indicate a novel way of interacting with the DNA, mediated by the NCP. Other proteins found that pique the interest were MCM22, MAD1 and TOP2 which are implicated in the regulation of chromosome segregation during cell division. RNA-binding proteins PRP43, PUF6 and YRA1 have roles in posttranscriptional regulation of mRNA. PDC1 plays an important role in the process of amino acid degradation. SGD1 regulates the expression of GDP1 through an unknown mechanism and has a previously established interaction with histone H4 as well as the ISWI remodelling complex. ECT1 is involved in phosphatidylethanolamine biosynthesis and is essential for sporulation. Then the uncharacterised proteins YHR127W which has been implicated in the process of mitotic spindle elongation and YNL108C with its phosphatase activity.

The great advantage of this pull-down approach is that it enables the identification of completely novel interactions as opposed to more guided approaches investigating specific interactions. This then offers a starting point from which to use the more directed approaches in order to confirm and characterise the interactions. Especially interesting will be to investigate the interactions between the transcription factors and the NCP as to date these factors have mostly been investigated in relation to specific DNA sequences.

None of the proteins originally expected to be identified were among the results. This might mean that the protein interactions do in fact not occur independent of the posttranslational modifications. However, SIR3 was also markedly absent from the results. SIR3 has been proven by previous studies to bind the globular domains and has also been used to verify the method. Thus, the most probable explanation was that the proteins of interest was present in low concentrations leading to them being missed by the mass spectrometer. To solve this future work needs to find a way to minimise abundant high concentration proteins that conceal the identification of lower concentration proteins. An investigation into the observed non-specific binding is needed to determine if a different pull down matrix other than magnetic beads would solve this, and whether it is possible to prepare a nuclear extract with low levels of cytoplasmic proteins.

With some refinement this approach can be a very good starting point in the exploration of novel interactions between the NCP and regulatory proteins and thus in the elucidation mechanisms of DNA regulation.

Chapter 5

5.1. Summary

Mutation studies have shown that the nucleosome surface is extremely important for proper DNA replication, transcription, chromatin remodelling and thus correct gene expression. Therefore it is expected that these surfaces of the nucleosome would bind some of the arsenal of regulatory proteins available in the cell. Despite the obvious functional importance of the nucleosome cores, little is known with regards to possible binding partners.

A few interactions with the nucleosome surface has been studied. Silent Information Regulator protein 3 (SIR3) is grouped in a family of chromatin regulators thought to facilitate transcriptional silencing via compaction of chromatin. Two viral proteins have also been shown to interact with chromatin by binding the acidic pocket formed by the H2A-H2B dimer. These are the latency-associated nuclear antigen (LANA) of Kaposi's sarcoma-associated herpesvirus and the 72kDa immediate early 1 (E1) protein of human cytomegalovirus. The nucleosome core surfaces are thus also assumed to play key roles in infectious cycles of these and possibly more viruses. Other known binding partners include members of the high mobility group (HMGN) proteins and regulator of chromosome condensation 1 (RCC1). These proteins play vital roles in the regulation of chromatin structure and thus DNA accessibility.

By identifying the binding partners of the nucleosome cores we will advance our understanding of the regulatory mechanisms of chromatin which will aid in understanding the onset and progression of many diseased states. This study aimed to identify proteins which bind to the non-tail parts of the nucleosome to further our understanding of the regulation of DNA function.

To this end Xenopus laevis histones were expressed in Escherichia coli, both as full-length, canonical histones as well as N-terminally truncated globular domains. These were reconstituted with a biotinylated DNA-fragment to pull down any proteins bound to the histone octamers following incubation with a nuclear extract from Saccharomyces cerevisiae. The resulting proteins were identified when analysed by nanoLC-MS/MS and searched against the SwissProt database using Mascot. In total 25 nuclear proteins were identified to bind exclusively to the globular domain of the nucleosomal core particle.

The results obtained provide a good starting point for further computer simulation studies as well as directed approaches to study specific interactions in vivo.

5.2. Opsomming

Mutasiestudies het gewys dat die nukleosoomoppervlak belangrik is vir suksesvolle DNA replisering, transkripsie, chromatien hermodellering en gevolglik korrekte geenuitdrukking. Dit word dus verwag dat hierdie oppervlaktes van die nukleosoom sekere lede van die regulatoriese proteïengroep sal bind.

Ten spyte van die ooglopende funksionele belang van die nukleosoom se globulêre domein is min inligting beskikbaar oor moontlike proteïen-interaksies. Die Silent Information Regulator proteïen 3 (SIR3) groepeer onder ‘n familie van chromatienreguleerders wat vermoedelik transkripsie onderdruk deur middel van chromatienkompaktering. Twee virale proteïene, Latency-Associated Nuclear Antigen (LANA) van die Kaposi se sarkoomverwante herpesvirus en Immediate Early 1 (E1) proteïen van die menslike sitomegaalvirus, is ook bekend daarvoor om met chromatien se suurresidues op die H2A-H2B dimeer se oppervlak te bind. Die nukleosoom se globulêreoppervlaktes word dus moontlik geïmpliseer in ‘n sleutelrol in die infeksiesiklus van hierdie en moontlik ander virusse. Ander proteïene met bekende interaksies met die nukleosoomoppervlak sluit in lede van die High Mobility Group (HMG) proteïene sowel as die Regulator of Chromosome Condensation 1 (RCC1). Hierdie proteïene speel belangrike rolle in die regulasie van chromatienstruktuur en dus DNA toeganklikheid.

Deur verdere identifisering van proteiene wat die globulêre areas bind word die kennis rondom chromatienreguleringsmeganismes uitgebrei. Kennis hieromtrent kan lei tot ‘n beter begrip van die aanvang en ontwikkeling van siektetoestande en sal bydra tot ‘n beter begrip van die regulasie van DNA-funksies.

Hierdie studie het gepoog om proteïene wat die globulêre nukleosoomareas bind te identifiseer. Hiervoor is *Xenopus laevis* histone (ongemodifiseerde sowel as globulêre histone waarvan die N-terminale areas afgesny is) in E.coli uitgedruk en met ‘n gebiotinileerde DNA-fragment hersaamgestel. Op hierdie wyse is die hersaamgestelde oktamere en enige proteïene wat hulle sou bind tydens inkubasie met ‘n *Saccharomyces cerevisiae* kernekstrak afgetrek. Die afgetrekte proteïene is met nanoLC-MS/MS geanaliseer en met Mascot vanuit die SwissProt databasis geidentifiseer. In totaal is 25 kernproteiene geidentifiseer wat uitsluitlik aan die globulere domein area van die NCP bind.

Die resultate verkry bied ‘n goeie wegspringpunt vir verdure ondersoeke met rekenaargebasseerde simulasies en meer direkte in vivo studies.

Bibliography

Akhtar, A., Zink, D. & Becker, P.B., 2000. Chromodomains are protein-RNA interaction modules. *Nature*, 407(6802), pp.405–409.

Akhtar, N. *et al.*, 2000. SGD1 encodes an essential nuclear protein of Saccharomyces cerevisiae that affects expression of the GPD1 gene for glycerol 3-phosphate dehydrogenase. *FEBS letters*, 483(2-3), pp.87–92.

