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CHAPTER 1

HISTONE EXCHANGE: SCULPTING THE EPIGENOME

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SUMMARY

Chromatin not only serves as a packaging material, but also functions as a platform for integrating signals that act upon the genome. Indeed, chromatin is a dynamic macromolecular structure that can be dramatically altered in many ways to facilitate the different transactions at the genome. Examples of such alterations are relocalization of genomic loci within the nucleus upon transcriptional activation or induction of DNA damage, adding or removing post-translational modifications on histones or other chromatin-binding factors, or altering the basic organization of chromatin by moving or removing nucleosomes, i.e. modifying the occupancy by histone octamers. New insights into the scope and mechanisms of chromatin dynamics have recently been obtained by the development of novel techniques to visualize chromatin protein mobility and stability. Here we discuss the developments in this area, with special emphasis on histone exchange, which we define as the replacement of histone proteins without a prerequisite change in occupancy. Although histone exchange may not affect chromatin organization per se, recent studies suggest that it can influence key epigenetic processes such as histone inheritance, the distribution of histone post-translational modifications, and the output of transcription factors. Importantly, errors in histone exchange in humans can contribute to malignant transformation.
INTRODUCTION: CHROMATIN PROTEIN DYNAMICS

The basic building block of chromatin is the nucleosome, which consists of DNA wrapped around an octamer of histone proteins. The histone octamer consists of one tetramer of histones H3-H4 and two dimers of H2A-H2B. The nucleosome is a robust structure. Indeed, at a bulk level histone proteins are stable proteins with long half-lives and relatively little mobility in the nucleus of the cell. This, however, does not mean that histone proteins are static entities. In fact, recent studies suggest that chromatin is a highly dynamic platform that coordinates many different processes in which the genome is involved, such as transcriptional regulation, RNA processing, DNA repair and replication. These processes can involve extensive and complex post-translational modification of histones, subnuclear genome organization, recruitment of regulatory factors, or alterations in nucleosome occupancy. In addition to these processes that can be readily visualized, more subtle chromatin rearrangements can occur by replacing resident histones for newly synthesized ones. This process of histone exchange or turnover has until recently remained unnoticed but may nevertheless have an important impact on the epigenome and its function. Here we discuss technologies that have been developed to measure histone dynamics and what they have taught us on the mechanisms and functions of this novel layer of epigenetic regulation.

METHODS TO MEASURE HISTONE PROTEIN DYNAMICS

There are various ways to capture the dynamics of proteins in the cell. Here we focus on methods that have been applied to histones and chromatin-bound proteins (reviewed in (Deal and Henikoff, 2010a)). A short overview of the different techniques and their applications will be given here. Some methods are aimed at visualizing spatial movement of chromatin proteins; others measure protein stability, turnover, or exchange. What these methods have in common is that they provide a means to distinguish between resident and newly assembled or newly synthesized proteins.

FRAP

Movement of chromatin proteins within the nucleus can be visualized by combining the use of fluorescent fusion proteins with live cell imaging. By Fluorescence Recovery After Photobleaching (FRAP) a discrete region of the nucleus is subjected to laser photo bleaching. The time it takes to regain fluorescence in that region is a measure for the replacement of resident proteins by proteins from non-bleached regions or newly synthesized proteins, thereby informing on the residence time of the labeled protein in chromatin. FRAP has been used to study a wide range of proteins including histones, transcription factors, and chromatin remodeling enzymes. These studies have shown that the residence time of regulatory proteins is in the order of seconds and of linker histone H1 in the order of minutes. The
residence time of bulk canonical histones is in the order of hours, with H2A and H2B showing more exchange than H3 and H4 (Catez et al., 2006; Kimura and Cook, 2001; Phair et al., 2004). Imaging approaches to measure protein dynamics or interactions using conditional time-controlled fluorescent proteins have recently been reviewed elsewhere (Toyama and Hetzer, 2013; Wu et al., 2011). Although very powerful and applicable to single cells, imaging based methods do not provide information about the dynamics of chromatin proteins at specific genomic regions.

**Conditional Fluorescent Labeling: SNAP-tag, FlAsh-ReAsh, and TimeSTAMP**

The SNAP-tag is a modified variant of O6-alkylguanine-DNA alkyltransferase. The normal function of this alkyltransferase is in DNA repair (Jansen et al., 2007). The SNAP-tag polypeptide can bind covalently to its substrate O6-benzylguanine. Derivatives of O6-benzylguanine are cell-permeable and can be fluorescent or non-fluorescent, allowing both labeling and quenching approaches. By alternating the substrates, pulse-chase assays can be performed to follow newly synthesized proteins (Figure 1A). Using this method, distinct mechanisms and timing of deposition have been described for canonical histone H3 and the variant histone H3.3; in HeLa cells histone variant H3.1 is incorporated during S phase while histone variant H3.3 is incorporated throughout the cell cycle (Ray-Gallet et al., 2011). The HaloTag, a protein fusion tag that covalently binds to a series of specific ligands, may offer similar approaches (Kovalenko et al., 2011). The FlAsh and ReAsh technique employs a small tetracysteine tag that can bind to biarsenical fluorescent tags (Adams and Tsien, 2008). These biarsenical tags can permeate the cell membrane and are not fluorescent until they are bound to the tetracysteine tag. Although this method may not be as sensitive as conventional GFP (Green Fluorescent Protein)-fusion protein labeling, an advantage of this method is that there is no need to wash away unbound dye. Moreover, the tag is relatively small when compared to GFP, avoiding problems caused by bulky tags. The TimeSTAMP method combines epitope tagging with protease cleavage (Lin, 2010). The protein of interest is tagged with a small epitope tag that contains a specific protease cleavage site. The specific protease cleaves off the epitope tag by default. Upon inhibition of the protease the tag remains stable, allowing detection of the newly synthesized protein, for example by immunoblotting or immunocytochemistry.

**Detection of protein dynamics by Mass Spectrometry**

Mass Spectrometry (MS) has been used extensively to identify post-translational modifications (PTMs) on histones. Using Stable Isotope Labeling by Amino acids in Cell culture (SILAC) in combination with MS, global dynamic changes in modifications can be monitored due to the mass differences of old and new proteins. With this method, and consistent with previous studies, histone acetylation and deacetylation have been found to be highly dynamic processes. Histone methylation generally is
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a more stable PTM. For example, in SILAC studies on HeLa cells, mono-methylation occurred on most lysines in H3 (K4, K9, K27, and K36) shortly after incorporation into the chromatin, whereas di- and tri-methylation accumulated more slowly during the cell cycle. Methylation of H3K79 even increases across multiple cell generation (Sweet et al., 2010). MS-based technologies are very powerful. They can not only be used to measure levels and dynamics of modifications occurring on proteins but also of the proteins themselves. SILAC-MS analysis of histone proteins in HeLa cells has shown long half-lives of canonical histones and somewhat shorter half-lives for histone H1, which is generally in agreement with the FRAP studies described above (Zee et al., 2010).

