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MINI-REVIEW

At the right place at the right time: novel CENP-A binding proteins shed light on centromere assembly

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Abstract Centromeres, the chromosomal loci that form the sites of attachment for spindle microtubules during mitosis, are identified by a unique chromatin structure generated by nucleosomes containing the histone H3 variant CENP-A. The apparent epigenetic mode of centromere inheritance across mitotic and meiotic divisions has generated much interest in how CENP-A assembly occurs and how structurally divergent centromeric nucleosomes can specify the centromere complex. Although a substantial number of proteins have been implicated in centromere assembly, factors that can bind CENP-A specifically and deliver nascent protein to the centromere were, thus far, lacking. Several recent reports on experiments in fission yeast and human cells have now shown significant progress on this problem. Here, we discuss these new developments and their implications for epigenetic centromere inheritance.

Introduction

The centromere is a specialized chromosomal locus that drives the assembly of the kinetochore, the structure to which spindle microtubules attach during mitosis and meiosis, allowing accurate chromosome segregation (Allshire and Karpen 2008; Cleveland et al. 2003). Although centromeres are directly associated with chromosomal DNA, specific DNA sequences are neither required nor sufficient for centromere identity in most eukaryotes with the exception of budding yeast (Cheeseman et al. 2002). Rather, centromeres appear to be inherited through an epigenetic mechanism. Key evidence in support of an epigenetically maintained centromere is the generation of neocentromeres in human patients and experimentally in fission yeast on non-centromeric DNA (Ishii et al. 2008; Warburton 2004). Once formed, neocentromeres are mitotically stable and are, in some cases, inherited across generations (Amor et al. 2004). Despite the divergence in size and sequence of centromeric DNA among eukaryotes, the nature of centromeric chromatin is similar across species. All eukaryotes, thus far, feature a unique histone H3 variant, identified in humans as CENP-A (Cse4 in *Saccharomyces cerevisiae*, Cnp1 in *Schizosaccharomyces pombe*, HCP-3 in *Caenorhabditis elegans*, CenH3 in plants and CenH3 or CID in *Drosophila*) that replaces canonical histone H3 in nucleosomes at active centromeres and is responsible for nucleating the centromere/kinetochore complex (Foltz et al. 2006; Liu et al. 2006; Palmer et al. 1987; Regnier et al. 2005; Yoda et al. 2000). Overexpression of CENP-A^{CID} in *Drosophila* leads to misincorporation outside the centromere locus which in turn triggers ectopic recruitment of centromere and kinetochore components supporting the idea that CENP-A is the primary candidate for generating an epigenetic mark that specifies centromere identity and function (Heun et al. 2006).

Amide proton exchange studies have shown CENP-A nucleosomes to be more rigid compared to their canonical counterpart suggesting that, indeed, centromeric chromatin is structurally divergent from the rest of the chromosome (Black et al. 2004, 2007a). Strikingly, structural rigidity can be recapitulated in canonical histone H3 when substituting the 22 amino acids specific to CENP-A within the 40 amino acids that comprise loop1 and α2 helix within its histone

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fold. This domain was termed the CENP-A centromere targeting domain (CATD) as it is sufficient to target histone H3 to the centromere and functionally replace CENP-A (Black et al. 2004, 2007b). Interestingly, the biophysical measurements mentioned above showed, by deuterium exchange, that this domain is locked inside the nucleosome suggesting that CENP-A function is derived from the structural changes it induces in the nucleosome.

If, indeed, the structurally divergent centromeric nucleosome identifies the centromere, two questions become critical. First, epigenetic memory of centromere inheritance across mitotic and perhaps even meiotic divisions implies a positive feedback loop where the assembly of the centromeric nucleosome into chromatin is dependent on nucleosomes inherited from previous divisions. The question of how CENP-A is assembled and maintained is, therefore, a relevant one. Secondly, if the CATD domain of CENP-A is essential yet buried inside the nucleosome, how then is centromeric chromatin recognized by the centromere complex that is assembled on it? Now, reports on experiments in fission yeast and human cells discussed below shed light on both questions.

