HIRA and Daxx Constitute Two Independent Histone H3.3-Containing Predeposition Complexes

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Histone H3.3 is a universal replacement histone in metazoans that has been implicated in diverse processes ranging from gene activation to heterochromatin silencing. Here, we show that, before deposition, H3.3 exists in two biochemically distinct complexes, associated with either Daxx or HIRA, Ubinuclein-1, and Cabin-1. Although the HIRA complex is evolutionarily conserved in yeast, Daxx is a novel histone chaperone unique to metazoans. Deletion of HIRA in mouse embryonic stem cells impairs the HIRA complex integrity but does not abolish Daxx association with H3.3/H4. Similarly, HIRA interacts with H3.3/H4 in the absence of Daxx. We hypothesize that these two H3.3 chaperone systems provide separate pools of H3/H4 units for incorporation at distinct sites within the genome. We provide evidence that the association of histone H3.3 with distinct assembly systems allows it to acquire unique posttranslational modifications before deposition that might affect its role after incorporation into chromatin.

Histone octamers package the eukaryotic genome, not only compacting the DNA, but also encoding and propagating a sophisticated additional layer of epigenetic information. Despite the inherent physical stability of the nucleosome particle, the resulting chromatin structure must be highly dynamic to allow access to the DNA-encoded information. H3/H4 and H2A/H2B units are deposited and evicted from nucleosomes by different histone chaperones and ATP-dependent chromatin remodelers. Histone chaperones thereby serve two major functions: They (1) buffer the pool of nonchromatin-associated histones in predeposition complexes and (2) facilitate favorable histone–DNA interactions during the stepwise assembly of the histone octamer–DNA complex from H3/H4 and H2A/H2B units. Consequently, mutants of histone chaperones in *Saccharomyces cerevisiae* cannot form proper chromatin structure (Sharp et al. 2002; Adkins and Tyler 2004; Andrews et al. 2010).

Metazoans have evolved histone variants to meet the diverse needs for establishing and maintaining complex epigenetic information. Histone H3.3 is a minor variant of the canonical S-phase histones H3.1 and H3.2 in metazoans. However, its role as a replacement histone outside of S phase is conserved to lower eukaryotes, and accumulating evidence suggests that, by virtue of a unique amino acid motif, H3.3 engages in several variant-specific chromatin assembly pathways. Whereas the amino acid sequence of H3.3 is >95% identical to H3.1/2, the small AAIG motif in the core histone fold (Fig. 1) allows targeting of the variant to specific genomic loci by a replication-independent mechanism (Ahmad and Henikoff 2002; Goldberg et al. 2010). The existence of identical H3 variants throughout the metazoan lineage underscores their importance as carriers of epigenetic information in somatic cells and the germline (for review, see Campos and Reinberg 2010; Elsaesser et al. 2010).

Reading out the subtle differences between H3.3 and H3.1/2 poses an intricate structural problem to variant-specific chaperones. Hence, a number of recent studies have aimed to elucidate which proteins confer specificity in chromatin assembly. By purifying H3.1- and H3.3-associated complexes from HeLa nuclear extracts, the replication-independent histone deposition factor HIRA was found to associate specifically with H3.3 (Tagami et al. 2004). Using the same approach, we and other investigators identified a different polypeptide, the Fas death-domain-associated protein Daxx, as an H3.3-specific binding protein (Drané et al. 2010; Goldberg et al. 2010; Lewis et al. 2010). We found that Daxx specifically interacts with the AAIG motif of H3.3 in vitro and is a bona fide H3.3-specific histone chaperone (Lewis et al. 2010). The ATP-dependent chromatin remodeler and α -thalassemia X-linked mental retardation protein (ATRX) formed a complex with Daxx–H3.3/H4 that was essential for H3.3 chromatin assembly in vivo (Goldberg et al. 2010; Lewis et al. 2010).

