Published online 18 September 2018

Nucleic Acids Research, 2018, Vol. 46, No. 19 ***9907–9917***
doi: 10.1093/nar/gky823

SURVEY AND SUMMARY

Mechanistic insights into histone deposition and nucleosome assembly by the chromatin assembly factor-1

Paul V. Sauer[1], Yajie Gu[2], Wallace H. Liu[3], Francesca Mattiroli[4], Daniel Panne[1,5], Karolin Luger[2,6] and Mair E.A. Churchill[7,*]

[1]European Molecular Biology Laboratory, 38042 Grenoble, France, [2]Department of Biochemistry, University of Colorado at Boulder, Boulder, CO 80309, USA, [3]Department of Pharmacology, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA, [4]Hubrecht Institute, Utrecht 3584 CT, the Netherlands, [5]Leicester Institute of Structural and Chemical Biology, Department of Molecular and Cell Biology, University of Leicester, Lancaster Road, Leicester LE1 7RH, UK, [6]Howard Hughes Medical Institute, Chevy Chase, MD 20815-6789, USA and [7]Department of Pharmacology and Program in Structural Biology and Biochemistry, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA

Received July 20, 2018; Revised August 28, 2018; Editorial Decision August 30, 2018; Accepted September 15, 2018

ABSTRACT

Eukaryotic chromatin is a highly dynamic structure with essential roles in virtually all DNA-dependent cellular processes. Nucleosomes are a barrier to DNA access, and during DNA replication, they are disassembled ahead of the replication machinery (the replisome) and reassembled following its passage. The Histone chaperone Chromatin Assembly Factor-1 (CAF-1) interacts with the replisome and deposits H3–H4 directly onto newly synthesized DNA. Therefore, CAF-1 is important for the establishment and propagation of chromatin structure. The molecular mechanism by which CAF-1 mediates H3–H4 deposition has remained unclear. However, recent studies have revealed new insights into the architecture and stoichiometry of the trimeric CAF-1 complex and how it interacts with and deposits H3–H4 onto substrate DNA. The CAF-1 trimer binds to a single H3–H4 dimer, which induces a conformational rearrangement in CAF-1 promoting its interaction with substrate DNA. Two CAF-1•H3–H4 complexes co-associate on nucleosome-free DNA depositing $(H3–H4)_2$ tetramers in the first step of nucleosome assembly. Here, we review the progress made in our understanding of CAF-1 structure, mechanism of action, and how CAF-1 contributes to chromatin dynamics during DNA replication.

INTRODUCTION

Cell division not only requires the accurate replication of DNA but also efficient propagation of chromatin and associated 'epigenetic' modification states. In eukaryotic organisms, this epigenetic information is encoded both in the DNA and the histone proteins of chromatin through complex patterns of posttranslational modifications. The basic building block of chromatin, the nucleosome, consists of an octamer of histone proteins wrapped with ∼147 bp of genomic DNA (1,2). The histone core consists of two H3–H4 dimers that assemble into a $(H3–H4)_2$ tetramer which organizes the central 70–80 bp of the DNA, flanked by two H2A–H2B dimers which organize the peripheral 30–40 bp on either side of the tetramer. The passage of the replication fork requires the temporary removal and subsequent reassembly of the parental nucleosomal histones along with a full complement of newly synthesized histones (3).

To enable propagation of chromatin, cells have evolved efficient nucleosome assembly machineries. These include components of the replication machinery, nucleosome remodelers, and a diverse class of proteins known as histone chaperones (4–8). The histone chaperones mediate the stepwise processes of disassembly and reassembly of nucleosomes (3). Histone chaperones are typically highly acidic

*To whom correspondence should be addressed. Tel: +1 303 724 3670; Email: mair.churchill@ucdenver.edu

proteins that bind dimers of H2A–H2B, H3–H4 or assemble intermediates such as $(H3–H4)_2$ tetramers; and several histone chaperones are specific for histone variants (9,10). Histone chaperones thus safeguard histones and prevent their off-pathway interactions and aggregation, ultimately guiding their ordered deposition onto DNA to form nucleosomes (11). Diverse and distinct histone chaperones drive nucleosome assembly and disassembly during replication-independent and replication-dependent processes. Thus, there is a division of labor among histone chaperones, as many only fulfill some of these functions (4,8,11,12).

During DNA replication, the importance of the histone chaperones is particularly evident, as, chemical inhibition or genetic ablation of replication-coupled chaperones slows down replication fork progression, and leads to checkpoint activation (13–17). At the replication fork, histone chaperones direct histones towards two different deposition pathways: nucleosome recycling and *de novo* nucleosome assembly from newly-synthesized histones (Figure 1) (17). During nucleosome recycling, parental H3–H4 histones are propagated 'conservatively' as $(H3–H4)_2$ tetramers and are reinstated close to their original position in the genome through the actions of histone chaperones that capture evicted histones and assist in their reassembly (18,19). In parallel, the *de novo* assembly of nucleosomes is required to account for the duplicated amount of DNA, for which histone chaperones transport a full complement of newly synthesized histones to the nucleus and aid in *de novo* nucleosome assembly. In all cases the assembly of nucleosomes is completed by the addition of recycled or newly synthesized H2A–H2B dimers, which is not directly coupled to DNA replication (20–22).

