Review

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Epigenetic inheritance: histone bookmarks across generations

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Multiple circuitries ensure that cells respond correctly to the environmental cues within defined cellular programs. There is increasing evidence suggesting that cellular memory for these adaptive processes can be passed on through cell divisions and generations. However, the mechanisms by which this epigenetic information is transferred remain elusive, largely because it requires that such memory survive through gross chromatin remodeling events during DNA replication, mitosis, meiosis, and developmental reprogramming. Elucidating the processes by which epigenetic information survives and is transmitted is a central challenge in biology. In this review, we consider recent advances in understanding mechanisms of epigenetic inheritance with a focus on histone segregation at the replication fork, and how an epigenetic memory may get passed through the paternal lineage.

Beyond the Mendelian rules of inheritance

Through classical genetics and the advent of modern sequencing, we have developed a comprehensive understanding of traditional modes of Mendelian inheritance, yet these advances cannot fully explain how organisms propagate vastly different phenotypes across generations independently of alterations in gene sequence, that is, epigenetically. Conservatively, epigenetic inheritance (see Glossary) requires that the transmitted phenotype be: (i) independent of changes in DNA sequence; (ii) conveyed in the absence of the initial stimulus that caused the phenotype in the parental cell or organism (F_0); and (iii) propagated via a *bona fide* mechanism. Despite a rich and growing literature on epigenetic inheritance in a multitude of species, uncovering phenomena that satisfy all of these criteria has been a challenge, with the mechanism itself often being the most controversial (Box 1 and [1,2]). In this review, we discuss possible mechanisms of epigenetic inheritance with an emphasis on recent insights derived from the chromatin level. First, we consider transmission of epigenetic memories by examining the most fundamental constituent of conveying information in a dividing cell, the nucleosome, with emphasis on the replication fork. Second, we examine the complexities of inheritance across generations in multi-cellular organisms by highlighting exciting new discoveries involving chromatin dynamics that may convey epigenetic inheritance through the paternal lineage. Through these two fronts, we intend to shed light on possible mechanisms guiding the transmission of an epigenetic memory across multiple developmental stages.

Dismantling and restoring chromatin throughout DNA replication

The post-replicative restoration of DNA methylation on the newly synthesized DNA via the maintenance DNA methyltransferase, DNMT1, is perhaps one of the better-understood examples of epigenetic inheritance (recently reviewed elsewhere [3]). By contrast, other epigenetic factors are thought to segregate onto replicated DNA to produce two phenotypically identical daughter cells at the end of mitosis. This is particularly true of histones – integral components of chromatin and the center of the following discussion.

Glossary

Epigenetic inheritance: the inheritance of a phenotype in a manner that is independent of the DNA sequence and that remains self-perpetuating in the absence of the initial stimulus that caused the phenotype in the parental cell or organism.

Histone variant: core canonical and linker histones are encoded by a number of different histone genes, resulting in a number of non-synonymous substitutions and divergent domains. This variation adds complexity to the epigenetic landscape.

Histone chaperone: proteins or protein complexes that specifically bind histones, thwarting non-specific interactions, and that promote their deposition or removal from DNA in an ATP-independent manner.

PcG: polycomb Group Proteins. A group of proteins involved in the regulation and transcriptional silencing of key developmental genes, including the *Homeotic* (or *Hox*) gene loci. Human PcG proteins assemble into Polycomb Repressive Complexes (PRCs), of which PRC2 catalyzes the methylation of H3K27 and PRC1 guides the ubiquitin ligation of H2AK119.

Protamine: low molecular weight proteins that tightly package DNA in late spermatids and mature sperm largely due to their arginine-rich DNA anchoring domains. Their precise function is unknown but might include protecting the paternal genome from DNA damage, facilitating formation of a small elongated sperm head for better motility and/or conveying epigenetic information.

Spermatogenesis: the process of generating mature, haploid sperm (spermatozoa) from a diploid spermatogonium. This process initially requires mitosis to create spermatocytes, their subsequent meiotic divisions to create spermatids and finally maturation of spermatids to spermatozoa. During this chain of events, chromatin undergoes dynamic changes whereby canonical histones are largely replaced by protamines through a number of intermediate steps, including histone variant incorporation, nucleosomal destabilization, histone eviction and replacement with transition proteins prior to protamine deposition.

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Box 1. Transgenerational inheritance; considering caveats and alternative mechanisms

Non-chromatin based mechanisms likely contribute to transgenerational inheritance. For example, some of these phenotypes might arise from cryptic genetic variation given that inbred strains, nearly identical clones or even neighboring cells in the same organism may possess marked genetic differences [108]. Such genetic variation could be passed on to offspring or arise *de novo* (e.g., transposable elements, mutations) and account for differences. Unfortunately, these alternatives are seldom examined in transgenerational studies. Furthermore, establishing transgenerational inheritance in its purest sense is often confounded by maternal care, social transmission, or other variables that may propagate a phenotype without requirement for epigenetic memory *per se*. Indeed recent studies suggest that maternal care may play a significant role even in the transmission of phenotypes originating from the father [109].