Although we were able to detect HIRA in H3.3-associated fractions, this association was unstable following exposure to the more stringent biochemical conditions used to identify Daxx. This raised the question of whether HIRA and its associated proteins Ubinuclein-1 and Cabin-1 were recruited indirectly, potentially via a secondary interaction with Daxx or another as yet unidentified protein. We addressed this issue by biochemically fractionating H3.3-containing subcomplexes from HeLa cells and purifying H3.3-associated proteins from mouse embryonic stem cells (ESCs) devoid of HIRA, Daxx, or ATRX. We find that the HIRA/Ubinuclein-1/Cabin-1 and ATRX/Daxx complexes are two separate biochemical entities that independently associate with H3.3. We have previously shown that HIRA is responsible for incorporation of H3.3 at genic regions, whereas ATRX is required for telomeric

Figure 1. A highly conserved AAIG motif in the core histone fold of metazoan H3.3. Schematic of noncentromeric histone H3 variants in mammals. H3.1 and H3.2 are commonly referred to as canonical or S-phase histone H3. Variable amino acids are shown with residue number. Three unique residues of H3.3 form the AAIG motif at the base of helix 2 of the histone H3 core (purple; note that isoleucine is hidden in the helical depiction). The molecular structure of a representative H3/H4 dimer ([H3] white, [H4] green) has been adapted from the nucleosome structure (PDB:1kx5). The H3:H3 interface of the H3/H4 tetramer is indicated.

deposition of H3.3 (Goldberg et al. 2010). Therefore, HIRA and Daxx/ATRAX constitute two separate histone H3.3-deposition machineries that act on distinct genomic loci.

MATERIALS AND METHODS

Nuclear extracts were prepared as described in Lewis et al. (2010) from HeLa cells stably expressing FLAG-HA-tagged H3.1 or H3 (Tagami et al. 2004) or mouse ESCs (Goldberg et al. 2010). Immunoprecipitations were performed in 20-mM HEPES•HCl (pH 7.5), 150 mM NaCl, 10% glycerol, 0.2 mM EDTA, 2 mM MgCl₂ in the presence of DNase and RNase, washed five times with wash buffer (20 mM HEPES•HCl [pH 7.5], 250 mM NaCl, 10% glycerol, 0.2 mM EDTA, 0.01% NP-40 [Nonidet P-40]) and eluted with either FLAG peptide or 1% SDS. Eluted material was separated by SDS polyacrylamide gel electrophoresis (SDS PAGE) and analyzed by western blotting with the following antibodies: α -FLAG-HRP (Sigma-Aldrich), α -HA-HRP A190-107P (Bethyl), α -HIRA WC119 (Peter Scambler), α -p150 sc-10772 (Santa Cruz), α -Daxx sc-7152 (Santa Cruz), α -p60 NB500-212 (Novus Biologicals), α -Cabin-1 ab3349 (Abcam), α -ATRAX H-300 (Santa Cruz), α -ASF1a/b 4A1/3 (Santa Cruz), α -H3K9Ac, No. 07-352 (Millipore), α -acetyl-H4, No. 06-598 (Millipore), and α -H4K5Ac, No. 06-759 (Millipore).

BIOCHEMICAL FRACTIONATION OF H3.3-CONTAINING COMPLEXES

Previous studies used double-affinity purification of FLAG-HA-tagged H3.3 (e-H3.3) from nuclear extracts to isolate H3.3-associated factors (Tagami et al. 2004; Drané et al. 2010; Lewis et al. 2010). However, this strategy did not allow us to identify putative subcomplexes that independently coimmunoprecipitate with H3.3. Thus, we developed an alternative biochemical approach to fractionate

H3.3-containing complexes from nuclear extracts based on their affinity to a negatively charged heparin matrix.