How the histone chaperones synergize and contribute to the various steps of chromatin replication remains unclear. However, a preliminary model for the assembly and disassembly of the H3–H4 core tetramer of the nucleosome is emerging. In this model, parental H3–H4 histones are transferred by 'Mini-chromosome maintenance' (MCM2) and Anti Silencing Factor 1 (ASF1) to the daughter strand –by transient disruption of the histone tetramer (14,23–27). Whereas, the histone chaperones Regulator of Ty1 transposition (Rtt106) (28), Facilitates chromatin transcription' (FACT) (29,30) and CAF-1 assemble $(H3–H4)_2$ tetramers *de novo* (27,31).

CAF-1 is a central histone H3–H4 chaperone, first identified in DNA replication experiments *in vitro*. Addition of purified biochemical fractions containing CAF-1 to cytosolic HeLa cell extracts reconstituted SV40 DNA replication and chromatin assembly *in vitro* (31). It soon became clear that the assembly of chromatin occurred in a stepwise manner, with CAF-1 specifically depositing H3–H4 first, followed by the deposition of H2A–H2B by other factors (3). The discovery of the yeast homologue of CAF-1 in the Stillman laboratory demonstrated the high degree of functional conservation of CAF-1 in eukaryotes (32).

CAF-1 primarily functions in the deposition of newly-synthesized H3–H4 onto newly synthesized DNA (27). During S-phase CAF-1 is present in foci of DNA replication (33). It localizes to sites of ongoing DNA synthesis by binding to the processivity factor for DNA polymerases known as Proliferating cell nuclear antigen (PCNA) (31,34–36). Subsequent studies revealed the interdependence between DNA replication and chromatin assembly, as CAF-1-mediated nucleosome assembly in cells is efficient only on newly replicated DNA, and as replication stalls without CAF-1 activity (15). Once recruited to the replication fork by PCNA, CAF-1 receives H3–H4 dimers from the histone chaperone ASF1 and deposits them onto DNA to form a DNA-$(H3–H4)_2$ complex known as the tetrasome, thereby initiating nucleosome assembly (37) (Figure 1). In addition to recruitment by PCNA, coordination of CAF-1 activity with DNA replication is further regulated by CDK-dependent phosphorylation of the p150 and p60 subunits of CAF-1 (38,39). Importantly, CAF-1 also forms a distinct complex with the essential replication kinase Cdc7-Dbf4 *in vivo*, suggesting that CAF-1 activity is regulated throughout the cell cycle by phosphorylation (40). However, the mechanism by which phosphorylation regulates CAF-1 activity or chromatin targeting is currently unclear.

CAF-1 also participates in other cellular processes. Upon DNA damage, CAF-1 localizes to the damaged foci, reassembling nucleosomes after nucleotide excision repair and double strand break repair. CAF-1 is also required for chromatin silencing and heterochromatin integrity (41–46). This involvement of CAF-1 in a wide variety of nuclear processes has consequences for cell fate decisions. As seen during development and in somatic cells, knockdown of CAF-1 leads to a more accessible chromatin state that promotes cellular reprogramming (47–49). Moreover, genetic deletion of CAF-1 is lethal in metazoans (15,50,51), and in contrast, elevated levels of CAF-1 have been linked to human disease (52,53). Taken together, these observations emphasize the critical connection between DNA synthesis-coupled nucleosome assembly, epigenetic inheritance, cell fate control and human disease.

Structural and mechanistic models for how CAF-1 mediates nucleosome assembly are only beginning to emerge. Here, we focus on the recent advances in elucidating the biochemical and structural features of CAF-1, which were primarily obtained from the *S. cerevisiae* orthologue, and discuss the implications for our general understanding of nucleosome assembly.

ARCHITECTURE OF CAF-1

CAF-1 consists of three subunits, referred here to as 'large', 'middle' and 'small' subunits (32,54). These subunits are functionally conserved throughout the eukaryotic domain (Table 1), and invariably form a 1:1:1 complex (44,55).

Large subunit

The primary sequence of the large subunit is moderately conserved between species, with the exception of short sequence insertions or deletions (Figure 2A). The DISOPRED plots (Figure 2A) (56) predict that the large subunit contains many intrinsically disordered protein regions (55,57,58). The N-terminal portion contains two important regions: a stretch that is enriched in K/E/R amino acids (KER), and a PCNA interacting peptide (PIP-box) motif. The conserved KER region is predicted to form a

