Nucleosome recognition and spacing by chromatin remodelling factor ISW1a

Timothy J. Richmond[1]
ETH Zürich, Institute of Molecular Biology and Biophysics, Schafmattstrasse 20, CH-8093 Zürich, Switzerland

Abstract

Nucleosomes are actively positioned along DNA by ATP-dependent, chromatin remodelling factors. A structural model for the ISW1a chromatin remodelling factor from *Saccharomyces cerevisiae* in complex with a dinucleosome substrate was constructed from the X-ray structures of ISW1a (ΔATPase) with and without DNA bound, two different cryo-EM (cryo-electron microscopy) structures of ISW1a (ΔATPase) bound to a nucleosome, and site-directed photo-cross-linking analyses in solution. The X-ray structure of ISW1a (ΔATPase) with DNA bound suggests that DNA sequence may be involved in nucleosome recognition and thereby specificity of promoter interaction. The model suggests how the highly ordered nucleosome arrays observed by mapping nucleosomes in genes and their promoter regions could be generated by a chromatin remodelling factor.

Introduction

In eukaryotic genomes, the DNA in genes and gene promoter regions is generally packaged in well-positioned nucleosomes [1] (Figure 1). DNA sequence can favour particular nucleosome positions by enhancing compatibility of DNA binding within the nucleosome core, and it can act to exclude nucleosomes by encoding DNA stretches resistant to bending (i.e. poly[dA · dT]) or by specifying the binding of one or more regulatory proteins (e.g. Rep1) [2–4]. Nevertheless, production of native arrays of regularly positioned nucleosomes in gene regions requires the activity of ATP-dependent chromatin remodelling factors [5]. The importance of well-positioned nucleosomes in these regions is probably two-fold. First, promoter elements such as the TATA-box and the TSS (transcription start-site) are generally found in the stretch of DNA just inside (yeast) or just outside (metazoans) the nucleosome core boundary of nucleosome +1 with respect to the TSS [6,7]. This location presumably facilitates gene regulation through modulation of the accessibility of these sites to the transcription machinery, either by blocking binding or impeding elongation of RNA polymerase II. The nucleosomes from +2 onward along the gene may also be important for regulation of polymerase elongation. Secondly, the nucleosome higher-order structure (i.e. the chromatin fibre), which provides another level of DNA occlusion beyond the nucleosome core, depends on arrays of regularly spaced nucleosomes [8].

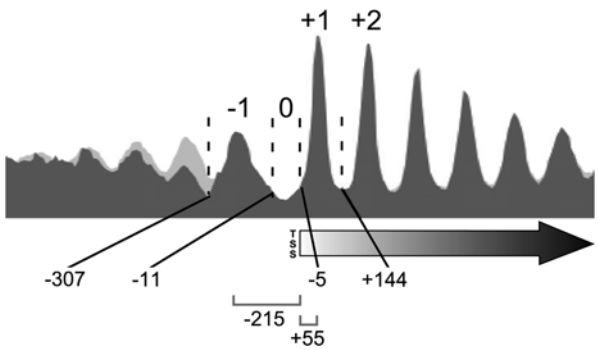
Two questions concerning the mechanism of chromatin remodellers in the context of promoter regions with their associated nucleosomes (e.g. −1, +1) are apparent. How are specific promoters and explicit nucleosomes within promoters recognized, and how is the length of linker DNA between two adjacent nucleosomes determined by these enzymes? The structural studies in my laboratory on the yeast chromatin remodelling factor ISW1a suggest possible answers to these questions of nucleosome recognition and spacing within gene and gene promoter regions packaged in chromatin [9].