Allan, J. *et al.*, 1982. Participation of core histone “tails” in the stabilization of the chromatin solenoid. *The Journal of cell biology*, 93(2), pp.285–297.

Allan, J. *et al.*, 1980. The structure of histone H1 and its location in chromatin. *Nature*, 288(5792), pp.675–679.

Amersham Biosciences, 2002. *Gel Filtration Principles and Methods*, Technical Manual

Arenas, J.E. & Abelson, J.N., 1997. Prp43: An RNA helicase-like factor involved in spliceosome disassembly. *Proceedings of the National Academy of Sciences of the United States of America*, 94(22), pp.11798–11802.

Armache, K.-J. *et al.*, 2011. Structural basis of silencing: Sir3 BAH domain in complex with a nucleosome at 3.0 A resolution. *Science (New York, N.Y.)*, 334(6058), pp.977–982.

Arnaudo, N. *et al.*, 2013. The N-terminal acetylation of Sir3 stabilizes its binding to the nucleosome core particle. *Nat Struct Mol Biol*, 20(9), pp.1119–1121.

Van Attikum, H. *et al.*, 2004. Recruitment of the INO80 complex by H2A phosphorylation links ATP-dependent chromatin remodeling with DNA double-strand break repair. *Cell*, 119(6), pp.777–788.

Bannister, A.J. & Kouzarides, T., 2011. Regulation of chromatin by histone modifications. *Cell Res*, 21(3), pp.381–395.

Bao, Y. & Shen, X., 2007. INO80 subfamily of chromatin remodeling complexes. *Mutation research*, 618(1-2), pp.18–29.

Barbera, A.J. *et al.*, 2006. The nucleosomal surface as a docking station for Kaposi’s sarcoma herpesvirus LANA. *Science (New York, N.Y.)*, 311(5762), pp.856–861.

Baumann, C. *et al.*, 2008. Association of ATRX with pericentric heterochromatin and the Y chromosome of neonatal mouse spermatogonia. *BMC molecular biology*, 9, p.29.

Bednar, J. *et al.*, 1998. Nucleosomes, linker DNA, and linker histone form a unique structural motif that directs the higher-order folding and compaction of chromatin. *Proceedings of the National Academy of Sciences of the United States of America*, 95(24), pp.14173–14178.

Bianchi, M.E. & Agresti, A., 2005. HMG proteins: dynamic players in gene regulation and differentiation. *Current opinion in genetics & development*, 15(5), pp.496–506.

Biterge, B. & Schneider, R., 2014. Histone variants: key players of chromatin. *Cell and tissue research*, 356(3), pp.457–466.

Bochar, D.A. *et al.*, 2000. BRCA1 is associated with a human SWI/SNF-related complex: linking chromatin remodeling to breast cancer. *Cell*, 102(2), pp.257–265.

Boon, K.-L. *et al.*, 2006. Yeast ntr1/spp382 mediates prp43 function in postspliceosomes. *Molecular and cellular biology*, 26(16), pp.6016–6023.

Bose, M.E. *et al.*, 2004. The origin recognition complex and Sir4 protein recruit Sir1p to yeast silent chromatin through independent interactions requiring a common Sir1p domain. *Molecular and cellular biology*, 24(2), pp.774–786.

Bouazoune, K. *et al.*, 2002. The dMi-2 chromodomains are DNA binding modules important for ATP-dependent nucleosome mobilization. *The EMBO journal*, 21(10), pp.2430–2440.

Bowen, N.J. *et al.*, 2004. Mi-2/NuRD: multiple complexes for many purposes. *Biochimica et biophysica acta*, 1677(1-3), pp.52–57.

Boyer, L.A. *et al.*, 2002. Essential role for the SANT domain in the functioning of multiple chromatin remodeling enzymes. *Molecular cell*, 10(4), pp.935–942.

Boyer, L.A., Latek, R.R. & Peterson, C.L., 2004. The SANT domain: a unique histone-tail-binding module? *Nature reviews. Molecular cell biology*, 5(2), pp.158–163.

Boyer, L.A. & Peterson, C.L., 2000. Actin-related proteins (Arps): conformational switches for chromatin-remodeling machines? *BioEssays : news and reviews in molecular, cellular and developmental biology*, 22(7), pp.666–672.

Brehm, A. *et al.*, 2004. The many colours of chromodomains. *BioEssays : news and reviews in molecular, cellular and developmental biology*, 26(2), pp.133–140.

Burgess, R.J. & Zhang, Z., 2013. Histone chaperones in nucleosome assembly and human disease. *Nature structural & molecular biology*, 20(1), pp.14–22.

Bustin, M. & Reeves, R., 1996. High-mobility-group chromosomal proteins: architectural components that facilitate chromatin function. *Progress in nucleic acid research and molecular biology*, 54, pp.35–100.

Bustin, M., Trieschmann, L. & Postnikov, Y. V, 1995. The HMG-14/-17 chromosomal protein family: architectural elements that enhance transcription from chromatin templates. *Seminars in cell biology*, 6(4), pp.247–255.

Butler, J.S. *et al.*, 2012. Histone-modifying enzymes: regulators of developmental decisions and drivers of human disease. *Epigenomics*, 4(2), pp.163–177.

Carazo-Salas, R.E. *et al.*, 1999. Generation of GTP-bound Ran by RCC1 is required for chromatin-induced mitotic spindle formation. *Nature*, 400(6740), pp.178–181.

Carriere, V. *et al.*, 2007. IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, 104(1), pp.282–287.

Carruthers, L.M. *et al.*, 1998. Linker Histones Stabilize the Intrinsic Salt-Dependent Folding of Nucleosomal Arrays: Mechanistic Ramifications for Higher-Order Chromatin Folding. *Biochemistry*, 37(42), pp.14776–14787.

Caterino, T.L. & Hayes, J.J., 2011. Structure of the H1 C-terminal domain and function in chromatin condensation. *Biochemistry and cell biology = Biochimie et biologie cellulaire*, 89(1), pp.35–44.

Chambers, A.L. *et al.*, 2013. The BAH domain of Rsc2 is a histone H3 binding domain. *Nucleic acids research*, 41(19), pp.9168–9182.

Chang, J.-F. *et al.*, 2003. Structure of the coiled-coil dimerization motif of Sir4 and its interaction with Sir3. *Structure (London, England : 1993)*, 11(6), pp.637–649.

Chang, M. *et al.*, 2002. A genome-wide screen for methyl methanesulfonate-sensitive mutants reveals genes required for S phase progression in the presence of DNA damage. *Proceedings of the National Academy of Sciences of the United States of America*, 99(26), pp.16934–16939.

Chen, P. & Li, G., 2010. Dynamics of the higher-order structure of chromatin. *Protein & cell*, 1(11), pp.967–71.

Chimura, T., Kuzuhara, T. & Horikoshi, M., 2002. Identification and characterization of CIA/ASF1 as an interactor of bromodomains associated with TFIID. *Proceedings of the National Academy of Sciences of the United States of America*, 99(14), pp.9334–9339.