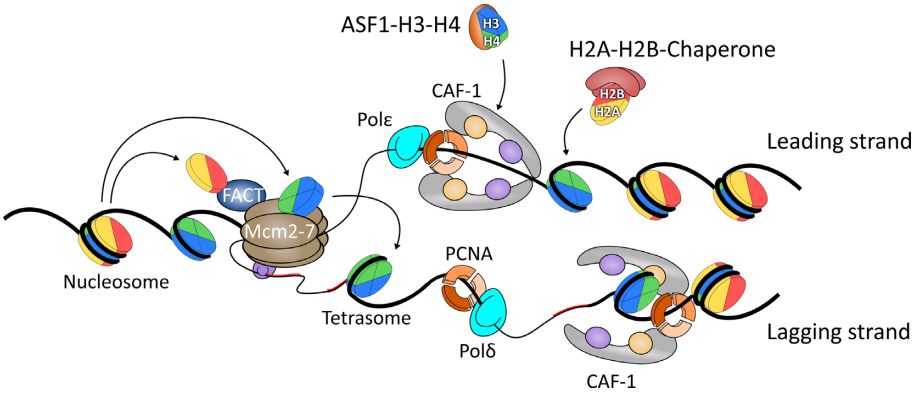

Figure 1. Histone chaperones orchestrate nucleosome assembly during DNA replication. Nucleosomes are removed ahead of the advancing MCM helicase complex and reassembled onto the daughter strands after the passage of the replisome. The transfer of $(H3–H4)_2$ tetramers occurs conservatively, i.e. is distributed as a single unit onto one of the two daughter strands. FACT, MCM2 and ASF1 are histone chaperones that might be involved here (23,24,30). De novo assembly of nucleosomes occurs primarily via CAF-1 which receives histone dimers from ASF1 and deposits $(H3–H4)_2$ tetramers through a mechanism described in the text. Other chaperones deposit H2A–H2B to complete recycled and *de novo* nucleosome assembly. The timely assembly of nucleosomes is critical for replication fork progression and lagging strand synthesis. After assembly, nucleosome positions are fine-tuned by ATP dependent chromatin remodelers (not shown) (7).

Table 1. Nomenclature of CAF-1 subunits in different species

Subunit	*H. sapiens*	*S. cerevisiae*	*D. melanogaster*	*C. elegans*	*X. laevis*	Function
Large	p150/CHAF1A	Rlf2/Cac1	p180	Chaf1	p150/CHAF1A	Scaffolding, recruitment, substrate binding (DNA and histones), possibly deposition
Middle	p60/CHAF1B	p60/Cac2	p105/p75	Chaf2	p60/CHAF1B	Histone binding, ASF1 binding, possibly deposition
Small	p48/RbAp48/RBBP4	Msi1/Cac3	p55/NURF	Rba1	p48/RbAp48/RBBP4	Possibly histone tail binding, also component of other complexes

coiled-coil domain that binds to long (~40 bp) DNA fragments, suggesting a function during substrate DNA recognition and potentially histone deposition (55) (see below). The PIP-box motif mediates the interaction with PCNA (35,36). Notably, more than one PIP motif is present in most CAF-1 complexes, implying a complex hierarchy of interactions between CAF-1 and the replication machinery (59). The C-terminal region is predicted to form a structured domain (Figure 2A) (32). Depending on the species, additional protein-protein interacting domains are present in the N- and C-terminal regions, including SUMO2/3 and HP1 binding regions in the human isoform (60,61) (Figure 2A).

Based on biochemical and biophysical experiments including chemical crosslinking mass spectrometry (XL-MS), hydrogen-deuterium exchange mass spectrometry (HX-MS) and mutagenesis, the central part of the large subunit was found to be responsible for binding the small subunit (35,55,62). The adjacent acidic ED domain is responsible for histone binding (55,58,62,63). A segment C-terminal to the ED domain is the binding site for the middle subunit (32,55,58). The C-terminal domain of the large subunit contains a conserved winged helix domain (WHD) which has been crystallized and structurally characterized (63,64). Crosslinking results show that the WHD is also located in proximity to H3–H4 (63). Importantly, it associates non-sequence specifically with 10–16 bp of DNA with a K_D of approximately 2 μM (64) and is involved in chromatin silencing. The WHD also participates in H3–H4 tetramerization and in regulating the nucleosome assembly activity of CAF-1 *in vitro* and *in vivo* (see section on mechanism) (58,64).

Middle subunit

Secondary structure predictions indicate that the middle subunit of CAF-1 adopts a WD40 fold. This subunit contributes to the interaction of CAF-1 with ASF1, the delivery vehicle for H3–H4 (Figure 1) (27,54,57,65,66). A major part of the interaction with ASF1 occurs via a C-terminal extension called the B domain (66). A similar B-domain is also found in the histone chaperone Histone cell cycle regulator (HIRA), which deposits H3–H4 in the context of DNA transcription and heterochromatin silencing (65,67). This suggests that the competition of the two B-domains (of CAF-1 and of HIRA) for the same binding site on ASF1 is part of the regulatory mechanism by which histone variants get distributed to their proper deposition pathways (54,65,66,68). The B-domain and the histones bind on opposite faces of ASF1, potentially enabling the formation of a transitional handover-complex in which ASF1 presents its cargo to the acceptor (CAF-1 or HIRA) before dissociating again (57,69). In addition to binding to ASF1, the middle subunit is also required for productive association with H3–H4, and thus for nucleosome assembly (32,62,63,70).

