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REVIEW

Split decision: why it matters?

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Abstract **The establishment and faithful maintenance of epigenetic information in the context of chromatin are crucial for a great number of biologic phenomena, including position effect variegation, Polycomb silencing, X-chromosome inactivation and genomic imprinting. However, mechanisms by which that the correct histone modification patterns propagate into daughter cells during mitotic divisions remain to be elucidated. The partitioning pattern of parental histone H3-H4 tetramers is a critical question toward our understanding of the epigenetic inheritance. In this review, we discuss why the histone H3-H4 tetramer split decision matters.**

Keywords histone, nucleosome, epigenetic inheritance

Introduction

"Epigenetics" refers to the study of inherited changes in gene expression caused by mechanisms other than alterations to the DNA sequence (Riggs et al., 1996). DNA methylation, which plays critical roles in the X-chromosome inactivation and genomic imprinting, is probably the most studied epigenetic mark (Li et al., 1992; Jaenisch, 1997; Jones and Takai, 2001). In the last two decades, ample evidences indicate that histone modifications play important roles in specifying chromatin domains and influencing gene expression (Grunstein, 1997; Martin and Zhang, 2005; Allis et al., 2006). It is generally believed that chromatin modifications, including DNA methylation and certain histone modifications, provide key epigenetic information (Margueron et al., 2005; Allis et al., 2006; Kouzarides, 2007; Probst et al., 2009). Once epigenetic status is established, by definition, it should be able to propagate through cell divisions to ensure the fidelity of committed cell fate. However, mechanism elucidating that how epigenetic information, especially the patterns of histone modifications, is inherited across cell generations remains to be unveiled.

In this review, we focus on the histone H3-H4 tetramer partition models and discuss what kind of insights toward understanding epigenetic inheritance could be provided by clarifying these models.

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Partition of the histone H3-H4 tetramers: why it matters?

Prior to the studies related to epigenetic inheritance mediated by histone modifications, scientists already elucidated the molecular mechanism that propagates DNA methylation. Newly synthesized DNA strand acquires its precise DNA methylation pattern by copying the pre-existing DNA methylation signature from the template strand with the maintenance DNA methyltransferase DNMT1, which is associated with the replication machinery (Chuang et al., 1997; Hermann et al., 2004; Sharif et al., 2007). This mechanism resembles semi-conservative DNA replication. However, the mechanism by which histone modifications are duplicated and propagated through mitotic cell divisions remains enigmatic. To gain insights into this process, it is essential to know how histone octamers themselves duplicate and assemble into chromatin during DNA replication.

Each nucleosome, the basic unit of chromatin, is composed of one octamer containing two copies of each of the core histones H2A, H2B, H3 and H4, wrapped by 147 base pairs of DNA (Luger et al., 1997). Within a nucleosome, the H3-H4 tetramer is considered as the core particle, with the addition of two H2A-H2B dimers (Luger et al., 1997). Interestingly, the most stable histone modifications (Zee et al., 2010), histone lysine methylations occur on either H3 (Lys 4/9/27/36/79) or

H4 (Lys 20) histones (Allis et al., 2006), suggesting that the H3-H4 tetramers are the main carriers of histone modification-based epigenetic information.

Nucleosomes must be disassembled and reassembled during DNA replication to allow the passage of the replication fork. Generally, the assembly of replicating chromatin can be divided into two fundamental steps, the partition or redistribution of the parental histones onto DNA during the passage of the replication fork and the deposition of newly synthesized histones onto DNA behind the replication fork. Understanding the partition pattern of the main epigenetic information carrier, H3-H4 tetramers, during DNA replication-dependent chromatin assembly is the first step toward understanding the mechanisms of epigenetic inheritance mediated by histone modifications. Because only the H3-H4 tetramer "split model" that one existing H3-H4 dimer pairs with one newly synthesized H3-H4 dimer to form the so-called "hybrid tetramer" would allow precise modification copying event within each nucleosome, like DNMT1 mediated DNA methylation copying on the newly synthesized DNA (Chuang et al., 1997; Hermann et al., 2004; Sharif et al., 2007). In contrast, the H3-H4 tetramer "non-split model" suggests that histone modifications cannot be faithfully maintained at the mono-nucleosome level; therefore, epigenetic inheritance requires much more sophisticated mechanisms to be achieved.

Taken together, the H3-H4 tetramer split decision is a key question toward understanding the mechanisms of epigenetic inheritance.

History about studies on nucleosome partition during DNA replication-dependent chromatin assembly

Nucleosome partition during DNA replication-dependent chromatin assembly, by no means, is a new question. In fact, shortly after the historical observation of chromatin fibers as "particles on a string" (Olins A L and Olins E D, 1974) and the milestone nucleosome theory (Kornberg, 1974; Kornberg and Thomas, 1974), an insightful model involving "two symmetrically paired half-nucleosomes" was proposed to explain "potential direct inheritance of information in the form of histone structure" (Weintraub et al., 1976). This was most likely the earliest attempt to dissect the mechanisms of mitotic inheritance of the epigenetic information carried by histones. Since then, many investigators were engaged in the studies of histone inheritance during chromatin replication.