Pulse-chase labeling combined with affinity purification: CATCH-IT and QuaNCAT

Recently, two new methods were developed that do not need the use of inducible gene copies or fusion proteins. CATCH-IT (Covalent Attachment of Tags to Capture Histones and Identify Turnover) has been used to capture histone exchange genome-wide. In this method, cells are treated with a methionine analog L-azidohomoalanine (AHA), which is incorporated into all newly synthesized proteins in the cell and which can subsequently be linked to a biotin tag. Newly synthesized proteins can be purified using streptavidin beads and histones and the DNA bound to these new histones can be extracted under stringent washing conditions (Deal and Henikoff, 2010b) (Figure 1B). With CATCH-IT all proteins in the cell will be labeled, which makes this method at the moment especially suited for studying the dynamics of (H3-H4) tetramers, since these remain associated with the DNA under conditions that lead to dissociation of most other proteins (Deal and Henikoff, 2010a). A recent variation to this method is QuaNCAT. It combines site selective labeling with AHA (BONCAT; bio-orthoganol non-canonical amino acid tagging) to enrich for newly synthesized proteins with quantitative SILAC-based mass spectrometry to provide a way for quantitative comparison of bulk protein dynamics between two conditions (Howden et al., 2013).

Internalization of ectopic exogenous histones

The cells of the slime mold Physarum polycephalum grow in a naturally synchronous manner and are capable of internalizing exogenous proteins. Taking advantage of these unique properties, incorporation of tagged histones has been determined. In this system, exogenous H2A-H2B dimers localize to the nucleus in the G2 phase of the cell cycle, regardless of transcriptional activity, however, assembly into the chromatin is dependent of transcription by RNA Polymerase II, whereby H2A-H2B exchange is higher in coding regions than in promoter regions and higher in active genes than in inactive genes. H3-H4 tetramers show very little replication-independent incorporation. The fate of resident (untagged) histones cannot be determined by this assay (Ejlassi-Lassallette et al., 2011; Thiriet and Hayes, 2005, 2006).
Figure 1. Methods to measure histone dynamics. A) SNAPtag can be used to follow newly synthesized proteins in the cell. The SNAPtag binds covalently to labels. The labels can be either fluorescent or non-fluorescent, to allow pulse-chase and quench-pulse-chase assays, by alternating the types of labels. TMR (tetramethylrhodamine) conjugated to SNAP substrate is used as fluorescent label to detect exchange; BG-block (O₆-benzylguanine) is used to block binding of TMR. B) CATCH-IT (Covalent Attachment of Tags to Capture Histones and Identify Turnover) can be used to measure dynamics of native histones across the genome. Newly synthesized proteins (depicted with a lighter color and dashed outline) are labeled with a methionine analog. After isolating nuclei, the label can be biotinylated and purified via streptavidin. Stringent washing strips off all proteins except H3-H4 from the DNA, which can subsequently be analyzed by microarray or deep sequencing. Green and yellow triangles depict H3-H4; blue and purple arches depict H2A-H2B. C) In the inducible ectopic expression approach the protein of interest is expressed from the endogenous, constitutive gene and from a second ectopic gene that is under control of an inducible promoter. D) RITE (Recombination-Induced Tag Exchange) can be
Inducible expression of epitope-tagged histones

In budding yeast, several methods have been developed by which newly synthesized and old chromatin proteins can be distinguished by genetically encoded differentially labeled chromatin proteins. A frequently used approach involves the conditional expression of an ectopically expressed version of the protein of interest in the background of the endogenous untagged or differently tagged protein (Cheng and Gartenberg, 2000; Jamai et al., 2007; Linger and Tyler, 2006; Rufiange et al., 2007; Schermer et al., 2005). This method, which typically involves an inducible promoter such as the galactose-inducible GAL1 promoter, has been particularly successful in combination with Chromatin Immuno Precipitation (ChIP) to map the incorporation of new chromatin proteins across the genome (Figure 1C). A drawback of the use of ectopically expressed histones is that the endogenously expressed copy represents a mixture of old and new proteins because of its ongoing synthesis. As a consequence, the endogenously expressed and induced histones will eventually reach a steady state. However, promoter shut-down strategies applied to the endogenous copy can overcome this limitation (Katan-Khaykovich and Struhl, 2011). Another point of consideration is that the introduction of an extra copy can lead to overexpression, which in turn can lead to altered dynamics (Au et al., 2008).

Recombination-Induced Tag Exchange

Another genetically encoded method that has been developed in budding yeast is RITE (Recombination-Induced Tag Exchange) (Verzijlbergen et al., 2010). RITE is a genetic pulse-chase assay that makes use of the site-specific Cre-recombinase to switch from an old tag to a new tag. DNA cassettes containing an epitope tag between two LoxP recombination sites and an orphan epitope tag downstream of the second LoxP recombination site can be targeted downstream of the gene of interest (Figure 1D). The LoxP sites can be induced to recombine by transient activation of Cre recombinase by adding the human hormone β-estradiol to the media. The recombination between the LoxP sites will result in a swap from the ‘old’ tag to the orphan (‘new’) tag in the coding sequence, leading to an epitope tag switch at the protein level (Verzijlbergen et al., 2010). RITE has several strengths. The gene of interest is expressed from the endogenous promoter, the switch is permanent and does not involve complex promoter-induction/shut-down strategies, and old and new proteins can be monitored simultaneously. RITE has been used to map the exchange and inheritance of histone proteins in the yeast genome. Since

▶ used to measure protein dynamics by distinguishing between old and new protein expressed from the same gene. The first epitope tag is flanked by LoxP recombination sites and has a stop codon; the second epitope tag is located downstream of the second LoxP site. Before inducing Cre recombinase, the protein will be tagged with the first epitope tag; after induction the first tag is removed and the newly synthesized proteins will have the second tag.
the recombination process can take several hours, RITE may be less suitable for proteins that have a very high turnover. However, RITE is a flexible method that can be applied to any protein of interest and combined with various downstream approaches. For example, RITE using fluorescent tag-switching has recently been employed to visualize the inheritance of organelles and macromolecular complexes in replicating yeast cells (Hotz et al., 2012; Menendez-Benito et al., 2012).