We noted that e-H3.3 eluted over a broad range of salt concentrations from immobilized heparin (Fig. 2A). Whereas HIRA and CAF-1 p150 complexes elute with the bulk of nuclear proteins at medium salt, Daxx only dissociated at higher salt concentrations from the heparin column (Fig. 2A). Therefore, this approach provided us with a means to separate a distinct subpopulation of e-H3.3 and associated proteins. We combined fractions containing almost exclusively HIRA/CAF-1 p150 or Daxx and immunoprecipitated e-H3.3 (Fig. 2B). HIRA, but not CAF-1 p150, copurified with H3.3. Because Daxx was absent from the immunoprecipitated material, we conclude that HIRA associates specifically with H3.3, independent of Daxx. As a control, we heparin-fractionated e-H3.1 nuclear extracts (data not shown) and performed immunoprecipitation from the corresponding HIRA/CAF-1 p150 fractions; this e-H3.1 immunoprecipitation yielded CAF-1 p150 (Fig. 2B). Daxx coimmunoprecipitated with H3.3 from the higher salt fractions in the absence of HIRA (Fig. 2B). Thus, we were able to isolate nonoverlapping H3.3 subcomplexes containing either HIRA or Daxx from heparin-fractionated nuclear extract.

In addition to HIRA, we found Cabin-1 and Ubinuclein-1 associated with the first pool of e-H3.3/H4 (Fig. 2C). HIRA, Cabin-1, and Ubinuclein-1 have previously been found to form a complex in the presence and absence of H3.3/H4 (Tagami et al. 2004; Drané et al. 2010). Iterative alignment searches identified Cabin-1 and Ubinuclein-1 as mammalian homologs of the yeast proteins Hir3 and Hcp2p, respectively, suggesting a HIRA complex analogous to the yeast Hir complex. The control H3.1 immunoprecipitation yielded all three subunits of the CAF-1 complex: p150, p60, and RbAp46/48.

Daxx coprecipitated with H3.3 from the higher salt fractions without apparent stoichiometric partners (Fig. 2C,D), in agreement with our previous observation that

Figure 2. HIRA and Daxx constitute two biochemically distinct H3.3-containing subcomplexes. (A) Biochemical fractionation of nuclear H3.3 complexes: 200–800-mM KCl fractions from a heparin column were probed for e-H3.3, HIRA, CAF-1, and Daxx by western blotting. (B) Affinity purification of e-H3.3 and e-H3.1 complexes. e-H3.3/H3.1 complexes were immunoprecipitated from indicated pools and analyzed by western blotting. Immunoprecipitation from the medium-salt e-H3.3 and e-H3.1 fractions yielded predominantly HIRA and CAF-1, respectively; Daxx coprecipitated with high-salt e-H3.3. (C) Silver staining of eluted CAF-1, HIRA, and Daxx complexes from above. Indicated bands were all identified by mass spectrometry and/or verified by western blotting. (D) Coomassie stain of the Daxx complex estimates protein abundance.

the direct interaction of Daxx with H3.3/H4 is not dependent on other proteins such as ATRX (Lewis et al. 2010). In conclusion, we find that the known H3.3-associated proteins fractionate into two biochemically distinct subcomplexes constituted by HIRA and Daxx.

GENETIC DISSECTION OF H3.3-CONTAINING COMPLEXES

Next, we assessed how the deletion of either HIRA or Daxx histone chaperones affects H3.3 and its associated complexes in respective knockout mouse ESC lines. We used a zinc-finger–based genome editing strategy to specifically tag one allele of the endogenous H3.3B gene in wild-type W9.5, HIRA^{−/−}, Daxx^{−/−}, and ATRX^{−/−} ESCs with a carboxy-terminal HA tag (H3.3-HA) (Goldberg et al. 2010), assuring endogenous expression levels of the tagged histone. We found elevated levels of H3.3-HA in nuclear extracts of HIRA^{−/−} ESCs, as previously reported (Meshorer et al. 2006), whereas the soluble pool of H3.3-HA was reduced in Daxx^{−/−} ESCs (Fig. 3, left). We speculate that Daxx buffers a considerable pool of H3.3 in the nucleus by forming a stable complex with H3/H4 units. Overall, H3 and H4 levels (accounting for the untagged H3.3 as well as H3.1/2) in the nucleoplasm were unchanged, indicating that reduced levels of H3.3 might be compensated by an increase in soluble H3.1/2. Levels of chromatin-bound H3.3-HA were comparable in all cell lines (Fig. 3, bottom).