Structural centromere components affecting CENP-A assembly

The core centromere is comprised of proteins that are associated with CENP-A chromatin throughout the cell cycle referred to as the constitutive centromere-associated network (CCAN) that so far include, in addition to centromeric nucleosomes, 15 proteins; CENP-C, CENP-H, CENP-I, CENP-K through CENP-U, and CENP-W (Cheeseman and Desai 2008; Hori et al. 2008). Three different subsets of this collection of proteins are known as the CENP-A Nucleosome Associated Complex (CENP-A^{NAC}; Foltz et al. 2006), the CENP-H–I complex (Okada et al. 2006; Table 1), and the Interphase Centromere Complex (Izuta et al. 2006). Several members of the CCAN have been shown to affect CENP-A levels at the centromere that include CENP-H, CENP-I, CENP-K, and CENP-M (Okada et al. 2006). In flies, another member of this complex, CENP-C is also required for maintaining CENP-A levels at the centromere, although this requirement appears specific for this species (Erhardt et al. 2008). Members of the human Mis12 complex, although not constitutively centromeric, also influence CENP-A centromere occupancy (Kline et al. 2006).

Now, in a recent issue of Nature Cell Biology, Carroll and co-workers reported that among eight members of the previously described CENP-A nucleosome associated complex (Foltz et al. 2006), only CENP-N directly binds CENP-A nucleosomes in vitro (Carroll et al. 2009). Strikingly, efficient binding occurs only when CENP-A or histone H3 carrying the CATD domain of CENP-A is assembled into nucleosomes. No specific binding is found to H3 nucleosomes or prenucleosomal CENP-A/H4 tetramers. Moreover, CENP-N binding to CENP-A nucleosomes is not dictated by any specific nucleosomal DNA sequence. This leads to the proposal that CENP-N directly recognizes a structural aspect specific to the CENP-A nucleosome induced by the CATD domain that constitutes the epigenetic centromere mark (Fig. 2a). CENP-N is bound in turn by CENP-L, leading the way to the formation of the rest of the centromere complex. Mutations that affect CENP-N binding to the CENP-A nucleosome while retaining the ability to bind CENP-L result in loss of centromere function indicating that CENP-A nucleosome binding is essential for CENP-N function. Like for other members of the CCAN mentioned above, depletion of CENP-N affects CENP-A levels at the centromere. This defect results from an inability to assemble or stabilize newly synthesized CENP-A as measured by SNAP-based pulse labeling experiments (Carroll et al. 2009). It is, at present, unclear how these apparent structural components affect CENP-A assembly. The fact that these proteins are found associated with CENP-A nucleosomes (either directly or indirectly) but not found in soluble fractions of CENP-A (Dunleavy et al. 2009; Foltz et al. 2009) indicates that they do not represent assembly factors per se. Instead, they may serve as a platform for specific CENP-A loading factors to target to centromeres or, alternatively, their complex formation onto centromeric chromatin may stabilize CENP-A nucleosomes. Importantly, all of these proteins are themselves dependent on CENP-A for their localization to the centromere (Carroll et al. 2009; Foltz et al. 2006; Liu et al. 2006). The intriguing implication of this is that one or more members of the CCAN may form the molecular basis for an epigenetic feedback loop to control the propagation of active centromeres (Fig. 2c).

The role of histone chaperones

If not structural centromere components, what then are the activities that escort nascent CENP-A to the centromere? The fission yeast Mis16 protein, homologous to human RbAp46 and RbAp48, is required for CENP-A localization at the centromere (Hayashi et al. 2004). Indeed, both human proteins also affect CENP-A assembly (Dunleavy et al. 2009; Hayashi et al. 2004). Both are members of the CAF-1 complex that is responsible for the assembly of canonical histone H3.1 during S phase (Verreault et al. 1996). RbAp48 is also a member of the HIRA complex, controlling assembly of the H3.3 variant that exchanges into chromatin preferentially at transcriptionally active regions (Tagami et al. 2004). CENP-A, when overexpressed, can interact with RbAp48 in *Drosophila* and human cells (Dunleavy et al. 2009;

