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MOLECULAR BIOLOGY

Histone Chaperones: Variety and Functions

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Abstract—Histone chaperones are required for the formation of the nucleosome—the basic unit of chromatin that consists of the DNA and histones. In this review, participation of histone chaperones CAF-1, ASF1, NAP1, and FACT in key cellular processes is discussed. Being multifunctional factors, histone chaperones take part in DNA replication, transcription, and repair. During replication, histone chaperones are required to form chromatin structure on both the mother and daughter DNA. They are involved at different stages of genome packaging: from histone transport into the nucleus to nucleosome formation. During transcription, histone chaperones reduce a nucleosome barrier for RNA polymerases accelerating the rate of RNA synthesis and promote nucleosome reassembly. During DNA repair, histone chaperones provide access to the damaged genome region for the repair enzymes, and participate in the chromatin assembly after DNA repair. Mutations in histone chaperones typically result in multiple defects in the cell, underlying the functional importance of these proteins.

Keywords: chromatin, nucleosome, histone, histone chaperone, replication, transcription, review

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INTRODUCTION

In eukaryotes, genetic information is encoded by the DNA molecule, which, in a complex with histone proteins, forms the chromatin. The structural unit of chromatin is the nucleosome, which consists of double-stranded DNA (147 bp, packed in 1.65 turns) wound around the histone octamer [1]. Histone octamer includes four pairs of proteins—H2A, H2B, H3, and H4—and tetramer (H3-H4)2 is flanked on both sides with H2A-H2B dimers [1].

An important role in the shaping of proper chromatin structure and in the prevention of aggregation of histone proteins with DNA during nucleosome formation belongs to histone chaperones (HC). The first such chaperone—nucleoplasmin—was described in 1978 [2]. Currently, more than fifteen different HC are known [3–8], which are involved in the storage of histones and their transport, in nucleosome formation and disassembly, as well as in transcription, and DNA replication and repair (Table). Operation of HC is ATP-independent. In this review, HC functions are described on the example of CAF-1, ASF1, NAP1, and FACT.

HISTONE CHAPERONES ARE INVOLVED IN DNA REPLICATION

Proper packaging of the DNA into chromatin is especially important after replication, the doubling of the genetic material prior to cell division, in which process the synthesized daughter strand of DNA must be structurally identical to the parent strand. Fractionation of extracts from human cells revealed that trisubunit protein complex CAF-1 (Chromatin Assembly Factor-1) that operates as a HC by placing the newly synthesized histones H3 and H4 on the replicating DNA in vitro [9]. CAF-1 is localized in the cell at the sites of DNA replication, which, indirectly, confirms its function in this process. Discovery of the physical interaction between CAF-1 and the replication apparatus confirmed that CAF-1 is indeed a factor that structures the chromatin during the doubling of genetic material [6]. Inactivation of CAF-1 causes elongation of the Okazaki fragments, whose size depends on the position of nucleosomes on the DNA. This also points to the CAF-1 role in replication in conjunction with chromatin assembly [10]. Furthermore, it was reported that CAF-1 is required for chromatin-dependent suppression of gene expression [6].

There are other factors that can substitute for CAF-1 function. For instance, yeast cells lacking CAF-1 remain viable and actively proliferate [6]. These factors include ASF1 (Anti-Silencing Factor 1) and Rtt106, which are involved in replication-associated construction of chromatin [11, 12]. In human cells, ASF1 associated with chromatin is localized in the complex with MCM helicase, an important component of the DNA replication machinery [13]. Studies

Histone chaperones, their partners and processes in which the chaperones are involved

Histone chaperones	Histones-chaperone partners	Processes involving chaperones
NAP1	H2A-H2B, H2A.Z-H2B, H3-H4	Transcription, import of histones into the nucleus from cytoplasm
Chz1	H2A.Z-H2B	Transcription
Swr1	H2A.Z-H2B	Transcription
ANP32E	H2A.Z-H2B	Response to DNA damage
FACT	H2A-H2B, H3-H4	Replication, transcription, repair
Spt6	H3-H4	Transcription
Asf1	H3-H4, H3.3-H4	Replication, transcription
Rtt106	H3-H4	Replication, transcription
CAF-1	H3-H4	Replication
ANP32E	H2A.Z-H2B	Transcription
DAXX	H3.3-H4	Formation of telomere chromatin
Hir	H3.3-H4	Transcription
HIRA	H3.3-H4	Transcription
HJURP	$CenH3^{CENP-A}$	Formation of centromere
Scm	$CenH3^{CSE4}$	Formation of centromere
CAL1	$CenH3^{CID}$	Formation of centromere

indicate that ASF1 binds H3-H4 and regulates histone accessibility to CAF-1 and other chaperones [6]. In particular, ASF1 is required for posttranslational acetylation of H3 at lysine 56 [14]. This modification is characteristic for the newly synthesized histones [15], and facilitates ubiquitination of H3, which, in turn, facilitates the transfer of H3-H4 from ASF1 to other chaperones [16].

ASF1 is not the only chaperone that forms a complex with MCM. MCM complex can also bind FACT factor (Facilitates Chromatin Transcription) [17], which is involved in the replication-associated chromatin remodeling together with CAF-1 and Rtt106 [18]. FACT has been shown to interact with DNA polymerase directly [19]. In addition, experimental studies in vitro established that FACT can specifically bind to the H2A-H2B dimers and to the H3-H4 tetramer and, thereby, stimulate nucleosome formation [5].

