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Biochimica et Biophysica Acta 1677 (2004) 3–11
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
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Review

Histone chaperones, a supporting role in the limelight

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Received 29 July 2003; received in revised form 25 September 2003; accepted 25 September 2003

Abstract

In eukaryotic cells, highly basic histone proteins are associated with the DNA to form the nucleosome, the fundamental unit of chromatin. Histones are closely escorted by histone chaperones from their point of synthesis up to their delivery site. We will present an overview of the histone chaperones identified to date with their various roles, in an attempt to highlight their importance in cellular metabolism. Nucleoplasmin will illustrate a role in histone storage and Nap-1, a histone translocator. CAF-1 and Hira will provide examples of distinct histone deposition factors coupled to and uncoupled from DNA synthesis, respectively, while Asf1 could act as a histone donor. We then will illustrate with two examples how histone chaperones can be associated with chromatin remodeling activities. Finally, we will discuss how the RbAp46/48 proteins, as escort factors, are part of multiple complexes with various functions. Based on these examples, we will propose a scheme in which the diverse roles of histone chaperones are integrated within an assembly line for chromatin formation and regulation. Finally, we discuss how these chaperones may have more than a supporting role in a histone metabolic pathway.
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Keywords: Histone chaperone; Nucleoplasmin; Nap-1; CAF-1; Hir/Hira; Asf1

1. Introduction

Histones are the main structural proteins associated with DNA in eukaryotic cells. They can be divided into two groups: the core or nucleosomal histones and the linker histones of the H1 family. The four core histones H2A, H2B, H3, and H4 associate as H2A/H2B dimers and H3/H4 tetramers to then form the histone octamer, containing two H2A/H2B dimers and a H3/H4 tetramer around which the DNA is wrapped within the nucleosome—the chromatin unit. These core histones are small highly basic proteins, ranging between 11 and 16 kDa, with more than 20% of their amino acid composition being lysines and arginines. They represent some of the most conserved proteins in eukaryotes, highlighting the importance of their role in organizing DNA folding (for recent reviews see Refs. [1,2]).

Structural studies have shown that core histones are comprised of two distinct domains. The histone fold domain, formed by three α-helices connected by two loops is involved in histone/histone and histone/DNA interactions, while the N-terminal tail domain is composed of about 15–30 highly basic amino acids, which protrude out of the nucleosome surface. The N-terminal tails are the main sites of several covalent post-translational modifications, including acetylation, methylation and phosphorylation, which are involved in the regulation of chromatin dynamics. These covalent modifications have been hypothesized to function as "a histone code", which theoretically could be used in two ways: as a short-term signal to activate or repress a specific gene in response to cellular demands; and as a stable marking system that could be used during cellular differentiation to determine specific chromatin states that are inherited in an epigenetic manner (for a recent review see Refs. [2,3]).

Importantly, the synthesis of core histones is highly regulated and balanced. Indeed, in *S. cerevisiae* over-expression of either H2A/H2B or H3/H4 pairs, leads to frequent chromosome loss [4]. Therefore, the stoichiometry between histone pairs has to be controlled to ensure the maintenance of genome integrity.

In proliferating cells, the bulk of histone synthesis occurs during the S phase of the cell cycle and is coupled to DNA synthesis, in order to ensure the assembly of chromatin on the newly replicated DNA. However, a lower level of histone synthesis also takes place outside of S phase, arising

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doi:10.1016/j.bbaexp.2003.09.012

from a set of histone genes called the "histone variants" or "replacement histones", including H3.3, H2A.X, H2A.Z, and CENP-A, among others. Based on the timing of their synthesis, it is believed that these variants can be deposited onto the DNA independently of DNA synthesis. Indeed, as an example, the histone variant H3.3 in *Drosophila* can be incorporated into chromatin outside of S phase, and the sites of incorporation correspond to genomic loci that are highly transcribed [5]. These histone variants might be used to mark specific regions or to act as a signal that helps recruit factors that activate/repress transcription, or both. Thus histone variants, along with the modifications of the histone tails, can be involved in the setting of an "epigenetic code". In any case, it is clear that the relative importance of replacement versus S phase histones will be directly related to the cellular proliferative state.