Cho, I. *et al.*, 2013. ATP-dependent chromatin remodeling by Cockayne syndrome protein B and NAP1-like histone chaperones is required for efficient transcription-coupled DNA repair. *PLoS genetics*, 9(4), p.e1003407.

Cho, K.S., Elizondo, L.I. & Boerkoel, C.F., 2004. Advances in chromatin remodeling and human disease. *Current opinion in genetics & development*, 14(3), pp.308–315.

Chodaparambil, J. V *et al.*, 2007. A charged and contoured surface on the nucleosome regulates chromatin compaction. *Nature structural & molecular biology*, 14(11), pp.1105–1107.

Chodosh, L.A., 2001. Purification of DNA-binding proteins using biotin/streptavidin affinity systems. *Current protocols in molecular biology / edited by Frederick M. Ausubel ... [et al.]*, Chapter 12, p.Unit 12.6.

Choi, H.-S. & Carman, G.M., 2007. Respiratory deficiency mediates the regulation of CHO1-encoded phosphatidylserine synthase by mRNA stability in Saccharomyces cerevisiae. *The Journal of biological chemistry*, 282(43), pp.31217–31227.

Chua, P. & Roeder, G.S., 1995. Bdf1, a yeast chromosomal protein required for sporulation. *Molecular and cellular biology*, 15(7), pp.3685–3696.

Citterio, E. *et al.*, 2000. ATP-dependent chromatin remodeling by the Cockayne syndrome B DNA repair-transcription-coupling factor. *Molecular and cellular biology*, 20(20), pp.7643–7653.

Clapier, C.R. & Cairns, B.R., 2009. The biology of chromatin remodeling complexes. *Annual review of biochemistry*, 78, pp.273–304.

Clarke, P.R. & Zhang, C., 2008. Spatial and temporal coordination of mitosis by Ran GTPase. *Nature reviews. Molecular cell biology*, 9(6), pp.464–477.

Connelly, J.J. *et al.*, 2006. Structure and function of the Saccharomyces cerevisiae Sir3 BAH domain. *Molecular and cellular biology*, 26(8), pp.3256–3265.

Corona, D.F. V & Tamkun, J.W., 2004. Multiple roles for ISWI in transcription, chromosome organization and DNA replication. *Biochimica et biophysica acta*, 1677(1-3), pp.113–119.

Correll, S.J., Schubert, M.H. & Grigoryev, S.A., 2012. Short nucleosome repeats impose rotational modulations on chromatin fibre folding. *The EMBO journal*, 31(10), pp.2416–2426.

Cosgrove, M.S., Boeke, J.D. & Wolberger, C., 2004. Regulated nucleosome mobility and the histone code. *Nature structural & molecular biology*, 11(11), pp.1037–1043.

Crippa, M.P. *et al.*, 1993. Deposition of chromosomal protein HMG-17 during replication affects the nucleosomal ladder and transcriptional potential of nascent chromatin. *The EMBO journal*, 12(10), pp.3855–3864.

Cunico, R.L., Gooding, K.M. & Wehr, T., 1998a. *Basic HPLC and CE of Biomolecules* 1st ed., Bay Bioanalytical Laboratory Richmond, CA.

Cunico, R.L., Gooding, K.M. & Wehr, T., 1998b. *Basic HPLC and CE of Biomolecules* 1st ed., Bay Bioanalytical Laboratory Richmond, CA.

Davey, C.A. *et al.*, 2002. Solvent mediated interactions in the structure of the nucleosome core particle at 1.9 a resolution. *Journal of molecular biology*, 319(5), pp.1097–1113.

Davis, P.K. & Brackmann, R.K., 2003. Chromatin remodeling and cancer. *Cancer biology & therapy*, 2(1), pp.22–29.

Delmas, V., Stokes, D.G. & Perry, R.P., 1993. A mammalian DNA-binding protein that contains a chromodomain and an SNF2/SWI2-like helicase domain. *Proceedings of the National Academy of Sciences of the United States of America*, 90(6), pp.2414–2418.

Deng, L. *et al.*, 2008. Manipulation of major membrane lipid synthesis and its effects on sporulation in Saccharomyces cerevisiae. *Bioscience, biotechnology, and biochemistry*, 72(9), pp.2362–2368.

Denslow, S.A. & Wade, P.A., 2007. The human Mi-2/NuRD complex and gene regulation. *Oncogene*, 26(37), pp.5433–5438.

Deuring, R. *et al.*, 2000. The ISWI chromatin-remodeling protein is required for gene expression and the maintenance of higher order chromatin structure in vivo. *Molecular cell*, 5(2), pp.355–365.

Dickinson, J.R. *et al.*, 2000. An investigation of the metabolism of isoleucine to active Amyl alcohol in Saccharomyces cerevisiae. *The Journal of biological chemistry*, 275(15), pp.10937–10942.

Dickinson, J.R., Harrison, S.J. & Hewlins, M.J., 1998. An investigation of the metabolism of valine to isobutyl alcohol in Saccharomyces cerevisiae. *The Journal of biological chemistry*, 273(40), pp.25751–25756.

Dickinson, J.R., Salgado, L.E.J. & Hewlins, M.J.E., 2003. The catabolism of amino acids to long chain and complex alcohols in Saccharomyces cerevisiae. *The Journal of biological chemistry*, 278(10), pp.8028–8034.

Dominguez, R. & Holmes, K.C., 2011. Actin structure and function. *Annual review of biophysics*, 40, pp.169–186.

Dorigo, B. *et al.*, 2004. Nucleosome arrays reveal the two-start organization of the chromatin fiber. *Science (New York, N.Y.)*, 306(5701), pp.1571–1573.

Duina, A.A. & Winston, F., 2004. Analysis of a mutant histone H3 that perturbs the association of Swi/Snf with chromatin. *Molecular and cellular biology*, 24(2), pp.561–572.

Dunham, W.H., Mullin, M. & Gingras, A.-C., 2012. Affinity-purification coupled to mass spectrometry: basic principles and strategies. *Proteomics*, 12(10), pp.1576–1590.

Dutta, B. *et al.*, 2012. Elucidating the temporal dynamics of chromatin-associated protein release upon DNA digestion by quantitative proteomic approach. *Journal of proteomics*, 75(17), pp.5493–5506.

Dyer, P.N. *et al.*, 2004. Reconstitution of nucleosome core particles from recombinant histones and DNA. *Methods in enzymology*, 375, pp.23–44.

Ebbert, R., Birkmann, A. & Schuller, H.J., 1999. The product of the SNF2/SWI2 paralogue INO80 of Saccharomyces cerevisiae required for efficient expression of various yeast structural genes is part of a high-molecular-weight protein complex. *Molecular microbiology*, 32(4), pp.741–751.

Eberharter, A. *et al.*, 2004. ACF1 improves the effectiveness of nucleosome mobilization by ISWI through PHD-histone contacts. *The EMBO journal*, 23(20), pp.4029–4039.

Eberharter, A. *et al.*, 2001. Acf1, the largest subunit of CHRAC, regulates ISWI-induced nucleosome remodelling. *The EMBO Journal*, 20(14), pp.3781–3788.

Fan, J.Y. *et al.*, 2004. H2A.Z alters the nucleosome surface to promote HP1alpha-mediated chromatin fiber folding. *Molecular cell*, 16(4), pp.655–661.