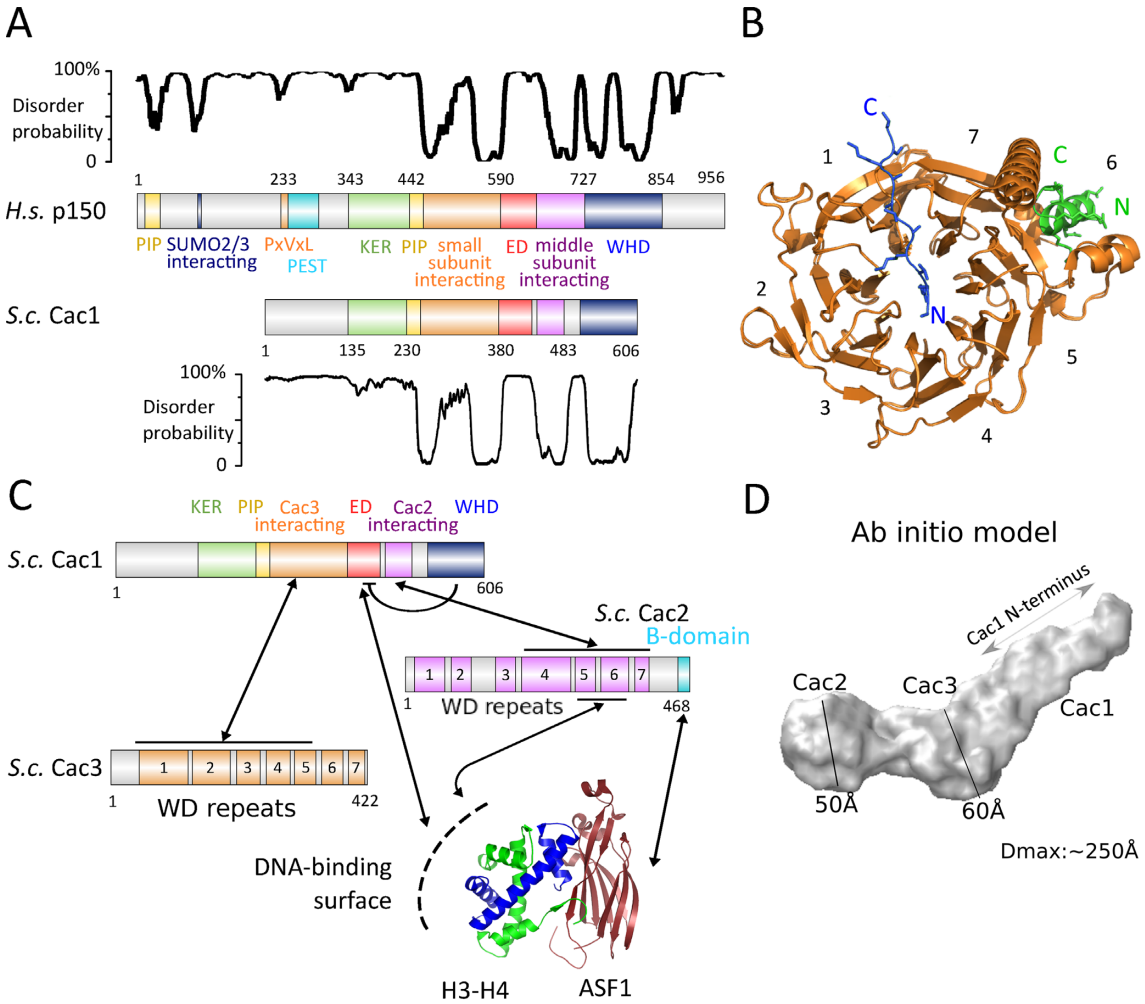

Figure 2. Architecture of the CAF-1 complex: (**A**) domain arrangement of the large CAF-1 subunit in humans and yeast. The primary structures of both homologues are shown with selected protein-protein and protein-DNA interaction domains highlighted. Sequence disorder predictions (obtained from DISOPRED3 (56)) are shown. PIP—PCNA interacting peptide (34–36); SUMO - small ubiquitin like modifier (60); PxVxL is a HP1 (Heterochromatin protein) interacting sequence motif (61); The PEST sequence is associated with reduced intracellular half-life (32); KER sequence binds to DNA (55); ED sequence binds to histones and WHD (58); WHD—Winged helix domain (64). (**B**) Superimposition of Drosophila RbAp48/p55 (orange) bound to H3 (green, residues 1–11; PDB 2YBA) or H4 (blue, residues 30–43; PDB 2XYI). The WD40 blades of p55 are numbered, starting from the N-terminus. Note that alignment of the H4 peptide to nucleosomal H4 results in clashes of the nucleosomal H3–H4 pair with p55 (78–80). (**C**) Overall organization of the CAF-1 complex and interacting regions, including ASF1-H3–H4. Individual domains or regions are connected with arrows and represent only approximations, based on XL-MS, HX-MS and mutagenesis experiments. Individual WD40 blades of Cac2 and Cac3 are indicated and were predicted by Phyre2. ASF1delivers H3–H4 to CAF-1 but is not part of the complex that carries out deposition. See text for details (26,55,58,62,63,83). (**D**) Ab initio model calculated from SAXS data. The predicted subunit regions within CAF-1 are labeled. The dimensions for the complex, and subunits are noted (62).