Interestingly, histones interact with a variety of proteins, among which the histone chaperones have regained interest recently. In general terms, a molecular chaperone is a protein that associates with a target protein and prevents its misfolding, thereby avoiding the production of inactive or aggregated forms. By extension, histone chaperones are proteins that associate with histones to facilitate their interaction with other molecules without being a component of the final reaction product. Since histones are found as H2A/H2B and H3/H4 pairs, histone chaperones have preferential affinity for one of these pairs. A list of histone chaperones and their presumed cellular role is presented in Table 1.

In this review we will use selected examples to illustrate the variety of roles that histone chaperones can play as supporting partners in regulating histone metabolism, as represented schematically in Fig. 1. We will focus on the following chaperones:

1. Nucleoplasmin, the founder member of the histone chaperone group, as a typical acceptor involved in histone storage.
2. Nap-1, a histone translocator acting as a shuttle from the cytoplasm to the nucleus.
3. CAF-1, a histone DNA-deposition factor linked to DNA synthesis.
4. Hira, a histone DNA-deposition factor independent of DNA synthesis.
5. Asf1, a histone donor at the crossroads of different assembly pathways.
6. Rsf-1 and Arps, associated to chromatin remodeling factors.
7. RbAp46/48 family, as histone escort proteins involved in various histone-related complexes.

We will then highlight the fact that histones appear constantly escorted and assisted in their various moves, and also use these paradigms to propose a scheme in which the diverse roles of histone chaperones are integrated within an assembly line.

2. Nucleoplasmin: the founder member of the histone chaperones, a histone acceptor for storage or a histone sink?

The histone chaperone nucleoplasmin represents the most abundant protein in the *Xenopus* oocyte nucleus. This acidic protein is complexed with the maternal pool of histones, mainly histones H2A/H2B, whereas N1/N2, another histone chaperone, is associated with histones H3/H4. These complexes allow the progressive release of the histones after fertilization, which ensures nucleosomal assembly during the rapid cell divisions in early development.

Table 1
Histone chaperones discussed in the text

Histone chaperones	Complex and main associated factors	Related function
(*x*) Nucleoplasmin (*m*) Nucleosplasmin 1–3	homomultimeric complex	storage of H2A/H2B pools in *Xenopus* oocyte to be used for chromatin assembly, sperm chromatin decondensation, histone acceptor
(*x*) N1/N2	–	storage of H3/H4 pools in *Xenopus* oocyte to be used for chromatin assembly
(*Sc*, *d*, *m*, *h*) NAP-1	–	cytoplasmic-nuclear H2A/H2B transfer, cell cycle regulator?
(*Sc*) Cac1, Cac2, Cac3 (*d*) p150, p105, p55 (*h*) p150, p60, p48	CAF-1 complex	
(*Sc*) Hir1, Hir2 (*d*, *x*, *m*, *h*) HIRA	2 MDa complex of unknown components	chromatin assembly independent of DNA replication
(*Sc*) Spt16/Cdc68, Pob3 (*d*, *x*, *h*) hSpt16, SSRP1	FACT	chromatin-specific RNA polymerase II transcription elongation factor
(*Sc*, *d*) ASF-1 (*m*, *h*) ASF1a and b	RCAF: Asf-1, histones H3 and H4	putative histone transfer, synergy with CAF-1 activities
Arps: (*Sc*) Arp4, (*Sc*) Arp8	SWI/SNF, RSC, NuA4, INO80 containing ISWI	regulation of chromatin structure, connecting histones to a nucleosome-remodeling complex, putative role in DNA repair and transcription
(*h*) Rsf-1	RSF: Rsf-1 and hSNF2H	chromatin assembly independent of DNA replication, ATPase, nucleosome spacing
(*Sc*) Cac3 (*d*, *x*) p55 (*h*) RbAp46/48	several complexes, for details, see text	histone-binding escort

(*nd*) not determined; (*Sc*) *S. cerevisiae*; (*d*) *Drosophila*; (*x*) *Xenopus*; (*m*) mouse; (*h*) human.

