Chromatin replication: **Finding the right connection**

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Nucleosomes are preferentially assembled on replicating DNA by chromatin assembly factor 1; recent studies have shown that replicated DNA is marked for assembly into chromatin by the replication-fork-associated protein PCNA.

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As the genomic DNA of a eukaryotic cell is replicated, the newly synthesised DNA daughter strands are efficiently assembled into chromatin fibres; this enables the requisite higher-order packaging of the genomic DNA within the confines of the nucleus. The repetitive subunits of chromatin, the nucleosomes, are not only architectural units — they are also important elements in the control of principle DNA transactions such as transcription, replication and repair. Control mechanisms must thus ensure that newly replicated DNA is rapidly and efficiently assembled into chromatin before it becomes a template for these transactions [1]. The spatial and temporal coordination of DNA replication and nucleosome assembly from newly synthesised histone proteins is thus a vital task for the cell.

The heterotrimeric protein chromatin assembly factor 1 (CAF-1) has a central role in the replication-dependent assembly of nucleosomes ([2], reviewed in [1]). CAF-1 was originally purified from human nuclear cell extracts, but homologues exist in organisms as diverse as yeast and *Drosophila*. CAF-1 acts as a molecular chaperone, mediating the assembly of nucleosomes from newly synthesised histone proteins onto replicating DNA *in vitro*. It binds to these histone proteins and targets them to the site of nucleosome assembly on the newly replicated DNA. CAF-1 appears to recognise some feature of replicating DNA as substrate, as it does not mediate nucleosome assembly on non-replicating DNA templates. In this respect, CAF-1 differs from other histone chaperones such as NAP-1 or nucleoplasmin, which facilitate nucleosome assembly on any DNA template, even in the absence of replication. For this reason, CAF-1 has attracted considerable interest as a candidate coordinator of nucleosome assembly and DNA replication.

The molecular mechanism of CAF-1 specificity for replicating DNA templates has, until recently, been elusive. It became possible to analyse this specificity after it was found that DNA replication and chromatin assembly by CAF-1 can be temporally uncoupled [3]. The key observation was that preferential nucleosome assembly on newly replicated DNA was still observed when CAF-1 was added to a reaction a short time after the elongation steps of DNA replication were blocked by the DNA polymerase inhibitor aphidicolin [3]. This suggested that newly replicated DNA contains an epigenetic marker for a short time following DNA replication, which can attract CAF-1 to mediate nucleosome assembly on this substrate [3].

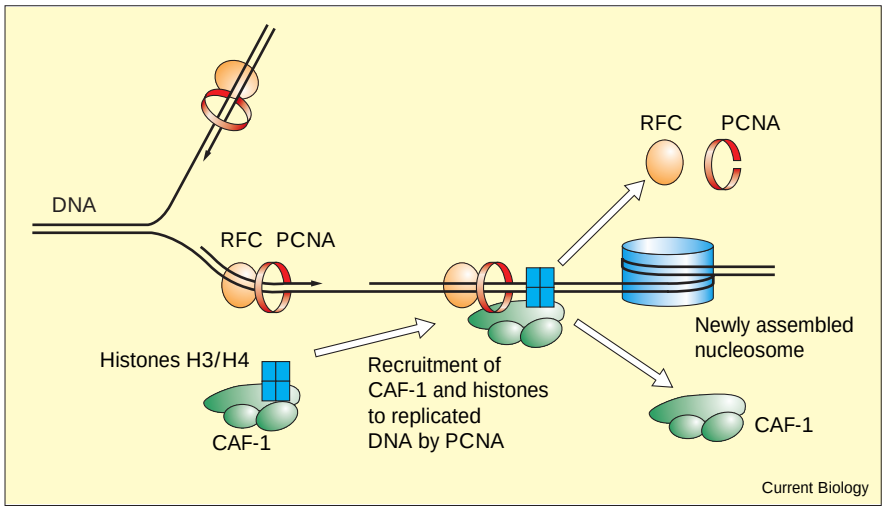
Shibahara and Stillman [4] have recently investigated the molecular nature of this marker. In their work, purified recombinant human CAF-1 was able to mediate chromatin assembly on DNA substrates after DNA replication was inhibited by aphidicolin. They found that the epigenetic marker on replicated DNA was stable when the replicated DNA templates were isolated and incubated on ice, before addition of CAF-1. The marker could, however, be removed from the templates, and subsequent chromatin assembly prevented, upon incubation at 37°C in the presence of purified replication factor C (RFC) and ATP. These data strongly suggested that RFC, or its interacting substrate partner, the proliferating cell nuclear antigen (PCNA), are involved in the epigenetic marking of newly replicated DNA.

PCNA is a protein associated with the DNA replication fork, and one of its major functions is that of a processivity factor for the replicative DNA polymerases (reviewed in [5–7]). The protein is a trimer of identical subunits, which can associate around double-stranded DNA to form a ring-like structure [8]. The loading and unloading of PCNA onto and off the DNA require another replication-fork-associated protein — RFC. RFC functions as a molecular matchmaker to generate the non-covalent, topological linkage between the PCNA ring and DNA; it acts at junctions between single-stranded and double-stranded regions of the DNA, as found at primer–template junctions in replication forks or sites of DNA repair.