HISTONE ASSEMBLY, DEPOSITION, AND EXCHANGE

Since the discovery of the nucleosome, a lot of research has been done to unravel its composition and complexity, revealing that alongside the four canonical histones and their PTMs, histone variants exist that have specific functions and PTMs. Whereas canonical histones are deposited into the chromatin mainly during DNA replication, variants can replace the canonical forms by replication-independent mechanisms. In this section, we will give an overview of the histone deposition pathways, histone exchange, and canonical histone replacement by histone variants.

DELIVERING HISTONES TO CHROMATIN

Following the production of histones in the cytoplasm, they undergo a multi-step maturation process before they are translocated to the nucleus and incorporated into the chromatin. Several protein complexes work together to deliver the histones to the chromatin and to facilitate nucleosome assembly, whereby H3-H4 and H2A-H2B are controlled by distinct pathways (Figure 2).

Immediately after translation, histone H3 in mammalian cells is bound by HSC70, a heat shock chaperone that assists in the proper folding of a number of proteins. It has been suggested that this protein is the first to bind to H3 after translation because H3 bound to HSC70 is monomethylated at lysine 9 (K9me1), a mark that is found exclusively on newly synthesized H3 in human cells (Campos et al., 2010; Loyola et al., 2006). Also, there is a lack of chromatin-related modifications on H3 bound to HSC70 and H4 is not found in this complex (Campos et al., 2010). Newly synthesized histone H4 is found in another complex containing HSP70 and HSP90. In these early complexes, histone H3 and H4 are poly(ADP-ribosylated) (Alvarez et al., 2011). It has been hypothesized that the poly(ADP-ribosylation) mark is removed from H3 and H4 when these proteins are brought together in a complex containing HSP90 and tNASP. In this complex H3K9me1 is still high and H4 in this complex is not yet acetylated (Campos et al., 2010). The H3-H4 dimer is subsequently handed down to another complex containing sNASP and the HAT1 histone acetyltransferase complex. In this complex histone H4 is acetylated on K5 and K12 (Campos et al., 2010). Next, the acetylated H3-H4 dimer is transferred to the nuclear import complex. It has been suggested that there are two different import complexes in human cells. Each complex associates with H3-H4 dimers, but with a different pattern of histone modifications,
and a different destination in the chromatin. One import complex contains importin-4 and ASF1a. This complex binds to dimers that have H3K14 acetylation as well as H3K9me1 and H4K5K12ac (Alvarez et al., 2011). The second import complex contains importin-4 and ASF1b. This complex binds H3-H4 dimers containing H3K9me1 and H4K5K12ac (Alvarez et al., 2011). It has been suggested that these different sets of H3-H4 dimers determine the modification pattern the dimers will eventually have in the chromatin. H3K9me1 is necessary to establish H3K9me3 in heterochromatin, whereas H3K14ac is found in active chromatin (Alvarez et al., 2011). The negative crosstalk observed between H3K14ac and H3K9me1 supports the hypothesis that these two sets have different destinations (Alvarez et al., 2011). In budding yeast H3-H4 dimers are bound by the histone chaperone Hif1, which binds to the HAT1 complex (and forms the NuB4 complex) that acetylates H4. The H3-H4 dimer is then handed down to the histone chaperone Asf1. Histone acetyltransferase Rtt109 can bind to Asf1 and acetylates H3K56, which is a hallmark of newly synthesized histone H3 (Verreault et al., 1996; Winkler et al., 2012). H3-H4 dimers are imported into the nucleus by Kap123, a member of the karyopherin/importin family (Avvakumov et al., 2011; Mosammaparast et al., 2002b).

In the nucleus, Asf1 hands H3-H4 dimers to factors that assemble them into tetrasomes or nucleosomes (Figure 3). These factors are the CAF-1 chromatin assembly complex and Rtt106 (Clemente-Ruiz et al., 2011; Fazly et al., 2012; Li et al., 2008). The transit of H3-H4 dimers is promoted by H3K56 acetylation, since this PTM increases the binding affinity of CAF-1 and Rtt106 with H3-H4. CAF-1 is implicated in replication-coupled H3-H4 deposition since it binds to the replication-coupled H3.1 variant, but not the replication-independent H3.3 variant in metazoans (Tagami et al., 2004). Also, CAF-1 interacts with PCNA (Proliferating Cell Nuclear Antigen) (Green et al., 2005; Shibahara and Stillman, 1999; Su et al., 2012; Winkler et al., 2012). The transfer of H3-H4 dimers from Asf1 to CAF-1 involves structural changes that alter the binding affinity of the H3-H4 dimers: binding of Asf1-H3-H4 to the CAF-1 complex, containing RbAP48, causes a structural change in the conformation of the H3-H4 dimer, which leads to a decreased affinity of Asf1 for H3-H4 (Zhang et al., 2013). Interestingly, like Asf1, the CAF-1 complex only binds H3-H4 dimers, and not (H3-H4)₂ tetramers, possibly due to a destabilized H3-H3 interface (Zhang et al., 2013). However, CAF-1 can bind two H3-H4 dimers to form and deposit an H3-H4 tetramer onto the DNA (Liu and Churchill, 2012; Winkler et al., 2012). It has been suggested that CAF-1 and DNA compete for binding to the H3-H4 tetramer and that efficient deposition of tetramers may require the action of ATP-dependent chromatin remodelers or post-translational modifications. For example, acetylation of H4K5 and K12 by HAT1 facilitates the dissociation of H3-H4 tetramers from CAF-1. H3K56ac also plays a role in this part of the assembly line. Binding of H3-H4 tetramers to DNA is reduced by acetylation of H3K56, leading to unstable tetrasomes. However, once the H2A-H2B dimers are loaded, H3K56ac has no effect on nucleosome stability (Andrews et al., 2010; Watanabe et al., 2013).
Figure 2. Transport of new histones. Overview of factors involved in shuttling of histone proteins from the cytoplasm to the nucleus.