Small subunit

Like the middle subunit, this subunit also adopts a WD40 propeller fold (Figure 2B). The human and *Drosophila* homologues, RbAp48 and p55 have been structurally characterized (71,72). RbAp48 is also a constituent of several other chromatin regulating complexes, namely Polycomb repressive complex (PRC2), Nucleosome remodeling factor (NURF), Nucleosome remodeling deacetylase (NURD), and Histone deacetylase (HDAC1), suggesting that this subunit serves as a molecular bridge between histone modifying enzymes and their substrates (73–77). The small subunit binds independently to fragments of H3 and H4 using different interactions surfaces (Figure 2B) (72,78,79). Moreover, the structures of the *Drosophila* p55 in complex with either H3 or H4 peptides, indicate that the histone binding locations are likely incompatible with a nucleosome-like configuration due to steric clashes (69,80). As these interactions exhibit a significantly lower affinity than full-length CAF-1 with intact H3–H4 dimers, their overall importance for H3–H4 dimer binding by CAF-1 is unclear. In fact, the yeast Cac3 exhibits no interaction with H3–H4, even at micromolar concentrations *in vitro* (63), and Cac3 is not required for robust histone binding in the context of CAF-1 (62). Therefore, the role of the small subunit and its mode of H3–H4 interaction in the context of CAF-1 are still not well defined. The small subunit also appears to be a non-essential subunit of the PRC2 chromatin modifying complex thus raising questions about the precise role of this subunit in different contexts (81,82).

Overall organization of the CAF-1 complex

While a high-resolution structure of the CAF-1 complex is not yet available, low-resolution methods have revealed the overall shape of the complex. Negative stain electron microscopy of the CAF-1•H3–H4 complex at approximately 30 Å resolution shows an elongated shape, in which the two globular WD40 subunits are connected by the large subunit (Figure 2C) (83). *In* solution, *ab initio* SAXS envelopes showed a slightly more elongated shape than the EM structure and also allowed the tentative placement of the WD40 subunits and the large subunit, which were in general agreement with the negative stain EM data (Figure 2D) (55,62). HX-MS and XL-MS data show that the middle and the small subunits directly interact with the large subunit, but not with each other (Figure 2C) (62). The binding sites of the two smaller subunits on the large subunit have been determined by several groups through HX-MS, XL-MS, mutagenesis and pull-down assays, and flank the acidic ED domain (32,35,55,62,63,83). Thus, the subunits of the CAF-1 complex perform an efficient division of labor - the large subunit provides a scaffold for the other CAF-1 subunits and mediates recruitment of the complex and interaction with other nuclear factors. The middle subunit is responsible for histone chaperone cross talk and histone loading, while the small subunit provides less-well characterized accessory interactions. The current model suggests that H3–H4 bind at the center of the histone chaperone complex, in close proximity to the acidic ED domain and WD40 subunits.

Histone binding

Initial reports indicated that CAF-1 binds H3–H4 in a dimeric conformation *in vivo* (27,84,85) and that CAF-1 could form homodimers (86). Later reports have suggested that CAF-1 is monomeric (55,57,58), and also that a tetrameric histone conformation might exist in the complex (57,87–89). However, the most recent reports have proposed an updated and unified model for how CAF-1 interacts with histones. In budding yeast, the CAF-1 complex forms a trimer containing one copy of each subunit, and there is no indication of higher order complex formation for yeast CAF-1 in the absence of the histones (55,57,58,63). Native mass spectrometry, fluorescence-based measurements and hydrogen-deuterium exchange data show that the trimeric CAF-1 complex binds to a single H3–H4 dimer with nanomolar to picomolar affinity (55,58,62,63). The H3–H4 dimer is bound in a conformation such that the H3 α 3 helix, which mediates H3-H3 interactions in the context of a $(H3–H4)_2$ tetramer, is available to engage the second copy of the H3–H4 dimer (58). Additionally, CAF-1•H3–H4 crosslinking results point to the proximity of CAF-1 to the DNA binding surface of H3–H4 but not the dimerization region (Figure 2C) (63). Together these binding properties allow for H3–H4 tetramerization and potential protection of DNA binding surfaces until necessary during the histone deposition reaction (see below) (58,63).

H3–H4 mainly binds to the large and middle subunits of CAF-1. HX-MS and mutagenesis experiments indicate that the histones might contact blade 5 and 6 of the WD40 fold of the middle subunit (Figure 2C) (63). For the large subunit, the highly acidic ED domain is involved in histone binding (62,63). This property is shared with other histone chaperones which often contain acidic regions that are thought to help neutralize the positive charges of the histones (11,90). Indeed, even a minimally designed CAF-1 complex comprised of only the middle subunit and the central region of the large subunit (consisting of the ED domain and the middle-subunit-binding domain) can provide the histone binding affinity of CAF-1 WT and carry out tetrasome assembly *in vitro* (62). Moreover, a small region of the large subunit alone, comprising only the ED and WHD domains, can form tetrasomes (63) albeit less efficiently than the Cac1–Cac2 complex. As mentioned above, the smallest subunit of CAF-1 is completely dispensable for tetrasome assembly *in vitro* (62,63).

