

Asf1, a Loveseat for a Histone Couple

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In this issue of *Cell*, English et al. (2006) present the first crystal structure of a histone chaperone (Asf1) bound to histones (the H3/H4 heterodimer). The structure provides insights into how histone chaperones participate in nucleosome disassembly. It reveals that Asf1 physically blocks (H3/H4)₂ tetramer formation and that the C terminus of H4 undergoes a dramatic conformational change upon binding to Asf1.

The fundamental structural unit of chromatin is the nucleosome, which is composed of 147 base pairs of DNA wrapped in 1.65 turns around a histone octamer formed by two H2A/H2B dimers tethered to each side of one (H3/H4)₂ tetramer (Luger et al., 1997). Nucleosome assembly and disassembly probably occurs during all DNA-associated processes, including replication, repair, transcription, and recombination. The basic steps of nucleosome assembly likely involve deposition of one histone (H3/H4)₂ tetramer (or two H3/H4 dimers) onto DNA, followed by the incorporation of two histone H2A/H2B dimers to form the nucleosome core. The key players in these processes are histones, histone chaperones, and ATP-dependent remodeling complexes (reviewed in Polo and Almouzni, 2006). Histone chaperones are a family of proteins that facilitate histone deposition and allow histone exchange and eviction during nucleosome assembly and disassembly. In this issue of *Cell*, English et al. (2006) solve the structure of the histone chaperone Asf1 (anti-silencing function 1) bound to the H3/H4 heterodimer, providing insights into how this chaperone promotes nucleosome assembly and disassembly.

Asf1 was initially characterized genetically in budding yeast as a suppressor of gene silencing when overexpressed. Deletion of Asf1 in budding yeast leads to defects in DNA replication, repair, transcription, and histone modification (Recht et al., 2006; Schwabish and Struhl, 2006;

Tyler et al., 1999). Its role as a histone chaperone in chromatin assembly emerged after it was shown in the fruit fly that Asf1 was part of the replication-coupling assembly factor (RCAF) complex together with newly synthesized histones H3 and H4 (Tyler et al., 1999). Studies thereafter indicated that Asf1—which is structurally and functionally conserved across eukaryotic species—is likely to be the major H3/H4 chaperone in cells. Asf1 is a common partner of the chromatin assembly complexes CAF-1 and HIRA and facilitates histone deposition through both replication-dependent and replication-independent chromatin assembly pathways (Tagami et al., 2004).

The histones H3 and H4 are highly conserved in eukaryotes and, like a good couple, the H3/H4 pair remains together faithfully under virtually all conditions. Although the atomic details of how this histone pair lives within a nucleosome is known (Luger et al., 1997), its behavior outside the “nucleo-home” has been something of a mystery. Merging structural and genetic studies, English et al. (2006) provide a high-resolution view of a histone H3/H4 dimer bound to the yeast Asf1 chaperone, revealing Asf1 as a fitting loveseat for the histone couple.

The structure of the Asf1-H3/H4 complex was solved by molecular replacement, using the models of free Asf1—first solved by the Kaufman group (Daganzo et al., 2003)—and the histone H3/H4 dimer from the nucleosome core particle (Luger

et al., 1997). The overall structure reveals that both H3 and H4 make extensive contacts with Asf1. The Asf1 core is an elongated β sandwich domain with three α helices in the loops. Comparison of the structures of unbound Asf1 and the Asf1-H3/H4 complex structure reveals that subtle conformational changes occur in Asf1 upon binding to the H3/H4 dimer. In addition, the conserved histone folds and the “handshake motif” of H3 and H4 are retained similar to those found in nucleosomes (Luger et al., 1997). However, several key interactions between Asf1 and the H3/H4 dimer suggest a possible mechanism of nucleosome disassembly and assembly.

Several β strands of Asf1 make extensive contacts with the C-terminal helix and other regions of histone H3. These interactions were confirmed by in vitro and in vivo mutagenesis studies. Interestingly, this antiparallel β sheet region is a common structural motif present in other histone chaperones, such as nucleoplasmin and Nap1 (Park and Luger, 2006), suggesting a similar mode of histone binding. Notably, all of the residues that distinguish the histone variants H3.3 and H3.1 are not involved in interactions with the Asf1-globular domain. This observation suggests that the Asf1 C terminus or other HIRA and CAF-1 subunits may be involved in recognizing the right histone variants.