ISW1a is a member of the ISWI family of ATP-dependent chromatin remodelling factors, and is found as demonstrated by CHIP [C-terminus of the Hsc (heat-shock cognate) 70-interacting protein]-seq mapping experiments in the vicinity of the −1 and +1 nucleosomes of many TSS [10,11]. Genetic and mapping studies on the yeast MET16 promoter *in vivo* have shown that ISW1a is involved in returning this gene to its repressed state, in part by binding and repositioning nucleosomes [12]. ISW1a comprises the two proteins Isw1 (131.1 kDa) and Ioc3 (90.1 kDa). Isw1 can be partitioned into its ATPase domain (72 kDa) and a second domain containing the subdomains HSS (HAND, SANT and SLIDE) with a 133-amino-acid linker connecting the two domains. For the purpose of analysis of ISW1a by high-resolution X-ray crystallography, the ATPase domain and part of the linker were deleted leaving ISW1a (ΔATPase) or the HSS–Ioc3 complex. The structure of HSS–Ioc3 was determined at 3.2 Å (1 Å = 0.1 nm) resolution on its own and again at 3.6 Å resolution with a 48 bp DNA oligonucleotide bound at two different sites [9]. Isw1–SLIDE makes extensive contacts with the Ioc3 subunit in an apparent rigid interaction that results in an elongated complex. Two structures of homologous HSS domains alone were determined previously, and those studies noted that Isw1–SANT and Isw1–SLIDE are potentially DNA-binding motifs on the basis of their homology with the SANT domain of cMyb [13,14]. However, as seen from the HSS–Ioc3/DNA structure, the mode of DNA-binding for Isw1–SANT

Key words: chromatin, chromatin remodelling, Ioc3, Isw1, ISW1a, nucleosome.
Abbreviations used: CLB, coil linker-binding; cryo-EM, cryo-electron microscopy; E-linker DNA, external-linker DNA; HLB, helix linker-binding; HSS, HAND, SANT and SLIDE; I-linker DNA, internal-linker DNA; I/E-nucleosome, nucleosome with two DNA extensions (I- and E-linker DNA); NCB, nucleosome core (DNA) boundary; TF, transcription factor; TSS, transcription start-site..
[1]email richmond@mol.biol.ethz.ch

Figure 1 | The nucleosome organization around the TSS

The superposition of mapped nucleosomes for genes in yeast reveals the regular positioning of nucleosomes in the promoter region is preserved. The nucleosomes -1, $+1$ and $+2$, as well as the nucleosome-free region (0) are labelled with their approximate boundaries indicated (TSS origin). The distance (bp) of the -1 and $+1$ nucleosome dyad or centre is from the TSS is noted (brackets). The Figure is adapted from Figure 3(a) of [1] with permission.



and Isw1–SLIDE is different than that seen for cMyb. HSS–Ioc3 has large, positively charged surfaces contributed by lysine and arginine amino acids involved in DNA binding [9]. Most notable is the region of Ioc3 extending over both the HLB (helix linker-binding) motif and the CLB (coil linker-binding) motif, as well as the molecular surface between them. A second region extends over Ioc3–HLB and across the Isw1–SLIDE and Isw1–SANT subdomains. Each of these elongated basic regions binds a separate DNA double-stranded oligonucleotide in the HSS–Ioc3/DNA structure.