DNA binding

The DNA binding properties of CAF-1 have gained much attention recently as they appear to play a central role both in CAF-1 recruitment to the replication fork and the histone deposition mechanism. Early experiments using CAF-1 purified from endogenous sources did not reveal any DNA binding activity (31). Later, two DNA binding domains were identified and characterized, namely the WHD and the KER domain, both located in the large subunit (55,64) (Figure 2). Both domains are important for the nucleosome assembly activity of CAF-1 (55,58). Whereas the isolated WHD binds to a minimal 10–16 bp of DNA, the full CAF-1 complex preferentially interacts with extended ∼40 bp DNA substrates, likely due to the additional DNA binding activity conferred by the KER domain (55,64). Binding of the full length protein to DNA is cooperative, indicating that more than one CAF-1 complex binds to its DNA substrate at the same time (55). CAF-1 preferentially binds linear but not nucleosomal DNA (55,58). Whereas linear DNA typically adopts regular B-form geometry, the conformation of nucleosomal DNA is distorted, and one face of the DNA double helix is occluded over the entire 147 bp (1,91). Together these results show that CAF-1 has preferences for DNA length, conformation, and accessibility. All of these properties could be important for CAF-1 activity at the replication fork by enabling CAF-1 to recognize linear target sequences of sufficient length to accommodate $(H3–H4)_2$ tetramers and release from the DNA once the histones have been deposited. Such extended, linear B-form DNA is present at replication forks, and in cooperation with the processivity clamp PCNA, could facilitate the recruitment of CAF-1 to these locations, to promote tetrasome assembly (64,92,93). In contrast, short DNA linker lengths between assembled nucleosomes could interfere with substrate recognition by CAF-1 and thus restrict its histone chaperoning function to replication forks. Such a recruitment mechanism could also in part explain why KER and WHD deletion mutants of CAF-1 are defective in assembling replicating chromatin in cell extracts under physiological conditions (32,55).

The ability of CAF-1 to bind to DNA is a characteristic which, upon closer examination, is shared with many other H3–H4 chaperones that deposit histones onto DNA. Rtt106, HIRA, Replication protein A (RPA), Holiday

junction recognition protein (HJURP) (and its yeast homologue Suppressor of chromosome missegregation (Scm3)) have all been shown to bind DNA with varying affinities but typically in the medium to low micromolar range, similar to CAF-1 (94–97). Virtually nothing is known about how this binding occurs. As such, future studies will address the mechanism of DNA recognition of histone chaperones, which enables nucleosome assembly during replication and transcription.

MECHANISM OF CAF-1 MEDIATED NUCLEOSOME ASSEMBLY

Tetrasome formation

Formation of the H3–H4 tetramer–DNA complex, known as the tetrasome, does not require ATP hydrolysis, but is instead driven by the high affinity of $(H3–H4)_2$ tetramers for DNA in a mechanism guided by CAF-1. The finding that CAF-1 binds to a single H3–H4 dimer and cooperatively binds to DNA in a length-dependent manner suggests a mechanism for tetrasome formation. Results from two groups suggest that the deposition step requires the association of two CAF-1•H3–H4 complexes, that co-assemble on DNA, followed by the concerted deposition of one H3–H4 tetramer (Figure 3) (55,58).

Using DNA fragments of varying lengths, the Luger lab was able to reveal intermediate steps in the mechanism of CAF-1-mediated H3–H4 deposition. In-solution cross-linking studies show that the association of two CAF-1•H3–H4 complexes is dependent on the DNA binding capacity of the large subunit (58). The WHD plays an important role in the mechanism that enables H3–H4 deposition. In the absence of the H3–H4 cargo, the positively charged DNA binding surface of the WHD engages the acidic ED domain, thus auto-inhibiting potential WHD–DNA interactions. Upon H3–H4 binding to the ED domain, this interaction is destabilized and the WHD becomes available for DNA engagement. Because the WHD binds DNA in a cooperative manner, this greatly enhances the subsequent association of two CAF-1•H3–H4 complexes, and mutations in the WHD that interfere with DNA binding abolish dimerization of CAF-1•H3–H4 complexes (58). The tetramerization of H3–H4 requires the interaction of H3 α3 helices. Notably, mutation of the H3 α3 tetramerization interface still allows H3–H4 interaction with CAF-1 or DNA but perturbs the concerted deposition of H3–H4 dimers and tetrasome assembly (55,58). This finding, together with crosslinking experiments, suggests that the two α3 helices of the H3–H4 dimers are positioned in close proximity to each other prior to deposition (58). In the final step, CAF-1 releases the histones once a tetrasome has successfully formed.

Regulated dimerization of histone chaperones guides nucleosome assembly

Histone chaperone dimerization is likely a means to control the oligomerization state of H3–H4 itself: presumably, maintenance of H3–H4 as dimers represents a response to the need to control and restrict the histone tetramerization reaction during critical tasks. As DNA is being replicated, the assembly of chromatin impacts the speed at which the replication fork progresses (13,98–100). Rapid deposition of new $(H3–H4)_2$ tetramers is therefore critical to prevent replication fork stalling and genomic instability. The concerted DNA-mediated association of histone-bound CAF-1 ensures a timely and controlled mechanism and reduces the possibility for unproductive interactions.

Thermodynamically, the free energy associated with H3–H4 tetramerization onto DNA is the sum of partial reactions, which exhibit opposing energetic expenditures: overall, the DNA must be substantially deformed at a high energetic cost. This must be offset by favorable energy from the establishment of contacts between H3–H4 and DNA, formation of the H3-H3 four-helix bundle and the hydrophobic effect. The presence of all of these would be required to provide the entire assembly pathway with the necessary free energy to proceed in an ordered fashion. Much like an enzyme, CAF-1 promotes an optimal micro-environment, which allows formation of these contacts. In addition, histone tetramerization and concerted DNA deposition could establish directionality of the reaction and explain why histone chaperones like CAF-1 do not catalyze nucleosome disassembly reactions - the energetic cost of tetrasome splitting and DNA unwinding is simply too high and can only be accomplished with the help of ATP-dependent remodelers or helicases. In conclusion, the exploitation of the directed DNA binding energy of H3–H4 by histone chaperones supports the mechanism that renders them independent of ATP hydrolysis, while still providing a high degree of directionality for the H3–H4 deposition process.