As previously reported, ATRX levels were reduced in Daxx^{−/−} cells (Lewis et al. 2010). Unexpectedly, Cabin-1 was depleted in HIRA^{−/−} nuclear extracts (Fig. 3, left panels) and whole-cell lysates (data not shown). Cabin-1 mRNA levels are not perturbed in HIRA^{−/−} cells (Goldberg et al. 2010), suggesting that the Cabin-1 protein might be unstable and degraded in the absence of HIRA. Therefore, Cabin-1 likely contributes directly to the function of the HIRA complex, as already shown for Ubinuclein-1 (Banumathy et al. 2009).

HIRA and Daxx Complexes Independently Bind H3.3

Based on our biochemical fractionation of H3.3-containing complexes, we hypothesized that association of HIRA and Daxx with H3.3 occurs independently. We tested this by immunoprecipitating H3.3-HA from the above ESC nuclear extract. Indeed, HIRA copurified with H3.3-HA in Daxx^{−/−} cells and vice versa (Fig. 3, right panels). ATRX coprecipitated with Daxx, but ATRX deletion did not influence binding of either histone chaperone, as expected from our earlier studies (Lewis et al. 2010). ASF1 was constitutively associated with H3.3-HA.

We therefore conclude that the HIRA and Daxx complexes independently interact with H3.3 and likely maintain distinct pools of H3.3/H4 units in the nucleus. This is in line with our recent observation that HIRA and ATRX/Daxx deposit histones at distinct genomic locations: HIRA at genic regions (Goldberg et al. 2010) and ATRX/Daxx at telomeres as well as pericentromeric heterochromatin (Drané et al. 2010; Lewis et al. 2010).

Although our *in vitro* studies confirmed that Daxx directly interacts with the H3.3 AAIG motif (Lewis et al. 2010), similar direct evidence is thus far missing for HIRA. Together with previous data, our finding that HIRA consistently copurifies with H3.3 in a complex with Cabin-1 and Ubinuclein-1 (Tagami et al. 2004; Lewis et al. 2010) raises the possibility that these components contribute to binding and specificity as well.

Figure 3. HIRA and Daxx independently associate with nucleoplasmic H3.3. Nuclear extracts were prepared from wild-type, HIRA^{-/-}, Daxx^{-/-}, and ATRX^{-/-} mouse ESC lines (Roberts et al. 2002; Lewis et al. 2010). Where indicated, one H3.3 allele has been endogenously tagged with an H3.3B-HA knockin. Protein levels in the nuclear extracts and insoluble chromatin were assessed by immunoblotting (left panels). e-H3.3 was immunoprecipitated with α-HA-agarose and eluted with 1% SDS. Copurifying proteins were analyzed by western blotting (right panels).

DO PREDEPOSITION COMPLEXES REPRESENT INDEPENDENT HISTONE POOLS?

Although H3.3 was originally characterized to be associated with actively transcribed genes (Ahmad and Henikoff 2002), the discovery of new H3.3-chaperone pathways and heterochromatic target regions (Hake et al. 2005; van der Heijden et al. 2007; Drané et al. 2010; Lewis et al. 2010; Santenard et al. 2010) poses some intriguing questions: Does H3.3 perform unique functions at distinct genomic locations? How can a single replacement variant contribute to gene activation or repression, depending on the chromatin context? How do the specialized deposition machineries contribute to the functional outcome of H3.3 incorporation?

The functional diversity of H3.3 could be explained if the distinct predeposition complexes would represent independent pools of H3.3/H4 units. Assuming that the exchange between those pools is slow due to the biochemical stability of the complexes or their distinct spatial or temporal distribution during the cell cycle, the associated histones could acquire specific posttranslational modifications (PTMs) that might prime them for their respective functions at the genomic target sites.

