

# SWRred Not Shaken: Mixing the Histones

## Minireview

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**The recently isolated SWR1 complex catalyzes an ATP-dependent histone exchange with specificity for the histone variant H2A.Z. This provides a new theme in chromatin remodeling mechanisms and an explanation of how histone variants are incorporated into the nucleosome outside of S phase. In connection with the isolation of histone variant specific chaperone complexes, we are beginning to see the mechanisms that mix the histone octamer with intriguing implications for epigenetic inheritance.**

Chromatin, the packaged form of eukaryotic DNA, influences all DNA-related nuclear processes like transcription, replication, repair, and recombination. At a basic level, chromatin is assembled from only four histone proteins, H2A, H2B, H3, and H4. An octamer of two of each builds a protein core around which the DNA is wound in about 1.7 turns to give the compact particle of a nucleosome. Nucleosomes are the basic packaging unit of chromatin, assembled like beads-on-a-string along the DNA to form the nucleofilament. When this substructure of chromatin was first detected, it was seen as a gray and monotonous array just required to provide a first level of compaction to the eukaryotic DNA, and the nucleosome itself was pictured more like a solid steel ball. Over the past ten years, this view has given way to a much more modulated and colorful picture in which each nucleosome may have its own distinctive make up. This individuality resides predominantly in the protruding N-terminal tails of the nucleosomal histones, which are covalently modified through a surprising variety of enzymatic activities. These findings have led to the proposal of a second code, the “histone code,” superimposed on the information encoded in the DNA sequence (Jenuwein and Allis, 2001; Turner, 2002).

Still additional complexity in the structure of the nucleosomes arises from the presence of so called variant histones, which are paralogous to the standard ones. At least some of the variant histones appear to be incorporated site specifically into chromatin outside of S phase of the cell cycle replacing the corresponding canonical histones which are deposited onto freshly replicated DNA. Special chromatin arrangements, like the centromere for example, benefit from a centromere specific H3 variant, called CENP-A (Smith, 2002) and another histone variant of H3, called H3.3, becomes specifically incorporated in the very actively transcribed rDNA locus (Ahmad and Henikoff, 2002). The presence of such

variants makes the nucleofilament look even less like a monotonous array but much more like a kaleidoscope which displays novel, ever changing colorful patterns each time it is shaken.

Shaking will not do for the nucleofilament, but an exciting set of three recent papers points out a way how the cell might change the composition of nucleosomes after the initial assembly, and insert variant histones in exchange for the regular ones (Krogan et al., 2003; Mizuguchi et al., 2004; Kobor et al., 2004). In all three studies, a multiprotein complex, the SWR1 complex, was isolated from yeast, which specifically exchanges histone H2A for its variant H2A.Z in nucleosomes. Just a year ago a strong enrichment of H2A.Z in chromatin adjacent to transcriptionally repressed, so called silenced regions was reported, and H2A.Z was found to prevent the ectopic spread of silencing into the neighboring euchromatin (Meneghini et al., 2003). In a comment several questions were raised (van Leeuwen and Gottschling, 2003): “Is H2A.Z deposited like canonical H2A in S phase, or is it deposited by a replication-independent pathway? ... If such a replacement occurs, does it require a particular chromatin remodeling machinery?”

The SWR1 complex seems to be the answer to these questions. The three groups started from quite different angles but arrived at the same protein complex. Krogan et al. (2003) used a genetic interaction only approach and crossed 4700 viable gene deletion mutants to each of three genes that are involved in transcriptional elongation and chromatin modification and looked for genes that had synthetic growth defects with all three query genes. Only five genes fulfilled those criteria. Amazingly, three of the five encoded components of what turned out to be the novel complex, the fourth one was H2A.Z, and only one was unrelated. Mizuguchi et al. (2004) extended their previous strategy to look at Snf2 (see below) paralogs in yeast (a strategy that had previously uncovered the INO80 complex) to now include Swr1. The multiprotein complex containing Swr1 was then found to interact with histones, notably H2A.Z. Kobor et al. (2004) used still another approach and searched directly for proteins interacting specifically with H2A.Z. Thirteen such proteins were identified and found to make up the SWR1 complex. Genetic disruption of SWR1 function reported by all three groups leads to defects in H2A.Z incorporation in vivo and shows extensive phenotypic overlap with deletions of H2A.Z itself. And excitingly, the SWR1 complex is indeed a chromatin-remodeling machine.

