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Chromatin remodeling: a complex affair

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ATP-dependent chromatin remodelers are multi-subunit enzymes that catalyze nucleosome dynamics essential for chromosomal functions, and their inactivation or dysregulation can lead to numerous diseases, including neuro-degenerative disorders and cancers. Each remodeler contains a conserved ATPase "motor" whose activity or targeting can be regulated by enzyme-specific, accessory subunits. The human ISWI subfamily of remodelers has been defined as a group of more than six different enzyme complexes where one of two related ATPase subunits (Snf2L/SMARCA1 and Snf2H/SMARCA5) is paired with one of six different accessory subunits. In this issue of *EMBO Reports*, Oppikofer *et al* [1] find that the human ISWI subfamily is even more polymorphic in nature—every known accessory subunit can interact and function with both ATPase isoforms. This raises the complexity of the human ISWI subfamily to > 12 distinct enzymes, with the possibility for much higher levels of combinatorial assemblies, and has the potential to create enzymes with novel biochemical activities, as well as novel regulatory wiring through differential interactions with locus-specific factors or histone modifications.

See also: **M Oppikofer *et al*** (October 2017)

The ISWI subfamily of chromatin remodelers is composed of multi-subunit enzymes conserved from yeast to man, each assembled around a catalytic ATPase subunit with high sequence similarity to the founding member, *Drosophila* ISWI. The ISWI ATPase is a member of the larger SF2 superfamily of RNA/DNA helicases, and although the ISWI ATPase lacks helicase activity, it does harbor DNA translocation activity, shared with other remodeler ATPases, which plays a central role in the assembly, mobilization, and spacing of nucleosomes during ISWI-catalyzed chromatin remodeling events (for a recent review, see [2]). Mammals contain two isoforms of the ISWI ATPase, encoded by two related genes, Snf2L/SMARCA1 and Snf2H/SMARCA5. In the mouse, Snf2H is essential for early development and is expressed fairly ubiquitously, whereas Snf2L is expressed highly in the brain and testes [3]. In contrast, mice lacking Snf2L are viable and fertile, but show delayed neurogenesis and an enhanced forebrain hyper-cellularity phenotype [4]. Interestingly, these neural defects are not rescued by Snf2H overexpression, suggesting that each isoform has unique functions. In humans, both ISWI isoforms are more uniformly expressed, and ISWI remodelers have been linked to cancers and stress-induced depressive-like behavior [5,6].

Although the isolated Snf2L or Snf2H ATPases have intrinsic chromatin remodeling activity, they have been purified from cells as complexes that contain at least one additional accessory subunit (for review, see [7]). For instance, human ACF was purified from HeLa cells based on its nucleosome assembly and spacing activity. It contains Snf2H and BAZ1A/ACF1, but a comparable Snf2L complex was not identified. Likewise, vertebrate WICH was purified via the BAZ1B/WSTF subunit and also found to contain only the Snf2H isoform. At least five other ISWI complexes have been purified from mammalian cells, and in nearly every instance, each ISWI isoform co-purified with its own unique set of accessory subunits. This was a satisfying result, as it was similar to what had been found in budding yeast where the two ISWI isoforms (Isw1 and Isw2) interact with different accessory subunits to form three unique complexes.

In this issue of *EMBO Reports*, Oppikofer *et al* [1] revisit the complexity of the ISWI remodeler subfamily by performing unbiased, immuno-affinity purifications of either Snf2L or Snf2H from human cell lines, followed by mass spectrometry. They find that each ISWI isoform co-purifies with all of the known accessory subunits (BAZ1A/Acf1, BAZ1B/Wstf, BAZ2A/Tip5, CECR2, BPTF/NURF301, Rsf-1), and they identify the BAZ2B protein as a seventh accessory factor for both ATPases. The co-purification with all accessory subunits is not due to contamination with the alternate ISWI isoform, as purifications were also carried out with cell lines where one of the two isoforms was deleted by CRISPR/Cas9 editing. Furthermore, interactions with each accessory subunit were observed in several different cell lines, and the abundance of an accessory subunit correlated well with its expression level, rather than a particular ATPase isoform.