Histone H2A and H2B follow a different transportation route. H2A-H2B dimers are imported into the nucleus by Kap114 in association with Nap1 (Mosammaparast et al., 2002a). Kap114 is a karyopherin that binds to the NLS of H2A. In the nucleus, RAN-GTP binds to Kap114 and releases its cargo upon GTP hydrolysis. Nap1 subsequently delivers H2A-H2B to chromatin for deposition (Mosammaparast et al., 2002a). Nap1 also binds to H3-H4. However due to the higher affinity of the H3-H4 for DNA, Nap1 readily deposits H3-H4 onto the DNA (Andrews et al., 2010). Similarly, Nap1 efficiently deposits H2A-H2B onto DNA containing H3-H4 tetramers and disfavors non-nucleosomal deposition of H2A-H2B on DNA (Andrews et al., 2010).
The FACT complex also plays a role in H2A-H2B deposition (Formosa et al., 2001; Owen-Hughes and Gkikopoulos, 2012). FACT has been shown to be a chaperone for H2A-H2B and can facilitate nucleosome assembly but also binds to H3-H4 (Belotserkovskaya et al., 2003; Belotserkovskaya et al., 2004). It is unclear whether the displacement of H2A-H2B by FACT is direct or indirect, but it is clearly involved in altering chromatin structure during DNA replication (Wittmeyer and Formosa, 1997). Interestingly, human FACT interacts with the Mini Chromosome Maintenance complex (MCM) (Tan et al., 2006) and yeast FACT interacts with Replication Protein A (RPA) (VanDemark et al., 2006), supporting genetic studies that link FACT to DNA replication (Gambus et al., 2006; Okuhara et al., 1999; Schlesinger and Formosa, 2000; Tan et al., 2006) (Figure 3).

REPLICATION-COUPLED HISTONE ASSEMBLY

Production and delivery of new histones peaks during S-phase, when not only DNA but also its packaging material is duplicated. Early studies on chromatin duplication
suggest that the existing, parental, nucleosomes are randomly distributed over the
two daughter strands (Sogo et al., 1986). The gaps need to be filled with newly
synthesized histones to maintain the same nucleosome occupancy as the parent cell
(Alabert and Groth, 2012; Annunziato, 2005). Interestingly, random distribution
of existing histones may not apply to all cell types. A recent study showed that
preexisting canonical histone H3 is preferentially retained in male germline stem
cells during asymmetric division, whereas the replicated DNA in the differentiating
daughter cell was assembled in newly synthesized histones (Tran et al., 2012).
This suggests that the stem cell maintains the epigenetic information and that the
differentiated cell has to establish its epigenome de novo.

The replication fork is flanked by short stretches of 250-300 bp of naked DNA
(Gasser et al., 1996; Sogo et al., 1986), indicating that DNA replication and chromatin
assembly are tightly coordinated. The coupling between DNA replication and
assembly of new histones is most likely facilitated by various interactions between
replication proteins and chromatin assembly factors (Burgess and Zhang, 2013; Li
et al., 2012). Interestingly, those interactions may also play a role in the reassembly
of old histones following passage of the replication fork, as has been suggested for
Asf1 (Groth et al., 2007). An Asf1-MCM complex has been described that binds
to H3-H4 dimers harboring chromatin-associated histone modifications during
replication stress, suggesting that Asf1 can deliver histones that were previously
present in chromatin of the parental DNA (Groth et al., 2007). The histone chaperone
NASP provides another mechanism to coordinate histone synthesis and supply with
DNA replication. NASP controls the pool of soluble histones H3-H4 by regulating the
activity of heat shock proteins Hsc70 and Hsp90, which direct H3-H4 for degradation
by autophagy (Cook et al., 2011). Thereby, NASP prevents the accumulation of free
histones and the possible negative consequences thereof during DNA replication
stress or malfunctioning of Asf1. In budding yeast the checkpoint kinase Rad53 is
involved in avoiding the buildup of soluble histones during misregulation of histone
expression or replication stress. Rad53 phosphorylates excess histones (H3 and H4),
which targets them for degradation by the proteasome (Gunjan and Verreault,
2003; Singh et al., 2009; Singh et al., 2010).

Early studies suggested that parental (H3-H4)$_2$ tetramers are segregated as
tetramers and not as dimers during DNA replication (Annunziato, 2005; Jackson
and Chalkley, 1981; Leffak et al., 1977), suggesting that epigenetic information
encoded on nucleosomes is not inherited equally by the two daughter cells. The
identification of histone chaperones bound to H3-H4 dimers (Asf1, HIR complex)
led to the suggestion that histone H3-H4 tetramers might segregate as dimers and
mix with newly synthesized H3-H4 proteins (Ray-Gallet et al., 2011; Tagami et al.,
2004). Splitting of nucleosomes or tetramers could provide a mechanism to transmit
epigenetic information to both daughter strands for PTMs that are present on each of
the two copies of the respective modified histone protein. Recent studies suggest that
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tetramer splitting can indeed occur, but is restricted to a small subset of nucleosomes and is not associated with DNA replication. In mammalian cells tetramers of canonical H3-H4 do not split, whereas a small fraction of H3.3-H4 tetramers are present as mixed (old+new) tetramers through replication-independent nucleosome assembly (Huang et al., 2013; Xu et al., 2010). In budding yeast tetramer splitting only occurs at highly active genes undergoing high levels of replication-independent histone exchange (Katan-Khaykovich and Struhl, 2011). These findings are in line with biochemical studies showing that chromatin assembly factors CAF-1 and Rtt106 deposit H3-H4 tetramers (Su et al., 2012; Winkler et al., 2012).

REPLICATION-INDEPENDENT HISTONE ASSEMBLY: EXCHANGE

Although DNA replication in S-phase represents the major pathway of histone deposition, histones can also be disassembled and re-assembled by replication-independent mechanisms, leading to histone turnover or exchange. Indeed, histone synthesis peaks during S-phase, but canonical histones are also substantially expressed outside S-phase. Moreover, variants of the canonical histones are typically expressed and assembled throughout the cell cycle. Variant histones do not generally use the assembly routes of canonical histones. Instead, each variant has dedicated chaperones, providing opportunities for differential regulation of the different assembly processes. Importantly, replication-independent histone exchange and deposition of histone variants can influence DNA accessibility and stability of histone PTMs and has been linked to critical cellular processes such as gene regulation, development, oncogenic transformation, and nuclear reprogramming.