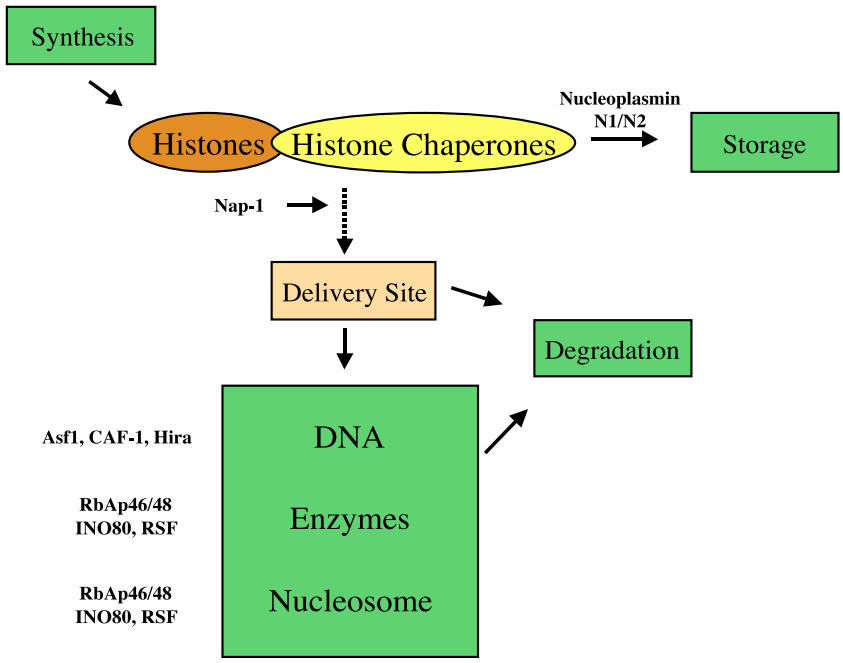

Fig. 1. Histones and their supporting partners. A schematic representation of histone metabolism is depicted. Starting from their synthesis in the endoplasmic reticulum, histones soon interact with a histone chaperone. The escorted histones are then taken to their destination site by specific chaperones. Among them, and as illustrated in the figure, *Nucleoplasmin* functions in histone storage, *NAP-1* as a histone nucleus-cytoplasm translocator, *CAF-1* as a nucleosome assembly factor during DNA synthesis and repair, *HIRA* as nucleosome assembly factor independent from DNA synthesis, *ASF1* as a histone donor, and the ISWI-associated chaperones and *RbAp46*/*48* family as links to enzymatic activities. Finally, in order to keep the necessary stoichiometry between histones, and also due to normal protein turnover, histones are degraded. We also speculate that their degradation must be under surveillance by chaperones.

Crystallographic studies of the nucleoplasmin core show that a pentamer of nucleoplasmin dimerizes to form a decamer that is competent for the association of five H2A/H2B dimers or five histone octamers, ideal for such a storage role [6]. By virtue of its charge, nucleoplasmin can promote in vitro nucleosome formation, yet this capacity for histone deposition has not been linked to any aspect of DNA synthesis. Therefore, a role of these kinds of chaperones during early development in *Xenopus* could be to maintain a histone pool available for other chaperones to deposit onto the DNA (i.e. N1/N2 would transfer histones H3/H4 to CAF-1 to load them onto the DNA, see below).

Nucleoplasmin also participates in the decondensation process that the highly compacted sperm chromatin undergoes after fertilization. Indeed, in sperm chromatin, canonical histones are usually replaced by histone variants, mostly protamines, which are removed during fertilization. In *Xenopus* sperm histones, H3 and H4 are maintained and H2A/H2B replaced by specialized histones X and Y. In this context, nucleoplasmin was shown to serve as an acceptor at the time of fertilization to remove the spermatic histones and facilitate the incorporation of maternal H2A/H2B dimers [7]. Similarly, if somatic cell nuclei are injected into the cytoplasm of an egg, for instance during cloning procedures, they also undergo a remodeling process that results in transcriptional reprogramming of the transplanted nucleus. Analysis of the changes associated with this reprogramming showed that the removal of the linker histone H1 from the somatic chromatin can be detected at early stages and depended on nucleoplasmin in *Xenopus* egg extracts [8].

The general "histone acceptor" properties of nucleoplasmin might also have implications in transcriptional regulation. Indeed, in vitro experiments have shown that nucleoplasmin can stimulate the binding of several transcription factors to DNA [9]. It probably carries out this function by acting as a "histone sink" that keeps the nucleosomes in a dynamic state. While this hypothesis still awaits in vivo confirmation, it is worth noting that it might also apply to other histone chaperones.