Notably, yeast cells are less sensitive to the disturbances in chaperone function than multicellular organisms. Yeast can survive even when both CAF-1 and ASF1 are inactive [11]. In contrast, mutations in CAF-1 in *C. elegans* disrupt development of its nervous system [20].

Histones H2A and H2B are incorporated into nucleosomes with the aid of the chaperone NAP-1 (Nucleosome Assembly Protein 1) [21]. NAP-1 binds these histones and facilitates their transport from the cytoplasm to the nucleus during cell cycle transition from G1 to S-phase [22]. This occurs simultaneously with the activation of nucleosome assembly during DNA replication. NAP-1 contributes to the flow of H2A and H2B in the nucleus by mediating their interaction with importin Kap114 [23]. In vitro experiments revealed that NAP-1 facilitates inclusion into the nucleosomes not only of the H2A and H2B histones but also H3 and H4 [24]. It was reported that NAP-1 is also able to disrupt already formed nonspecific complexes between DNA and histones [25]. This activity is fundamental to the chaperone mechanism of action during chromatin assembly.

HISTONE CHAPERONES ARE INVOLVED IN DNA REPAIR

Eukaryotic DNA repair is followed by its subsequent correct layering within the chromatin, and HC are directly involved in this process. For instance, CAF-1 is involved in DNA repair after damage by UV radiation. It promotes proper chromatin assembly subsequent subsequent to base excision repair [26].

ASF1 is also involved in the DNA repair process [6]. It has been shown that ASF1 is involved in cell response to UV light irradiation, which is manifested especially clearly in the absence of CAF-1. Yeast cells lacking a functional ASF1 display a significant increase in the sensitivity to substances that cause double stranded DNA breaks, which points to the ASF1 role in DNA repair [6]. In the absence of stress, cellular ASF1 is associated with Rad53 protein [27, 28], which is presumed to prevent ASF1 interaction with histones [27]. DNA damage leads to the phosphorylation of Rad53, which causes the release of

ASF1 complex. Thereafter, ASF1 is able to bind histones and package the DNA into chromatin [6].

The role of FACT factor in the DNA repair has been established [29]. It is believed that FACT promotes the movement of RNA polymerase along the damaged portion of the chromatin [4]. It is possible that the chaperone also increases DNA accessibility to other molecules involved in the repair. It has also been suggested that FACT promotes incorporation of H2A-H2B histones dimers into the repaired regions of chromatin.

HISTONE CHAPERONES ARE INVOLVED IN CHROMATIN TRANSCRIPTION

During their function, RNA polymerases disrupt chromatin structure, whose nucleosome organization represents a barrier to their advancement on the DNA [30]. HC actively participate in the process of chromatin transcription. First, they facilitate the passage of enzymes through the nucleosomes, and second, they restore chromatin structure after the passing of the polymerases. FACT was the first factor for which it was shown that it facilitates chromatin transcription in vitro [31]. It is believed that FACT functions during transcription elongation by competing with DNA for the interaction with histones. This facilitates the dissociation of histones from DNA and reduces the occurrence of unproductive elongation complexes [32].

It is known that RNA polymerase can displace both the H2A-H2B histone dimer, as well as $(H3\text{-}H4)_2$ tetramer, from the nucleosomes during transcription, and this process is particularly effective on actively transcribed genes. In the presence of yeast FACT, efficiency of the RNA polymerase in the histone displacement and exchange are significantly reduced [33]. In the cell, FACT shows the same kinetics of chromatin binding and movement through transcribed genes as the RNA polymerase II [34, 35]. Thus, an important function of the chaperone FACT is to maintain the nucleosomes on the DNA during transcription. FACT could perform this role either by keep the histones in the same place or by restoring their relationship with the DNA immediately after the passage of the RNA polymerase. Mutations in genes encoding chaperones FACT and Spt6 lead to the activation of transcription from cryptic promoters as the result of nucleosome loss [36], which also indicates the important role of chaperones in maintaining the chromatin structure.

During transcription, chaperone Asf1 promotes elimination of histones from gene promoters and coding regions, thereby facilitating the movement of the RNA polymerase. Asf1 also aids in the acetylation of H3K56, which makes the nucleosome less stable. The signal-antagonist for this Asf1 activity is the methylation of H3K36, which prevents the loss of histones [3]. This H3 modification takes place with the involvement of HC Spt6 described above [37].

Chaperones NAP1 and Chz1 stimulate the inclusion into the chromatin of the histone variant H2A.Z, which facilitates transcription [38, 39]. In turn, NAP1 function is controlled by the chromatin-remodeling factor RSC. Histone variant H2A.Z is an antagonist of DNA methylation, and its presence in the chromatin leads to the rapid gene activation [40, 41]. The signal for the inclusion of H2A.Z into promoters is the emergence of a variant histone H3.3 at the enhancers [42]. Apparently, this step is a part of the mechanism of transcription activation.

HISTONE CHAPERONES HAVE OTHER FUNCTIONS

The accumulated data on the HC suggests that their role is not limited to such processes as DNA replication, transcription, and repair. For example, the FACT chaperone shows a significant ability to modify the nucleosome canonical structure. This process takes place without energy consumption, is reversible, and the nature of this phenomenon is not fully understood [5]. There are, apparently, other yet unknown HC in the cells, and their search and study, in addition to the fundamental aspects, also have a practical significance, since disruptions in the HC function are associated with various pathologies, for example, with the development of cancer [43].

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