In order to confirm that Snf2L and Snf2H do indeed assemble into at least 14 different remodeler complexes, Oppikofer *et al* [1] express and purify 12 of the possible pairwise ISWI ATPase/accessory subunit combinations from a baculoviral expression system, followed by nucleosome remodeling and ATPase assays. Each heterodimeric remodeling enzyme was purified at apparently similar yields and stoichiometries, consistent with a simple model where both Snf2L and Snf2H are fully competent to interact with all accessory subunits. But does assembly of the same accessory subunit with a different ATPase lead to distinct biochemical activities? Here, the CECR2 subunit provides a tantalizing example. Whereas members of the BAZ family of accessory subunits create ISWI remodelers that slide mononucleosomes to a centralized position irrespective of the ISWI isoform, the CECR2/Snf2H complex tends to slide mononucleosomes to more end-localized positions, while the CECR2/Snf2L complex slides mononucleosomes toward the center. In both cases, the final equilibrium positions are more heterogeneous than those catalyzed by BAZ/ISWI complexes. Interestingly,

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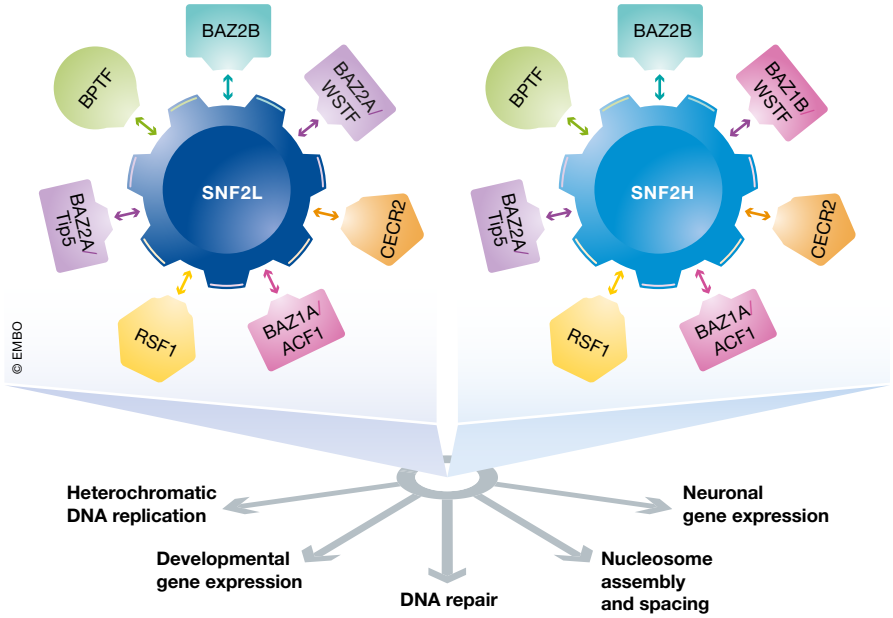

Figure 1. Complexity of the human ISWI subfamily of remodeling enzymes.
Schematic depicts the interactions of two different ISWI ATPase isoforms—Snf2L and Snf2H—with seven different accessory subunits. Each of the resulting remodelers may be involved in a host of nuclear functions.

the CECR2/Snf2L remodeler is also a more robust ATPase as compared to the CECR2/Snf2H complex, suggesting that differential accessory subunit interactions can alter how an enzyme couples nucleosome binding to ATP hydrolysis. These biochemical differences may be key for cell type-specific roles for CECR2, as the CECR2/Snf2L complex is found in neurons, while the CECR2/Snf2H complex is found in testes and embryonic stem cells [8,9].

With at least 14 different remodeler assemblies, the human ISWI subfamily is clearly the most polymorphic of all remodeling enzyme subfamilies (Fig 1). Why the complexity? As noted above for CECR2-containing complexes, different accessory subunit/ATPase isoform combinations may alter the biochemical outcome of the chromatin remodeling reaction. Indeed, previous studies found that the BAZ1A/Acf1 subunit plays a key role in defining the nucleosome spacing activity of ACF (BAZ1A/Snf2h) [2]. In many cases, different accessory subunits also provide protein–protein interaction surfaces that target an ISWI enzyme to different gene sets or to different nuclear processes. For instance, the BAZ2A/Tip5 subunit interacts with the TTF-1 transcription factor, targeting the NoRC complex (BAZ2A/Snf2H) to ribosomal genes [10]. Likewise, the BAZ1B/Wstf subunit interacts with the PCNA sliding clamp to target the WICH complex (BAZ1B/Snf2H) to replication forks [11]. Members of the BAZ accessory subunit family all contain domains that interact with histone modifications (e.g., bromodomains and PHD fingers), and thus ISWI complexes that harbor different BAZ subunits be regulated or targeted by distinct nucleosomal epitopes.

And finally, one must remember that two of the founding members of the ISWI family of remodelers, *Drosophila* NURF and CHRAC, contain more than just one accessory subunit and an ATPase isoform—CHRAC also contains two developmentally regulated histone fold proteins, CHRAC14 and CHRAC17, in addition to BAZ1A and Snf2H; the NURF complex contains two subunits, p38 and p55, in addition to BPTF and Snf2L [7]. Thus, ISWI remodelers are likely to be even more complex, with different accessory subunits providing a platform for assembly of larger ISWI complexes that may have novel activities or regulatory mechanisms. Given that the Snf2L isoform is also known to have multiple splice variants, the combinatorial assembly of ISWI enzymes seems complex indeed.

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