Newly synthesized chromatin and parental chromatin exhibit comparable micrococcal nuclease digestion kinetics, indicating that newly synthesized chromatin contains full nucleosomes at regularly spaced intervals (Seale, 1976). Sedimentation studies on heavy isotope-labeled histones indicated that histone octamers segregate in a conserved manner (Leffak et al., 1977). Together with many other studies that we cannot cite individually, these studies supported a conservative nucleosome segregation model.

The above mentioned model was amended several years later, due to overwhelming evidences indicating the broad existence of "hybrid nucleosomes" containing old H3/H4 tetramers and new H2A/H2B dimers, or vice versa (Jackson and Chalkley, 1981; Russev and Hancock, 1981; Annunziato et al., 1982).

However, H3/H4 tetramers, the core particles of nucleosomes, appeared to be intact without inter-nucleosome exchanges during replication-coupled nucleosome assembly (Prior et al., 1980; Jackson 1988; Jackson 1990; Yamasu and Senshu 1990). Prior et al. (1980) first demonstrated that exogenous H3-H4 tetramers delivered into microplasmodia were assembled into nucleosomes and remained intact *in vivo* after several rounds of DNA replication. In their experiments, physarum H3 histones were fluorescently labeled with a cysteine-specific reagent iodoacetoxypyrene. H3-H4 tetramers with both H3 histones labeled emit green fluorescence, whereas H3-H4 tetramers containing only one labeled H3 emit blue fluorescence. The green fluorescence emitting H3-H4 tetramers were delivered into microplasmodia. Interestingly, the green fluorescence persisted for several rounds of DNA replication, whereas the blue fluorescence which indicates "hybrid tetramers" never appeared. Their observations suggest that H3-H4 tetramers do not split during DNA replication-dependent chromatin assembly. Several years later, Jackson improved the initial stable-isotope labeling and sedimentation studies by offering resolution for each individual core histones, and reexamined the segregation of H3-H4 tetramers *in vivo*. In his pulse-chase experiments, old H3-H4 tetramers dissociated from the old H2A/H2B dimers and reformed "hybrid octamers" with new H2A/H2B dimers, but old H3-H4 tetramers did not split and form "hybrid tetramers" with newly synthesized H3-H4 histones even after a long chase period (Jackson, 1988, 1990). Yamasu and Senshu (1990) independently obtained the same conclusions with similar strategy.

Besides the above mentioned *in vivo* studies supporting the non-split model, H3-H4 dimers were only observed in extreme non-physiologic conditions *in vitro* (Baxevanis et al., 1991), suggesting that the formation of H3-H4 tetramers is far more thermodynamically favored (Isenberg, 1979; Baxevanis et al., 1991) and that H3-H4 tetramers instead of H3-H4 dimers are probably the basic units for chromatin assembly. These were also important supporting evidences for the H3-H4 tetramer "non-split model."

Finally, FRAP experiments performed in living cells demonstrated that the exchange of H3/H4 tetramers is minimal compared to the rapid exchange of H2A/H2B dimers (Kimura and Cook, 2001), which was also in line with the H3-H4 "non-split model."

Taken together, historical studies related to the stability of H3-H4 tetramers provided overwhelming evidence for the H3-H4 tetramer non-splitting model. Considering that all six

major lysine methylation sites are present on either H3 or H4 (Allis et al., 2006), this H3-H4 tetramer non-split dogma suggests that newly deposited nucleosomes cannot obtain precise histone modification patterns by "copying" modifications within each mono-nucleosome.

Discoveries that re-evoked the tetramer "split model"

However, expectations for the H3/H4 tetramer splitting model have revived recently, after the unexpected discoveries demonstrating that H3-H4 histones are deposited as dimers, rather than tetramers (Tagami et al., 2004; English et al., 2005; Benson et al., 2006). In these studies, histone H3-H4 pre-deposition complexes were affinity purified using epitope tagged H3 or H4 histone. The expression levels of these epitope tagged exogenous H3 or H4 histones were much lower than the endogenous histones. However, endogenous histones were only co-purified with the exogenous histones in mono-nucleosome fractions, but not in the non-chromatic fractions; indicating that H3 and H4 histones exist as dimers, not tetramers in the pre-deposition chaperone complexes (Tagami et al., 2004; English et al., 2005; Benson et al., 2006). These studies were further supported by crystal structure evidences that histone chaperone Asf1 forms heterotrimeric complex with H3 and H4 (English et al., 2006; Natsume et al., 2007). Most interestingly, histone chaperone Asf1 is capable of disrupting preassembled H3-H4 tetramers to form H3-H4-Asf1 heterotrimers (Natsume et al., 2007). Although no direct evidence was provided, these studies certainly raised the possibility of H3-H4 tetramer splitting events.