In mouse, three nucleoplasmin proteins have been identified. Knocking out the *nucleoplasmin-2* gene gives rise to sterile females [10]. Microscopic analysis of the oocyte chromatin derived from mutant females showed an amorphous and diffuse DNA staining, with no condensation around the nucleolus. Further analysis of the nucleolus marker fibrillarin showed a dispersed staining pattern, confirming the abnormal nucleolar structures. Upon fertilization of these mutant oocytes, the sperm chromatin showed a normal initial decondensation, perhaps due to redundant properties provided by one of the two other nucleoplasmin-like proteins. Nevertheless, when the zygote was analyzed, it showed the same structural defects as the oocyte [10], arguing for a unique role of nucleoplasmin-2 in nuclear organization in vivo. The inactivation of nucleoplasmin-1 and -3 will reveal whether these mutants display similar or distinct phenotypes.

These data obtained with nucleoplasmin illustrate how a chaperone can function as a histone acceptor and regulate histone exchange among different pools in a variety of situations. In *Xenopus*, nucleoplasmin functions as storage factor in oocytes to maintain the highly concentrated histone pools; while during fertilization, it helps decondense the sperm chromatin. Furthermore, in mouse development, one of the nucleoplasmins is important for normal nuclear organization.

3. Nap-1: a histone shuttle from the cytoplasm to the nucleus and a cell cycle regulator?

Nap-1 (nucleosome assembly protein-1) was originally identified in HeLa cell extracts as an activity that facilitates the in vitro reconstitution of nucleosomes using pure histones in combination with other factors [11]. Based on sequence homology, a family of Nap-1 proteins has been described, many of them with unknown functions.

Two in vivo studies point towards a role for Nap-1 in the regulation of gene expression, possibly through the maintenance of chromatin in a dynamic state. A genome-wide analysis in *S. cerevisiae* showed that the expression of about 10% of all genes were affected in a *nap-1*-deficient yeast strain, with some genes being up-regulated and others down-regulated. A detailed analysis of chromosome IV showed that 35% of the genes affected were clustered together, suggesting that Nap-1 might function in large chromosomal domains rather than at local restricted sites [12]. In mouse, knocking out the neuron-specific *nap1/2* gene resulted in embryonic lethality at the mid-gestation stage. This is accompanied by an overproduction of neural precursor cells, arguing for a role of Nap-1 in the regulation of neuronal cell proliferation [13]. In *S. cerevisiae*, Nap-1 also participates in the control of mitotic events through an interaction with Clb2, a protein belonging to the cyclin B family. This interaction seems to be conserved in other species, including *Xenopus* [14]. Taken together, these data argue for a role of Nap proteins in cell cycle control. Whether this role is direct or indirect, remains to be determined.

Intriguingly, microscopic analyses showed that while Nap-1 is located in the cytoplasm during the G2 phase of the cell cycle, it is found in the nucleus in S phase [15], suggesting that Nap-1 could shuttle histones between the cytoplasm and the nucleus. This hypothesis is further supported by the finding that Nap-1 interacted with Kap114p, a member of the karyopherin/importin family of proteins, responsible for the nuclear import of H2A and H2B [16]. Based on its higher affinity for histones H2A/H2B, Nap-1 could act specifically to shuttle histones H2A/H2B. An open issue, then, is whether a specific H3/H4 translocator can also be found.

Obviously, further studies are necessary in order to understand the role of Nap-1 in mitotic control and histone translocation, and to determine if the two properties are conserved in various species and whether they are interrelated.

Therefore, Nap-1 is a family of histone chaperones with a potential role in the transfer of histones from the cytoplasm into the nucleus and in the control of the cell cycle. It is tempting to speculate that Nap-1 could serve as a link between cell cycle control and the nuclear localization of histones. Further studies are needed to clarify this point.

4. CAF-1: nucleosome deposition linked to DNA synthesis

To date, CAF-1 (chromatin assembly factor-1) is the only chromatin assembly factor whose function is tightly associated with DNA replication and DNA repair (for a review see Ref. [17]). CAF-1 was initially identified by biochemical fractionation of extracts derived from human HeLa cells as an activity that allowed chromatin assembly coupled to DNA replication in the SV40 viral system [18] and was later found to be also required during DNA repair [19].