Replication-independent deposition of histone H3.3

In metazoans, deposition of canonical H3 is restricted to S-phase. In contrast, histone H3 variant histone H3.3 is expressed throughout the cell cycle and incorporated by replication-independent mechanisms (Ahmad and Henikoff, 2002). In Drosophila, H3.3 is assembled into chromatin in promoters, transcribed regions, and regulatory regions (Mito et al., 2005; Teves et al., 2012). A similar profile has been described in Arabidopsis (Stroud et al., 2012). These patterns are consistent with the idea that deposition of H3.3 is linked to disruption of chromatin by the act of transcription. In mammals, H3.3 is deposited mainly in promoter regions of active and silent genes (Chow et al., 2005) and is also found in regulatory regions (Ray-Gallet et al., 2011) and telomeric and centromeric regions (Goldberg et al., 2010; Wong et al., 2009). Three dedicated H3.3 chaperones have been described: HIRA, Daxx/ATRX and DEK (Campos et al., 2010; Drané et al., 2010; Elsaesser and Allis, 2010; Sawatsubashi et al., 2010; Tagami et al., 2004). HIRA is responsible for depositing H3.3 in euchromatic regions. It has been proposed to act by a gap-filling mechanism at regions where canonical H3 is absent. Daxx/ATRX mediates H3.3 deposition in heterochromatic regions while DEK may target
H3.3 to specific regulatory regions (Campos et al., 2010; Drané et al., 2010; Elsaesser and Allis, 2010; Goldberg et al., 2010; Sawatsubashi et al., 2010; Wong et al., 2009). H3.3 deposition by HIRA is promoted by phosphorylation of histone H4S47 (histone H4 serine 47), which is catalyzed by the kinase PAK2. This phosphorylation event acts by increasing the binding affinity of HIRA for H3.3-H4 and reducing the binding of CAF-1 with H3-H4 (Kang et al., 2011).

Replication-independent deposition of histone H3 in yeast

Unlike higher eukaryotes, yeast only has a canonical H3 and lacks H3 variants, with the exception of the centromere-specific H3 variant Cse4. However, the one histone H3 protein is involved in replication-dependent deposition as well as replication-independent exchange. Several studies in yeast showed that histone H3 is a dynamic entity in chromatin and that resident histone H3 molecules can be evicted and replaced by new histones in-trans upon activation and subsequent inactivation of inducible genes (Boeger et al., 2003; Rando and Winston, 2012; Reinke and Hörz, 2003; Schermer et al., 2005). This model system has uncovered roles for Asf1, Spt6, Spt16 (FACT), the HIR complex, Rtt106, H2B ubiquitination, Chd1, and the proteasome in reassembling chromatin in the wake of RNA polymerase (Fleming et al., 2008; Imbeault et al., 2008; Ivanovska et al., 2011; Jamai et al., 2009; Kim et al., 2007; Lee et al., 2012; Ransom et al., 2009; Schwabish and Struhl, 2006). Using inducible copies of tagged histones (as described above) the process of histone exchange in yeast has subsequently been delineated in fine detail. It was first shown in fission yeast that replication-independent exchange occurs preferentially in euchromatic regions (Choi et al., 2005). These findings are in agreement with studies in budding yeast, which showed high levels of transcription-independent histone exchange in gene promoter regions, as well as transcription-dependent exchange of H3 in coding regions and boundary-associated regions (Dion et al., 2007; Jamai et al., 2007; Rufiange et al., 2007). While there is a relationship between replication-independent H3 exchange and RNA polymerase density in coding regions, genes with the same transcription rate can still have different amounts of H3 exchange. The amount of exchange that is not explained by transcription rate has been referred to as relative exchange (Gat-Viks and Vingron, 2009), a feature that describes gene-specific rather than transcription-related effects. A critical gene-specific feature is the type of promoter that drives the expression of a coding sequence: histone exchange in coding regions is higher in regulated or inducible genes (also called stress genes) than in constitutive genes (also called growth genes or housekeeping genes) (Dion et al., 2007; Jamai et al., 2007; Radman-Livaja et al., 2011; Rufiange et al., 2007). Whereas growth genes are typically regulated by TFIID, have no canonical TATA boxes, and contain promoters with highly organized nucleosomes flanking a nucleosome depleted region, stress genes are typically dependent on SAGA, have canonical TATA boxes, and show a non-stereotypical nucleosome organization around the transcription start site (Rando
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and Winston, 2012). It has been suggested that the differences in histone exchange may be related to the bursty nature of transcription of stress genes (Dion et al., 2007; Jamai et al., 2007; Radman-Livaja et al., 2011; Rufiange et al., 2007).

In yeast, histone exchange has also been studied by using the RITE genetic pulse-chase assay (Verzijlbergen et al., 2010). Using this assay, H3 exchange has been detected in cells arrested in G1, G2/M, and starvation conditions. Upon exit from starvation, yeast cells show extensive replication-independent histone exchange across the genome, suggesting large scale epigenetic remodeling. A major determinant of H3 exchange is transcription. Active genes display a higher replication-independent exchange in their promoter and coding regions than inactive genes (Verzijlbergen et al., 2010). The RITE assay does not only allow the following of newly synthesized histones, but also the following of the old histones. Radman-Livaja et al. investigated the retention of old, ancestral histones over many cell generations in a genome-wide manner (Radman-Livaja et al., 2011). In replicating yeast cells, H3 is preferentially retained at the 5’ end of long lowly transcribed genes. A model that has been proposed to explain the ancestral H3 patterns contains three components: 1) histone exchange, which is higher in promoter regions than in ORFs; 2) lateral movement of the histones towards the 5’ end, called ‘passback’ (possibly due to the passing of the transcription machinery); 3) spreading of the histones via dissociation and re-association during replication (Radman-Livaja et al., 2011).

