Broken Silence Restored—Remodeling Primes for Deacetylation at Replication Forks

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Faithful propagation of chromatin structures requires assimilation of new histones to the modification profile of individual loci. In this issue of *Molecular Cell*, Rowbotham and colleagues identify a remodeler, SMARCAD1, acting at replication sites to facilitate histone deacetylation and restoration of silencing.

Maintenance and transmission of proper chromatin organization is fundamental for genome stability and function in eukaryotes. The challenge of propagating both genetic and epigenetic information is met in S phase, entailing genome-wide disruption and restoration of chromatin coupled to faithful copying of DNA. How chromatin domains are restored on new DNA and transmitted through mitotic cell division remains a fundamental question in biology, with implications for development, somatic cell reprogramming, and complex diseases like cancer. In this issue of *Molecular Cell*, Rowbotham and colleagues identify a function of the SWI/SNF-like remodeling factor SMARCAD1 in restoring silenced heterochromatin domains in dividing human cells. SMARCAD1 is recruited to newly synthesized DNA and facilitates histone deacetylation, histone H3K9 tri-methylation (H3K9me3), and efficient HP1 recruitment through a mechanism involving ATP hydrolysis (Rowbotham et al., 2011) (Figure 1). This work places nuclesome remodeling as an early, perhaps priming, event in the step-wise action of enzymes modifying new histones to restore functional chromatin domains.

To dissect SMARCAD1 function in human cells, Rowbotham et al. (2011) profile interactors by mass spectrometry and identify a transcriptional repressor complex containing KAP1 along with histone deacetylases HDAC1 and HDAC2 and the histone H3K9 methyltransferase G9a/GLP. Knockdown of SMARCAD1 leads to global loss of H3K9me3 and accumulation of acetylated histones, consistent with a function in silencing heterochromatin "integrity" is challenged in every S phase when the domain is replicated and parental histones carrying repressive marks are "diluted" by acetylated new histones (Figure 1). To meet this challenge, a crowd of repressive activities arrive to restore the configuration of the domain (reviewed in Jasencakova and Groth, 2010). SMARCAD1 localizes to replication sites during both early and late S phase (Rowbotham et al., 2011), supporting a general role in chromatin replication. The same is true for HDAC1, HDAC2, and G9a as also reported by others (reviewed in Jasencakova and Groth, 2010), while KAP1 appears to reside constitutively in heterochromatin. Key questions concern how SMARCAD1 is recruited and how its activity is directed toward specific regions in the genome. Rowbotham et al. (2011) show that SMARCAD1 interacts with PCNA both in vitro and in vivo. PCNA, a processivity clamp part of the replication machinery, has emerged as a central factor linking replication, chromatin assembly, and propagation of epigenetic information (reviewed in Jasencakova and Groth, 2010). However, since PCNA is present at all replication forks, additional levels of regulation must be at play if SMARCAD1 has specificity for distinct chromatin domains. In the case of the maintenance DNA methyltransferase, DNMT1, PCNA-dependent recruitment appears to ensure a high local concentration, while UHRF1 (Np95 in mouse) directs specificity by recognizing hemimethylated CpG (reviewed in Jones and Liang, 2009). Similarly, SMARCAD1 association with chromatin could be regulated via PCNA and yet to be identified PCNA-independent mechanism(s) acting in parallel. Interestingly, H3K9 histone methyltransferase G9a can also be recruited to replication sites via interaction with DNMT1, and another H3K9 methyltransferase, SETDB1, is recruited to replicating heterochromatin as part of a CAF-1-containing complex (reviewed in Jasencakova and Groth, 2010). It will thus be important to resolve the functional differences and potential loci-specific function of these distinct complexes. Given the role of KAP-1 in DNA repair (reviewed in Goodarzi et al., 2010), it would also be interesting to know whether SMARCAD1 participates in the large-scale chromatin reorganization during DNA repair.

How new histones acquire the modification profile of the loci where they are incorporated is a matter of intense studies. While deacetylation of new histones is a relatively fast process correlating with restoration of a "nuclease-resistant" chromatin structure, establishment of methylation marks can be rather slow spanning several hours (reviewed in Jasencakova and Groth, 2010). SMARCAD1 function is central for maintenance of heterochromatin organization as its depletion leads to accumulation of H3 acetylation and loss of HP1, KAP1, and H3K9me3 at centric, pericentric, and telomeric repeats. This triggers severe chromosome segregation defects, resembling the situation in cells experiencing heterochromatin defects upon treatment with HDAC inhibitors (Taddei et al., 2001). Interestingly, *S. pombe* SMARCAD1 homolog Fft3 also protects silent regions from being invaded by euchromatic marks (Strålfors et al., 2011). Genome-wide analysis showed high Fft3 occupancy at boundaries between euchromatin and heterochromatin in pericentromeric and subtelomeric regions (Strålfors et al., 2011). Transfer RNA genes are often found in boundary regions and can function as

