Geneviève Almouzni

Chromatin Dynamics

Introduction

Chromatin organization is a critical key in the control of the multiple functions within the eukaryotic nucleus. Importantly genome organization provides in addition to genetic information another layer of information, so called epigenetic, which by definition means that it is stably inherited throughout cellular divisions, yet it is not encoded genetically. Thus each cell type will display a specific "epigenome". It becomes thus important to understand how to preserve not only genetic but also epigenetic information. In the past years, we uncovered a potential important link between chromatin assembly and DNA repair which may contribute in the restoration of the original chromatin organisation. To study chromatin organization and its dynamics, our main working model is an amphibian, Xenopus laevis. This experimental system is ideal for developmental approaches in vivo and biochemical studies in vitro. Based on our findings in this system, we explore our working hypothesis in other systems such as mammalian cells or Drosophila embryos.

Factors involved in chromatin dynamics

To analyse parameters involved in chromatin assembly, first we focused our attention on the initial step involving histone deposition to form a nucleosomal particle (Mello and Almouzni, 2001). To date one of the best candidate in promoting nucleosome formation during S phase is Chromatin Assembly Factor 1, CAF-1, a complex first identified in human cell extracts in the laboratory of Bruce Stillman (CSH). Remarkably, Xenopus egg extracts have proven extremely efficient to carry out chromatin assembly on an exogenous DNA and nuclear formation under cell-cycle control. Using these extracts we developed a model system coupling chromatin assembly to DNA synthesis as observed in vivo during DNA replication. We also observe coupling between DNA repair and chromatin assembly, a process that should be important to maintain chromatin integrity within the cell.

Lab members

Geneviève Almouzni
Group Leader
Institut Curie/Section de recherche UMR218
26, rue d'Ulm, 75231 Paris CEDEX 05, France

Tel: + 33 1 4234 6701, Fax: + 33 1 4633 3016
Email: Genevieve.Almouzni@curie.fr

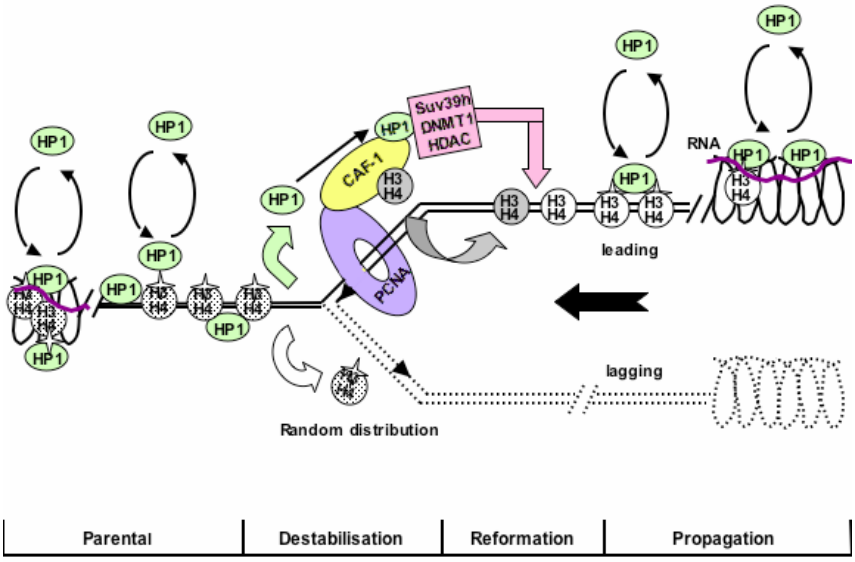
Staff:

Delphine Bailly
Dominique Chardonnieras
Leanne De Koning Annabelle
Gérard Sébastien Huart
Stéphane Koundrioukoff
Patricia Le Baccon Alejandra
Loyola Christèle Maison
Martijn Moné
Sophie Polo
Jean-Pierre Quivy
Dominique Ray-Gallet
Danièle Roche
Barbara Spadilero

This coupling was observed in systems derived from three organisms Xenopus, human and Drosophila. In Xenopus egg extracts a CAF-1 activity was revealed (Gaillard et al., 1996). Following the cloning of the largest subunit of this complex in Xenopus, our data helped to support a specific role of CAF-1 to facilitate nucleosome formation coupled to DNA synthesis in vitro. Furthermore, using a dominant negative strategy based on the properties of the largest subunit of CAF-1 to dimerize, we could demonstrate a critical role for this factor during the rapid divisions of the early development in Xenopus (Quivy et al., 2001). Several histone chaperones may also function within an assembly line for histone deposition with either specific or redundant function (Loyola (Ray-Gallet et al., 2002) and ASF-1. To address how replicative histones variant H3.1 and constitutive variant H3.3 are deposited into chromatin through distinct pathways, we have purified deposition machineries for these histones. The H3.1 and H3.3 complexes contain distinct histone chaperones, CAF-1 and HIRA, that we show are necessary to mediate DNA synthesis-dependent and -independent nucleosome assembly, respectively. Notably, these complexes possess one molecule each of H3.1/H3.3 and H4, suggesting that histones H3 and H4 exist as dimeric units that are important intermediates in nucleosome formation. This finding provides new insights into possible mechanisms for maintenance of epigenetic information after chromatin duplication (Tagami, Ray-Gallet et al., 2004).

