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Chromatin dynamics at the replication fork: there's more to life than histones

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Before each division, eukaryotic cells face the daunting task of completely and accurately replicating a heterogeneous, chromatinized genome and repackaging both resulting daughters. Because replication requires strand separation, interactions between the DNA and its many associated proteins — including histones — must be transiently broken to allow the passage of the replication fork. Here, we will discuss the disruption and re-establishment of chromatin structure during replication, and the consequences of these processes for epigenetic inheritance.

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Introduction: the epigenetic challenge

In our understanding, the term epigenetics is used to describe the inheritance of patterns of gene expression that are not based on changes in DNA sequence [1]. Epigenetic processes need not rely on histones or post translational modifications [2], but they should fulfill three key requirements: first, to regulate gene expression; second, to be maintained through cell division; and third, to template their own duplication; thus, epigenetic factors must be heritable in the absence of ongoing inducing signals [3].

Chromatin is frequently associated with epigenetics; chromatin structure is determined by myriad histone modifications, histone variants, transcription factors, remodeling enzymes, and RNAs [4–8]. The primary repeating unit of chromatin is the nucleosome, which contains ~147 bp of DNA wrapped around an octameric core composed of two copies of each of the core histone proteins: H2A, H2B, H3, and H4 [9]. The core histones interact to form H2A–H2B and H3–H4 dimers; two H3–H4 dimers associate to form the $(H3–H4)_2$ tetramer, and H2A–H2B dimers bind on either side of the tetramer to form the octamer. The large number of histone post-translational modifications found on histones within transcriptionally active or repressed chromatin [10,11], and the associated 'histone code' hypothesis [8], leads intuitively to the attractive possibility that histones may act as global carriers of regulatory information to control gene expression through generations. However, if histone modifications are to serve as epigenetic vectors they must surmount the challenge of DNA replication, in which chromatin is disassembled ahead of the replication fork and reassembled onto two daughter genomes.

Eukaryotic replisomes contain up to five distinct histone chaperones (extensively reviewed [12]), which together minimize the amount of non-nucleosomal DNA at the replication fork and ensure timely redeposition of nucleosomes on nascent DNA. After displacement, H3–H4 dimers and/or $(H3–H4)_2$ tetramers are passed to PCNA-associated CAF-1 [13,14] via FACT [15–17], Asf1 [18,19] or other chaperones [5] for deposition. Electron microscopy has suggested the disruption of 1–2 nucleosomes in front of the replication machinery [20], and we have shown that ongoing lagging-strand synthesis is intrinsically linked to the immediate redeposition of histones in *Saccharomyces cerevisiae* [21••], suggesting that the location of parental histones on the daughter strand to which they have segregated should closely correspond to their position within the parental genome. Consistent with this, a clever epitope switching method that allows parental and nascent histone H3 to be distinguished has shown that the parental histones are generally redeposited within ~400 bp of their prereplication position in *S. cerevisiae* [22•]. The reassembly of nucleosomes close to their parental locations renders theoretically feasible the inheritance of histone post-translational modifications within relatively discrete domains (H2A and H2B are deposited after H3 and H4 [23,24] and will not be discussed further).

Importantly, $(H3–H4)_2$ tetramers generally remain intact during replication fork passage [25•,26,27•] and appear to segregate randomly to the two daughter genomes [28]. Some tetramers, containing variants of H3 whose deposition is independent of S-phase [29], split [27] but the significance of this is presently unclear. It is notable that key histone chaperones [30,31] — including CAF-1 [32] — interact with two H3–H4 dimers most likely in the form of a $(H3–H4)_2$ tetramer [32]. The production of two daughter genomes necessitates that nucleosomes

containing parental $(H3–H4)_2$ cores are intermixed with an equal number containing newly synthesized H3–H4, devoid of parental modifications but carrying characteristic deposition-related marks [33,34]. *Trans*-acting maintenance modification complexes (see below), targeted by marks on parental histones, can subsequently modify newly synthesized histones to harmonize the chromatin modification state across domains in the two daughter genomes.

