Catch me if you can
How the histone chaperone FACT capitalizes on nucleosome breathing

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Nucleosomes confer a barrier to processes that require access to the eukaryotic genome such as transcription, DNA replication and repair. A variety of ATP-dependent nucleosome remodeling machines and ATP-independent histone chaperones facilitate nucleosome dynamics by depositing or evicting histones and unwrapping the DNA. It is clear that remodeling machines can use the energy from ATP to actively destabilize, translocate or disassemble nucleosomes. But how do ATP-independent histone chaperones, which “merely” bind histones, contribute to this process? Using our recent structural analysis of the conserved and essential eukaryotic histone chaperone FACT in complex with histones H2A-H2B as an example, we suggest that FACT capitalizes on transiently exposed surfaces of the nucleosome. By binding these surfaces, FACT stabilizes thermodynamically unfavorable intermediates of the intrinsically dynamic nucleosome particle. This makes the nucleosome permissive to DNA and RNA polymerases, providing temporary access, passage, and read-out.

Introduction

The eukaryotic FACT complex is an essential and highly conserved eukaryotic histone chaperone. It assists the progression of DNA and RNA polymerases, for example, by facilitating transcriptional elongation. The structure together with biochemical and in vivo data give much support to the hypothesis that FACT stabilizes a nucleosome state where the DNA is partially peeled off the histone octamer. In this model, FACT would stabilize partly unfolded nucleosome intermediates in

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order to catalyze the passage of nucleic acid polymerases on the chromatinized DNA template.

How does FACT Mediate Its Vital Engagement with Histones H2A-H2B?

FACT is composed of two subunits: Spt16 and Pol3 in yeast, or Spb1 and SsR1 in humans. In our recent analysis, which includes the 2.35 Å crystal structure of a complex between the C. thermophilum yeast Spt16M domain with the globular core domains of histone H2A (13–106) and H2B (24–122) (Fig. 1A), we show that the Spt16 module of FACT’s Spt16 subunit establishes the evolutionarily conserved histone H2A-H2B binding and chaperoning function.5 Spt16M adopts a tandem PHL (pleckstrin homology-like) fold, with a novel α-helical feature at the C-terminus, which we termed the “U-turn.” This extension engages in a strong hydrophobic interaction ($K_{D} \sim 400$ nM) with the N-terminal α1 helix of histone H2B. Yeast cells carrying mutations in U-turn residues were unable to grow, therefore the U-turn motif represents a protein surface that is essential to FACT’s function and cell viability. We also found that two other surfaces of Spt16 contribute to H2A-H2B binding electrostatically and stabilize the complex kinetically. First, a conserved acidic patch on the second PHL domain of Spt16M interacts with the unstructured basic N-terminal tail of histone H2B. Yeast cells carrying mutations in U-turn residues were unable to grow, therefore the U-turn motif represents a protein surface that is essential to FACT’s function and cell viability.

The high-resolution snapshot of the Spt16M–H2A-H2B complex serves as a structural platform for determining the mechanism(s) by which FACT couples the recognition of hydrophobic and electrostatic features of H2A-H2B to productive nucleosome reorganization. Combining the structure and biochemistry, we show that Spt16M interacts with three proximal surface patches of the histone octamer that organize the first 30 bp of nucleosomal DNA (Fig. 1B):6 the H2B N-terminal tail, the H2B α1 helix and the H3 αN helix. Further, we envision the acidic Spt16C domain interacting with exposed, positively charged histone surfaces.

Electrostatic Interactions Drive Rapid Complex Formation

Many protein–protein interactions are known to be driven by initial electrostatic interactions (which are not necessarily part of the main interaction interface) that then help successive, stable hydrophobic interfaces to form, since the attractive forces of electrostatic interactions have greater reach in solution and promote faster complex assembly.4 The Spt16M–H2A-H2B interactions we have described perfectly fit this model: the highly charged basic H2B N-terminal tail and the unstructured acidic Spt16C domain could mediate the first electrostatic interactions between chaperone and histone, facilitating the successive (hydrophobic) binding of the Spt16M U-turn to H2B α1. In fact, the solvent-accessible N-terminal tail of H2B exits from the nucleosome between the gyres of DNA (Fig. 1B) and is quite accessible. It is therefore possible that it would mediate some of the first interactions of FACT with the nucleosome (ref. 7 and our data), and indeed we observe that while only a minority of histone H3-H4 binding proteins. It is quite unexpected, the structure of the nucleosome core particle reveals that the tandem PHL domains strongly interacts with H2A-H2B ($K_{D} \sim 30$ nM) and might also make initial contacts. However, the other two interaction sites, the H2B α1 and H3 αN helices, are DNA-covered in the folded, canonical structure of the nucleosome core particle (Fig. 1B).7 So how should the chaperone FACT get access?