Because of these unexpected findings, the previously believed unbreakable nature of H3-H4 tetramers fell apart. Therefore, the tetramer "non-split" dogma predominantly relies on the stable-isotope labeling and sedimentation studies performed in the late 1980s (Jackson, 1988; Jackson, 1990; Yamasu and Senshu, 1990). However, the significance of different histone H3 variants became more and more appreciated after these studies. Mammalian cells contain four distinct histone H3 variants, termed H3.1, H3.2, H3.3 and the centromere specific histone H3 variant, CENP-A (Henikoff et al., 2004). H3.1 and H3.2 histones are so-called canonical H3 histones, which are mainly synthesized and deposited during DNA replication-dependent chromatin assembly at S phase (Franklin and Zweidler, 1977). In contrast, H3.3 that differs at only four amino acids from the canonical H3 histones is expressed and deposited into chromatin throughout the cell cycle (Wu et al., 1982; Ahmad and Henikoff, 2002). The sedimentation studies cannot distinguish the different histone variants; in addition, sedimentation cannot offer absolute resolution between the light and heavy histones. Hence, debates and discussions about the H3-H4 tetramer splitting model arose in many subsequent reviews (Nakatani et al., 2004; Henikoff et al., 2004; Annunziato, 2005; Hake and Allis, 2006; Groth et al., 2007; Martin and Zhang 2007; Henikoff, 2008; Probst et al., 2009). Indeed, the latest textbook dedicated to epigenetics anxiously calls for new evidences to validate (or disprove) the H3-H4 tetramer non-split model (Allis et al., 2006).

Split or not: an interesting choice?

To address the above mentioned debates, a new study with variant-specific, high-resolution approach was performed (Xu et al., 2010). In this report, the combination of inducible expression of the Flag tagged H3 and affinity purification allowed selective purification of mono-nucleosomes containing one old H3 or one new H3; SILAC (stable isotope labeling by amino acid in cell culture, Ong et al., 2002)-based quantitative mass spectrometry offered full resolution and accurate quantification between the old and new histones. In addition, H3.1-H4 and H3.3-H4 tetramers were studied individually due to the non-mixing nature of H3.1 and H3.3 histones in mono-nucleosomes (Tagami et al., 2004).

In this recent report, the vast majority of histone H3.1 containing tetramers clearly demonstrated their non-split characteristics (Xu et al., 2010). However, a portion of H3.3 containing tetramers did split and about 20% of old H3.3 histones formed "hybrid tetramers" with new H3.3 histones after approximately two rounds of DNA replication (Xu et al., 2010). This is likely the first direct observation that detected such "hybrid tetramers" *in vivo*. Interestingly, treatment with DNA replication inhibitors such as aphidicolin and hydroxyurea greatly reduced the H3.3-H4 tetramer splitting events, suggesting the split decision is replication-dependent (Xu et al., 2010).

Perspective

Epigenetic information copied from neighboring nucleosomes?

The fact that the vast majority of H3-H4 tetramers follow the "non-split" dogma is a clear indication that histone modification mediated epigenetic information cannot be precisely copied at mono-nucleosome level. Instead, epigenetic inheritance may be achieved by reproducing the modification pattern at individual gene locus to ensure the faithful inheritance of the transcription status. Modification copying from neighboring nucleosomes containing pre-existing modifications might be one of the mechanisms involved (Allis et al., 2006). Indeed, such modification copying or spreading events have been documented. Clr4 (Suvar39)-Swi6(HP1) mediated H3K9 methylation spreading (Nakayama et al., 2001) and Eed mediated PRC2 allosteric activation by H3K27me3 peptides (Margueron et al., 2009) are both good examples that support this model.

However, modification copying likely is not the sole mechanism for epigenetic inheritance. Alternative mechanisms such as chromatin modification reinforcement may also be involved. Notably, different chromatin modifiers participate in the same regulatory pathway, such as gene repression, are often organized into functional complexes. These combinations include, but are not limited to, the Lsd1-HDAC complex (Shi et al., 2004), the UTX-MLL2/3 complex (Lee et al., 2007), etc.

Is H3.3-H4 splitting event a regulated process?

The surprising observation of partial splitting of the H3.3-H4 tetramers (Xu et al., 2010) led to an important question: whether this is a regulated process for a particular function? If this is a regulated process that is of functional significance, we would expect: 1) A dedicated chromatin remodeler and/or chaperone might mediate this process; 2) Such splitting events might be enriched at specific regions of the genome. Addressing these questions in future studies might provide functional insight for this unexpected discovery (Ray-Gallet and Almouzni, 2010; Xu et al., 2010).

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