### **Chromatin Remodeling Complexes— a Growing Family**

The recognition of ATP-dependent chromatin-remodeling complexes is the other success story, in addition to the identification of histone-modifying enzymes, that we have witnessed over the past ten years in the chromatin field (reviewed in Becker and Hörz, 2002). These multiprotein complexes are generally defined by the catalytic ATPase subunit. The SWI/SNF complex, for example, is named after Swi2 or Snf2, the prototype of the ATP-

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hydrolyzing “motor,” first discovered in *S. cerevisiae*. Also the SWR1 complex is named after its ATPase subunit Swr1 (for *Swi2/Snf2* related). ATP-dependent remodeling machines have grown to be a large family of related proteins in all eukaryotic organisms. As the name implies, they can remodel the structure of chromatin and support a dynamic state of the nucleofilament. The precise mechanism of remodeling appears to be distinct from one family to another, however. Complexes of the SWI/SNF family appear to alter the path of the DNA around the histone core such that the nucleosome adopts a stably altered form, and the DNA becomes more accessible. On the other hand, complexes of the so-called ISWI family (*I*mitation *S*wi2) relocate nucleosomes by sliding the histone octamers along the DNA. This process yields canonical nucleosomes at different positions without generating stably altered forms that are typical of SWI/SNF action. Maybe both mechanisms have in common the generation of DNA loops on the histone octamer surface and differ just by the size of the loops and the speed of their propagation along the nucleosome (Längst and Becker, 2004). The common theme in both cases is the breaking of histone DNA interactions.

The SWR1 complex adds a new theme to chromatin remodeling: the breaking of histone-histone interactions, thereby affecting the integrity of the octamer core itself. Mizuguchi et al. (2004) show directly in a novel *in vitro* assay that, in the absence of replication, catalytic amounts of SWR1 complex can transfer substantial amounts of H2A.Z-H2B dimers in exchange for H2A-H2B dimers to nucleosomal arrays but not to free DNA. This reaction is dependent on ATP and the integrity of the ATPase site of the Swr1 subunit. Importantly, a dual specificity was observed: only the SWR1 complex but not other remodeling machines catalyzes the transfer of H2A.Z, and, secondly, it efficiently transfers only H2A.Z and not H2A.

An obvious question raised by the new findings is how the SWR1 complex is targeted to the appropriate genomic regions. The identification of Bdf1 (*Bromodomain factor 1*) as a member of the SWR1 complex by both Krogan et al. (2003) and Kobor et al. (2004) might give a clue. This protein owes its name to the fact that it contains two bromodomains, protein modules that have been found to interact specifically with acetylated histones. An interesting report last year showed that the absence of a functional Bdf1 protein has a similar phenotype as a *SWR1* or H2A.Z deletion (Ladurner et al., 2003), namely the ectopic spread of heterochromatin into nearby euchromatin regions. Histone acetylation levels are higher in euchromatin, and therefore Bdf1 might be involved in targeting the SWR1 complex to the appropriate regions. It is not clear, however, if a specific acetylation signal exists that Bdf1 recognizes to allow recruitment to select regions within euchromatin, or if other signals also contribute, and what sets the signal in the first place. Further evidence linking acetylation to the functioning of the SWR1 complex comes from the finding by Kobor et al. (2004) of genetic interactions between the SWR1 complex and NuA4, a histone acetylase complex, which is responsible for histone H4 acetylation.