Maintenance of chromatin organization

DNA repair and cell-cycle. The cell under constant genotoxic stresses has developped specific DNA repair mechanisms to ensure the maintenance of genome integrity. During these repair events chromatin rearrangements occur (Moggs and Almouzni, 1999a ; Kaufman and Almouzni, 2000 ; Green and Almouzni, 2002 ; Gontijo et al., 2003). Thus, epigenetic information largely transmitted through chromatin organization must be considered and mechanisms ensuring its proper maintenance will be critical in order to maintain a fully competent genetic and epigenetic



For simplification, only the leading strand is detailed on which a continuous DNA synthesis is observed. During replication, the dynamics of histones involves (i) the random distribution between the two daughter strands of the parental histones (circles with dotted pattern), and (ii), concomitantly, the deposition of *de novo* synthesized histones (acetylated on histone H4; grey circles). Starting from parental heterochromatic DNA on which heterochromatin protein 1 (HP1) is associated with multiple partners (methylated histone H3-K9, DNA, RNA), the passage of the replication fork triggers the release of parental modified nucleosomal histones (methylated on histone H3, star) ahead of the fork, thereby leading to local destabilization and the loss of HP1 nucleosomal binding sites. New binding sites are subsequently created through the proliferating cell nuclear antigen (PCNA)-mediated recruitment of chromatin assembly factor 1 (CAF-1), which assists *de novo* histone deposition in the 'reformation' step. The continual exchange between free and bound forms of HP1 ensures the plasticity of the domain. The CAF-1-bound HP1 recruits the DNA methyltransferase DNMT1 and its binding partner histone deacetylase (HDAC) to methylate DNA on CpG and deacetylate histones, respectively. CAF-1-bound HP1 also recruits Suv39h to methylate histone H3 on K9 (star). This latter modification in turn provides new HP1-binding sites to propagate the heterochromatin state ('propagation' step). Soon after, newly synthesized DNA in the form of chromatin bound to HP1 can associate with the 'structural RNA' (purple) that helps to ensure the architectural organization of the entire domain.

information. We initially focused our attention on Nucleotide Excision Repair, NER, a pathway highly conserved throughout evolution which allows to eliminiates major DNA lesions due to UV irradiation. Our results support a mechanism for chromatin assembly coupled to NER which involves CAF-1. The importance of this factor at the crossroads of DNA repair and replication as well as epigenetic inheritance (Ridgway and Almouzni, 2000) led us to search for its partners. Two human homologs of the yeast protein ASF1 (anti silencing factor 1) were found to interact with the midsize subunit of CAF-1 and synergize with it to promote nucleosome formation during NER (Mello et al., 2002).

One of the interactors of CAF-1 identified to date, the protein PCNA (Proliferating Cell Nuclear Antigen), has been the subject of more extensive biochemical studies (Moggs et al., 2000). Most importantly, the interaction with PCNA gives us the first molecular determinant directly connecting repair and chromatin assembly.

Other interactors, such as ASF-1 (anti-silencing factor-1), HP1 (Heterochromatin Protein 1), identified in our laboratory appear to be interesting candidates linking CAF-1 to DNA metabolism and to cell cycle control (for review see Koundrioukoff et al., in press). These interacting proteins could be highly relevant to tumorigenesis. Our current current results underly the hypothesis for CAF-1 as a potential proliferation marker of clinical value in breast cancer (Polo et al., 2004).

Chromatin dynamics at a cellular level and during development.

We have followed in vivo changes affecting Chromatin Assembly Factor 1 following UV irradiation of human cells (Martini et al., 1998) and recently developed an approach using local irradiation combined with the detection of UV lesions using specific antibodies as initially described (Moné et al., 2001). This strategy enabled us to support a role for CAF-1 in the maintenance of the chromatin organisation during NER (Green and Almouzni, 2003).

In parallel, we have characterized the subcellular distribution of acetylated isoforms of the histones by comparison with replication foci. The analysis of the distribution of a protein of interest using confocal microscopy, combined to the labeling of the replication foci with pulse/chase experiments, enables us to establish a link between the protein and the synthesis event. The application of this method to the study of CAF1, HP1 (Heterochromatin protein 1) and the acetylation of histones, gave us means to built up a model for the propagation and heritability of heterochromatic regions (Taddei et al., 1999).

One characteristic conserved between regions of heterochromatin is the presence of under-acetylated histones. Our studies using deacetylase inhibitors have shown that they induce a spatial reorganisation of pericentric heterochromatin, accompanied by mitotic defects (Taddei et al., 2001). These results prompted us to examine the stability of pericentric heterochromatin, using various treatments in combination with the use of an antibody raised against a branched peptide correponding to the Lysine 9 methylated N-terminal portion of histone H3 (collaboration with T. Jenuwein, Vienna). Our data point to the importance of an RNA and histone modification dependent organization of pericentric heterochromatin required for the accumulation of HP1 proteins (Maison et al., 2002 ; Maison and Almouzni, 2004). We examine in more details the fate of subdomains (centric and pericentric) and their importance for centromeric function (Guenatri et al., in press). Most recently, we also examined how pericentric regions enriched in HP1 proteins replicate in order to maintain an overall stability of the domain (Quivy et al., in press). We propose a model in which pericentric heterochromatin duplication bodies act as factories where both DNA replication and its assembly into chromatin would be coordinated.

We have followed chromatin properties during Xenopus development. We observed a major transition in the modification of core histones (acetylation) as well as in the nature of the linker histone together with a modification of the major subunit of RNA polymerase II at the time of zygotic activation (Palancade et al., 2001). Exogenous DNA can be injected into the egg after fertililization. The fate of the molecules can then be followed at the level of their structural organization as well as their transcriptional properties, all throughout development. The possibility to use an inductible transcription system has been explored (Ridgway and Almouzni, 2000). We have also pursued our investigations concerning the role during development of putative chromatin assembly factors. We could establish the importance of CAF-1 during the rapid division at the early stages of development in Xenopus (Quivy et al., 2001).