Importantly, in the nucleosome structure, the C-terminal helix, and the C-terminal part of the central

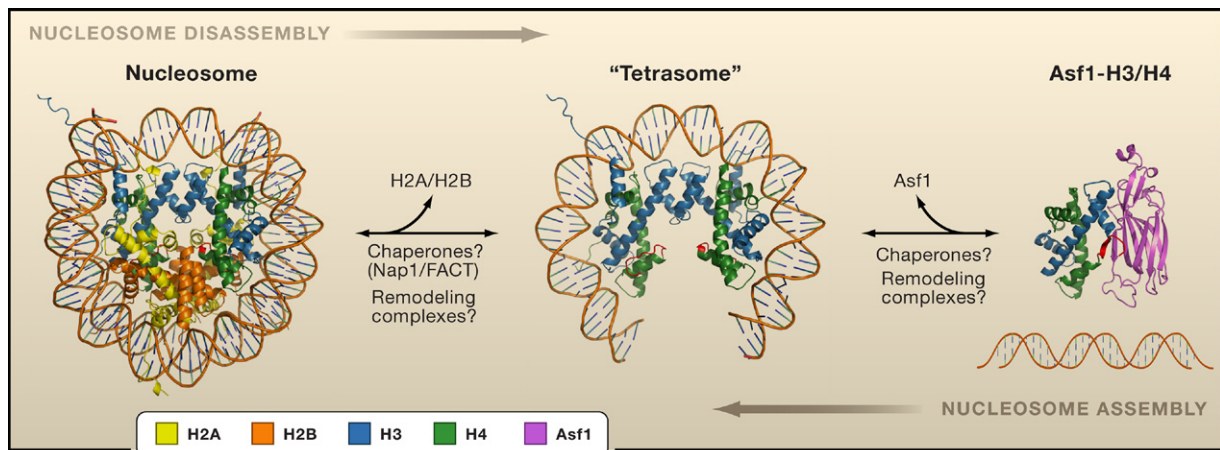


Figure 1. The Strand-Capture-Split Model of Nucleosome Assembly/Disassembly

In the nucleosome structure (PDB ID: 1AOI [Luger et al., 1997]), the C terminus of H4 (residues 93–102 in red) makes contacts with H2A and is buried in the nucleosome. During nucleosome disassembly, the H2A/H2B dimers are removed by other histone chaperones and ATP-dependent remodeling complexes, exposing the C terminus of H4 in the “tetrasome.” The tetrasome model in this figure is based on the nucleosome structure (Luger et al. 1997). Asf1 recognizes and captures the C terminus of H4 in the tetrasome (note the conformational change of the H4 C terminus in the Asf1-H3/H4 complex). Asf1 then dissociates the (H3/H4)₂ tetramer into a H3/H4 dimer bound to Asf1 (English et al., 2006). Other histone chaperones and chromatin remodeling complexes may also be involved in the dissociation of the (H3/H4)₂ tetramer. The reverse reaction could represent nucleosome assembly. Still images of nucleosome and tetrasome were generated using MacPymol (<http://www.pymol.org>). The structure of Asf1-H3/H4 complex was adapted from English et al. (2006).

helix of each histone H3 are involved in the formation of a 4-helix bundle, which drives the two H3/H4 dimers to form the (H3/H4)₂ tetramer (Luger et al., 1997). However, in this histone-chaperone complex structure, the formation of the (H3/H4)₂ heterotetramer is completely blocked because those regions of H3 are enveloped by the globular domain of Asf1. This observation suggests that binding of Asf1 to H3 within a nucleosome (or a tetrasome) may “split” the (H3/H4)₂ tetramer, thus providing a potential mechanism for dissociating this tetramer. Given that the association/dissociation of the (H3/H4)₂ tetramer are potentially involved in many DNA transactions, such a splitting mechanism provides a way to regulate (H3/H4)₂ tetramer dynamics.

Further surprises come from the interactions between Asf1 and H4. In the nucleosome structure, the C terminus of H4 forms a parallel β sheet with the docking domain of histone H2A and stabilizes the interaction between the (H3/H4)₂ tetramer and the H2A/H2B dimer (Luger et al., 1997). Unexpectedly, the C terminus of H4 in the Asf1-H3/H4 structure rotates about 180° to form an antipar-

allel β sheet with one β strand of Asf1. Additionally, the five final residues of H4 make contacts with a hydrophobic region formed by the two β strands of Asf1. Remarkably, one residue (F100) in the C terminus of H4 fits snugly into the pocket on the surface of Asf1, suggesting a “lock-and-key” binding between H4 and Asf1. Extensive mutagenesis analysis by the authors confirmed that these H4-Asf1 interactions are required for Asf1 histone chaperone function *in vitro* and *in vivo*.