Furuyama et al. 2006). However, the fact that RbAp48 is involved in the assembly process of all histone H3 variants suggests that it serves as a general histone chaperone possibly through direct binding to histone H4 that is common to all H3 prenucleosomal complexes. These findings have led to proposals that perhaps the CENP-A assembly process occurs through a default pathway. Centromere specificity is then achieved either by targeting competing histone H3.1 and H3.3 elsewhere (Furuyama et al. 2006) or by generating available sites for CENP-A assembly (e.g., by evicting histone H3 nucleosomes) only at the centromere (Dalal 2009). While these are rather unlikely models, they obviate the need for a specific CENP-A assembly factor. In fission yeast, the Sim3 protein not only binds CENP-A^{Cnp1} but also displays affinity for canonical H3, like RbAp48/46 as well as the Sim3-related human NASP (Dunleavy et al. 2007; Tagami et al. 2004). Although Sim3 appears to prefer CENP-A^{Cnp1} as a partner, it is not centromere-localized and, therefore, has been proposed to act as an intermediate histone chaperone that delivers CENP-A to assembly factors (Dunleavy et al. 2007).

Cell cycle control of CENP-A assembly

A critical advance in determining the mechanism of CENP-A assembly has been the identification of a discrete cell cycle window during which CENP-A loading into chromatin occurs. GFP-CENP-A^{CID} photo-bleaching experiments in rapidly cycling *Drosophila* syncytial embryos showed fluorescence recovery only during a brief window following mitotic exit concurrent with an increase of overall GFP-CENP-A^{CID} levels at the centromere (Schuh et al. 2007). This suggests that centromeric chromatin is assembled during this time window. Direct evidence for the timing of CENP-A assembly came from fluorescent pulse labeling experiments based on SNAP-tagging in human cells that allow for specific visualization of a newly synthesized pool of CENP-A (Jansen et al. 2007). These experiments showed that CENP-A can be expressed at any stage during the cell cycle but will target to centromeres only following exit from mitosis during the first hours of G1 phase (Figs. 1 and 2). This finding was confirmed by photo-bleaching experiments and is consistent with the results in fly embryos (Hemmerich et al. 2008; Schuh et al. 2007). Early G1 loading of new CENP-A, thus, appears to be a general phenomenon across animals. Strikingly, once assembled, CENP-A nucleosomes are extremely stable and are quantitatively recycled during subsequent rounds of DNA replication consistent with a role in heritably maintaining centromere identity (Jansen et al. 2007). Fission yeast cells enter S phase almost immediately following exit from mitosis. It is at this time that CENP-A^{Cnp1} is expressed and assembled suggesting a temporal control similar to that found in higher eukaryotes. However, unlike in fly embryos and human tissue culture cells, there is a second wave of assembly during the fission yeast G2 phase (Takahashi et al. 2005).

Being at the right place at the right time

Given the conserved cell cycle control of CENP-A assembly, one would expect that if specific factors exist that propagate centromeric chromatin, they would be centromere-localized within the appropriate cell cycle window. Indeed, a set of proteins has recently been identified that meet this requirement. One of the founding members of this group, Mis18, was identified in fission yeast and was shown to be required for CENP-A^{Cnp1} localization to the centromere (Hayashi et al. 2004). Strikingly, Mis18 is absent from centromeres during mitosis but accumulates there following mitotic exit. This temporal localization pattern is conserved in a complex of human proteins that include the Mis18 homologs hMis18α and hMis18β as well as an associated protein, M18BP1 that was independently identified as the human homolog of the *C. elegans* KNL-2 protein (Fujita et al. 2007; Maddox et al. 2007). All three proteins arrive at the centromere during anaphase and leave 2–3 h later in mid-G1 (Fujita et al. 2007). Depletion of any of these proteins severely impacts CENP-A localization at the centromere. Importantly, CENP-A assembly closely follows hMis18/HsKNL2 targeting in anaphase ensuing shortly thereafter in telophase (Fig. 1; Fujita et al. 2007; Jansen et al. 2007; Maddox et al. 2007). Despite their suggestive localization pattern and clear role in CENP-A assembly, none of the yeast or human Mis18/HsKNL2 proteins appear to bind to CENP-A directly (Fujita et al. 2007; Hayashi et al. 2004). In addition, they have not been found in proteomic screens for CENP-A nucleosome or prenucleosome binding factors (Dunleavy et al. 2009; Foltz et al. 2006, 2009).