Histone variants have been found enriched in PTMs that correlate with the local chromatin environment at their target sites (Hake et al. 2006). A set of specific modifications has been identified for the soluble pool of histones composed of newly synthesized histones but possibly also evicted histones “recycled” from chromatin: ubiquitous H4K5/K12 diacetylation (H4K5/K12diAc) (Sobel et al. 1995) and H3K9 monomethylation (H3K9me1) on both H3.1/2 and H3.3, as well as H3K9 dimethylation (H3K9me2) and H3K9 acety-

lation (H3K9Ac) enriched on H3.3 (Loyola et al. 2006). Given the overall heterogeneity of predeposition PTMs on the single H3.3K9 residue, we wondered if any of these marks might be characteristic of a subset of predeposition H3.3 associated with a specific chaperone complex.

Distinct Histone Posttranslational Modifications Are Present in Predeposition Complexes

Using heparin fractionation to separate e-H3.3-containing complexes from HeLa cells, we identified a subpopulation of e-H3.3 that was enriched in H3K9Ac (Fig. 4A), a modification that is thought to be established on non-nucleosomal H3 by the histone acetyltransferase Gcn5 (Adkins et al. 2007). Because the enrichment of H3K9Ac tracked well with the HIRA complex, we further analyzed the PTMs present on histones within the CAF-1, HIRA, and Daxx complexes (Fig. 4B). We found that H3K9Ac on e-H3.3 associated with the HIRA complexes but was undetectable in the Daxx complex. Both complexes contained low levels of acetylated H4. e-H3.1 in the CAF-1 complex was enriched in H4 acetylation, with H3K9Ac present at a lower level than on e-H3.3. With the differential H3K9Ac as a first indication, we conclude that unique H3.3/H4 PTMs can be associated with predeposition complexes.

A MODEL FOR THE DIFFERENT PATHWAYS OF H3.3 CHROMATIN ASSEMBLY

Our biochemical characterization of the distinct H3.3 deposition machineries allows us to propose a model for the journey of histones from synthesis to incorporation at specific genomic location. Newly synthesized histones in

Figure 4. H3.3 predeposition complexes are associated with distinct histone posttranslational modifications. (A) Western blot analysis of e-H3.3 lysine 9 acetylation (K9Ac) in the heparin salt gradient. K9Ac on e-H3.3 is enriched in fractions that correspond to the HIRA complex. (B) The CAF-1, HIRA, and Daxx complexes were analyzed for selected H3 and H4 posttranslational modifications. K9Ac is enriched on e-H3.3 within the HIRA complex but is undetectable in the Daxx complex.

the cytosol are likely immediately chaperoned by abundant general histone chaperones, such as ASF1 and/or NASP (Fig. 5, top). Furthermore, H4 is diacetylated on K5 and K12. ASF1-bound H3.3/H4 dimers then serve as substrates for various assembly pathways. Although a Daxx-H3.3/H4 complex has been found in the cytosol (Drané et al. 2010), HIRA seems to be exclusively localized to the nucleus and has not been found in a cytosolic complex with H3.3/H4 (Lorain et al. 1998; Tagami et al. 2004). ASF1 is ubiquitously detectable with H3.3/H4 (Tagami et al. 2004; Drané et al. 2010), but our biochemical data suggest that it is not a required component of HIRA and Daxx predeposition complexes (Fig. 2C,D) (Lewis et al. 2010).

HIRA Complex and Histone Deposition at Transcribed Gene Bodies and Promoters

HIRA histone chaperone activity has been well studied in *Xenopus* egg extracts, where it is necessary and sufficient for replication-independent chromatin assembly on exogenous DNA (Ray-Gallet et al. 2002, 2007; Tagami et al. 2004). However, in vivo, it accounts for only a subset of replication-independent chromatin assembly (Fig. 5, pathway 1), namely, within transcribed regions, at promoters and some regulatory elements (REs) (Goldberg et al. 2010). Evidence from yeast showing that the Hir complex and Asf1p genetically and physically interact with the Set2 methyltransferase suggests that histone deposition might be functionally linked to H3K36 trimethylation. Subsequently, histone deacetylases remove acetylation marks from nucleosomes after RNA Pol passage.