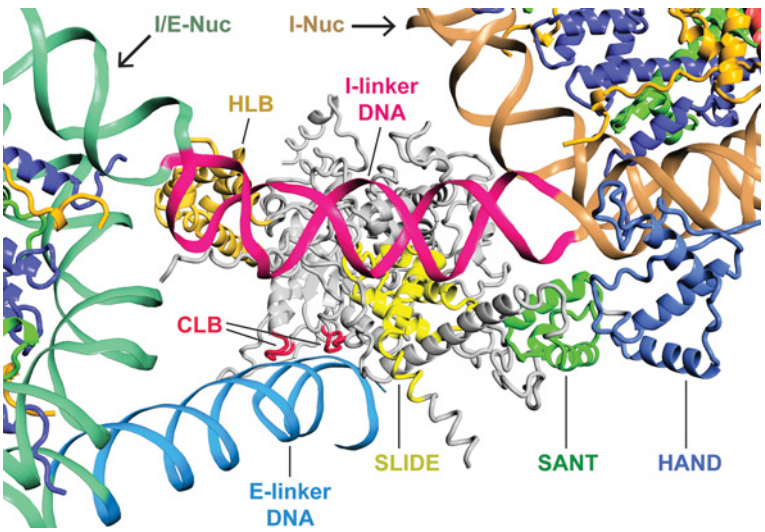
HSS–Ioc3 is capable of binding two nucleosomes, one at each end of its elongated shape. These nucleosomes were visualized by cryo-EM (cryo-electron microscopy) in separate HSS–Ioc3/mononucleosome complexes. One complex contains a nucleosome with a single DNA extension of 45 bp, and the other contains a nucleosome with two DNA extensions of 45 and 29 bp. In parallel with the cryo-EM work, an analysis of these two HSS–Ioc3/nucleosome complexes was done in solution by protein–DNA photo-cross-linking using site-specific attachment of the cross-linking reagent 4-azidophenacyl bromide to sites where cysteine had been introduced into HSS–Ioc3 [9]. These cross-linking experiments corroborated the binding of the nucleosomes to the Isw1–SANT and Isw1–SLIDE subdomains and the Ioc3–HLB and Ioc3–CLB motifs as observed by cryo-EM. A dinucleosome model was constructed by superposition of the two cryo-EM structures via their common HSS–Ioc3 moiety (Figure 2). The resulting model showed that the two 45 bp DNA extensions, one from each mononucleosome complex, superimpose in opposite orientations with their major and minor grooves aligned correctly. Together, the superimposed extensions represent an I-linker DNA (internal linker DNA) connecting the two nucleosomes and contacting Ioc3–HLB, Isw1–SLIDE and Isw1–SANT over approximately three turns of the double helix. The 29 bp extension or E-linker DNA (external linker DNA) is bound to the Ioc3–CLB and does not overlap with the second nucleosome. Owing to their appearance in the model, the nucleosome with a single DNA extension was designated the I-nucleosome (having only I-linker DNA), and the nucleosome with two DNA extensions was designated the I/E-nucleosome [nucleosome with two DNA extensions (I- and E-linker DNA)]. These results strongly suggest that the substrate for the ISW1a nucleosome spacing reaction is a dinucleosome.

The general question of how chromatin remodelling factors recognize a limited set of promoters may have several possible answers. Localization of a remodelling factor may depend on preceding recognition of the promoter by a TF (transcription factor) to which the chromatin remodeller binds directly, or via binding histone modifications introduced by a TF. For example, ISW2 is capable of binding the TF Ume6 at a specific UAS (upstream activating sequence) near a promoter, and SWI/SNF is capable of binding acetylated histone tails of promoter nucleosomes via its bromodomain [15,16]. Additionally, the remodelling complex may itself contain a sequence-specific DNA-recognition motif as has been suggested for the Rsc3/30 component of the RSC (remodelling the structure of chromatin) complex [17].

ISW1a, which is found as a subset of promoters in yeast, may have a preference for binding nucleosomes adjacent to an NFR (nucleosome-free region) or relatively long linker DNA in order to facilitate its role as a transcription silencer. The quantity of DNA in these more accessible stretches is limited compared with the expansion of the genome packaged in nucleosomes. Therefore ISW1a would need only to distinguish the features of these sites from each other to regularize the nucleosomes in proximity to a TSS. In detail, two features of the I/E-nucleosome, which may be initially nucleosome -1 or $+1$, are possibly important for recognition of chromatin by HSS–Ioc3[9]. With regard to the first feature, an α-helix of the Ioc3–HLB is in a position in the I/E-nucleosome of dinucleosome model to bind the first DNA double helical turn of the I-linker DNA outside the NCB [nucleosome core (DNA) boundary], while another surface of the Ioc3–HLB comes sufficiently close to the central turn of the I/E-nucleosome superhelix to allow electrostatic interaction. This constellation of linker DNA and central turn is unique to a nucleosome and probably requires the full-length of the I-linker DNA for complex formation initially. The second feature entails the Ioc3–CLB binding to the E-linker DNA in a stretch 21–26 bp from the other I/E-nucleosome NCB. The X-ray structure suggests that the Ioc3–CLB may have a DNA sequence preference to satisfy full interaction. Therefore, on the basis of the distance of a preferred Ioc3–CLB binding sequence from an NCB and the requirement of making both the Ioc3–HLB and Ioc3–CLB interactions simultaneously, recognition of this previously positioned nucleosome would be achieved. The importance of accurate prior positioning may be reduced somewhat by adjustment of the DNA length between