Field, C.M. & Lenart, P., 2011. Bulk cytoplasmic actin and its functions in meiosis and mitosis. *Current biology : CB*, 21(19), pp.R825–30.

Filippakopoulos, P. *et al.*, 2010. Selective inhibition of BET bromodomains. *Nature*, 468(7327), pp.1067–1073.

Fischle, W. *et al.*, 2003. Molecular basis for the discrimination of repressive methyl-lysine marks in histone H3 by Polycomb and HP1 chromodomains. *Genes & development*, 17(15), pp.1870–1881.

Flanagan, J.F. *et al.*, 2005. Double chromodomains cooperate to recognize the methylated histone H3 tail. *Nature*, 438(7071), pp.1181–1185.

Fletcher, T.M. & Hansen, J.C., 1995. Core histone tail domains mediate oligonucleosome folding and nucleosomal DNA organization through distinct molecular mechanisms. *The Journal of biological chemistry*, 270(43), pp.25359–25362.

French, C.A. *et al.*, 2001. BRD4 bromodomain gene rearrangement in aggressive carcinoma with translocation t(15;19). *The American journal of pathology*, 159(6), pp.1987–1992.

French, C.A. *et al.*, 2003. BRD4-NUT fusion oncogene: a novel mechanism in aggressive carcinoma. *Cancer research*, 63(2), pp.304–307.

Fujita, N. *et al.*, 2003. MTA3, a Mi-2/NuRD complex subunit, regulates an invasive growth pathway in breast cancer. *Cell*, 113(2), pp.207–219.

Fussner, E., Ching, R.W. & Bazett-Jones, D.P., 2011. Living without 30nm chromatin fibers. *Trends in biochemical sciences*, 36(1), pp.1–6.

Gasteiger, E. *et al.*, 2005. Protein Identification and Analysis Tools on the ExPASy Server. In *The Proteomics Protocols Handbook*. pp. 571–607.

Gavin, A.-C. *et al.*, 2006. Proteome survey reveals modularity of the yeast cell machinery. *Nature*, 440(7084), pp.631–636.

Goytisolo, F.A. *et al.*, 1996. Identification of two DNA-binding sites on the globular domain of histone H5. *The EMBO Journal*, 15(13), pp.3421–3429.

Grigoryev, S.A. *et al.*, 2009. Evidence for heteromorphic chromatin fibers from analysis of nucleosome interactions. *Proceedings of the National Academy of Sciences of the United States of America*, 106(32), pp.13317–13322.

Grigoryev, S.A. & Woodcock, C.L., 2012. Chromatin organization - the 30 nm fiber. *Experimental cell research*, 318(12), pp.1448–1455.

Grune, T. *et al.*, 2003. Crystal structure and functional analysis of a nucleosome recognition module of the remodeling factor ISWI. *Molecular cell*, 12(2), pp.449–460.

Gu, M. *et al.*, 2015. H2A.Z marks antisense promoters and has positive effects on antisense transcript levels in budding yeast. *BMC Genomics*, 16(1), p.99.

Gu, W. *et al.*, 2004. A new yeast PUF family protein, Puf6p, represses ASH1 mRNA translation and is required for its localization. *Genes & development*, 18(12), pp.1452–1465.

Guidi, C.J. *et al.*, 2001. Disruption of Ini1 leads to peri-implantation lethality and tumourigenesis in mice. *Molecular and cellular biology*, 21(10), pp.3598–3603.

Hadjebi, O. *et al.*, 2008. The RCC1 superfamily: From genes, to function, to disease. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1783(8), pp.1467–1479.

Hanahan, D., 1983. Studies on transformation of Escherichia coli with plasmids. *Journal of molecular biology*, 166(4), pp.557–80.

Hediger, M.A., 1986. High resolution preparative gel electrophoresis of DNA fragments and plasmid DNA using a continuous elution apparatus. *Analytical biochemistry*, 159(2), pp.280–286.

Henikoff, S., Furuyama, T. & Ahmad, K., 2004. Histone variants, nucleosome assembly and epigenetic inheritance. *Trends in genetics : TIG*, 20(7), pp.320–326.

Ho, C.S. *et al.*, 2003. Electrospray ionisation mass spectrometry: principles and clinical applications. *The Clinical biochemist. Reviews / Australian Association of Clinical Biochemists*, 24(1), pp.3–12.

Hoelder, S., Clarke, P.A. & Workman, P., 2012. Discovery of small molecule cancer drugs: successes, challenges and opportunities. *Molecular oncology*, 6(2), pp.155–176.

Hoffman, A.R. & Vu, T.H., 2004. The histone code and epigenetic inheritance. In *Encyclopedia of Genetics, Genomics, Proteomics and Bioinformatics*. John Wiley & Sons, Ltd.

Hou, Z. *et al.*, 2005. Structural basis of the Sir1-origin recognition complex interaction in transcriptional silencing. *Proceedings of the National Academy of Sciences of the United States of America*, 102(24), pp.8489–8494.

Hsu, H.-C., Stillman, B. & Xu, R.-M., 2005. Structural basis for origin recognition complex 1 protein-silence information regulator 1 protein interaction in epigenetic silencing. *Proceedings of the National Academy of Sciences of the United States of America*, 102(24), pp.8519–8524.

Hyland, E.M. *et al.*, 2005. Insights into the role of histone H3 and histone H4 core modifiable residues in Saccharomyces cerevisiae. *Molecular and cellular biology*, 25(22), pp.10060–10070.

Ivy, J.M., Klar, A.J. & Hicks, J.B., 1986. Cloning and characterization of four SIR genes of Saccharomyces cerevisiae. *Molecular and Cellular Biology*, 6(2), pp.688–702.

Jenuwein, T. & Allis, C.D., 2001. Translating the histone code. *Science (New York, N.Y.)*, 293(5532), pp.1074–1080.

Jiang, C. & Pugh, B.F., 2009. Nucleosome positioning and gene regulation: advances through genomics. *Nat Rev Genet*, 10(3), pp.161–172.

Johnson, A. *et al.*, 2009. Reconstitution of heterochromatin-dependent transcriptional gene silencing. *Molecular cell*, 35(6), pp.769–781.

Jonsson, Z.O. *et al.*, 2004. Rvb1p/Rvb2p recruit Arp5p and assemble a functional Ino80 chromatin remodeling complex. *Molecular cell*, 16(3), pp.465–477.

Kagalwala, M.N. *et al.*, 2004. Topography of the ISW2–nucleosome complex: insights into nucleosome spacing and chromatin remodeling. *The EMBO Journal*, 23(10), pp.2092–2104.

Kalab, P. & Heald, R., 2008. The RanGTP gradient - a GPS for the mitotic spindle. *Journal of cell science*, 121(Pt 10), pp.1577–1586.

Kalashnikova, A.A. *et al.*, 2013. The role of the nucleosome acidic patch in modulating higher order chromatin structure. *Journal of the Royal Society, Interface / the Royal Society*, 10(82), p.20121022.

Kan, P.-Y., Caterino, T.L. & Hayes, J.J., 2009. The H4 tail domain participates in intra- and internucleosome interactions with protein and DNA during folding and oligomerization of nucleosome arrays. *Molecular and cellular biology*, 29(2), pp.538–546.