Recently, Rsf-1, a component of the ATP-dependent chromatin remodeling and spacing factor RSF, was added to the list of proteins temporally transiting the centromere. Rsf-1 localization occurs in mid-G1, several hours after the hMis18/HsKNL2 proteins arrive there (Perpelescu et al. 2009). Unlike the (h)Mis18/HsKNL2 proteins, Rsf-1 as well as its partner SNF2h associates with CENP-A chromatin at the mononucleosome level. RSF does not appear to be involved in targeting CENP-A as Rsf-1 localization occurs well after nascent CENP-A arrives at the centromere and Rsf-1 depleted cells show normal CENP-A levels at centromeres. However, centromeric CENP-A is sensitive to salt extraction in these cells. This has led Perpelescu and colleagues to suggest a model in which CENP-A is initially targeted to centromeres in early G1 after which it is assembled into

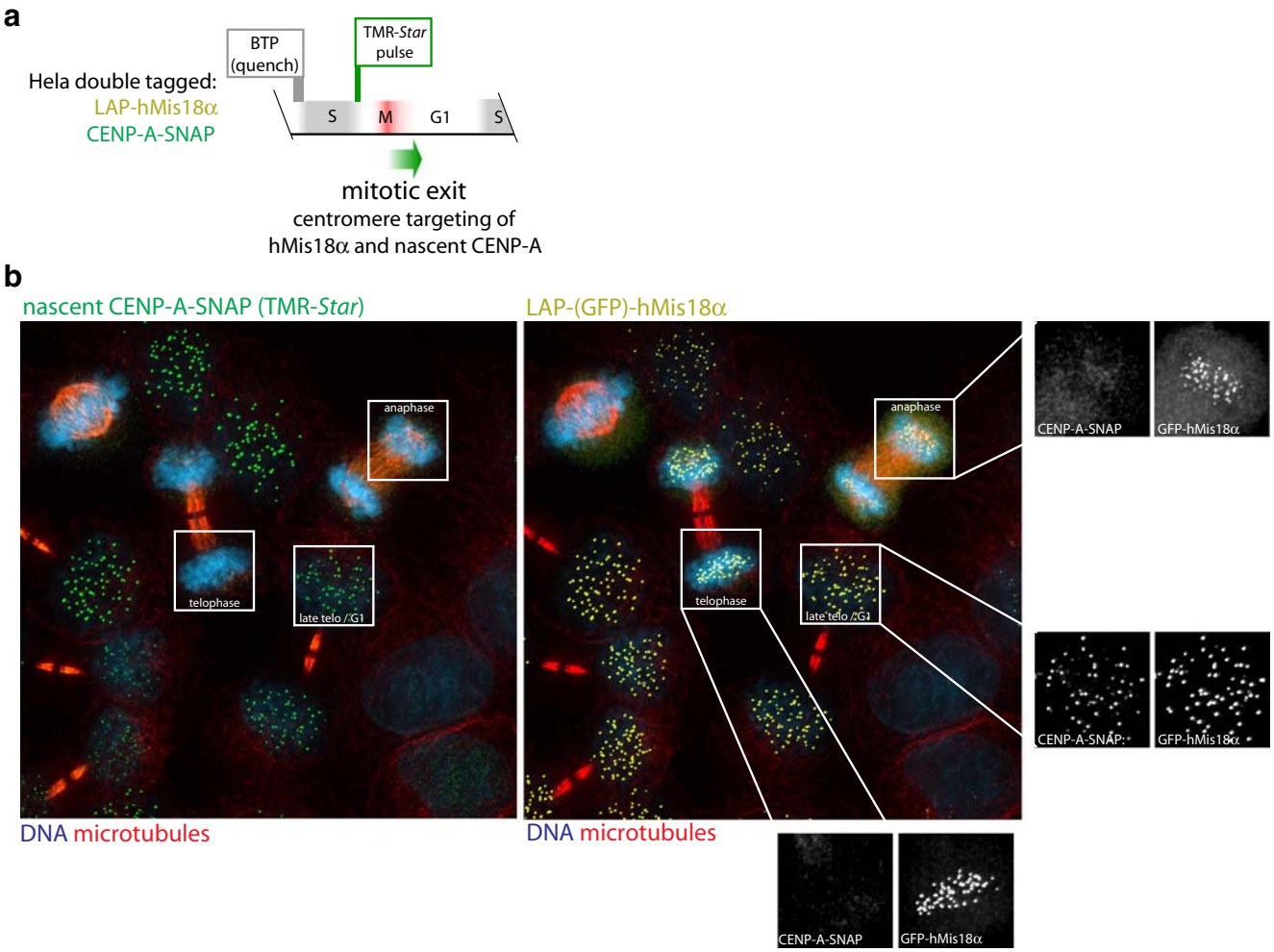

Fig. 1 hMis18α targets to centromeres prior to CENP-A assembly. **a** An S-phase synthesized pool of CENP-A-SNAP was specifically labeled with fluorescent TMR-Star by quench-chase-pulse labeling as described (Jansen et al. 2007) in HeLa cells stably expressing both CENP-A-SNAP and LAP-(GFP)-hMis18α. **b** A single four channel field is shown as two separate images with TMR-labeled nascent CENP-A-SNAP in green and LAP-tagged hMis18α in yellow. *Grayscale blowups* show differential timing of centromere loading (hMis18α in anaphase, newly synthesized CENP-A in telophase). Microtubules and DNA are shown in *red* and *blue*, respectively