In cells, CAF-1 is found associated with histones H3/H4 carrying the acetylation pattern of newly synthesized histones, i.e. acetylated lysines 5 and 12 of the histone H4 tail. In most species, CAF-1 consists of three components: the large, mid, and small subunits (see Table 1). In humans, the subunits correspond to p150, p60, and p48, and in budding yeast to Cac1, Cac2, and Cac3 (for a review see Ref. [20]). The small subunit, p48/Cac3, a member of the RbAp46/48 family, directly interacts with histones and is found in several other chromatin-related complexes, as discussed below.

In humans, p150, the largest subunit of CAF-1, interacts directly with proliferating cell nuclear antigen (PCNA), an accessory factor of the DNA polymerase complex. This result provided the first molecular link between chromatin assembly and DNA replication and repair [21,22]. Interestingly, two findings suggest that the mouse CAF-1 could play a role in heterochromatin formation. First, CAF-1-p150 interacts directly with the heterochromatic protein HP1α [23], and second, CAF-1 interacts with MBD1, a methyl-CpG binding protein that recruits histone deacetylases (HDACs) to their binding sites [24]. How these interactions might be exploited within the cell in order to play a role in the formation and/or maintenance of heterochromatin is a current matter of interest.

In yeast, a CAF-1-like activity was identified using the in vitro chromatin assembly assay coupled to DNA replication [25]. The corresponding *CAC* genes are not essential for viability, but deletion of any of them lead to an increased sensitivity to UV radiation and defects in the silencing of genes that are flanking telomeric DNA. In addition, mutations in PCNA that impair its interaction with Cac1 lead to a reduced repression of genes located near telomeres, supporting a role for the Cac complex in silenced chromatin

structure. Interestingly, there are genetic interactions between *CAC1* and both *ASF1* (anti-silencing function 1) and *SAS2* (*s*omething *a*bout *s*ilencing 2), a yeast-specific histone acetyltransferase that promotes silencing at the telomeres and HML [26,27]. The Sas-2 containing complex acts in vivo to contribute to silencing by limiting spreading of the Silent mating type information regulator, Sir, proteins onto non-heterochromatic loci [28,29]. Interestingly, mutations in *CAC1* or *ASF1* induce gross chromosomal rearrangement (GCRs) [30]. These rearrangements are postulated to be a consequence of errors occurring during DNA replication. As a result, *cac1* and *asf1* mutants activate DNA-damage checkpoints in the absence of exogenous damage. In addition, *asf1* mutants also activate replication checkpoint pathways [30]. Although the significance of the differences between *cac* and *asf1* mutants will have to be clarified, these experiments in *S. cerevisiae* suggest a link between histone chaperone function and genomic stability, and it will be critical to understand how the histones themselves fit into the picture. A defect in histone deposition should cause (i) an excess of non-DNA bound histones and (ii) nucleosome-free DNA regions, which might be more prone to DNA damage. Whether one of these two situations is sensed directly or indirectly by the checkpoints, or whether they both are, will be an interesting question to address.

Some insights into the in vivo importance of CAF-1 in higher eukaryotes have been obtained by interference and knock out experiments. In *Arabidopsis*, expression of the gene *FASCIATA*, corresponding to CAF-1-p150, is highly associated with the G1/S transition, marking actively proliferating cells, as found in apical meristems. Remarkably, the knock out of the gene *FASCIATA* dramatically affects the organization of those structures, sustaining a critical role in proliferating tissues [31]. CAF-1-p150 contains a conserved dimerization domain in human and *Xenopus* that is critical for the ability of CAF-1 to stimulate chromatin assembly of newly synthesized DNA in vitro. A dominant negative form of CAF-1-p150 lacking this domain was expressed in *Xenopus* embryos. The resulting embryos showed defects in cell cycle progression during the rapid cleavage stages of early development [32]. Recently, a dominant negative CAF-1-p150 introduced into a human cell line caused inhibition of DNA synthesis in S phase with induction of DNA damage and activation of the S phase checkpoint [33]. These latter results show that the interference with a histone chaperone involved in chromatin assembly can result in defect in cell cycle progression. Importantly, in contrast to the situation in *S. cerevisiae*, a knock out of the largest subunit of the *Drosophila* CAF-1 shows that it is an essential gene (B. Dietrich, N. Dostatni, G. Almouzni, personal communication).