Kanemaki, M. *et al.*, 1999. TIP49b, a new RuvB-like DNA helicase, is included in a complex together with another RuvB-like DNA helicase, TIP49a. *The Journal of biological chemistry*, 274(32), pp.22437–22444.

Kaplan, N. *et al.*, 2010. Nucleosome sequence preferences influence in vivo nucleosome organization. *Nat Struct Mol Biol*, 17(8), pp.918–920.

Kaplan, N. *et al.*, 2009. The DNA-encoded nucleosome organization of a eukaryotic genome. *Nature*, 458(7236), pp.362–366.

Kapoor, P. & Shen, X., 2014. Mechanisms of nuclear actin in chromatin-remodeling complexes. *Trends in cell biology*, 24(4), pp.238–246.

Kashyap, A.K. *et al.*, 2005. Biochemical and genetic characterization of Yra1p in budding yeast. *Yeast (Chichester, England)*, 22(1), pp.43–56.

Kastenmayer, J.P. *et al.*, 2005. The C-terminal half of Saccharomyces cerevisiae Mad1p mediates spindle checkpoint function, chromosome transmission fidelity and CEN association. *Genetics*, 170(2), pp.509–517.

Kato, H. *et al.*, 2011. Architecture of the high mobility group nucleosomal protein 2-nucleosome complex as revealed by methyl-based NMR. *Proceedings of the National Academy of Sciences of the United States of America*, 108(30), pp.12283–12288.

Kelbauskas, L. *et al.*, 2009. Intrinsic promoter nucleosome stability/dynamics variations support a novel targeting mechanism. *Biochemistry*, 48(20), pp.4217–4219.

Kelley, D.E., Stokes, D.G. & Perry, R.P., 1999. CHD1 interacts with SSRP1 and depends on both its chromodomain and its ATPase/helicase-like domain for proper association with chromatin. *Chromosoma*, 108(1), pp.10–25.

Kim, J. *et al.*, 2006. Tudor, MBT and chromo domains gauge the degree of lysine methylation. *EMBO reports*, 7(4), pp.397–403.

Klochendler-Yeivin, A. *et al.*, 2000. The murine SNF5/INI1 chromatin remodeling factor is essential for embryonic development and tumor suppression. *EMBO reports*, 1(6), pp.500–506.

Klose, R.J. & Bird, A.P., 2006. Genomic DNA methylation: the mark and its mediators. *Trends in biochemical sciences*, 31(2), pp.89–97.

Kobor, M.S. *et al.*, 2004. A protein complex containing the conserved Swi2/Snf2-related ATPase Swr1p deposits histone variant H2A.Z into euchromatin. *PLoS biology*, 2(5), p.E131.

Kouzarides, T., 2007. Chromatin modifications and their function. *Cell*, 128(4), pp.693–705.

Kristjuhan, A. *et al.*, 2003. Spreading of Sir3 protein in cells with severe histone H3 hypoacetylation. *Proceedings of the National Academy of Sciences of the United States of America*, 100(13), pp.7551–7556.

Krogan, N.J., Keogh, M.-C., *et al.*, 2003. A Snf2 family ATPase complex required for recruitment of the histone H2A variant Htz1. *Molecular cell*, 12(6), pp.1565–1576.

Krogan, N.J. *et al.*, 2006. Global landscape of protein complexes in the yeast Saccharomyces cerevisiae. *Nature*, 440(7084), pp.637–643.

Krogan, N.J., Kim, M., *et al.*, 2003. Methylation of histone H3 by Set2 in Saccharomyces cerevisiae is linked to transcriptional elongation by RNA polymerase II. *Molecular and cellular biology*, 23(12), pp.4207–4218.

Krogan, N.J. *et al.*, 2002. RNA polymerase II elongation factors of Saccharomyces cerevisiae: a targeted proteomics approach. *Molecular and cellular biology*, 22(20), pp.6979–6992.

Kruithof, M. *et al.*, 2009. Single-molecule force spectroscopy reveals a highly compliant helical folding for the 30-nm chromatin fiber. *Nature structural & molecular biology*, 16(5), pp.534–540.

Kumar, R. *et al.*, 2002. A naturally occurring MTA1 variant sequesters oestrogen receptor-alpha in the cytoplasm. *Nature*, 418(6898), pp.654–657.

Kuo, A.J. *et al.*, 2012. The BAH domain of ORC1 links H4K20me2 to DNA replication licensing and Meier-Gorlin syndrome. *Nature*, 484(7392), pp.115–119.

Ladurner, A.G. *et al.*, 2003. Bromodomains mediate an acetyl-histone encoded antisilencing function at heterochromatin boundaries. *Molecular cell*, 11(2), pp.365–376.

Lambert, J.-P. *et al.*, 2010. Defining the budding yeast chromatin-associated interactome. *Molecular systems biology*, 6, p.448.

Lee, K.K. *et al.*, 2011. Combinatorial depletion analysis to assemble the network architecture of the SAGA and ADA chromatin remodeling complexes. *Molecular systems biology*, 7, p.503.

Li, G. & Reinberg, D., 2011. Chromatin higher-order structures and gene regulation. *Current opinion in genetics & development*, 21(2), pp.175–186.

Lin, H., Nguyen, P. & Vancura, A., 2002. Phospholipase C interacts with Sgd1p and is required for expression of GPD1 and osmoresistance in Saccharomyces cerevisiae. *Molecular genetics and genomics : MGG*, 267(3), pp.313–320.

Loo, S. & Rine, J., 1995. Silencing and heritable domains of gene expression. *Annual review of cell and developmental biology*, 11, pp.519–548.

Luger, K., Rechsteiner, T.J., *et al.*, 1997. Characterization of nucleosome core particles containing histone proteins made in bacteria. *Journal of molecular biology*, 272(3), pp.301–11.

Luger, K., Mäder, A.W., *et al.*, 1997. Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature*, 389(6648), pp.251–60.

Luger, K., Dechassa, M.L. & Tremethick, D.J., 2012. New insights into nucleosome and chromatin structure: an ordered state or a disordered affair? *Nature reviews. Molecular cell biology*, 13(7), pp.436–447.

Luger, K., Rechsteiner, T.J. & Richmond, T.J., 1999. Expression and Purification of Recombinant Histones and Nucleosome Reconstitution. *Chromatin Protocols*, 119, pp.1–16.

Luger, K., Rechsteiner, T.J. & Richmond, T.J., 1999. Preparation of nucleosome core particle from recombinant histones. *Methods in Enzymology*, 304, pp.3–19.

Lusser, A., Urwin, D.L. & Kadonaga, J.T., 2005. Distinct activities of CHD1 and ACF in ATP-dependent chromatin assembly. *Nature structural & molecular biology*, 12(2), pp.160–166.

Lygerou, Z. *et al.*, 1994. The yeast BDF1 gene encodes a transcription factor involved in the expression of a broad class of genes including snRNAs. *Nucleic acids research*, 22(24), pp.5332–5340.