Figure 2 | **Model of ISW1a interaction with a dinucleosome substrate**

The I-linker DNA (pink) bridges between NCB of the I/E-nucleosome (green DNA) and I-nucleosome (brown DNA). Contacts made between the I-linker DNA and the subdomains Ioc3–HLB (gold), Isw1–SLIDE (yellow) and Isw1–SANT (green). The HAND subdomain (blue) does not contact with DNA. The other NCB of the I/E-nucleosome connects to the E-linker DNA (light blue) which binds Ioc3–CLB (red). The remainder of Ioc3 and the Isw1–HSS domain are shown in grey.



the NCB and the Ioc3–CLB DNA-binding site via DNA stretching on the nucleosome [18]. Moreover, recognition of the I/E-nucleosome could also be augmented by interactions of the N-terminal tails of the core histone proteins or Ioc3 with the complementary binding partner. In the case of the Ioc3 tail, an interaction with a localized histone variant may augment recognition.

The I-nucleosome would be recognized by virtue of being at the opposite end of the I-linker DNA from the I/E-nucleosome. With a modelled internal linker of approximately 25 bp, Isw1–SANT contacts a few base pairs of the I-nucleosome DNA inside its NCB [9]. This interaction does not appear to impart any specificity, however, and may be dispensed with altogether if the I-linker were shortened to 15 bp (sterically acceptable). Hydroxyl radical DNA footprinting of ISW1a indicates that the ATPase domain would bind the I-nucleosome DNA in a stretch 20–30 bp from the centre of the nucleosome DNA (dyad position), which is roughly 40 bp from the I-linker DNA/NCB junction [19]. Currently, there is no structure of an ATPase domain bound to a nucleosome, and therefore details for this type of interaction are not available. The X-ray structure of a *Sulfolobus solfataricus* ATPase that shares 30% identity with the ISW1a ATPase was determined with DNA bound, but does not indicate that DNA sequence recognition is important for interaction [20]. Other experiments show that the H4 N-terminal tail is required for ATPase activity in ISWI remodellers, but thus far, the H4 tail has not been implicated in promoter specificity [21,22]. The low-complexity sequence of the long polypeptide chain connecting the ATPase domain with the Isw1–HAND domain suggests that it acts simply to tether the ATPase. However, it may possibly bind the surface of the I-nucleosome taking on a regular structure, which aids nucleosome recognition.

Recent studies of nucleosome position in yeast with resolution better than 10 bp reveal that for the majority of gene promoter regions, nucleosomes -1 and $+1$ are precisely positioned to either side of a nucleosome free region of approximately 140 bp [1,23]. Positioning occurs for further nucleosomes in both directions, and is particularly regular over gene-encoding regions. For such nucleosome organization, positioning information from DNA sequence, local interacting factors, or pre-existing chromatin structure must come into play. A chromatin remodelling factor could then initiate or lengthen an array of regularly positioned nucleosomes established by pre-existing chromatin landmarks. For nucleosomes in the most highly ordered regions, it is unlikely that chromatin remodelling could be responsible if the remodelling mechanism relied simply on positioning nucleosomes randomly between pre-existing barriers, as has been suggested for ACF1 [24,25]. In contrast, the remodelling mechanism envisioned for ISW1a on the basis of the structural studies described is compatible with generation of the highly organized arrays observed in mapping studies. Remodelling would begin with recognition of a pre-existing, well-positioned nucleosome, possibly augmented by DNA sequence recognition, and is followed by positional adjustment of the histone octamer of a second nucleosome via the ATPase domain tethered to the remodelling factor. Translocation of this second nucleosome along the DNA would continue until a DNA-binding surface of the remodeller measures off the preferred length of linker DNA connecting the two nucleosomes. For ISW1a, the HSS structural unit provides this 'protein ruler' [9]. Propagation