Regulation of CAF-1 recruitment through post-translational modifications

Histone deposition by CAF-1 is likely regulated not only by other proteins but also by post-translational modifications on the histone chaperone as well as on the histones. While phosphorylation regulates recruitment of CAF-1 to the replication fork in a cell cycle dependent manner (see introduction) (40), posttranslational modifications of the histones have the potential to directly affect the deposition mechanism. In yeast, H3–H4 dimers that are directed towards incorporation at the replication fork are acetylated by the acetyltransferase Rtt109 on lysine 56 of H3 ($H3K56^{ac}$); this modification serves as one of the marks for newly synthesized histones (101,102). In contrast, acetylation of H3K56 in humans only appears to be required for nucleosome assembly associated with DNA repair, while like yeast, histones targeted to the replication fork are acetylated by the cytosolic HAT1 on H4K5 and H4K12 (77,103–108) and by HAT4 on H4K91 (109). CAF-1 is thought to recognize the $H3K56^{ac}$ modification, based on biochemical studies reporting that CAF-1 binds to $H3K56^{ac}$-H4 with higher affinity than to unmodified histones (101,105) and XL-MS showing numerous crosslinks between CAF-1 and the H3 N-helix containing K56 (63). The N-terminal tails of both histones are not required for nucleosome assembly by CAF-1 even though they are essential for chromatin formation in a physiological context (70,77,110). Therefore, if and

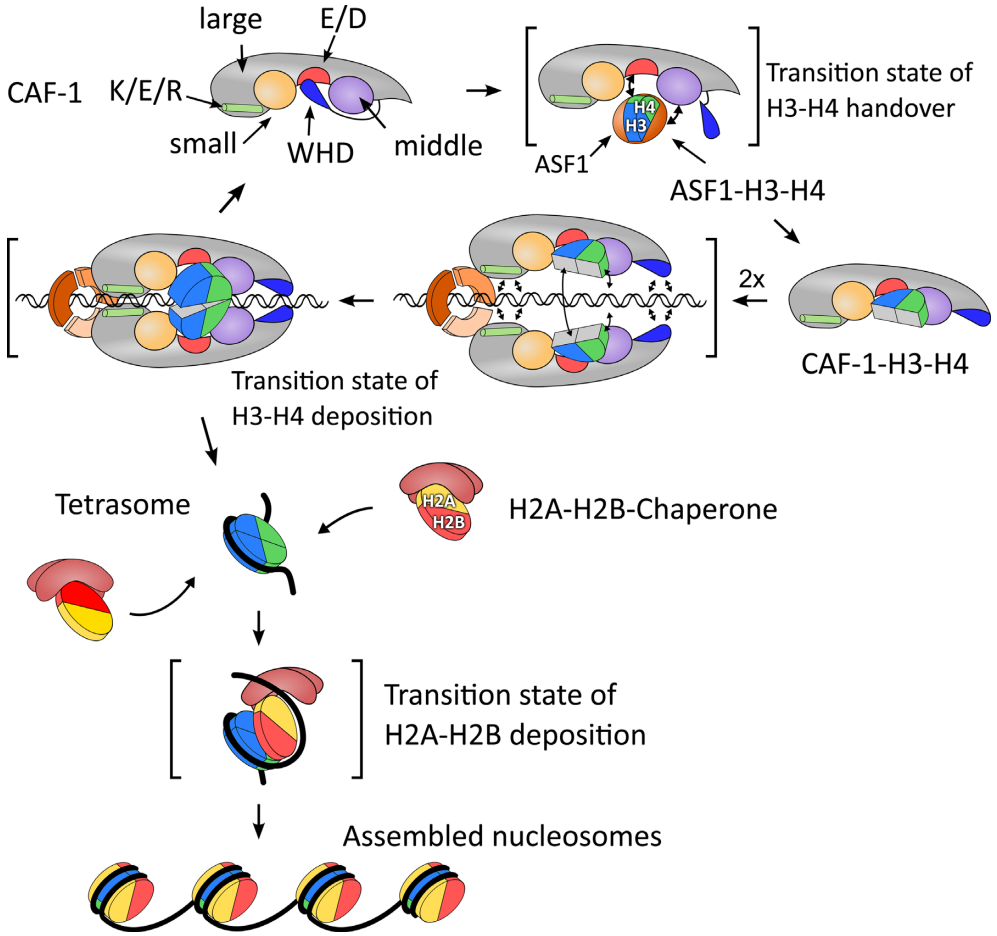

Figure 3. Model for CAF-1 mediated nucleosome assembly. In absence of histones, the C-terminal WHD is inaccessible due to sequestration by the ED domain. ASF1 transfers a single H3–H4 dimer to CAF-1, resulting in the liberation of the WHD. Two CAF-1•H3–H4 complexes associate in close proximity to each other, mediated by PCNA–CAF-1 and DNA–CAF-1 contacts. The transient association of two CAF-1•H3–H4 complexes on DNA allows for H3-H3 contacts to form and the two histone chaperone complexes concertedly deposit one $(H3–H4)_2$ tetramer onto the DNA prior to being released from the DNA. During the second assembly step, H2A–H2B histone chaperones mediate H2A–H2B deposition onto the preexisting tetramer forming full nucleosomes. For details see text.

how CAF-1 specifically recognizes modifications on H4 is not clear and future work should address how histone modifications contribute mechanistically to CAF-1 function.