H3.3 enrichment in gene bodies was consistently lost in HIRA^{-/-} ESCs, but gene-expression patterns and the transcription elongation mark H3K36me3 were only mildly affected (Goldberg et al. 2010). Several other histone

chaperones including Spt6 and FACT have also been implicated in histone exchange during transcription. Thus, the purpose of the transcription-associated H3.3 deposition and possible compensatory mechanisms in the absence of HIRA have yet to be defined.

The homology of HIRA to the yeast Hir corepressor complex subunits Hir1/2p suggests that its histone deposition activity might suppress basal transcription (Spector et al. 1997; Anderson et al. 2009). Furthermore, it will be necessary to define the roles of the remaining complex members Cabin-1 and Ubinuclein-1. Cabin-1 has recently been shown to act as a corepressor at p53 target genes, and it will be interesting to see if this activity requires HIRA (Jang et al. 2009). Ubinuclein-1 cooperates with HIRA in a chromatin assembly pathway specific to establishment of facultative heterochromatin domains in senescent cells (Fig. 5, pathway 2) (Banumathy et al. 2009).

Daxx and ATRX Assemble H3.3 Chromatin at Pericentric Heterochromatin and Telomeres

A considerable fraction of H3.3 is localized to telomeres in mouse ESCs, and we have recently shown that this enrichment is dependent on the Daxx/ATRX complex but not HIRA (Goldberg et al. 2010; Lewis et al. 2010). Whereas Daxx readily assembles H3.3/H4 chromatin in vitro, ATRX is required in vivo to target Daxx and H3.3/H4 to the telomeres (Lewis et al. 2010) as well as to pericentric heterochromatin (Drané et al. 2010). Therefore, a diffusible Daxx-H3.3/H4 complex likely delivers histones to an ATRX-containing complex associated with telomeric chromatin (Fig. 5, pathway 3) (Wong et al. 2010). Furthermore, the chromatin-bound complex might contain the histone chaperone Dek and histone deacetylase HDACII, as suggested by biochemical studies (Hollenbach et al. 2002), to

Figure 5. Predeposition complexes mediate H3.3 chromatin assembly. A model for the flow of histones from their synthesis in the cytosol (*top*) to their target genomic regions in the nucleus (*bottom*). Newly synthesized histones H3.3/H4 are chaperoned by ASF1 and a cytosolic pool of Daxx. HAT1 acetylates H4K5/K12 in the cytosol. Predeposition complexes HIRA.com and Daxx.com chaperone distinct pools of H3.3/H4. (1) HIRA.com mediates deposition of H3.3 at promoters and transcribed regions together with RNA polymerase (RNAP)-associated FACT (Spt16/SSRP1) and Spt6 histone chaperones. (2) HIRA.com deposits histones at facultative heterochromatin in senescent cells. Histone methyltransferases (HMTs) establish a repressive chromatin state. (3) Daxx.com cooperates with ATRX and possibly other factors in incorporating H3.3 into pericentric heterochromatin and telomeres. The chromatin-bound deposition complex might also contain the histone chaperone Dek and the histone deacetylase HDACII (4). An unknown factor delivers H3.3 to regulatory elements (REs). (5) In the absence of Daxx.com or HIRA.com, H3.3 associates with the replication-dependent CAF-1 complex that mainly deposits histones after the DNA polymerase (DNAP) replication fork.

prime a repressive chromatin state. This notion is supported by our finding that H3.3 does not carry the activating K9Ac mark in the Daxx complex (Fig. 4B).