FACT Captures “Breathing” Nucleosomes

The nucleosome is not a static particle, but rather a dynamic complex: the first –30 basepairs of DNA constantly and progressively unwrap and rewrap with the histone octamer,8 a process that has been termed “nucleosome breathing.” We suggest that FACT can capitalize on these intrinsic nucleosome dynamics to gradually invade the nucleosome particle and develop stronger interactions with the two DNA-covered Spt16M binding patches on the histone octamer (Fig. 2A). Shielding of the histones’ DNA-interaction sites and prevention of unproductive DNA-histone interactions are a characteristic feature of histone chaperones (reviewed in 19, 20). The H3 αN helix stabilizes the first 10 bp of DNA at the nucleosomal superhelix location 6.5. This stretch of DNA is detached about 20–60% of the time,9,10 and the H3 αN helix is therefore quite accessible. The detachment probability decreases to 10% at a position 27 bp into the nucleosome (superhelical location 4.5), where the hydrophobic patch on H2B encompassing the region α1/L1α2 forms the second-strongest histone-DNA contact. This would be expected to greatly decrease the probability of diffusion-mediated interactions with Spt16M.

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However, if the chaperone domain is already captured by the H2B tail or by the H3 αN helix, Spt16M could afford to just "sit and wait" until the H2B α1 site becomes accessible.

In summary, we showed that Spt16M interacts with the two major DNA-histone octamer contacts that coordinate the outermost ~30 base pairs of DNA. Therefore, we and others7-9 suggest that FACT could passively stabilize a state of the dynamic nucleosome complex where this stretch of DNA is displaced, capturing an alternative nucleosome arrangement that occurs upon spontaneous nucleosome breathing. In support of this model, there is good evidence to show that this region of nucleosomal DNA becomes hypersensitive to enzymatic and chemical degradation in the presence of FACT.9 The resulting nucleosome particle would be reorganized to make DNA more accessible, without removing a H2A-H2B dimer or disassembling the nucleosome core particle. This is further supported by the fact that the structure of the Spt16M-H2A-H2B complex suggests no direct interference of the interactions between H2A-H2B and H3-H4 by Spt16M. The mechanism we propose is ATP-independent and clearly distinct from the nucleosome remodeling machinery. Rather, we suggest that FACT could function as a "wedge" that passively capitalizes on the spontaneous movements of the DNA in order to creep into the nucleosome.

Where Does the DNA Go when Displaced by Spt16?

If FACT is able to peel off some of the DNA from the histone proteins within the canonical nucleosome core particle, then is the (lost) binding energy between DNA and histones somehow compensated for? The Spt16M module displays distinct positively charged surface patches on the PHL-1 domain that could neutralize the negative charges of the dissociated DNA (see Supplementary Figure 2 of ref 10). Indeed, both the tandem PHL domain Spt16M or the isolated PHL-1 domain bind DNA in EMSA assays, just like the structurally homologous Pob3M and Rtt106 domains,14 while the PHL-2 domain does not bind DNA (Hondele M and Laudurner AG, unpublished data). In the superposition of the Spt16M-H2A-H2B complex onto the nucleosome structure (Figure 2A), the basic patches on PHL-1 would be in a good position to catch, neutralize and stabilize the displaced DNA, thereby compensating for some of the broken histone-DNA binding energy. In functional Spt16 sequences, an unstructured loop in PHL-1 is enriched for positively charged residues. A homologous stretch is absent or less distinctive in higher eukaryotes (Figure 3). However, this loss could be evolutionarily compensated for by the DNA-binding HMG box of SSRP1, which is not present in the yeast Pob3 homolog. The chaperone FACT could thus easily capture DNA surfaces normally involved in histone interactions, helping to prevent unproductive protein-DNA interactions.

FACT Breaks Strong Octamer-DNA Contacts to Allow Polymerase Passage

The enzyme RNA polymerase II by itself cannot generate enough force to transcribe through nucleosomes in vitro, since breaking DNA-histone contacts poses a strong energetic barrier (see ref. 21 for review). Yet if contacts between the H2A-H2B dimer and nucleosomal DNA are already broken, this sufficiently lowers the energetic barrier to permit polymerase passage.33 Recently, Hsieh et al11 showed that FACT does exactly this; it helps to partially unfold DNA from H2A-H2B and its presence is sufficient to facilitate transcription through the nucleosome. Since chemically crosslinking the histone octamer core does not inhibit FACT action, nor transcription, the authors assume that FACT does not evict the H2A-H2B dimer or introduce major changes in the overall structure of the histone octamer, such as the interface between H2A-H2B.