Several recent studies in yeast have already uncovered some of the mechanisms and functions of histone exchange (Figure 4). In yeast, newly synthesized H3 is acetylated at K56 by Asf1/Rtt109, and regions of the genome with high H3 exchange show enrichment for this modification. Deletion of Rtt109 or Asf1 or mutation of H3K56 reduce histone exchange rates in coding sequences (Kaplan et al., 2008; Rufiange et al., 2007; Schwabish and Struhl, 2006; Smolle et al., 2012; Venkatesh et al., 2012). The chromatin assembly factor Rtt106 associates with coding regions, binds H3K56 acetylated histones, and also promotes histone exchange in transcribed regions (Imbeault et al., 2008). H3K56ac has intimate connections to another PTM on histone H3. Methylation of histone H3K36 by Set2 represses histone exchange by disfavoring the interactions of histone H3 with chaperones Asf1, Spt16 (FACT) and Spt6 (Venkatesh et al., 2012). In a set2Δ mutant, where no H3K36 methylation is present, replication-independent histone exchange, H3K56ac (a hallmark of new H3) and H4ac are all increased over ORFs. Disrupting histone exchange by deleting Asf1 or Rtt109 in the set2Δ background decreases this accumulation of acetylation, thereby establishing histone exchange as a way to load pre-acetylated histones onto ORFs in the absence of Set2-mediated H3K36me (Venkatesh et al., 2012). Interestingly, H3K36me also recruits and/or activates the Rpd3S histone deacetylase complex to the 3’ end of coding regions. Therefore, Set2/H3K36me keeps acetylation in transcribed regions low by two mechanisms: it prevents the assembly of new, pre-acetylated histones and it recruits an active deacetylase complex to reset co-transcriptional
acetylation events. This cleaning up of chromatin in the wake of transcription is required to prevent the firing from cryptic promoters in coding regions. H3K36me has more functions in histone exchange because it also recruits the Isw1b chromatin remodeler to coding regions, which together with the remodeler Chd1 prevents histone exchange (Radman-livaja et al., 2012; Smolle et al., 2012), especially at long lowly transcribed genes. By combining RITE with a barcode-screen called Epi-ID, several other histone exchange factors have been identified. Whereas Gis1 and Nhp10 are negative regulators, Hat1 and its partners Hat2 and Hif1, which together form the NuB4 complex, are positive regulators of histone exchange (Verzijlbergen et al., 2011). Whether the NuB4 complex affects histone exchange via acetylation of new histones on H4K5K12 remains to be established.

The HIR complex in yeast has been suggested to reassemble H3 in cis (Kim et al., 2007) (Figure 4). The HIR complex is a conserved key player in histone exchange. In higher eukaryotes the HIRA complex deposits H3.3 (Pchelintsev et al., 2013; Rai and Adams, 2012; Tagami et al., 2004). In yeast, it was initially identified as a histone chaperone complex (containing Hir1-3 and Hpc2) involved in regulation of histone gene expression, but is also involved in replication-independent nucleosome assembly (Green et al., 2005; Kim et al., 2007; Lopes da Rosa et al., 2011; Silva et al., 2012). Interestingly, also the replication-coupled nucleosome assembly complex CAF-1 has been shown to affect histone exchange (Lopes da Rosa et al., 2011).

FACT (Facilitate Chromatin Transcription) plays a complex role in nucleosome dynamics during transcription. In yeast, the FACT complex contains two proteins, Spt16 and Pob3, aided by a third protein that is involved in DNA binding and contains a HMG-box-like structure, Nhp6 (Formosa et al., 2001). In mammalian cells, FACT consists of only two proteins, Spt16 and SSRP1, where the last protein contains an HMG-box for DNA binding (Belotserkovskaya and Reinberg, 2004). FACT travels along with RNA polymerase during transcription (Orphanides et al., 1999). It was first shown that FACT can displace one H2A-H2B dimer from the nucleosome, thereby creating a hexasome (Belotserkovskaya et al., 2003). RNA polymerase can transcribe through this hexasomal structure (Kulaeva et al., 2010), however Kulaeva et al. show that multiple closely spaced RNA polymerases will evict the hexasome from the DNA (Kulaeva et al., 2010). It has also been shown that FACT can act in the opposite way, reassembling the nucleosome in the wake of RNA polymerase (Fleming et al., 2008; Formosa, 2011). In histone exchange studies no increase in H2B exchange has been observed, while H3 exchange increased in the Spt16 deletion mutant (Jamai et al., 2009). This raises the hypothesis that old H3 might be reassembled into nucleosomes by FACT. In human cells Spt16 is recruited by H3K36 methylation by Set2D (Carvalho et al., 2013). Upon transcriptional activation Set2D methylates H3K36, which recruits FACT and leads to a decrease in H2B occupancy. Thus, Set2D and FACT seem to work together to reassemble nucleosome after RNA polymerase has passed, thereby preventing transcription from cryptic promoters within coding regions (Carvalho et al., 2013).
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Replication-independent deposition of histone H4 and H2B

Using variants of the inducible expression strategy, extensive replication-independent histone exchange has been observed for histone H4 and H2B in budding yeast (Jamai et al., 2007; Linger and Tyler, 2006). A comparison of the behavior of H3 and H2B suggests that the rate of exchange of H2B is higher than that of H3 and occurs equally in coding regions and promoters (Jamai et al., 2007). This finding is in agreement with the biochemical properties of the nucleosome, in which the H3-H4 tetramer forms a stable complex and from which the H2A-H2B dimers readily dissociate under conditions of high ionic strength or after passage of RNA polymerase (Hansen, 2002; Kulaeva et al., 2010). Recent in vitro transcription studies have implicated the H2A-H2B chaperone Nap1 and the nucleosome remodeling complex RSC (Remodels Structure of Chromatin) in nucleosome dynamics during transcription. RSC displaces a whole nucleosome and facilitates elongation by RNA polymerase. Nap1 assists RSC in promoting transcription through a nucleosome. Its positive effect on transcription coincides with the capturing of an H2A-H2B dimer, allowing RSC-dependent transcription to proceed through a hexasome and partially retaining chromatin architecture (Kuryan et al., 2012).

Exchange of other histone types

Although most research has been directed at histone H3 exchange in yeast or at the replacement of histone H3.1 for the replication-independent variant H3.3 in multicellular organisms, histone H2A also undergoes exchange and can be replaced by a range of variant histones. Here we briefly summarize the findings on exchange of histone H2A.

Replacement of H2A by H2A.Z

Histone H2A.Z is the major variant of H2A. This variant is common for all eukaryotes, and the high sequence conservation suggests that it arose early in evolution (Kusch and Workman, 2007; Skene and Henikoff, 2013). H2A.Z has been linked to a wide range of functions, including transcriptional activation, repression, and chromosome segregation. H2A.Z is localized at promoters, where it is believed to maintain a dynamic or accessible chromatin structure. In addition, H2A.Z might have an important role at the boundaries between heterochromatin and euchromatin (Kusch and Workman, 2007; Venkatasubrahmanyam et al., 2007). Structural studies have shown that nucleosomes containing H2A.Z have an extended acidic patch on the nucleosome core surface. Incorporation of H2A.Z is believed to make the nucleosome more unstable; however, results from in vitro studies are contradictory (Kusch and Workman, 2007; Watanabe et al., 2013). It has been proposed that nucleosomes containing both variants H3.3 and H2A.Z are very unstable and may in fact be lost during conventional chromatin extraction methods (Jin et al., 2009). Methods that preserve H3.3-H2A.Z nucleosomes show that double variant nucleosomes are predominantly located in the NDR of TSSs.
of active promoters, enhancers, and insulator regions. Jin et al. suggest that the region around the TSS is most of the time occupied, either by transcription factors, or by double variant nucleosomes, and only at the transition between those two a ‘nucleosome-free’ region will appear (Jin et al., 2009).