Notably, Daxx is a highly conserved histone chaperone that appeared with the emergence of metazoans. Therefore, unlike the H3.3-chaperone activity of HIRA that is shared in yeast, Daxx likely evolved to diversify the functionality of H3.3 within the more complex chromatin structure of higher eukaryotes. A specific example is the asymmetric incorporation of histone H3.3 between the male and female pronucleus in the fertilized egg (Loppin et al. 2005; Torres-Padilla et al. 2006). Specific incorporation of H3.3 into the paternal genome is required for establishing heterochromatin in the decondensed sperm nucleus (Santenard et al. 2010). It is tempting to speculate

that Daxx has taken on this and other metazoan-specific challenges.

H3.3 Deposition at Regulatory Elements

Many DNA sequences with regulatory functions including transcription factor-binding sites (TFBSs), insulator elements, and origins of replication are characterized by fast histone exchange dynamics. These epigenetic regulatory elements acquire newly synthesized histones and are constitutively enriched in H3.3 (Mito et al. 2007; Nakayama et al. 2007; Jin et al. 2009; Deal et al. 2010; Goldberg et al. 2010). Neither HIRA nor ATRX deletion significantly altered the global incorporation of H3.3 at these sites (Goldberg et al. 2010), suggesting that a yet unknown assembly

pathway is used. Although general histone chaperones such as ASF1 or NASP could provide the histones for ATP-dependent chromatin remodelers that occupy these locations, it is important to note that this pathway is also specific to H3.3 (Goldberg et al. 2010). We therefore speculate that Daxx, Dek, or yet unknown factors mediate the deposition of H3.3 at regulatory elements (Fig. 5, pathway 4). Dek has recently been found to cooperate with ATP-dependent chromatin remodelers and to act as a coactivator for nuclear receptors (Sawatsubashi et al. 2010).

Intriguingly, H3K4 monomethylation is another hallmark of regulatory elements; it tracks well with exchange hotspots and H3.3 enrichment in genome-wide analyses (Deal et al. 2010; Goldberg et al. 2010). Which histone methyltransferase activity is responsible for establishing the H3K4me1 mark remains elusive, but we speculate that methylation and histone exchange are functionally linked at these genomic regions.

Alternate Pathways for H3.3 Deposition

As is apparent from the imperfect discrimination that can be observed in pure in vitro assays with variant-specific chaperones (Drané et al. 2010; Lewis et al. 2010), only the interplay and competition of variant-specific histone chaperones can explain the exquisite specificity of H3.3 incorporation observed in vivo. Whereas in unperturbed systems, H3.1 and H3.3 associate exclusively with their cognate chaperone systems (CAF-1, HIRA, and Daxx, respectively), we did observe CAF-1 copurifying with e-H3.3 in the absence of Daxx and HIRA (Fig. 5, pathway 5) (Drané et al. 2010; Lewis et al. 2010). Furthermore, there is evidence that low levels of H3.3 are broadly incorporated into chromosomes by the replication-dependent machinery in *Drosophila* cells (Ahmad and Henikoff 2002; Schwartz and Ahmad 2005). Therefore, similar to the single yeast H3.3-like histone, H3.3 might represent a universal variant for replication-dependent and -independent deposition pathways, i.e., simply outnumbered by the large pool of H3.1/2 present during S phase. Additionally, studies in flies suggest that H3.1/2 can take over some but not all functions of H3.3 (Hödl and Basler 2009; Sakai et al. 2009).

CONCLUSION

As illustrated by the replacement histone H3.3, rapidly emerging literature points to remarkable complexity for the use of relatively minor histone variants in punctuating epigenomes in organisms ranging from yeast to man. Multiple predeposition and assembly systems are called on to deposit histone variants in select genomic locations. Although these variants often differ in only a small number of amino acids, mounting evidence suggests that, once assembled, they perform specialized functions that remain poorly defined. It has been proposed that selective use of histone variants may contribute to a “nucleosome code” (Bernstein and Hake 2006), providing additional variation to that provided by chromatin remodeling and posttranslational modifications. We look forward to future experiments identifying and characterizing the predeposition complexes that engage

these variants, giving the field more mechanistic insights into how these variants make their complicated journeys from synthesis to distinct chromatin functions.

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