Histone-mediated inheritance of chromatin domains

Histone modifications are clearly maintained in specific domains through the action of factors that target modification enzymes. Studies have shown that methylation of histone H3 lysine-9 (H3K9me) and lysine-27 (H3K27me) is catalyzed by methyltransferases that reside in complexes containing subunits that also bind methylated histone tails [35–38]. This provides a simple mechanism by which maintenance modification complexes can propagate a modification to nearby histones. The prototypic example of this behavior is provided by H3K9me3: this is bound by HP1, which recruits H3K9-specific methyltransfereases and, by oligomerizing, can spread this modification across a domain [39]. Thus, provided that modifications recognized in *cis* can be propagated in *trans* to nearby nucleosomes, domains of modified chromatin can potentially be maintained via simple mechanisms that do not rely on signaling or sequence-specific DNA binding proteins, and that are robust in the face of twofold dilution during replication [40].

Recently, a series of elegant experiments by the Crabtree laboratory [41••] has lent experimental support to the assertion that H3K9 methylation can be transmitted via truly epigenetic mechanisms. Through the small-molecule-induced recruitment of HP1α, Hathaway and coworkers were able to induce H3K9me3 at the Oct4 locus in mouse embryonic stem cells (ES) and embryonic fibroblasts (MEFs); the ensuing methylation spanned ~10 kb, making it remarkably similar in size to endogenous H3K9me3 domains. Critically, in MEFs, which do not express ES cell pluripotency factors, the induced H3K9me3 domain was heritably transmitted through DNA replication and persisted through multiple cell divisions after removal of the HP1α stimulus. Further *in silico* modeling demonstrated that a simple equilibrium between modification and replication-independent histone turnover or demethylation could give rise to stable, inherently bounded domains where spreading of the mark along the chromosome was opposed by removal of the modification [41,42] (see Figure 1a). According to the model proposed by the authors, steady state levels of H3K9me3 are the result of the opposing influences of histone methylation and turnover; this equilibrium can clearly be altered, which sits well with experiments in *Drosophila* that have shown that pericentromeric heterochromatin domains containing H3K9me3 are sensitive to the dosage of factors that either promote or oppose heterochomatin [43]. Consistent with this, targeting an activator to the ectopically methylated chromatin generated by Crabtree and colleagues was sufficient to disrupt the steady state and favor unmethylated H3K9 [41••]. Earlier studies in human fibroblasts have described a similar scenario for the persistence of H3K27me3. Hansen *et al.* [36] showed that H3K27me3, which is catalyzed by the Polycomb Repressive Complex 2 (PRC2), is also bound by PRC2; and, using a heterologous reporter system, found that transient recruitment of PRC2 led to H3K27me3 and gene repression that persisted through cell divisions. Importantly, methylation of H3K27 appears to be important for the long-term persistence of the repressed state as targeting a catalytically inactive methyltransferase greatly diminished the long-term silencing of the reporter.

Euchromatin and heterochromatin are spatially distinct, and are replicated at different times in S-phase [44,45]. Recent work has indicated that the spatial organization of chromatin within the nucleus directly impacts the time at which it replicates, and that proximity to early-replicating or late-replicating domains [46] is by far the best predictor of when a given region of the genome will be replicated [47••,48••]. The broad division of the genome into early-replicating and late-replicating regions based on spatial proximity presumably provides a simple means to increase the robustness with which domains of common modifications can be inherited, as the binding of euchromatin-specific or heterochromatin-specific maintenance factors to the replisome can be both spatially and temporally controlled. Consistent with this hypothesis, microinjection experiments have shown that the assembly of transcriptionally competent chromatin is dependent upon the timing of the injection, with DNA injected early in S-phase being assembled into acetylated chromatin and expressed at higher levels [49,50]. Temporally separating the replication of active and repressed chromatin can therefore be sufficient to establish distinct chromatin types, even on naked DNA. Spatial segregation of chromatin domains likely adds a layer of redundancy, ensuring that chromatin domains are properly inherited by providing a high local concentration of certain modifying factors; replication in this context will likely ensure that the resulting chromatin on the daughters will be modified by physically associated factors, regardless of the fate of parental histones and their modifications.

Inheritance mediated by nonhistone proteins

Although histones and their modifications are often described as epigenetic, in most cases there is in fact no direct evidence to support the assertion that modifications themselves are the key determinants of epigenetic processes. Indeed, several observations make it