Once formed, the PCNA ring is topologically linked to the DNA and it can move along the double-stranded DNA, serving as a ‘sliding clamp’. The physical association of the replicative DNA polymerases with this sliding clamp greatly enhances the processivity of DNA replication at the fork, and also facilitates the coordination of continuous and discontinuous DNA synthesis — that is, the ‘leading’ and ‘lagging’ strand synthesis that is required to generate the two daughter strands, given the polarity of DNA synthesis [7]. The recruitment of DNA polymerases to

Figure 1

An illustration of how PCNA may target CAF-1 to replicated DNA. The deposition of the PCNA ring (red) onto replicating DNA by RFC (orange) provides a marker of replicated DNA for a recruitment of the chromatin assembly complex, consisting of CAF-1 (green) and newly synthesised histones (blue squares). Following assembly of a nucleosome (blue cylinder), CAF-1 and PCNA–RFC dissociate from the DNA again. For simplification, other components of the replication fork, its topography and the possible involvement of parental chromatin structures are omitted from this scheme (for more details see [1,5,7]).



sites of DNA excision repair synthesis is also believed to be mediated by PCNA and RFC [5,6].

In addition to serving as an anchor point for DNA polymerases, PCNA also interacts with a multitude of other proteins that are involved in DNA replication, repair, recombination and modification, as well as proteins involved in the regulation of the cell-division cycle. Examples of such proteins that have been identified so far include DNA polymerases δ and ε, RFC, the 'flap' endonuclease Fen-1 (which is involved in processing the Okazaki fragments that are intermediates of discontinuous DNA synthesis), uracil-DNA glycosylase, DNA ligase 1 and DNA methyltransferase, as well as cyclin D and the cyclin-dependent kinase inhibitors p21 and p57 [5,6].

The properties of PCNA made it an attractive candidate for being the marker of replicated DNA for chromatin assembly, particularly given the observation that RFC can facilitate erasure of the marker. Shibahara and Stillman [4] found that, in their system, PCNA was indeed bound to replicated DNA and that its removal by RFC and ATP abrogated the ability of the substrates to support chromatin assembly by CAF-1. Further evidence that PCNA is at least part of the marker came from the observation that antibodies against PCNA prevented CAF-1 action on replicated substrates [4]. A direct physical interaction between PCNA and the largest subunit of CAF-1 was demonstrated in protein-binding assays. Further supporting evidence came from the observation that PCNA co-localised with CAF-1 in human cell nuclei [4], both PCNA and CAF-1 having previously been shown to colocalise with newly replicated DNA during S phase [9,10]. Taken together, these results indicate that PCNA has an essential, but not necessarily sufficient, role in coupling chromatin assembly and DNA replication (Figure 1).

CAF-1 has thus been added to the list of PCNA-interacting proteins, suggesting that PCNA can attract the nucleosome assembly machinery, as well as the various enzymes mentioned above, to DNA [4]. This broad array of interacting partners makes PCNA a central communicator, or adaptor, for proteins that interact with DNA without necessarily being DNA-binding proteins [5,6]. As PCNA and RFC are also involved in DNA excision repair, it is reasonable to extrapolate from the mechanism of nucleosome assembly at replication forks to the reassembly of chromatin after DNA excision repair. CAF-1 has been shown to mediate nucleosome assembly at these sites of DNA repair [11]. Furthermore, CAF-1 and PCNA are recruited to a chromatin-bound fraction in response to UV-irradiation and induction of DNA damage in intact cells [12]. These data suggest that PCNA targets CAF-1 to DNA templates undergoing DNA replication or repair by similar mechanisms [4].

Shibahara and Stillman [4] suggested a model for asymmetric chromatin assembly during replication, based on the observation that PCNA is asymmetrically distributed on the two DNA daughter strands at the replication fork. On the leading strand, only one PCNA ring is required as a sliding clamp, whereas on the lagging strand, the continuous priming and synthesis of Okazaki fragments leads to a higher number of PCNA rings in the vicinity of the fork. This number could be further increased by reducing the extent to which PCNA is unloaded by RFC, so that the PCNA rings remain on the replicated DNA for some time after replication. This suggests that CAF-1-mediated nucleosome assembly might occur more quickly and efficiently on the lagging strand than the leading strand during DNA replication.

This replication-linked asymmetry in chromatin assembly could give rise to different chromatin states on the two

daughter strands. If one assumes that DNA replication is initiated from a defined point with respect to a particular gene of interest, the asymmetry could potentially be exploited to generate daughter cells with different developmental fates, depending on which of the two daughter strands a cell inherits [4]. This possibility is complicated *in vivo*, however, by the fact that nucleosomes derived from the parental chromosome are simultaneously transferred to the replicated DNA, in addition to those assembled *de novo* by CAF-1. Further experiments will be required to test whether this interesting possibility really is used *in vivo*.

I would like to finish by turning the topic around and looking at new insights into how chromatin is connected to the regulation of DNA replication. In work recently published in *Current Biology* [13], a novel factor was purified and cloned from *Xenopus* which is required for establishing DNA replication *in vitro*; it has DNA unwinding activity, and so has been dubbed 'DNA unwinding factor' (DUF). Interestingly, one subunit of this heterodimeric factor is homologous to a mammalian high-mobility group (HMG) protein, whereas the other is homologous to a yeast transcriptional activator and chromatin modulator. Chromatin remodelling factors have, until recently, been considered largely in relation to the regulation of transcription, but the new results on DUF [13], together with recent studies on the stimulation of simian virus 40 DNA replication *in vitro* by HMG-17 protein [14] and by the chromatin remodelling factor CHRAC [15], indicate that chromatin modulating proteins also have important roles in the regulation of DNA replication.

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