Therefore, CAF-1 exemplifies another role of histone chaperones, as key regulators of histone metabolism by depositing histones onto newly synthesized DNA during DNA replication and repair. The fact that CAF-1 links chromatin assembly to DNA replication means it is ideally placed to act as a sensor during this window of opportunity. In this way, CAF-1 might have the potential not only to control the maintenance of epigenetic marks, but also to participate in generating a new epigenetic pattern in daughter cells, thereby contributing to developmental switches.

5. Hir/Hira: chromatin assembly independent of DNA synthesis

HIR/*HIRA* (histone regulation) genes were initially identified in yeast as negative regulators of histone gene expression [34]. They represent a conserved family of proteins found in different species including yeast, *Drosophila*, *Xenopus*, mouse, and human. Here, we will focus on the *S. cerevisiae HIR1* and *HIR2*, since they are the closest homologues to Hira in higher eukaryotes.

Yeast genetic data showed that *HIR* genes are not necessary for gene repression when histones H2A/H2B are tethered to promoters [35]. However, they are necessary when these histones are overproduced [35], giving the first hint of an interaction between Hira and histones. The involvement of Hir and Cac proteins in silenced structures is illustrated by several yeast mutations of *HIR* and *CAC*, in which a dramatic increase in the rate of transposition of the yeast retrotransposon Ty1 was observed. Moreover, telomeric and mating type loci silencing were also reduced in these mutants, suggesting that chromatin is less compacted [36]. Defects in kinetochore function also arise, as revealed by the increased mis-segregation of chromosomes during mitosis, and loss of viability. Moreover, when *hir*/*cac* mutations were combined with mutations in the centromeric histone variant gene *CSE4* or the kinetochore gene *CBF3*, the strains were no longer viable [37].

Further analysis of the molecular role of Hir/Hira proteins came mostly from studies in vertebrates, where there is only one Hir homologue, called Hira. In mouse, Hira is essential for proper embryonic development, particularly during mesoderm differentiation [38]. In humans, the gene is found at chromosome 22q11, within a locus encompassing a critical region associated with the developmental disorder DiGeorge syndrome (DGS), although Hira is probably not the main factor responsible for this syndrome [39]. Hira is a nuclear protein that can be phosphorylated by cyclin-cdk2 [40], and its ectopic over-expression led to a transcriptional down-regulation of the histone genes [41]. Analysis of Hira-interacting proteins showed histones among associated proteins [42]. A functional relevance for this interaction with core histones is highlighted by the demonstration that *Xenopus* Hira facilitates chromatin assembly in vitro [43]. This activity is independent of DNA synthesis, as Hira-immunodepletion abolished chromatin assembly that was uncoupled from DNA replication without affecting the DNA replication-dependent pathway [43]. This clearly shows that Hira is acting as a histone chaperone in a specific nucleosome deposition pathway.

Consistent with the role of Hira as a histone chaperone in *Xenopus*, in yeast a *hir* mutant was shown to be synthetically lethal with a mutation affecting FACT (facilitates chromatin transcription, for a recent review concerning FACT, see Belotserkovskaya and Reinberg in this issue) [44]. FACT is a chromatin-specific RNA polymerase II transcription elongation factor that helps RNA Polymerase II to elongate a transcript on a chromatin template. It is proposed that FACT does this through binding H2A/H2B dimers and removing them from the nucleosome [45]. In yeast, a reduction of FACT activity results in a requirement for Hir proteins [44], which may compensate for the diminished H2A/H2B histone chaperone activity of FACT.

Taken together, the current data support a role for the *HIR*/*HIRA* gene products as histone chaperones that promote chromatin assembly independently of DNA synthesis. In differentiated cells that do not replicate, such a DNA synthesis-independent assembly activity could be used to ensure histone turnover, possibly using ''replacement'' histones in vertebrates, for which no specific chaperones have been discovered to date.

6. Asf1: a histone donor and more?

Asf1 belongs to the H3/H4 family of histone chaperones. It was initially identified in yeast as a gene that, when over-expressed, derepressed the silent mating type loci [46]. Later, biochemical studies in *Drosophila* identified a complex called replication-coupling assembly factor (RCAF), which allowed for efficient nucleosome assembly on replicated DNA when CAF-1 activity was limiting [47]. This complex contains Asf1 and histones H3 and H4 and, in contrast with CAF-1 complex, RCAF/Asf1 is unable on its own to promote nucleosome deposition coupled to DNA replication [47]. The *Drosophila* Asf1 and the two Asf1 forms found in humans, Asf1a and -b, function synergistically with CAF-1 complex during in vitro chromatin assembly coupled to DNA replication and repair [47,48].