Maeshima, K., Hihara, S. & Eltsov, M., 2010. Chromatin structure: does the 30-nm fibre exist in vivo? *Current opinion in cell biology*, 22(3), pp.291–297.

Makde, R.D. *et al.*, 2010. Structure of RCC1 chromatin factor bound to the nucleosome core particle. *Nature*, 467(7315), pp.562–566.

Malawer, E. & Senak, L., 2003. Introduction to Size Exclusion Chromatography. In *Handbook Of Size Exclusion Chromatography And Related Techniques*. CRC Press.

Marfella, C.G.A. & Imbalzano, A.N., 2007. The Chd Family of Chromatin Remodelers. *Mutation research*, 618(1-2), pp.30–40.

Marmorstein, R., 2004. Structure and chemistry of the Sir2 family of NAD+-dependent histone/protein deactylases. *Biochemical Society transactions*, 32(Pt 6), pp.904–909.

Martino, F. *et al.*, 2009. Reconstitution of yeast silent chromatin: multiple contact sites and O-AADPR binding load SIR complexes onto nucleosomes in vitro. *Molecular cell*, 33(3), pp.323–334.

Matangkasombut, O. & Buratowski, S., 2003. Different sensitivities of bromodomain factors 1 and 2 to histone H4 acetylation. *Molecular cell*, 11(2), pp.353–363.

Matsubara, K. *et al.*, 2007. Global analysis of functional surfaces of core histones with comprehensive point mutants. *Genes to cells : devoted to molecular & cellular mechanisms*, 12(1), pp.13–33.

McBryant, S.J. *et al.*, 2008. The Silent Information Regulator 3 Protein, SIR3p, Binds to Chromatin Fibers and Assembles a Hypercondensed Chromatin Architecture in the Presence of Salt . *Molecular and Cellular Biology*, 28(11), pp.3563–3572.

McBryant, S.J., Adams, V.H. & Hansen, J.C., 2006. Chromatin architectural proteins. *Chromosome research : an international journal on the molecular, supramolecular and evolutionary aspects of chromosome biology*, 14(1), pp.39–51.

Mellado, R.P., Murray, K. & Chambon, P., 1983. Synthesis of yeast histone 3 in an Escherichia coli cell-free system. *Journal of Molecular Biology*, 168(3), pp.489–503.

Meza, J.E. *et al.*, 2014. Improved Tryptic Digestion of Proteins Improved T ryptic Digestion of Proteins. In *Agilent Technologies ABRF Poster*.

Miller, A.M., 2011. Role of IL-33 in inflammation and disease. *Journal of inflammation (London, England)*, 8(1), p.22.

Min, J., Zhang, Y. & Xu, R.-M., 2003. Structural basis for specific binding of Polycomb chromodomain to histone H3 methylated at Lys 27. *Genes & development*, 17(15), pp.1823–1828.

Mizuguchi, G. *et al.*, 2004. ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. *Science (New York, N.Y.)*, 303(5656), pp.343–348.

Mohrmann, L. & Verrijzer, C.P., 2005. Composition and functional specificity of SWI2/SNF2 class chromatin remodeling complexes. *Biochimica et biophysica acta*, 1681(2-3), pp.59–73.

Morrison, A.J. *et al.*, 2004. INO80 and gamma-H2AX interaction links ATP-dependent chromatin remodeling to DNA damage repair. *Cell*, 119(6), pp.767–775.

Moshkin, Y.M. *et al.*, 2012. Remodelers organize cellular chromatin by counteracting intrinsic histone-DNA sequence preferences in a class-specific manner. *Molecular and cellular biology*, 32(3), pp.675–88.

Mucke, K. *et al.*, 2014. Human cytomegalovirus major immediate early 1 protein targets host chromosomes by docking to the acidic pocket on the nucleosome surface. *Journal of virology*, 88(2), pp.1228–1248.

Munakata, T. *et al.*, 2000. A human homologue of yeast anti-silencing factor has histone chaperone activity. *Genes to cells : devoted to molecular & cellular mechanisms*, 5(3), pp.221–233.

Nair, S.S. & Kumar, R., 2012. Chromatin remodeling in cancer: a gateway to regulate gene transcription. *Molecular oncology*, 6(6), pp.611–619.

Narlikar, G.J., Sundaramoorthy, R. & Owen-Hughes, T., 2015. Mechanisms and Functions of ATP-Dependent Chromatin-Remodeling Enzymes. *Cell*, 154(3), pp.490–503.

Nishino, Y. *et al.*, 2012. Human mitotic chromosomes consist predominantly of irregularly folded nucleosome fibres without a 30-nm chromatin structure. *The EMBO Journal*, 31(7), pp.1644–1653.

Ohsaki, E. & Ueda, K., 2012. Kaposi's Sarcoma-Associated Herpesvirus Genome Replication, Partitioning, and Maintenance in Latency. *Frontiers in microbiology*, 3, p.7.

Olave, I.A., Reck-Peterson, S.L. & Crabtree, G.R., 2002. Nuclear actin and actin-related proteins in chromatin remodeling. *Annual review of biochemistry*, 71, pp.755–781.

Old, R.W. *et al.*, 1982. Organization and expression of cloned histone gene clusters from Xenopus laevis and X. borealis. *Nucleic Acids Research*, 10(23), pp.7561–7580.

Pamblanco, M. *et al.*, 2001. Bromodomain factor 1 (Bdf1) protein interacts with histones. *FEBS letters*, 496(1), pp.31–35.

Park, J.-H. *et al.*, 2002. A core nucleosome surface crucial for transcriptional silencing. *Nature genetics*, 32(2), pp.273–279.

Perisic, O., Collepardo-Guevara, R. & Schlick, T., 2010. Modeling studies of chromatin fiber structure as a function of DNA linker length. *Journal of molecular biology*, 403(5), pp.777–802.

Poddar, A., Roy, N. & Sinha, P., 1999. MCM21 and MCM22, two novel genes of the yeast Saccharomyces cerevisiae are required for chromosome transmission. *Molecular microbiology*, 31(1), pp.349–360.

Poot, R.A. *et al.*, 2004. The Williams syndrome transcription factor interacts with PCNA to target chromatin remodelling by ISWI to replication foci. *Nature cell biology*, 6(12), pp.1236–1244.

Pray-Grant, M.G. *et al.*, 2005. Chd1 chromodomain links histone H3 methylation with SAGA- and SLIK-dependent acetylation. *Nature*, 433(7024), pp.434–438.

Price, B.D. & D'Andrea, A.D., 2013. Chromatin remodeling at DNA double-strand breaks. *Cell*, 152(6), pp.1344–1354.

Prymakowska-Bosak, M. *et al.*, 2001. Mitotic phosphorylation prevents the binding of HMGN proteins to chromatin. *Molecular and cellular biology*, 21(15), pp.5169–5178.

Pugh, B.F., 2010. A preoccupied position on nucleosomes. *Nat Struct Mol Biol*, 17(8), p.923.

Qiu, X.B. *et al.*, 1998. An eukaryotic RuvB-like protein (RUVBL1) essential for growth. *The Journal of biological chemistry*, 273(43), pp.27786–27793.

Radman-Livaja, M. & Rando, O.J., 2010. Nucleosome positioning: how is it established, and why does it matter? *Developmental biology*, 339(2), pp.258–266.