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![Figure 1: Crystal structure of the Spt16M-H2A-H2B complex and overview of three surfaces of the histone octamer recognized by Spt16M. (A) The 2.35 Å crystal structure of the trimeric complex between Ch. thermophilum Spt16M and the globular cores (g) of histone H2A and histone H2B reveals the interactions between H2A-H2B dimer and nucleosomal DNA. (B) Close-up view of the histone octamer surface recognized by Spt16M (sphere representation of the amino acid side chains). The H2A-H2B terminal tail (light pink) extends between the gyres of DNA and might serve as an initial, solvent-accessible point of attachment for Spt16 to the nucleosome. The αN helix of H3 (light blue) and the α1 helix of H2B (dark pink) are DNA-covered in the structure of the canonical nucleosome but become accessible upon DNA breathing.](image)
and H3-H4. The authors propose that by shielding or “breaking” DNA-histone contacts the chaperone FACT lowers the thermodynamic barrier sufficiently to facilitate polymerase passage. Our structure of the Spt16M–H2A-H2B complex shows that a nucleosome-engaged Spt16M module would fulfill these mechanistic criteria. On a related note, we speculate that the torsional forces generated by RNA Polymerase II could further help to lift DNA off the histone octamer core and thereby facilitate access of Spt16M to the H2B α1 helix.

**FACT could Destabilize the Histone Octamer Core**

The superposition model also identifies a steric clash between a surface bulge of Spt16M’s PHL-2 and the H3-H4 dimer of the other nucleosome half-disc, which
locates below the H2B α1 helix bound by the Spt16M U-turn (Fig. 2B). This steric clash could be resolved by flexibility in the Spt16M-H2A-H2B interface, by nucleosome “gaping,” an oyster shell-like movement of two nucleosome half-discs around a H3-H3′ interface hinge, or by moving the H2A-H2B dimer away from the octamer. In physiological salt, contacts between H2A-H2B and H3-H4 are weak and the octamer is mainly stabilized by the presence of DNA. Therefore, although Spt16M does not actively disturb contacts between the H2A-H2B dimer and the H3-H4 heterotrimer, Spt16M-induced displacement of DNA and steric clashes with the other nucleosome half-disc might be sufficient to release the H2A-H2B dimer from the nucleosome, as observed in vitro.25

Spt16M Likely Chaperones Canonical, Variant, and Modified H2A-H2B Alike

The read-out of chromatin is regulated by post-translational modifications of histone proteins. These modifications can recruit or stabilize the chromatin binding of “reader” proteins or selectively block the binding of others. Several of these modifications have been well characterized for histone H3, but H2B in contrast only shows a few modifications that may be functional. Some of these localize directly to the interface with Spt16M and might therefore regulate the interaction between histone and chaperone.24,25 For example, phosphorylation of H2B Ser33 on the H2B α1 helix was found to promote transcriptional activation in D. melanogaster.26 In the Spt16M-H2A-H2B crystal structure, this residue is hydrogen bonded to a chloride ion, which is further coordinated by Spt16M Asn916 and H2B Ile36. The negatively charged phosphate group of Ser33 might well substitute the chloride, and thus we suggest that H2B Ser33 phosphorylation could stabilize the FACT–histone complex. In contrast, our structure suggests that Spt16M binding to histones might not be altered by H2A or H2B ubiquitination. During transcription elongation, FACT shows functional interaction with histone ubiquitin modifications: ubiquitination of H2A (at K119 in mammals) blocks FACT recruitment and inhibits transcription elongation.26 In contrast, FACT stimulates the ubiquitination of H2B K123 in yeast (K120 in mammals) and is in return retained at actively transcribed ORFs. Together with the PAF complex, FACT and H2B mono-ubiquitination (H2Bub1) cooperate to promote transcription elongation27 and preserve chromatin structure.28 Neither H2A K119 nor H2B K123 are visible in the structure of the complex. However, both ubiquitination-sites locate to the C-terminus of the histone proteins, opposite to where Spt16M binds H2B α1. Thus, the modifications will probably not directly block FACT binding and direct recognition of ubiquitin is also unlikely, since the H2A and H2B modifications have opposite effects. Since H2Bub, but not H2Aub, interferes with chromatin compaction and maintains an open and accessible chromatin fiber,29,30 ubiquitination may instead promote a chromatin state that is more accessible (H2Bub) or inaccessible (H2Aub) to the binding of the chaperone and other factors.