chromatin or otherwise stabilized through RSF action. It remains to be demonstrated, however, whether the salt-sensitive pool observed represents newly targeted CENP-A. Nevertheless, consistent with the model proposed, RSF was found to have general nucleosome assembly and/or spacing activity in vitro (LeRoy et al. 1998; Perpelescu et al. 2009). Interestingly, a similar ATP-dependent chromatin remodeling factor, Hrp1 in fission yeast has previously been shown to associate with centromeric repeats and affect centromeric CENP-A^{Cnp1} levels (Walfridsson et al. 2005).

Novel factors binding soluble CENP-A and their function in centromere assembly

If targeting of CENP-A to the centromeres is mediated by a specific assembly factor one would predict such a component to bind nascent CENP-A specifically as well as deliver it to centromeres at the right moment, a requirement not met in an obvious way by any component described, thus far. Two recent reports in *Molecular Cell* and two reports in *Cell* now make clear progress on this problem in fission yeast and human cells, respectively.

Pidoux et al. and Williams et al. identify the *S. pombe* Scm3 homolog of the budding yeast protein of the same name and demonstrate its requirement for CENP-A^{Cnp1} recruitment to centromeres and its direct interaction with CENP-A^{Cnp1}, both in vivo and in vitro (Pidoux et al. 2009; Williams et al. 2009). The interaction between Scm3 and CENP-A^{Cnp1} is dependent on Sim3, suggesting that this histone chaperone may hand over CENP-A^{Cnp1} to Scm3^{Sp} or form a complex with these two proteins (Pidoux et al. 2009). Furthermore, Scm3^{Sp} localizes to the centromere in a cell-cycle-dependent manner, resembling Mis16 and