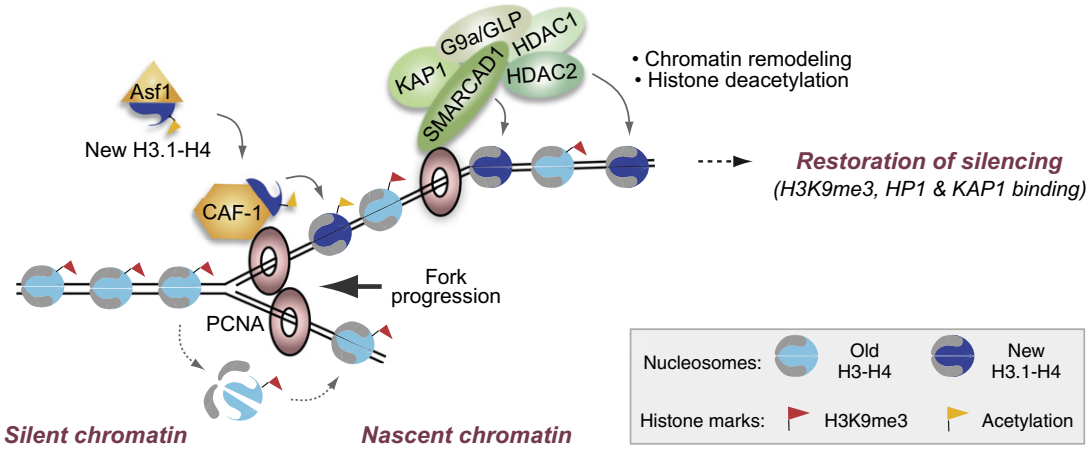

Figure 1. SMARCAD1 Function in Chromatin Replication

New histones, assembled through the Asf1-CAF-1 pathway, confer freshly replicated chromatin with high levels of H3-H4 acetylation. SMARCAD1 is recruited to new DNA via PCNA likely as part of a multiprotein complex containing KAP1, HDAC1/2, and G9a. Lack of SMARCAD1 function impairs heterochromatin restoration; H3-H4 acetylation accumulates, and repressive features like H3K9me3 and HP1 are lost. An attractive model is that acetylation is removed in a replication-coupled fashion facilitated by SMARCAD1 nucleosome remodeling. In turn, this could prime de novo assembled nucleosomes for further modifications guided by marks on parental histones (i.e., H3K9me3) in a replication-independent fashion.

strong replication pause sites. It is thus appealing to picture a link between Fft3 function and replication.

Several chromatin remodeling complexes, including mammalian ISWI complexes and yeast INO80, are recruited to replication sites and serve important functions in chromatin replication and/or stabilization of stalled forks (reviewed in Clapier and Cairns, 2009). These factors could act ahead of the fork to disrupt chromatin or in the restoration process on new DNA, where proper nucleosome spacing is required to establish higher order chromatin organization. Nucleosome sliding, disruption/assembly, histone exchange, and incorporation of histone variants—all of these functions are probably relevant when newly assembled chromatin is maturing. An attractive idea emerging from the study of Rowbotham and colleagues (2011) is that acetylation marks are removed in a replication-coupled fashion facilitated by SMARCAD1 nucleosome remodeling. This could in turn prime histones for further modifications guided by marks on parental histones in a replication-independent fashion. A key cue to SMARCAD1 function comes from the observation that acetylation marks characteristic for new histones accumulate during S phase in SMARCAD1-depleted cells (Rowbotham et al., 2011). All new histone H4 brought to replicating chromatin by the histone chaperone Asf1 is acetylated at lysines 5 and 12, while a fraction of histone H3 carries acetylation at lysines K14 and/or K18 (Jasencakova et al., 2010). The increase in histone acetylation observed in SMARCAD1-depleted cells (Rowbotham et al., 2011) is consistent with perturbed HDAC1/2-mediated deacetylation of newly deposited histones. Exactly how SMARCAD1 promotes histone deacetylation and H3K9me3 is unclear. Rowbotham and colleagues (2011) could not demonstrate in vitro remodeling activity of SMARCAD1, but the ATPase activity is required to rescue aberrant H3 acetylation and loss of H3K9me3 in SMARCAD1-depleted cells. Moreover, the budding yeast homolog Fun30 shows histone H2A-H2B dimer exchange and nucleosome sliding activity in vitro (Awad et al., 2010). Taken together, these data suggest that chromatin remodeling primes newly assembled nucleosomes for deacetylation. An important task ahead is to understand mechanistically how these processes are linked. Does SMARCAD1 serve a role in establishing proper nucleosome spacing? Or could this remodeler function to "clean up" improper assemblies of DNA and histones and hereby serve as a chromatin "caretaker" or "fidelity" guard?