The interactions we observed between Spt16M and the H2A-H2B are mediated by H2B residues that are conserved in all other isoforms of this histone heterodimer, such as complexes formed between H2B and H2A variants. In fact, most sequence variance for H2A variants localizes to the C-terminus of the histone protein, which is far from the interaction site on the H2B α1 helix. Similarly, only very few and rare histone variants of H2B are known, and none of these sequence variations localize to the interfaces with Spt16M. Thus, in summary, FACT may engage with canonical and variant modified H2A-H2B heterodimers alike, acting as a very general H2A-H2B chaperone.

The Hydrophobic Patch of H2B has so far Escaped Functional Analysis

Thus far, large-scale genetic screens using scanning alanine mutagenesis of all histone residues have found no requirement of the hydrophobic H2B α1 patch, which interacts with the Spt16M U-turn, for several tested biological functions (e.g., transcription, replication).29,30 This is somewhat surprising, but the lack of obvious phenotype might be explained by the rather hydrophobic character of the alanine residue used as replacement; single point mutants of this type would be predicted to conserve the overall hydrophobic properties of this FACT-interacting surface. In the case of Spt16M, for example,
we found that mutation of four hydrophobic U-turn residues to alanine is not sufficient to disrupt the complex with H2A-H2B. However, mutation to serine residues clearly is. Hydrophobic-to-charged H2A mutants in the H2A α helix might thus be necessary for functional analysis of histone mutants.

Holo-FACT acts as a Scaffold to Integrate Nuclear Processes

We also reported the structure of FACT's heterodimerization domain. Like Spt16M, Pob3M or Rtt106, the Spt16D and Pob3N complex is composed of PHL domains. However, the Spt16D-Pob3N heterodimer does not bind histones, rather our preliminary biochemical analysis showed that this region connects FACT to replicative DNA polymerases. Since Pob3M also interacts with RPA, the single-stranded DNA binding protein, this suggests that coupling of the chaperone FACT to the replication machinery might be important, promoting nucleosome (dis-)assembly during DNA replication.

Together with the published structures of Spt16N(3,4) and Pob3M,5 the structures of all globular domains of FACT have now been solved. Apart from Spt16N, which adopts the canonical "pita-bread" fold of amino-peptidases, all domains are composed of pleckstrin homology-like modules. It is possible that these histone-binding modules may have originated from domain fold amplification during evolution. All published structure are from yeast proteins, but since the sequences and secondary structures of FACT subunits are very conserved, we can assume that the observed features also apply to the proteins from human and other species. In fact, the human Sppt16M module readily binds H2A-H2B.1

We believe that the modular nature of the FACT complex may allow the chaperone to effect multiple interactions with distinct histones, DNA and a variety of other nuclear factors. The linkers connecting the globular FACT domains are predicted to be (at least) partially α-helical, but we believe that they do not rigidly connect the globular modules. Rather, each domain may act as a more or less "independent" unit that binds histones or other nuclear factors. To summarize, only one globular domain of FACT, Spt16M, binds histones H2A-H2B. However, three out of the four globular domains—Spt16N, Pob3M, and Sppt16M—interact with H3-H4 at least in vitro. Spt16M seems to be the strongest binder and primarily recognizes a peptide spanning H3 residues (46–60).6 Further, unstructured domains such as the highly acidic Spt16C region, promote further histone interaction through vital electrostatic interactions. We suggest that the tethering of all these domains together might have a recruiting function, to "keep things in place" as a scaffold, thus increasing the processivity of many distinct nuclear processes involving the dynamic reconformation of the chromatin template.

Where Are We Now?

Our ultimate goal is to understand how the holo-FACT complex reorganizes the nucleosome, and therefore to analyze how the individual modules of the multi-domain chaperone interact together with the various features of the nucleosome core particle to successfully reorganize DNA-histone contacts. To gain a full picture of this highly dynamic process, methods such as cryo-electron microscopy, cross-linking mass spectrometry and single-molecule in vitro and in vivo FRET will probably be most useful to capture "snapshots" of the dynamic complex of the full-length chaperone bound to the nucleosome, at different stages of nucleosome reorganization.

Further work is also necessary at the cellular level in order to reveal the temporal aspects of how FACT promotes nucleosome dynamics. In particular, it will be interesting to study its interactions with the DNA and RNA polymerase machineries and how FACT integrates these diverse functions. This will lead us to an understanding of how this apparently passive, "non-aggressive" protein complex has sufficient energy to facilitate the progression of DNA and RNA polymerases, and at the same time helps to ensure that chromatin structure is established properly and remains as intact as possible. In short: faithful to its function as a genuine chaperone.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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