Genetic analyses in *S. cerevisiae* further support this scheme, but also reveal distinct separate functions for Asf1 and Cac proteins. This is illustrated as follows:

(i) Mutations in the yeast Cac1 protein that impair its binding to PCNA cause minor telomeric silencing defects, but render silencing strongly dependent on Asf1 and Hir proteins [49]. Thus, the latter factors can be used in a compensating mechanism.

(ii) Yeast *asf1* mutants have different sensitivities to various DNA damaging agents when compared to *cac1* mutant strains [50]. Additionally, double mutations are more sensitive to DNA damage than either single mutant, suggesting that they have overlapping, but also independent roles [50].

(iii) Asf1 interacts with the DNA damage checkpoint protein Rad53 in *S. cerevisiae*, whereas no physical and genetic interaction has been identified between Rad53 and Cac1 [51,52]. Analysis of genomic instability in *CAC* and *ASF1* mutant strains point towards a mechanism involving the activation of checkpoint pathways in both backgrounds, but in a distinct manner [30]. The exact nature of these differences, at a molecular level, remains to be elucidated.

In humans, the checkpoint protein mostly related to Rad53 is Chk2 (checkpoint kinase 2), yet it has not yet been shown to interact with human Asf1a/b. Another link with checkpoint pathway is potentially interesting. Indeed, upon double strand breaks, the ATM/Chk1 DNA damage checkpoint pathway inhibits the Tlk (tousled-like kinase), unique to higher eukaryotes, for which Asf1 is the only known substrate [53]. The consequence of Asf1 phosphorylation by the Tlks now requires elucidation, specifically to examine how this could potentially modulate some of the Asf1 interactions. The Asf1 interactors known at present include histones, the middle subunit of CAF-1 [47,49], and Hir proteins in *S. cerevisiae* [54], as well as with Hira in *Xenopus* and mammalian cells (D. Ray-Gallet, J. Mello and G. Almouzni, unpublished).

Based on all these observations, we propose that Asf1 proteins might transfer histones to a specialized histone deposition factor, either the CAF-1 complex using a DNA replication-dependent pathway or Hir/Hira using a DNA replication-independent pathway. Asf1 proteins would thus be placed in a perfect position to monitor the fluctuation in the histone pools in a cycling somatic cell and, by virtue of their post-translational modifications, could perhaps activate one assembly pathway over the other.

In summary, the Asf1 proteins represent the prototype of a histone donor chaperone that could effectively ensure a constant supply of histones at sites of nucleosome assembly, as well as provide a connection with checkpoint control.

7. Chaperones associated with chromatin remodeling complexes

Arps (*a*ctin-*r*elated *p*roteins) represent a protein family highly homologous to actin and conserved from yeast to human [55]. Recently, they have also been found in the nucleus as components of several chromatin-related complexes. Interestingly, ARP4, a component of the NuA4 histone acetyltransferase and the INO80 (inositol 80) chromatin-remodeling complexes, is an essential gene in yeast. Arp4 proteins can interact with core histones in vitro. Conditional mutations in this gene lead to defects in the chromatin structure of episomal DNA, suggesting a possible in vivo role of Arp4 in chromatin organization [56]. The recent studies of the yeast INO80 complex, which contains Arp4, Arp5, and Arp8, provided an opportunity to examine

the properties of this complex when the Arps were eliminated by mutagenesis. The complex lacking Arp5 or 8 subunits was deficient for chromatin remodeling in biochemical assays. Furthermore, Arp8 was shown to bind to core histones. Thus, Arps might assist INO80 complex in chromatin remodeling by providing a histone chaperone function during the process of nucleosome reorganization.