of array ordering with respect to nucleosome +1 or −1 would proceed with ISW1a visiting each pair of adjacent nucleosomes in the array, probably reiteratively, generating the observed regularity.

By providing insight into near-atomic resolution, the structural studies on ISW1a suggest avenues of investigation to further test chromatin remodelling mechanisms. Structural analysis with chromatin substrates incorporating specific promoter sequences in conjunction with remodelling assays *in vivo* will be required to gain a definitive understanding of promoter and nucleosome specificity in the context of chromatin structure. The design of these experiments would benefit considerably from nucleosome mapping results at single nucleotide resolution, free from the errors incurred by the nuclease cleavage used to produce mononucleosomes.

Funding

I appreciate the financial support from the Swiss National Fund and the NCCR (National Center of Competence in Research) Structural Biology.

References

1 Jiang, C. and Pugh, B.F. (2009) A compiled and systematic reference map of nucleosome positions across the *Saccharomyces cerevisiae* genome. Genome Biol. **10**, R109
2 Lowary, P.T. and Widom, J. (1998) New DNA sequence rules for high affinity binding to histone octamer and sequence-directed nucleosome positioning. J. Mol. Biol. **276**, 19–42
3 Sekinger, E.A., Moqtaderi, Z. and Struhl, K. (2005) Intrinsic histone–DNA interactions and low nucleosome density are important for preferential accessibility of promoter regions in yeast. Mol. Cell **18**, 735–748
4 Hartley, P.D. and Madhani, H.D. (2009) Mechanisms that specify promoter nucleosome location and identity. Cell **137**, 445–458
5 Gkikopoulos, T., Schofield, P., Singh, V., Pinskaya, M., Mellor, J., Smolle, M., Workman, J.L., Barton, G.J. and Owen-Hughes, T. (2011) A role for Snf2-related nucleosome-spacing enzymes in genome-wide nucleosome organization. Science **333**, 1758–1760
6 Lee, W., Tillo, D., Bray, N., Morse, R.H., Davis, R.W., Hughes, T.R. and Nislow, C. (2007) A high-resolution atlas of nucleosome occupancy in yeast. Nat. Genet. **39**, 1235–1244
7 Mavrich, T.N., Jiang, C., Ioshikhes, I.P., Li, X., Venters, B.J., Zanton, S.J., Tomsho, L.P., Qi, J., Glaser, R.L., Schuster, S.C. et al. (2008) Nucleosome organization in the *Drosophila* genome. Nature **453**, 358–362
8 Schalch, T., Duda, S., Sargent, D.F. and Richmond, T.J. (2005) X-ray structure of a tetranucleosome and its implications for the chromatin fibre. Nature **436**, 138–141
9 Yamada, K., Frouws, T.D., Angst, B., Fitzgerald, D.J., DeLuca, C., Schimmele, K., Sargent, D.F. and Richmond, T.J. (2011) Structure and mechanism of the chromatin remodelling factor ISW1a. Nature **472**, 448–453
10 Tsukiyama, T., Palmer, J., Landel, C.C., Shiloach, J. and Wu, C. (1999) Characterization of the imitation switch subfamily of ATP-dependent chromatin-remodeling factors in *Saccharomyces cerevisiae*. Genes Dev. **13**, 686–697