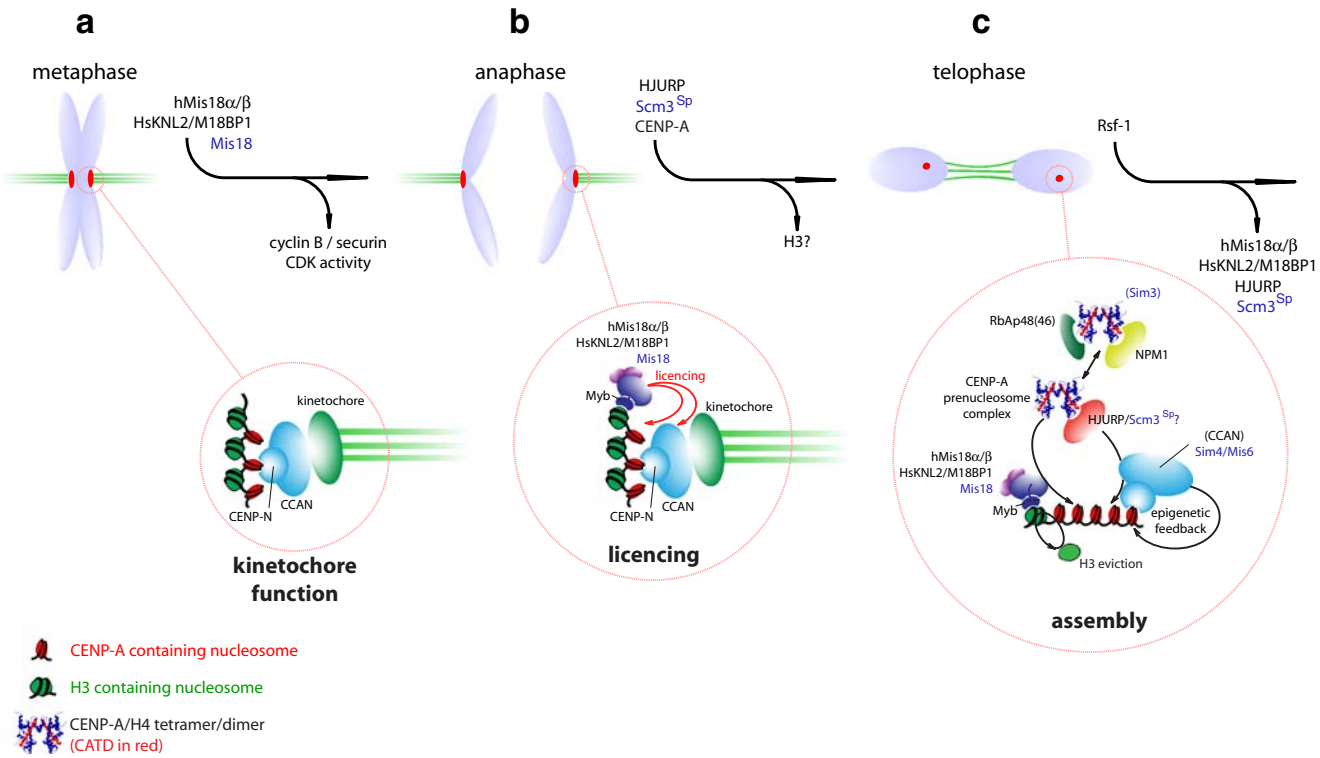

Fig. 2 **a**–**c** Model of the CENP-A assembly process emphasizing temporal control and focusing primarily on the early steps in centromeric chromatin assembly and the role of recently identified components. The human situation is depicted. Functional homology between human and fission yeast proteins (*in blue*) are indicated where appropriate

Mis18 temporal localization consistent with a direct role in CENP-A assembly.

Two independent efforts by Foltz et al. and Dunleavy et al. in human cells identify Holliday junction recognizing protein (HJURP; Kato et al. 2007) as a specific component of the CENP-A prenucleosomal complex but not H3.1 complexes by purification of soluble non-chromatin-bound histone fractions (Dunleavy et al. 2009; Foltz et al. 2009). This complex also includes nucleophosmin (NPM1) and histone H4. While both NPM1 and HJURP were found at centromeric chromatin at low levels in a previous study (Foltz et al. 2006), they are abundant in the CENP-A prenucleosomal complex. HJURP depletion severely impacts nascent CENP-A assembly as shown by SNAP-based pulse labeling and transient expression of GFP-CENP-A. Moreover, HJURP localizes to centromeres during late telophase through early G1, suggesting that it acts as a specific chaperone that can function as a CENP-A targeting factor. HJURP sequences reveal five conserved tryptophan residues similar to the tryptophan-aspartate (WD40) repeats found in other chromatin assembly factors, such as CAF1p60 and HIRA. Consistent with a role as a CENP-A chaperone, HJURP readily binds to CENP-A/H4 in vitro but not to H2A/H2B dimers (Foltz et al. 2009). This binding is mediated through the CATD that is responsible for maintaining centromeric chromatin structure (Foltz et al. 2009). The role of NPM1 in CENP-A assembly is unclear as it has affinity to a broad set of histones. In addition, modest depletions do not affect CENP-A assembly, and it appears to be present in prenucleosomal complexes distinct from those containing HJURP (Dunleavy et al. 2009). Perhaps similar to Sim3 and RbAp48, NPM1 may complex with an inactive pool of nascent CENP-A that transfers CENP-A/H4 to HJURP (Fig. 2c). Intriguingly, it has been previously reported that HJURP can relocalize to nuclear foci upon induction of DNA damage and can bind to holiday junctions in vitro (Kato et al. 2007). This suggests that HJURP may bind DNA directly, perhaps through secondary structures. It will be of interest to determine whether such conditions exist at the centromere region induced either by DNA damage or otherwise.