Rando, O.J. *et al.*, 2002. Phosphatidylinositol-dependent actin filament binding by the SWI/SNF-like BAF chromatin remodeling complex. *Proceedings of the National Academy of Sciences of the United States of America*, 99(5), pp.2824–2829.

Reisman, D.N. *et al.*, 2003. Loss of BRG1/BRM in human lung cancer cell lines and primary lung cancers: correlation with poor prognosis. *Cancer research*, 63(3), pp.560–566.

Renault, L. *et al.*, 2001. Structural basis for guanine nucleotide exchange on Ran by the regulator of chromosome condensation (RCC1). *Cell*, 105(2), pp.245–255.

Renault, L. *et al.*, 1998. The 1.7 A crystal structure of the regulator of chromosome condensation (RCC1) reveals a seven-bladed propeller. *Nature*, 392(6671), pp.97–101.

Richmond, T.J., Searles, M.A. & Simpson, R.T., 1988. Crystals of a nucleosome core particle containing defined sequence DNA. *Journal of Molecular Biology*, 199(1), pp.161–170.

Rill, R.L. *et al.*, 1975. Heterogeneity of chromatin fragments produced by micrococcal nuclease action. *Nucleic Acids Research* , 2 (9), pp.1525–1538.

Roberts, C.W. *et al.*, 2000. Haploinsufficiency of Snf5 (integrase interactor 1) predisposes to malignant rhabdoid tumors in mice. *Proceedings of the National Academy of Sciences of the United States of America*, 97(25), pp.13796–13800.

Roberts, C.W.M. *et al.*, 2002. Highly penetrant, rapid tumorigenesis through conditional inversion of the tumor suppressor gene Snf5. *Cancer cell*, 2(5), pp.415–425.

Roberts, C.W.M. & Orkin, S.H., 2004. The SWI/SNF complex--chromatin and cancer. *Nature reviews. Cancer*, 4(2), pp.133–142.

Robinson, K.M. & Schultz, M.C., 2003. Replication-independent assembly of nucleosome arrays in a novel yeast chromatin reconstitution system involves antisilencing factor Asf1p and chromodomain protein Chd1p. *Molecular and cellular biology*, 23(22), pp.7937–7946.

Robinson, P.J.J. & Rhodes, D., 2006. Structure of the “30 nm” chromatin fibre: a key role for the linker histone. *Current opinion in structural biology*, 16(3), pp.336–343.

Robinson, R.C. *et al.*, 2001. Crystal structure of Arp2/3 complex. *Science (New York, N.Y.)*, 294(5547), pp.1679–1684.

Rohs, R. *et al.*, 2009. The role of DNA shape in protein-DNA recognition. *Nature*, 461(7268), pp.1248–1253.

Rosa, S. & Shaw, P., 2013. Insights into chromatin structure and dynamics in plants. *Biology*, 2(4), pp.1378–1410.

Roussel, L. *et al.*, 2008. Molecular mimicry between IL-33 and KSHV for attachment to chromatin through the H2A-H2B acidic pocket. *EMBO reports*, 9(10), pp.1006–1012.

Rusche, L.N., Kirchmaier, A.L. & Rine, J., 2003. The establishment, inheritance, and function of silenced chromatin in Saccharomyces cerevisiae. *Annual review of biochemistry*, 72, pp.481–516.

Sanchez, R. & Zhou, M.-M., 2009. The role of human bromodomains in chromatin biology and gene transcription. *Current opinion in drug discovery & development*, 12(5), pp.659–665.

Schafer, D.A. & Schroer, T.A., 1999. Actin-related proteins. *Annual review of cell and developmental biology*, 15, pp.341–363.

Schmidt, B.H., Osheroff, N. & Berger, J.M., 2012. Structure of a topoisomerase II-DNA-nucleotide complex reveals a new control mechanism for ATPase activity. *Nature structural & molecular biology*, 19(11), pp.1147–1154.

Schoner, D. *et al.*, 2008. Annotating novel genes by integrating synthetic lethals and genomic information. *BMC systems biology*, 2, p.3.

Schwarz, P.M. *et al.*, 1996. Reversible oligonucleosome self-association: dependence on divalent cations and core histone tail domains. *Biochemistry*, 35(13), pp.4009–4015.

Segal, E. *et al.*, 2006. A genomic code for nucleosome positioning. *Nature*, 442(7104), pp.772–778.

Shen, X. *et al.*, 2003. Involvement of actin-related proteins in ATP-dependent chromatin remodeling. *Molecular cell*, 12(1), pp.147–155.

Shimahara, H. *et al.*, 2013. Nucleosome structural changes induced by binding of non-histone chromosomal proteins HMGN1 and HMGN2. *FEBS open bio*, 3, pp.184–91.

Shimizu, K. *et al.*, 2002. The fifth essential DNA polymerase phi in Saccharomyces cerevisiae is localized to the nucleolus and plays an important role in synthesis of rRNA. *Proceedings of the National Academy of Sciences of the United States of America*, 99(14), pp.9133–9138.

Shirakawa, H. *et al.*, 2000. Targeting of high mobility group-14/-17 proteins in chromatin is independent of DNA sequence. *The Journal of biological chemistry*, 275(48), pp.37937–37944.

Sims, R.J. 3rd *et al.*, 2005. Human but not yeast CHD1 binds directly and selectively to histone H3 methylated at lysine 4 via its tandem chromodomains. *The Journal of biological chemistry*, 280(51), pp.41789–41792.

Srinivasan, S. *et al.*, 2005. The Drosophila trithorax group protein Kismet facilitates an early step in transcriptional elongation by RNA Polymerase II. *Development (Cambridge, England)*, 132(7), pp.1623–1635.

Srivastava, S., Mishra, R.K. & Dhawan, J., 2010. Regulation of cellular chromatin state: insights from quiescence and differentiation. *Organogenesis*, 6(1), pp.37–47.

Stockdale, C. *et al.*, 2006. Analysis of nucleosome repositioning by yeast ISWI and Chd1 chromatin remodeling complexes. *The Journal of biological chemistry*, 281(24), pp.16279–16288.

Strahl, B.D. & Allis, C.D., 2000. The language of covalent histone modifications. *Nature*, 403(6765), pp.41–5.

Struhl, K. & Segal, E., 2013. Determinants of nucleosome positioning. *Nat Struct Mol Biol*, 20(3), pp.267–273.

Szerlong, H., Saha, A. & Cairns, B.R., 2003. The nuclear actin-related proteins Arp7 and Arp9: a dimeric module that cooperates with architectural proteins for chromatin remodeling. *The EMBO Journal*, 22(12), pp.3175–3187.

Tan, M. *et al.*, 2011. Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification. *Cell*, 146(6), pp.1016–1028.

Tan, S. & Davey, C.A., 2011. Nucleosome structural studies. *Current opinion in structural biology*, 21(1), pp.128–136.

Taverna, S.D. *et al.*, 2007. How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. *Nature structural & molecular biology*, 14(11), pp.1025–40.

Taylor, N.M.I. *et al.*, 2013. Structural and functional characterization of a phosphatase domain within yeast general transcription factor IIIC. *The Journal of biological chemistry*, 288(21), pp.15110–15120.