The new theme of histone exchange or displacement

has come up in other recent work as well. FACT, a complex involved in transcription elongation, was recently shown to remove and reassemble H2A-H2B dimers during the act of transcription (Belotserkovskaya et al., 2003). This puts FACT into a similar category as Spt6 which has been known as a histone chaperone for a while and was now found to restore chromatin in the wake of RNA polymerase II elongation (Kaplan et al., 2003). Neither FACT nor Spt6 are chromatin remodeling machines, however, and they do not have ATPase subunits. Perhaps more closely related to the action of the SWR1 complex is the ATP-dependent exchange of H2A-H2B dimers in an *in vitro* model system (Bruno et al., 2003). These authors showed an exchange of H2A-H2B dimers in a remodeling reaction, but only for complexes of the SWI/SNF and not the ISWI family. Apparently only a subgroup of remodeling complexes is able to break up the histone octamer. This could have to do with the extent to which the DNA is spooled off the histone octamer during the remodeling process, i.e., with the size of the generated loop. It is well known that a histone octamer without DNA falls apart into one (H3-H4)<sub>2</sub> tetramer and two H2A-H2B dimers at physiological ionic conditions *in vitro*. One could imagine that the nucleosome becomes more unstable if the DNA is looped out to a certain extent and one of the two H2A-H2B dimers, which are at the periphery of the octamer, may be replaced by a different copy when the DNA moves back into place. If one takes the aspect of histone exchange and displacement one step further, the possibility arises that not only H2A and H2B but also H3 and H4 lose contact with the DNA, which could lead to the disruption and reassembly of entire nucleosomes. This kind of phenomenon could explain the replication-independent removal of four nucleosomes from the promoter of the *PHO5* gene upon activation (Boeger et al., 2003; Reinke and Hörz, 2003) and its reassembly upon repression, again in the absence of replication.

#### **Specific Chaperones for Histone Variants**

Where do the histones go when they are not part of the nucleosome? Histones not bound to DNA are usually in association with chaperones. Histone chaperones are an abundant and diverse class of proteins that are thought to carry and shield free histones prior to their assembly into chromatin, especially in order to prevent the aggregation of the highly positively charged histones. The laboratories of Genevieve Almouzni and Yoshihiro Nakatani extend and complement the “variant histone” theme by looking at the chaperones involved rather than the enzymatic activities that incorporate them (Tagami et al., 2004). In a mammalian system, Tagami et al. (2004) have used the same strategy that led to the identification of the SWR1 complex in yeast, i.e., they used tagged versions of histone H3 and its H3.3 variant to search for associated proteins and have succeeded in isolating two distinct histone chaperone complexes. Similar to the molecular chaperones involved in protein folding the histone chaperones are usually considered a redundant system with low substrate specificity. Therefore the finding of Tagami et al. (2004) that the CAF1 histone chaperone complex prefers the S phase histone H3 whereas the HIRA chaperone complex appears to be specific for the H3.3 variant was unexpected. This finding is especially gratifying since

the Almouzni lab recently demonstrated in a *Xenopus* in vitro system that CAF1 delivers histones in a replication-dependent and HIRA in a replication-independent chromatin assembly pathway (Ray-Gallet et al., 2002).

In their new study, Tagami et al. (2004) find that both histone chaperone complexes carry only one copy of the respective H3 histone type together with an H4 histone. This makes a H3-H4 dimer which is in contrast to the stable (H3-H4)<sub>2</sub> tetramer which so far has been assumed to be assembled into chromatin (reviewed in Wolffe, 1998). According to the textbook view, histone octamers of the parental DNA strand are thought to be disassembled into one (H3-H4)<sub>2</sub> tetramer each and two H2A-H2B dimers upon passage of the replication fork. The (H3-H4)<sub>2</sub> tetramers would be randomly distributed onto the two DNA daughter strands so that each daughter strand would receive a parental copy only at some nucleosome positions. The finding of a H3-H4 dimer in the CAF1 complex, which presumably delivers the histones during replication-dependent nucleosome assembly, raises the possibility that also histones H3 and H4 are deposited as dimers, just like H2A and H2B. Tagami et al. (2004; admittedly without further experimental support) now postulate that not only during assembly of the new nucleosomes but also during disassembly of the parental ones, octamers are broken up into H3-H4 dimers which would then be evenly distributed onto the daughter strands. Although at face value this idea is in contrast to the established concept (Wolffe, 1998), an intermediate involving H3-H4 dimers may not have been recognized as such in the early studies. The beauty of the new concept is that each strand would inherit at each single nucleosome position half a histone complement with the epigenetic make up of the parental chromatin structure. The replication-dependent assembly machinery would complement the other half with new histones leading to a transient hybrid state. By a subsequent mechanism, the nucleosome could be covalently modified and/or rebuilt with the parental half serving as a template. Such a mechanism would solve many haunting questions of how epigenetic information can be stably transmitted even in the absence of DNA sequence specific factors in much the same way as how DNA methylation can be maintained over many generations.

It seems clear that we will discover more collaborations between nucleosome-remodeling complexes, specific histone variants or modification states, and dedicated histone chaperones. New insights into the mechanism of the nucleosomal kaleidoscope will be an exciting prospect.

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