Functional conservation of the CENP-A assembly process

Although Scm3^{Sp} and HJURP do not display any obvious sequence homology, they appear to play strikingly similar roles in CENP-A assembly. Both Scm3^{Sp} and HJURP are targeted to centromeres following mitotic exit. Scm3^{Sp} stays centromere-localized through the fission yeast S and G2 phases (Pidoux et al. 2009) while HJURP localization

Table 1 Overview of fission yeast and human proteins involved in CENP-A localization or assembly

S. pombe	Human	Centromere localized	CENP-A associated	References
Ams2	–	Central core and inner repeats	Not reported	(Chen et al. 2003; Takahashi et al. 2005)
Hrp1	CHD1[a]	Transiently to inner and outer repeats in *S. pombe*	Not reported	(Walfridsson et al. 2005)
–	Rsf-1	Transient centromere component in mid G1	Yes (CENP-A chromatin)	(Perpelescu et al. 2009)
Sim3	NASP[a]	No	Yes in *S. Pombe*, not reported in human	(Dunleavy et al. 2007)
Sim4	CENP-K	Constitutive centromere component	Indirect	(Okada et al. 2006; Pidoux et al. 2003)
Mis6	CENP-I	Constitutive centromere component	Indirect	(Okada et al. 2006; Takahashi et al. 2000)
Mis15	CENP-N	Constitutive centromere component	Yes in human (nucleosomal CENP-A only)	(Carroll et al. 2009; Foltz et al. 2006; Hayashi et al. 2004; McClelland et al. 2007)
Mis17	CENP-M	Constitutive centromere component	Indirect	(Fukagawa and De Wulf 2009; Hayashi et al. 2004)
Mis12[a]	hMis12	Constitutive but in human cells absent during G1	Indirect	(Cheeseman and Desai 2008; Kline et al. 2006)
Mis16	RbAp46/RbAp48	Transient in fission yeast, absent in human cells.	Yes in human cells and Drosophila	(Dunleavy et al. 2009; Furuyama et al. 2006; Hayashi et al. 2004)
–	M18BP1/HsKNL2	Transient centromere component in early G1	Not detected	(Fujita et al. 2007; Maddox et al. 2007)
Mis18	hMis18α and β	Transient centromere component in early G1	Not detected	(Fujita et al. 2007; Hayashi et al. 2004)
–	HJURP	Transient centromere component in early G1	Yes	(Dunleavy et al. 2009; Foltz et al. 2009)
Scm3	–	Transient centromere component	Yes	(Pidoux et al. 2009; Williams et al. 2009)

Proteins are listed according to their ability to localize to centromeres and/or to bind CENP-A

[a] Ortholog for which no role in CENP-A assembly has been found or reported

occurs only in hMis18α centromere-positive cells in early G1 (Foltz et al. 2009), roughly consistent with the times of CENP-A assembly in the respective organism. Both proteins interact directly with CENP-A. HJURP is part, primarily, of the CENP-A prenucleosomal complex. Whether $Scm3^{Sp}$ binds to soluble prenucleosomal CENP-A has not been directly tested. However, in vitro binding experiments show $Scm3^{Sp}$ binding can occur to recombinant non-nucleosomal CENP-A. Indeed, the budding yeast Scm3 protein forms a trimeric complex with Cse4 and H4 in vitro in striking similarity to the HJURP prenucleosomal complex (Mizuguchi et al. 2007). In addition, both HJURP and $Scm3^{Sp}$ appear to cooperate with RbAp48/46 (Mis16 in the case of $Scm3^{Sp}$). HJURP and RbAp48 can both bind prenucleosomal CENP-A, although they do not appear to be in the same complex (Dunleavy et al. 2009). Nevertheless, depletions of RbAp48 and RbAp46 result in loss of HJURP and consequently in a CENP-A assembly defect suggesting that RbAp48/46 are required for maintaining the prenucleosomal CENP-A assembly complex (Dunleavy et al. 2009). Similarly, $Scm3^{Sp}$ was shown to interact with Mis16 at least transiently and dependents on Mis16 for its centromere localization (Williams et al. 2009). Taken together, at least at a functional level, the HJURP and $Scm3^{Sp}$ proteins appear to have analogous roles in recruiting CENP-A to centromeres. It should be noted though that several homologs of Scm3 carry Myb domains (Aravind et al. 2007). It is, therefore, possible that at least in some fungal species, Scm3 may serve a role reminiscent of the Myb domain containing M18BP1/HsKNL2 protein (discussed below). Current evidence, however, does not favor this possibility since fission yeast Scm3 lacks a Myb domain, and the M18BP1/HsKNL2 protein does not appear to bind to CENP-A.