Mechanistic implications for propagation of chromatin structure

During DNA replication, $(H3–H4)_2$ tetramers are propagated as intact units from the parental DNA randomly to one of the two daughter strands (19,111). This finding raised questions about a possible copying mechanism to re-establish chromatin marks on newly deposited histones when the template histone is not located within the same nucleosome (112–115). In relation to that, the histone chaperones not only perform assembly of $(H3–H4)_2$ tetramers onto the newly-synthesized DNA, but could also regulate the initial tetrasome formation in response to certain histone modifications. Candidate chaperones involved in recycling of such modified H3–H4 are MCM2 and ASF1, as outlined in the introduction. Thus, the recent advances outlined here are compatible with a model in which CAF-1 acts as an acceptor of modified or native parental H3–H4 dimers for histone recycling during replication. Because of the coordinated deposition mechanism, the biochemical properties of CAF-1 could help to ensure that simultaneously transferred histone dimers are rapidly reassembled on DNA. In such a case, CAF-1 could fulfill a dual role, namely *de novo* assembly as well as recycling of parental $(H3–H4)_2$ tetramers.

A CONSERVED MECHANISM FOR CHAPERONING OF H3–H4?

The molecular deposition mechanism of histone chaperones has been the subject of much discussion, but common principles of chaperone mediated nucleosome assembly have yet to be elucidated. This may in part be due to the difficulty of obtaining atomic structures of histones in complex with their full-length chaperones, and the absence of structures of intermediates during the deposition process. The modular nature and prevalent disorder within both types of proteins (i.e. histone complexes and histone

chaperones) may be responsible for this. In addition, beyond the presence of acidic stretches and intrinsically disordered domains (90), the structural determinants of histone chaperones differ vastly and do not appear to correlate with chaperone function or histone preference: among H3–H4 chaperones, one can find Ig-folds (ASF1), WD40 repeats (CAF-1, HIRA), NAP1 folds (Nap1 / Vps75), PH domains (Rtt106) and more (26,78,116–118). This lack of common molecular architecture and structural parameters precludes prediction of histone chaperone functionality and histone assembly mechanisms. As such, histone chaperones are still primarily understood in terms of their context-specific function and their ability to prevent unproductive histone-DNA interactions.

To what extent then can the insights gained from our investigations of CAF-1 contribute to our general understanding of histone chaperones? One feature might be the proposed transient dimerization of CAF-1 during histone deposition. Many H3–H4 chaperones that are responsible for the *de novo* assembly of tetrasomes either are dimers or have the potential to dimerize. Rtt106, another replication dependent H3–H4 chaperone which has overlapping roles with CAF-1, dimerizes in response to acetylation of H3K56 (117). The human, centromeric histone chaperone HJURP forms a heterotrimer with CENP-A-H4 in solution, yet dimerization of the complex is essential for the formation of CENP-A nucleosomes and potentially for recruitment to the centromeres (119,120).

Insight from the finding that the CAF-1 interacting surfaces of H3–H4 generally localize to surfaces that interact with DNA points to a shared mechanism of histone chaperone function. The chaperones protect the H3–H4 surface that will be bound in the subsequent step of nucleosome assembly. For example, ASF1 blocks the H3-H3 dimerization surface, but leaves the DNA binding surface of H3–H4 exposed (26,121). Whereas, after hand off of H3–H4 from ASF1 to CAF-1, the H3–H4 DNA binding surface appears to be protected and the H3-H3 dimerization interface becomes exposed (63). Similarly, in the ASF1-MCM2 handoff, MCM2 protects the DNA binding surface of the H3–H4 tetramer. This pattern of protection indicates that histone chaperones shield H3–H4 not only from non-specific off-pathway interactions, but shield specific surfaces of H3–H4. These H3–H4 surfaces are the ones needed for the subsequent step along the assembly pathway, which ensures that histone hand off occurs to the correct chaperone (or DNA) at the correct time and place.

CONCLUSIONS

Recently, studies from several labs have led to novel insights underlying the molecular mechanism of CAF-1 function. The CAF-1 mediated H3–H4 tetrasome assembly is one of the first H3–H4 deposition mechanisms described in molecular detail. A theme is emerging where the oligomerization state of H3–H4 is tightly regulated during histone storage and *de novo* nucleosome assembly, through controlled multimerization of the histone chaperone and controlled access to H3–H4 binding surfaces. Such a mechanism would confer specificity to the reaction, ensure rapid deposition, and promote the formation of co-chaperone complexes during histone transfer.

FUNDING

National Institutes of Health (NIH) [R01GM111902 to M.E.A.C.]; Howard Hughes Medical Institute (to K.L., Y.G. and F.M.); European Molecular Biology Organization [ALTF 1267-2013 to F.M.]; The Dutch Cancer Society [KWF 2014-6649 to F.M.]; The ANR Grant 'Replicaf' [ANR-16-CE11-0028-02 to D.P.]. Funding for open access charge: NIH.

Conflict of interest statement. M.E.A.C. is Executive Editor of *Nucleic Acids Research*.

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