Based on the dramatic conformation changes in the C-terminal end of H4 upon binding of Asf1 and the interactions between Asf1 and H3, the authors propose a “strand-capture-split” model for disassembly of the histone (H3/H4)₂ tetramer (Figure 1). The reverse reaction would result in nucleosome assembly. First, the histone H2A/H2B dimers in the nucleosome are removed, potentially by histone chaperones such as Nap1 or FACT. Next, the C terminus of each H4 can be readily captured by Asf1 followed by interactions with H4 and H3 that trigger the dissociation of the (H3/H4)₂ tetramer into Asf1 bound H3/H4 dimers. These steps are prob-

ably facilitated by other histone chaperones and/or ATP-dependent chromatin remodelers. Notably, the recently solved structure of a complex between Asf1a (one of two human orthologs of Asf1) and the HIRA B domain revealed that the binding of the HIRA B domain to Asf1 does not interfere with H3/H4 binding because it occurs on a different side of Asf1 (Tang et al., 2006). Interestingly, a similar hydrophobic pocket to the one where the H4 C terminus inserts into Asf1 is also present in nucleoplasmin, suggesting that the “strand-capture” mechanism could be a common feature of histone chaperones. Future biochemical, genetic, and structural studies are needed to fully address the accuracy of this model.

These exciting new insights also raise further questions. For example, what are the mechanistic relationships among Asf1, histone variants, histone modifications, and DNA in the assembly reactions? What regulates Asf1 to carry out forward (disassembly) or reverse (assembly) reactions? Biologically, how is Asf1 activity targeted to distinct nuclear functions? What are

the functional relationships among Asf1, other chaperones, and chromatin modifiers? The candid picture of the H3/H4 couple sitting in the Asf1 loveseat represents a milestone in our understanding of how chromatin is assembled.

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Worms Clear the Smoke Surrounding Nicotine Addiction

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In this issue of *Cell*, Feng et al. (2006) report a worm model of nicotine dependence that shows behavioral adaptations surprisingly similar to those in humans. These authors show a critical link between nicotinic receptors and TRP channels, which may represent a new therapeutic target for treating nicotine addiction.

There is often striking phylogenetic conservation of function within the rich array of chemical signaling molecules in the brain. In the lobster, the neurotransmitter serotonin regulates dominance behavior, and in humans, serotonin is thought to be a key modulator of mood, impulse, and aggression. Dopamine receptors are important in reward learning in honeybees, molluscs, mice, and primates. An additional feature of neurochemical evolution is that many of these shared substrates also serve as targets for drugs of abuse, some of which are plant alkaloids that coevolved with animals. In this issue, Feng and colleagues (2006) report nicotine-dependent behavior in the nematode *Caenorhabditis elegans*, which mimics

that observed in mammals, including humans (Feng et al., 2006). Through elegant experiments, the authors pinpoint the genetic and biochemical mechanisms underlying this behavior in worms and discover that TRP (transient receptor potential) channels modulate the activity of nicotinic acetylcholine receptors (nAChRs). As nicotine addiction is a major cause of morbidity and mortality worldwide (Laviolette and van der Kooy, 2004), a simple animal model of nicotine addiction may reveal new therapeutic targets for treating this health problem.

Nicotine, the component in tobacco smoke that leads to addiction, acts on nAChRs in the brain. These receptors, which are ligand-gated ion channels, are widely distributed in the cen-

tral and peripheral nervous system in nearly all invertebrate and vertebrate species. These receptors consist of a variety of pentameric combinations of α and β subunits that form cation-selective pores (Dani and Bertrand, 2006). Receptors composed of $\alpha 7$ and $\alpha 4\beta 2$ subunits have received particular attention with regard to the cognitive and reinforcing effects of nicotine. Indeed, in rodents, the $\beta 2$ subunit is a critical player for releasing dopamine in response to nicotine and for reinforcing nicotine's effects (Maskos et al., 2005; Picciotto et al., 1998). Nicotine also has potent effects on glutamatergic transmission in brain regions important for learning, memory, and attention in rodents (Gray et al., 1996). In the mammalian brain, the widespread distribu-