How does the prenucleosomal loading complex target to the centromere?

$Scm3^{Sp}$ localization at centromeres depends on Mis18 as well as on the constitutive centromere components Sim4 and Mis6 (Pidoux et al. 2009; Williams et al. 2009). Similarly, CENP-A assembly in human cells depends on members of the constitutive centromere complex (CCAN)

as well as the hMis18/HsKNL2 proteins (Carroll et al. 2009; Fujita et al. 2007; Maddox et al. 2007; Okada et al. 2006). What emerges is a model where the CENP-A prenucleosome complex is targeted to centromeres by HJURP in human cells or Scm3 in fission yeast through an interaction either with the CCAN or by binding the Mis18/KNL2 proteins (Fig. 2c). Indeed, in support of the latter possibility, $Scm3^{Sp}$ has been shown to interact with Mis18 both by pull downs as well as by in vitro assays providing a molecular link between CENP-A and the Mis18 proteins that was, thus far, lacking (Pidoux et al. 2009). The extended question becomes how the Mis18/KNL2 proteins are targeted to the centromere themselves. Intriguingly, the human hMis18/HsKNL2 proteins arrive at the centromere before CENP-A assembly (Fig. 1; Fujita et al. 2007; Jansen et al. 2007; Maddox et al. 2007) in a manner that is largely unaffected by severe depletions of CENP-A chromatin (Fujita et al. 2007; Hayashi et al. 2004). Consistent with this, the M18BP1/HsKNL2 protein sports a divergent Myb/SANT domain suggesting the complex may be targeted directly to DNA or histone tails (Boyer et al. 2004). Although complete loss of CENP-A may ultimately lead to complete centromere disruption, these experiments suggest that the Mis18/KNL2 proteins target to centromeres distal from CENP-A nucleosomes and "license" the centromere for recruitment of new CENP-A nucleosomes that ensues shortly thereafter (Fig. 2b). The term "licensing" or "priming" refers here to some action at a distance fuelled largely by the lack of any detectable interaction between (h)Mis18/HsKNL2 and CENP-A. Fission yeast mis18 (and mis16) mutants display elevated H3 and H4 acetylation levels specifically in the central core where $CENP\text{-}A^{Cnp1}$ binds (Hayashi et al. 2004). In human cells, the consequences of hMis18α depletion can be alleviated by experimentally increasing global acetylation levels (Fujita et al. 2007). Although, at first glance, the fission yeast and human cell experiments appear to give exactly opposite results, acetylation of an as of yet unknown target may be involved in centromere licensing for CENP-A assembly. The target for licensing may be centromeric nucleosome components or member of the CCAN which would in turn allow for recruitment of the CENP-A/HJURP prenucleosomal complex (Fig. 2b, c).

How the HJURP-CENP-A/H4 complex targets to the centromere in human G1 cells remains to be tested. It is tempting to speculate that analogous to fission yeast the hMis18/HsKNL2 proteins may recruit HJURP directly to the centromere. Alternatively, HJURP binds a centromere compartment that is modified through hMis18/HsKNL2 licensing. Members of the CCAN that bind CENP-A through CENP-N and have been shown to affect CENP-A assembly would be obvious candidates. In this way, the epigenetic feedback loop would be closed explaining how epigenetic centromere identity provided by the CENP-A nucleosome is transferred to the next generation of nucleosomes set to survive into subsequent cell divisions. Further in vitro binding studies reminiscent of those conducted by Carroll and colleagues for CENP-A nucleosomes (Carroll et al. 2009) are bound to shed light on this issue.

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