Another example of chaperone activity linked to a chromatin-remodeling complex can be illustrated with remodeling and spacing factor (RSF). Initially identified in human cells for its ability to allow in vitro transcription initiation by RNA polymerase II on chromatin templates [57], RSF contains two subunits: the *Drosophila* ISWI homologue hSNF2H, and Rsf-1, encoded by a recently cloned human gene [58]. RSF was shown to bind histones H3/H4 and to promote chromatin assembly in vitro [59], with the Rsf-1 subunit potentially involved in the chaperone activity [59]. How this chaperone property can be exploited for the in vivo function of the complex will be a matter of future research.

8. RbAp46/48 family: histone chaperones with an escort function associated with different complexes

RbAp46/48 (retinoblastoma-associated protein 46/48) proteins were first characterized in human cells as proteins that bind to the tumor suppressor retinoblastoma (Rb) [60]. They are related in their sequence to negative regulators of the Ras pathway in yeast [60].

RbAp46/48 are components of several chromatin-related complexes (reviewed in Ref. [61]), including: (i) the histone acetyltransferase Hat1, involved in the acetylation of newly synthesized histones, (ii) NuRD (nucleosome remodeling histone deacetylase complex), which is a multisubunit complex containing an ATP-dependent chromatin remodeling and HDAC activity, postulated to help maintain genes in a specific repressed state, (iii) NURF (nucleosome remodeling factor), an ATP-dependent nucleosome-remodeling factor involved in regulating transcription in *Drosophila*, (iv) the Sin3 complex, which contains HDACs and is involved in transcriptional repression, and (v) CAF-1, a chromatin assembly factor coupled to DNA synthesis, as previously discussed. Recently, a new RbAp46/48-containing complex was identified, polycomb repressive complex 2 (PRC2), a histone methyltransferase-containing complex, associated with transcriptional silencing [62–64].

Since all these factors have histones as their substrates, the general hypothesis is that they interact with histones through the common RbAp46/48 subunit.

A knock out of *Arabidopsis* MSI1, one of the five RbAp46/48 members in this species and the most similar to RbAp46 protein, disrupts many developmental aspects. This broad phenotype can be explained by the participation of MSI1 in several distinct complexes [65].

Therefore, RbAp46/48 and the chaperones associated with ISWI illustrate another family of histone chaperones, those that are associated with complexes containing enzymatic activities, giving a new angle to the chaperone family of proteins.

9. Perspectives

In this review, we have emphasized the fact that histones appear constantly under surveillance by specific chaperones potentially involved in each step of the histone metabolism. Such chaperones appear to play essentially a supporting role that can be exploited to promote many aspects of histone metabolism, including cytoplasmic to nuclear transport, formation of defined chromatin structures, storage, post-translational modifications, nucleosome-remodeling activities, and perhaps the modulation of other histone or nucleosome-associated events.

In addition to this supporting role, we would like to point out that histone chaperones might have an active role in the metabolism of histones. They may do this through the other interactions that histone chaperones can make with important regulators of the cellular metabolism. This is perhaps best illustrated by the interaction of Nap-1 with Clb2 and Asf1 with Rad53. The CAF-1 complex and Hira may well also serve to integrate cell cycle information with chromatin assembly activity. Therefore, in a general way, these chaperones can serve as regulators of histone function.

As summarized in our simplified scheme shown in Fig. 1, chaperones accompany histones from their site of synthesis all the way up to the places where they are used and finally degraded. Throughout its lifetime, an individual histone may be passed between different chaperones that dictate its movement within the cell, its assembly into specific chromatic structures and its covalent modifications.

Clearly, our scheme still contains gaps to be filled and the full complexity of the histone chaperone story has not yet been elucidated. Although they initially appear to have an indirect role in each of these different aspects, histone chaperones can also provide a connection with other potential regulators of cellular metabolism. Therefore, histone chaperones might have more than a ‘‘supporting’’ role, integrating and possibly transferring information concerning the histone status.

Acknowledgements

We thank Bruce Dietrich, Catherine Green and Dominique Ray-Gallet for helpful comments and discussions on the manuscript. A.L. is supported by an Institute Curie fellowship. The team of G.A. is supported by grants from Programme Collaboratif Institut Curie/CEA, la Ligue Nationale contre le Cancer (Equipe labellisée la Ligue), Euratom (FIGH-CT-1999-00010 and FIGH-CT-2002-

00207), the Commissariat à l'Energie Atomique (LRC no. 26) and RTN (HPRN-CT-200-00078 and HPRN-CT-2002-00238).

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