H2A.Z is incorporated by dedicated complexes (Figure 5). Two chaperones have been identified for H2A.Z, Nap1, which is also a chaperone for H2A-H2B, and Chz1, which seems to be a chaperone exclusively for H2A.Z-H2B (Billon and Côté, 2012; Luk et al., 2010; Straube et al., 2010). In the cytosol, Nap1 associates with H2A.Z-H2B dimers (as well as H2A-H2B dimers) and shuttles them into the nucleus. In the nucleus, Nap1 hands the H2A.Z-H2B dimer to the SWR1 (SWI/SNF Related) complex (SWR-C). Chz1 has been shown to bind to H2A.Z in the nucleus, and is also able to deliver H2A.Z to the SWR-C (Straube et al., 2010). The deposition of H2A.Z has been proposed to be replication-independent (Svotelis et al., 2010). A recent study in mouse trophoblast stem cells is in agreement with this idea. This
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Figure 5. Pathways for the replacement of H2A and H2A.Z. Role of chaperones and remodelers in the nuclear import, deposition, and eviction of H2A.Z.

study revealed changes in the composition and abundance of H2A.Z-containing nucleosomes during the cell cycle (Nekrasov et al., 2012) and suggests that new H2A.Z in promoter regions is not assembled during S-phase but after completion of mitosis (Nekrasov et al., 2012). SWR-C incorporates H2A.Z in a stepwise manner, replacing one H2A-H2B dimer at the time (Luk et al., 2010). This can lead to the existence of heterotypic nucleosomes containing one H2A and one H2A.Z (Luk et al., 2010; Nekrasov et al., 2012). Heterotypic nucleosomes might be more unstable than homotypic nucleosomes (H2A.Z only or H2A only), as crystal structure analyses also suggest (Henikoff, 2009; Suto et al., 2000). The activity of the SWR-C in the genome is increased when the tails of H2A or H4 are acetylated (Altaf et al., 2010; Ishibashi et al., 2009; Shia et al., 2006). Acetylation of H2A and H4 in yeast is performed by the NuA4 complex (Altaf et al., 2010; Keogh et al., 2006); in the absence of a functional NuA4 complex, nucleosomal H2A.Z decreases in yeast. In yeast, the SWR-C is the only known H2A.Z assembly factor. In mammalian cells, H2A.Z assembly is carried out by two complexes: TIP60/P400 and SCRAP. The TIP60/P400 complex has an intrinsic acetylation ability for the tails of H2A and H4, and this is necessary for incorporation of H2A.Z. The SCRAP complex, however, is able to incorporate H2A.Z without the
need for H2A and H4 acetylation (Altaf et al., 2010). The SWR-C incorporates H2A.Z in the promoter regions of most genes in yeast. It has been proposed that SWR-C incorporates H2A.Z by default, resulting in H2A.Z incorporation around promoter sites and into gene bodies and that H2A.Z is removed from non-promoter regions by the INO80 complex (INO80-C) (Papamichos-Chronakis et al., 2011). INO80-C, which is closely related to SWR-C, is involved with the exchange of H2A.Z by canonical H2A, thereby opposing the action of SWR-C. The specificity of H2A.Z localization also involves its acetylation. Acetylation of H2A.Z in yeast occurs on the N-terminal tail and is carried out by NuA4 and SAGA histone acetyltransferase complexes and reversed by Hda1 (Babiarz et al., 2006; Ishibashi et al., 2009; Keogh et al., 2006; Lin et al., 2008; Mehta et al., 2010; Millar, 2006). H2A.Z is acetylated in promoter regions and INO80-C preferentially removes unacetylated H2A.Z form the DNA. In the absence of INO80-C, unacetylated H2A.Z aberrantly accumulates, which has a negative effect on DNA damage repair and replication fork stability (Papamichos-Chronakis et al., 2011). Finally, the chromatin-remodeler Fun30 contributes to H2A.Z positioning; deletion of Fun30 leads to a redistribution of H2A.Z from promoters to coding regions (Durand-Dubief et al., 2012).

Interestingly, the specificity of SWR-C for H2A.Z is under control of multiple levels of trans-histone crosstalk. Whereas in unmodified nucleosomes SWR-C is activated by DNA-bound H2A, in nucleosomes containing H3K56ac, SWR-C is also activated by H2A.Z and exchanges the variant for H2A (Watanabe et al., 2013). This finding and previous findings strongly link H2A.Z to exchange of histone H3 in yeast: 1) exchange of histone H3 leads to incorporation of H3 pre-acetylated on K56, 2) H3K56ac promotes turnover of H3 in promoter nucleosomes, 3) H3K56ac reverses the substrate-specificity of SWR-C, leading to removal of H2A.Z, and 4) H2A.Z enhances turnover of histone H3 in promoter-proximal nucleosomes. A model has been proposed in which an H3K56ac nucleosome may be subject to multiple rounds of SWR-C-catalyzed exchange of H2A and H2A.Z, and this rapid H2A-H2B/H2A.Z-H2B dimer exchange might promote H3-H4 exchange (Watanabe et al., 2013).

Other H2A variants

H2A.X is a variant of H2A that is present throughout the genome and involved in DNA double strand break (DSB) repair. Yeast does not encode H2A.X but uses canonical H2A instead. When a DSB occurs, H2A.X is quickly phosphorylated (γ-H2A.X). After repair, γ-H2A.X is replaced by non-phosphorylated H2A.X (Kusch and Workman, 2007). No specific deposition factors have been described for H2A.X to date but FACT has been found to bind to H2A.X (Skene and Henikoff, 2013). A quickly evolving variant of H2A has been discovered about ten years ago (Chadwick and Willard, 2002). This variant, called H2A.Bbd (Barr body deficient), only exists in mammals and not in invertebrates. H2A.Bbd is enriched in active gene bodies, and excluded from the inactive X chromosome, suggesting a role in transcription. It has also been suggested that H2A.
Bbd is involved in mRNA processing, although it is unclear if this involvement is direct or indirect (Tolstorukov et al., 2012). It has been shown that nucleosomes containing H2A.Bbd are less stable than nucleosomes containing canonical H2A (Gautier et al., 2004). This could be because nucleosomes containing this shorter variant of H2A wrap only about 120bp, leaving more DNA accessible for the binding of transcription factors. In agreement with the finding that H2A.Bbd-containing nucleosomes are less stable, it was found using FRAP, that exchange of H2A.Bbd is faster than H2A exchange (Gautier et al., 2004). Mouse H2A variant H2A.Lap1 may also act to destabilize nucleosome organization (Soboleva et al., 2012). Another variant of H2A, macroH2A is mainly found on the inactive X chromosome and near the promoters of inactive genes, suggesting that this variant is involved in gene repression (Gamble and Kraus, 2010). Exchange mechanisms of this variant are currently unknown.