Intriguingly, the level of H4K16ac also increased upon SMARCAD1 depletion (Rowbotham et al., 2011), despite its absence on histones delivered by Asf1 (Jasencakova et al., 2010). This could reflect elevated levels of transcription due to impaired silencing. Given that SMARCAD1 is recruited to replication factories throughout S phase, the prediction is that this remodeler, in addition to heterochromatin restoration, maintains gene silencing in euchromatin. SMARCAD1 knockout mice display developmental defects (Schoor et al., 1999), and the SMARCAD1 gene is found within a region rich in breakpoints and deletions in cancer (Adra et al., 2000). It will thus be important to address whether loss of SMARCAD1 function, by impairing chromatin restoration, promotes aberrant transcription and hereby challenges developmental and antitumorigenic programs.

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A Web of Interactions at the Ends

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The synthesis of telomeric DNA by telomerase entails repeated cycles of reverse transcription on a short RNA template. In this issue of *Molecular Cell*, Robart and Collins (2011) describe a set of interactions between human telomerase RNA, protein domains, and the substrate DNA that drives the intricate reaction cycle.

Telomerase, the enzyme dedicated to the synthesis of telomere repeat units, has attracted considerable attention owing to its critical function in maintaining chromosome ends and extending cellular life span (Artandi and Cooper, 2009). Though telomerase was referred to as telomere terminal transferase upon its initial discovery, it soon became clear that the enzyme uses an integral RNA component (TER) as the template for DNA synthesis, and is thus by definition a reverse transcriptase (RT) (Greider and Blackburn, 1989). Its evolutionary kinship to other RTs, however, was not resolved until more than a decade later, when the catalytic protein component (TERT) was cloned (Lingner et al., 1997). The initial TERT sequences from yeast and a ciliated protozoon, as well as from numerous homologs subsequently identified, reveal a core RT domain that clearly shares common ancestry with other prototypical RTs. The ensuing biochemical analyses and the recent crystal structures a TERT homolog from *Tribolium castaneum* (*Tc*TERT) further reinforce the notion that telomerase utilizes similar chemical mechanisms as other RTs to catalyze the nucleotidyl transfer reaction (Autexier and Lue, 2006; Gillis et al., 2008). A central question for devotees of this "special" RT then shifted to how a core RT domain can be elaborated and joined with other protein and RNA domains to perform its dedicated biochemical function. Considerable "tweaking" of the basic RT reaction is evidently necessary, given that telomerase (1) captures and extrudes single stranded DNA, and (2) repetitively reverse transcribes the same limited template region within a much larger RNA molecule (Figure 1). Through the efforts of many groups working on disparate systems, the outline of the answer to the central question is coming into closer focus; the RNA and protein domains necessary for telomere repeat synthesis are reasonably well defined and, in some cases, their contributions to specific steps of the reaction cycle characterized. The dissection of different systems was productive because of the vagaries of expressing and manipulating telomerase components and the distinct genetic and cell biological tools available for each organism. It also yielded a greater appreciation of the variability and diversity of telomerase structures and properties. For instance, the *Tetrahymena* telomerase has a greater propensity to reverse transcribe the template iteratively, thus producing long DNA products, whereas others generate mainly short products (Cohn and Blackburn, 1995; Greider, 1991). Having data from multiple systems, though, makes the task of integrating the findings and deriving common themes all the more challenging. In this regard, the work by Robart and Collins (2011) in the current issue of *Molecular Cell* helps to resolve a number of uncertainties and yield a more unifying picture of how telomerase works.

The authors set out to define the interactions between human TERT and TER (telomerase RNA) domains and assess the roles of these interactions in promoting telomere repeat synthesis. Their basic strategy was to express tagged human TERT and TER fragments in human cell lines, prepare cell extracts, and then examine the interactions between fragments by affinity purification, as well as determine the activities of the isolated fragments or complexes. Similar issues were addressed in earlier studies, but primarily through in vitro expression and reconstitution of telomerase fragments. An important finding by Robart and Collins (2011) was the detection of a robust interaction between the TRBD and CTE domain of TERT (Figure 1). In a sense, this interaction was not unexpected; the crystal structure of *Tc*TERT revealed a ring-shaped structure involving an extensive interface between precisely these two domains. However, many of the residues at the interface are not well conserved. Uncertainty as to the general