11 Venters, B.J., Wachi, S., Mavrich, T.N., Andersen, B.E., Jena, P., Sinnamon, A.J., Jain, P., Rolleri, N.S., Jiang, C., Hemeryck-Walsh, C. et al. (2011) A comprehensive genomic binding map of gene and chromatin regulatory proteins in *Saccharomyces*. Mol. Cell **41**, 480–492
12 Morillon, A., Karabetsou, N., O'Sullivan, J., Kent, N., Proudfoot, N. and Mellor, J. (2003) Isw1 chromatin remodeling ATPase coordinates transcription elongation and termination by RNA polymerase II. Cell **115**, 425–435
13 Grune, T., Brzeski, J., Eberharter, A., Clapier, C.R., Corona, D.F., Becker, P.B. and Muller, C.W. (2003) Crystal structure and functional analysis of a nucleosome recognition module of the remodeling factor ISWI. Mol. Cell **12**, 449–460
14 Horton, J.R., Elgar, S.J., Khan, S.I., Zhang, X., Wade, P.A. and Cheng, X. (2007) Structure of the SANT domain from the *Xenopus* chromatin remodeling factor ISWI. Proteins **67**, 1198–1202
15 Goldmark, J.P., Fazzio, T.G., Estep, P.W., Church, G.M. and Tsukiyama, T. (2000) The Isw2 chromatin remodeling complex represses early meiotic genes upon recruitment by Ume6p. Cell **103**, 423–433
16 Hassan, A.H., Neely, K.E. and Workman, J.L. (2001) Histone acetyltransferase complexes stabilize SWI/SNF binding to promoter nucleosomes. Cell **104**, 817–827
17 Badis, G., Chan, E.T., van Bakel, H., Pena-Castillo, L., Tillo, D., Tsui, K., Carlson, C.D., Gossett, A.J., Hasinoff, M.J., Warren, C.L. et al. (2008) A library of yeast transcription factor motifs reveals a widespread function for Rsc3 in targeting nucleosome exclusion at promoters. Mol. Cell **32**, 878–87
18 Luger, K., Maeder, A.W., Richmond, R.K., Sargent, D.F. and Richmond, T.J. (1997) Crystal structure of the nucleosome core particle at 2.8 Å resolution. Nature **389**, 251–260
19 Gangaraju, V.K. and Bartholomew, B. (2007) Dependency of ISW1a chromatin remodeling on extranucleosomal DNA. Mol. Cell. Biol. **27**, 3217–3125
20 Durr, H., Korner, C., Muller, M., Hickmann, V. and Hopfner, K.P. (2005) X-ray structures of the *Sulfolobus solfataricus* SWI2/SNF2 ATPase core and its complex with DNA. Cell **121**, 363–373
21 Clapier, B.R., Nightingale, K.P. and Becker, P.B. (2002) A critical epitope for substrate recognition by the nucleosome remodeling ATPase ISWI. Nucleic Acids Res. **30**, 649–655
22 Ferreira, H., Flaus, A. and Owen-Hughes, T. (2007) Histone modifications influence the action of Snf2 family remodelling enzymes by different mechanisms. J. Mol. Biol. **374**, 563–579
23 Venters, C.J. and Pugh, B.F. (2009) A canonical promoter organization of the transcription machinery and its regulators in the *Saccharomyces* genome. Genome Res. **19**, 360–371
24 Blosser, T.R., Yang, J.G., Stone, M.D., Narlikar, G.J. and Zhuang, X. (2009) Dynamics of nucleosome remodelling by individual ACF complexes. Nature **462**, 1022–1027
25 Racki, L.R., Yang, J.G., Naber, N., Partensky, P.D., Acevedo, A., Purcell, T.J., Cooke, R., Cheng, Y. and Narlikar, G.J. (2009) The chromatin remodeller ACF acts as a dimeric motor to space nucleosomes. Nature **462**, 1016–1021

Received 24 October 2011
doi:10.1042/BST20110748