OUTLOOK

It is becoming clear that the nucleosome is a very dynamic structure that is much more than a structural packaging mechanism for eukaryotic DNA. Histone proteins within the nucleosome are being modified by numerous PTMs on numerous residues and canonical histones can be replaced by variants. These variations on the nucleosome can influence chromatin structure and function in many ways. Indeed, histone variants have been linked to a diverse set of functions (Skene and Henikoff, 2013). However, important functions are also emerging for the act of histone exchange itself.

Replication-independent histone exchange provides an important mechanism to maintain epigenome integrity in non-replicating cells (Ray-Gallet et al., 2011; Skene and Henikoff, 2013). It allows the cell to replace histones that are evicted by transcription or repair and thereby repair disrupted chromatin structures. This may explain why histone H3.3 accumulates in terminally differentiated non-replicating rat neurons (Piña and Suau, 1987). However, histone exchange may not go without consequences since it leads to loss of existing PTMs and deposition of histones containing PTMs associated with newly synthesized histones. In fact, histone exchange may provide the cell with a mechanism to remove damaged histones or histones containing certain PTMs. Several recent studies support the idea that histone dynamics and histone PTMs can influence each other. One example is methylation of H3K79 by Dot1. No H3K79 demethylases have been identified, suggesting that removal of this PTM requires dilution of modified histones by replication-coupled or –independent mechanisms. Indeed, H3K79me3 levels increase when yeast cells grow slower and aged histones have higher average levels of H3K79me3 than young histones (De Vos et al., 2011). When examined in the epigenome, H3K79me3 positively correlates with inheritance of ancestral histones in replicating cells and negatively correlates with histone exchange and relative exchange levels (Radman-Livaja et al., 2011). Together, these findings strongly indicate that histone exchange
and inheritance fine-tune the H3K79me landscape laid down by Dot1. The observed slow buildup of H3K79me3 over successive generations also challenge models that propose that histone PTMs are rapidly copied following DNA replication to maintain epigenetic states of the parental cell.

Rapid exchange of histones in promoter regions is incompatible with the idea that cells transmit epigenetic information by passing on histones with specific PTMs. An alternative model proposes that histone exchange might perpetuate active or silent gene expression states by increasing or decreasing accessibility to sequence-specific binding proteins (Deal et al., 2010). Histones in coding regions are more stable than promoter histones and may therefore provide better opportunities for epigenetic memory mechanisms. However, tracking ancestral histone H3 in replicating yeast cells suggests that histone H3 does not associate with the exact same locus as where it came from prior to DNA replication (Radman-Livaja et al., 2011). This argues against inheritance of chromatin state at a single nucleosome resolution, but rather suggests that inheritance can occur in multi-nucleosome domains (Radman-Livaja et al., 2011). The fact that less exchange is observed in yeast coding regions does not mean that the chromatin in coding regions is less disrupted. It cannot be excluded that histones are evicted and recycled, a process that remains undetected with most assays that measure histone dynamics. For example, under conditions of replication stress, Asf1-bound H3-H4 dimers have been found to carry modifications that are associated with chromatin (Avvakumov et al., 2011), suggesting a recycling function of this chaperone. In yeast, the HIR complex has been found to be associated with old, chromatin-derived H3-H4 (Kim et al., 2007).

The rapid exchange seen at promoters could transiently expose otherwise occluded transcription factor binding sites or it could influence the outcome of a transcription factor binding event. An important example is provided by the transcriptional regulator Rap1. Mapping of Rap1 dynamics by applying the inducible expression strategy to Rap1 has shown that long Rap1 residence correlates much better with transcriptional activation than steady-state levels of Rap1 binding (Lickwar et al., 2012). Fast Rap1 turnover is linked with low transcriptional output. Moreover, long Rap1 residence time correlates with slow H3 exchange (Lickwar et al., 2012). This finding suggests that nucleosomes and transcription factors dynamically compete for DNA binding and opens up the possibility that histone exchange influences the transcriptional output of a transcription factor binding event. Systematic measuring of the dynamics of chromatin proteins and regulators and the identification of histone exchange factors and mechanisms will be critical to determine in future studies how chromatin protein dynamics and histone exchange influence the epigenetic landscape, gene regulation, and epigenetic inheritance.

Although the mechanisms and functions of histone exchange are not yet fully understood, recent studies indicate that histone H3.3 deposition is involved in key cellular processes such as epigenetic reprogramming and cancer development.
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(Filipescu et al., 2013). A large fraction of human pancreatic neuroendocrine tumors (PanNETs) has been found to harbor mutations that inactivate ATRX and Daxx. Based on the telomere disfunctioning observed in these tumor cells, it has been proposed that loss of ATRX/Daxx activity and deposition of H3.3 at telomeres leads to telomere destabilization (Heaphy et al., 2011). In human pediatric glioblastomas, driver mutations in ATRX/Daxx have been found as well as mutations in the H3.3 gene itself (Schwartzentruber et al., 2012; Sturm et al., 2012). Interestingly, some of the glioblastoma driver mutations in H3.3 reduce global H3 methylation levels in cells by inhibition of SET-domain enzymes, suggesting that the mutant H3.3 proteins act in a dominant manner to also influence canonical H3 (Lewis et al., 2013). Finally, replication-independent deposition of H3.3 by HIRA has recently been found to be important for transcriptional reprogramming of nuclei transplanted to Xenopus oocytes. During reprogramming, H3.3 deposition occurs in regulatory regions, among which that of Oct4, which may destabilize chromatin and facilitate a shift to different epigenetic states (Jullien et al., 2012). Together, these studies demonstrate that histone exchange is a novel layer of epigenetic control with key roles in important cellular processes.

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