Thomas, J.O., 1999. Histone H1: location and role. *Current opinion in cell biology*, 11(3), pp.312–317.

Thompson, J.S. *et al.*, 2003. Identification of a functional domain within the essential core of histone H3 that is required for telomeric and HM silencing in Saccharomyces cerevisiae. *Genetics*, 163(1), pp.447–452.

Tolkunov, D. *et al.*, 2011. Chromatin remodelers clear nucleosomes from intrinsically unfavorable sites to establish nucleosome-depleted regions at promoters. *Molecular biology of the cell*, 22(12), pp.2106–18.

Tremethick, D.J., 2007. Higher-order structures of chromatin: the elusive 30 nm fiber. *Cell*, 128(4), pp.651–654.

Tremethick, D.J. & Hyman, L., 1996. High mobility group protein 14 and 17 can prevent the close packing of nucleosomes by increasing the strength of protein contacts in the linker DNA. *The Journal of biological chemistry*, 271(20), pp.12009–12016.

Trieschmann, L. *et al.*, 1995. Incorporation of chromosomal proteins HMG-14/HMG-17 into nascent nucleosomes induces an extended chromatin conformation and enhances the utilization of active transcription complexes. *The EMBO Journal*, 14(7), pp.1478–1489.

Trifonov, E.N., 2011. Cracking the chromatin code: precise rule of nucleosome positioning. *Physics of life reviews*, 8(1), pp.39–50.

Tse, C. & Hansen, J.C., 1997. Hybrid trypsinized nucleosomal arrays: identification of multiple functional roles of the H2A/H2B and H3/H4 N-termini in chromatin fiber compaction. *Biochemistry*, 36(38), pp.11381–11388.

Tsukiyama, T. *et al.*, 1999. Characterization of the imitation switch subfamily of ATP-dependent chromatin-remodeling factors in Saccharomyces cerevisiae. *Genes & development*, 13(6), pp.686–697.

Tsukiyama, T. *et al.*, 1995. ISWI, a member of the SWI2/SNF2 ATPase family, encodes the 140 kDa subunit of the nucleosome remodeling factor. *Cell*, 83(6), pp.1021–1026.

Tsukuda, T. *et al.*, 2005. Chromatin remodelling at a DNA double-strand break site in Saccharomyces cerevisiae. *Nature*, 438(7066), pp.379–383.

Twyman, R., 2004. *Principles of Proteomics*, Taylor & Francis, London., pp.42-46.

Verreault, A. *et al.*, 1998. Nucleosomal DNA regulates the core-histone-binding subunit of the human Hat1 acetyltransferase. *Current biology : CB*, 8(2), pp.96–108.

Versteege, I. *et al.*, 1998. Truncating mutations of hSNF5/INI1 in aggressive paediatric cancer. *Nature*, 394(6689), pp.203–206.

Vestner, B., Bustin, M. & Gruss, C., 1998. Stimulation of replication efficiency of a chromatin template by chromosomal protein HMG-17. *The Journal of biological chemistry*, 273(16), pp.9409–9414.

Visa, N. & Percipalle, P., 2010. Nuclear functions of actin. *Cold Spring Harbor perspectives in biology*, 2(4), p.a000620.

Vissers, L.E.L.M. *et al.*, 2004. Mutations in a new member of the chromodomain gene family cause CHARGE syndrome. *Nature genetics*, 36(9), pp.955–957.

Walker, J.E. *et al.*, 1982. Distantly related sequences in the alpha- and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. *The EMBO journal*, 1(8), pp.945–951.

Wang, G.G., Allis, C.D. & Chi, P., 2007. Chromatin remodeling and cancer, Part II: ATP-dependent chromatin remodeling. *Trends in molecular medicine*, 13(9), pp.373–380.

Wang, W., 2003. The SWI/SNF family of ATP-dependent chromatin remodelers: similar mechanisms for diverse functions. *Current topics in microbiology and immunology*, 274, pp.143–169.

White, C.L., Suto, R.K. & Luger, K., 2001. Structure of the yeast nucleosome core particle reveals fundamental changes in internucleosome interactions. *The EMBO Journal*, 20(18), pp.5207–5218.

Widom, J., 1998. Chromatin structure: linking structure to function with histone H1. *Current biology : CB*, 8(22), pp.R788–91.

Wilm, M., 2011. Principles of electrospray ionization. *Molecular & cellular proteomics : MCP*, 10(7), p.M111.009407.

Wolffe, A.P. & Guschin, D., 2000. Review: chromatin structural features and targets that regulate transcription. *Journal of structural biology*, 129(2-3), pp.102–22.

Woodage, T. *et al.*, 1997. Characterization of the CHD family of proteins. *Proceedings of the National Academy of Sciences of the United States of America*, 94(21), pp.11472–11477.

Woodcock, C.L. & Dimitrov, S., 2001. Higher-order structure of chromatin and chromosomes. *Current opinion in genetics & development*, 11(2), pp.130–135.

Woudstra, E.C. *et al.*, 2002. A Rad26-Def1 complex coordinates repair and RNA pol II proteolysis in response to DNA damage. *Nature*, 415(6874), pp.929–933.

Wu, W.-H. *et al.*, 2009. N terminus of Swr1 binds to histone H2AZ and provides a platform for subunit assembly in the chromatin remodeling complex. *The Journal of biological chemistry*, 284(10), pp.6200–6207.

Xu, F., Zhang, K. & Grunstein, M., 2005. Acetylation in histone H3 globular domain regulates gene expression in yeast. *Cell*, 121(3), pp.375–385.

Xue, Y. *et al.*, 2003. The ATRX syndrome protein forms a chromatin-remodeling complex with Daxx and localizes in promyelocytic leukemia nuclear bodies. *Proceedings of the National Academy of Sciences of the United States of America*, 100(19), pp.10635–10640.

Yang, N. & Xu, R.-M., 2013. Structure and function of the BAH domain in chromatin biology. *Critical reviews in biochemistry and molecular biology*, 48(3), pp.211–221.

Zhang, Y. *et al.*, 2009. Intrinsic histone-DNA interactions are not the major determinant of nucleosome positions in vivo. *Nature structural & molecular biology*, 16(8), pp.847–852.

Zhang, Y. *et al.*, 2002. Noc3p, a bHLH protein, plays an integral role in the initiation of DNA replication in budding yeast. *Cell*, 109(7), pp.849–860.

Zhang, Z. *et al.*, 2011. A packing mechanism for nucleosome organization reconstituted across a eukaryotic genome. *Science (New York, N.Y.)*, 332(6032), pp.977–980.

Zhang, Z. & Pugh, B.F., 2015. High-Resolution Genome-wide Mapping of the Primary Structure of Chromatin. *Cell*, 144(2), pp.175–186.

Zhou, J. *et al.*, 2007. The nucleosome surface regulates chromatin compaction and couples it with transcriptional repression. *Nature structural & molecular biology*, 14(11), pp.1070–1076.

Zhou, Y.B. *et al.*, 1998. Position and orientation of the globular domain of linker histone H5 on the nucleosome. *Nature*, 395(6700), pp.402–405.