Even if a phenotype is transmitted in a transgenerational epigenetic fashion, chromatin events may not always be responsible for their propagation. Transcriptional loops are one example [110]. As in somatic tissue, noncoding RNAs such as siRNA, piRNAs as well as miRNA contribute to inheritance and might function independently of changes at the level of chromatin (recently reviewed by [63]). In fact, a recent study showed that miRNAs are important for transmitting the experience of trauma to progeny through the paternal lineage [65]. Studying the importance of these varied contributions to transgenerational inheritance is important in understanding whether they are truly epigenetic.

Pioneering studies established that pre-existing, parental nucleosomes contribute to approximately half of the histones on nascent DNA, suggesting that parental histones likely contribute to shaping the epigenome of daughter cells [4]. The segregation of histones is cooperative and dispersive resulting in the equal and random distribution of histones, in clusters, onto both daughter DNA strands (reviewed by Annunziato [4]). Deposition is closely coupled to the replication machinery, as nucleosomes re-appear ~200–300 bases behind the replication fork on both leading and lagging strands [5,6]. This relationship with the replication machinery is further apparent with Okazaki fragments in yeast that are nearly nucleosomal in size, with junctions clustering over nucleosomal dyads [7]. The exact molecular mechanism by which nucleosomal histones and their associated post-translational modifications (PTMs) redistribute behind the replication fork is believed to involve epigenetic processes. A number of histone chaperones have been proposed to contribute to the segregation of histones, yet their respective modus operandi is quite distinctive.

Nucleosomal histones predominantly dissociate as two H2A–H2B histone dimers and a central $(H3–H4)_2$ tetramer *in vitro* and *in vivo* at the replication fork [8–10]. Because H2A–H2B dimers are susceptible to internucleosomal exchange throughout interphase, the $(H3–H4)_2$ tetrameric core of the nucleosome at the replication fork is the likely candidate for transmitting epigenetic information. Evidence suggests that parental $(H3–H4)_2$ nucleosomal cores are immediately re-assembled behind the replication fork, followed by deposition of H2A–H2B dimers and linker histone H1 [4]. Pulse-chase analyses of isotope-labeled histones recently confirmed long-established biochemical data that the bulk of H3–H4 is transferred onto replicating DNA as intact $(H3–H4)_2$ tetrameric units [9,10]. This is in stark contrast to newly-synthesized histones, which are brought onto replicating DNA as H3–H4 dimers. The Anti-Silencing Factor 1 (ASF1) histone chaperone extensively binds the histone dimer, hindering the formation of H3–H3′ contacts seen within $(H3–H4)_2$ tetramers [11]. ASF1 associates with new cytoplasmic histones, which translocate into the nucleus as cargo on the importin-4 karyopherin [12,13]. In the nucleus, ASF1 channels the replication-coupled H3.1/H3.2 and replication-independent H3.3 histone variants through different deposition pathways [14] (the deposition of various histone variants is reviewed elsewhere [15]). Dimers consisting of newly synthesized replication-coupled histone H3.1 are transferred from ASF1 to the Chromatin Assembly Factor 1 (CAF-1) chaperone [14,16] to counteract the dilution of segregating parental histones. CAF-1 associates with the PCNA scaffold ring and is responsible for the *de novo* assembly of $(H3–H4)_2$ tetrasome intermediates (nucleosomes lacking histones H2A–H2B) on replicated DNA (Figure 1) [17]. Recent thermodynamic analyses established increasing binding affinities towards histones from ASF1, to CAF-1, and DNA, nicely illustrating the chain of successive handoffs [18,19]. The same studies further imply the likely formation of tetramers on CAF-1, immediately prior to deposition. CAF-1 handles newly synthesized histone molecules that are largely unmodified save for H4 acetylation [20], and doubts remain as to whether CAF-1 deposits parental nucleosomal histones under normal conditions. Hence, once tetrameric cores are formed, they likely remain as such through subsequent rounds of replication and may no longer be channeled through CAF-1.

Histone chaperones and the replicative helicase

In addition to interacting with CAF-1, ASF1 has also been co-purified with other components of the replication machinery, such as the replicative clamp loader RFC [21], and the MCM subunits of the replicative helicase [22]. The latter led to the compelling suggestion that ASF1 disassembles and splits nucleosomal $(H3–H4)_2$ tetramers to transfer epigenetic information in the form of two equivalent H3–H4 dimers onto both nascent DNA strands. This semi-conservative model is now however, countered in favor of a conservative segregation of histones, because the $(H3–H4)_2$ tetramers (notably H3.1-containing nucleosomes) largely remain intact through cell division [9,10]. Moreover, it is uncertain whether ASF1 merely associates with inactive MCM subunits or an actual processive helicase (see below). Further mass spectrometry studies revealed that nucleosomes do not necessarily harbor symmetric epigenetic information on their two sister H3–H4 dimers [23]. While the central $(H3–H4)_2$ tetramer is unlikely to be severed over the bulk of replicating chromatin (readers are directed to further views on H3–H4 segregation models [2,9,24]), the semi-conservative, partition-based model may still operate on a specific subset of nucleosomes given that a fifth of post-replicative H3.3 nucleosomal pools contain mixed parental and newly-synthesized species after two rounds of replication [10]. These nucleosomes cluster on active, tissue-specific enhancers [25], implying a unique and restricted route to partitioning at these important regulatory sites.

If ASF1 does not dissociate the bulk of nucleosomal histones, and CAF-1 handles newly-synthesized histones,