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ATP-facilitated Chromatin Assembly with a Nucleoplasmin-like Protein from *Drosophila melanogaster**

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Takashi Ito, Jessica K. Tyler‡, Michael Bulger, Ryuji Kobayashi§¶, and James T. Kadonaga‖

From the Department of Biology and Center for Molecular Genetics, University of California, San Diego, La Jolla, California 92093-0347 and §Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

To gain a better understanding of the factors that can mediate chromatin assembly, we have purified and cloned a core histone-binding protein from *Drosophila melanogaster* embryos. This protein resembles *Xenopus laevis* nucleoplasmin, and it has therefore been termed dNLP, for *Drosophila* nucleoplasmin-like protein. dNLP is a nuclear protein that is present throughout development. Both purified native and recombinant dNLP bind to core histones and can function in the assembly of approximately regularly spaced nucleosomal arrays in a reaction that additionally requires DNA, purified core histones, ATP, and a partially purified fraction (containing at least one other assembly activity). We also analyzed the properties of an N-terminally truncated version of dNLP, termed dNLP-S, and found that the deletion of the N-terminal 31 residues of dNLP results in a loss of the specificity of the interaction of dNLP with core histones. We then compared the abilities of dNLP and *Drosophila* nucleosome assembly protein-1 (dNAP-1) to promote the decondensation of *Xenopus* sperm chromatin, a process that can be mediated by nucleoplasmin. We observed that dNAP-1, but not dNLP, was able to promote the decondensation of sperm chromatin. These and other data collectively suggest that dNLP may participate in parallel with other histone-binding proteins such as dNAP-1 in the assembly of chromatin.

Chromatin assembly is a fundamental process that is involved in a broad range of biological phenomena such as gene regulation, recombination, DNA repair, and progression through the cell cycle, and it is therefore important to investigate the factors that participate in the formation of chromatin (for reviews, see Refs. 1–7). The analysis of chromatin assembly has revealed that the deposition of the core histones H3 and H4 precedes the incorporation of H2A and H2B into chromatin, and that both the pre-existing and newly synthesized histones are randomly distributed among the daughter DNA strands. Moreover, the newly synthesized histones, which are more highly acetylated in their lysine-rich N-terminal tails relative to bulk histones, become deacetylated after assembly into chromatin. Chromatin assembly commences immediately after DNA replication, and DNA replication and chromatin assembly appear to be coupled, although possibly by an indirect mechanism, as chromatin assembly appears to occur preferentially, but not obligatorily, with newly replicated DNA (see, for instance, Ref. 8).

Biochemical studies of chromatin assembly have led to the identification of several core histone-binding factors, which include nucleoplasmin (9–13), N1/N2 (14–17), nucleosome assembly protein-1 (NAP-1)[1] (18–21), and chromatin assembly factor-1 (CAF-1) (22–24). The current data suggest that nucleoplasmin and NAP-1 interact preferentially with H2A and H2B relative to H3 and H4 (17, 19, 21) and that N1/N2 and CAF-1 bind to H3 and H4 (14, 15, 17, 23, 24). These factors may therefore act, at least in part, as histone chaperones that deliver the core histones to the newly replicated DNA. Moreover, because of their related biochemical properties, it is possible that there may be some redundancy in the function of these histone-binding factors in chromatin assembly.

The mechanism of chromatin assembly is likely, however, to be more complex than the random deposition of histones that is mediated by the core histone-binding factors alone. For instance, it is known from studies of chromatin assembly activities in crude extracts derived from *Xenopus laevis* oocytes (25), HeLa cells (26), or *Drosophila melanogaster* embryos (27, 28) that the assembly of approximately regularly spaced nucleosomal arrays is an ATP-dependent process. Biochemical fractionation of a chromatin assembly extract from *Drosophila* embryos led to the identification of two fractions, termed dCAF-1 and dCAF-4, which, when combined, were able to reconstitute the ATP-facilitated assembly of nucleosomal arrays, as was observed with the crude extract (29). One component in the dCAF-4 fraction was purified and cloned, and found to be the *Drosophila* homologue of NAP-1 (dNAP-1) (21). Purified recombinant dNAP-1 was observed to function in a cooperative manner with the active component(s) in the dCAF-1 fraction to mediate the ATP-facilitated assembly of nucleosomal arrays. The ATP-utilizing chromatin assembly factor(s) in the dCAF-1 fraction has not yet been identified, although it is known that the *Drosophila* homologue of CAF-1 (termed dCAF-1 protein, which should not be confused with the dCAF-1 fraction) is

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‖ Presidential Faculty Fellow. To whom correspondence should be addressed: Dept. of Biology, 0347, Pacific Hall, Rm. 2212B, University of California, San Diego, 9500 Gilman Dr., La Jolla, CA 92093-0347. Tel.: 619-534-4608; Fax: 619-534-0555; E-mail: jkadonaga@ucsd.edu.

[1] The abbreviations used are: NAP-1, nucleosome assembly protein 1; CAF, chromatin assembly factor; dCAF-1, *Drosophila* chromatin assembly factor 1; dCAF-1 fraction, a protein fraction derived from *Drosophila* embryos that contains dCAF-1; dCAF-4 fraction, a protein fraction derived from *Drosophila* embryos that contains dNAP-1 and dNLP; dNAP-1, *Drosophila* nucleosome assembly protein 1; dNLP, *Drosophila* nucleoplasmin-like protein; dNLP-S, short N-terminally truncated version of the *Drosophila* nucleoplasmin-like protein; PBS, phosphate-buffered saline; PCR, polymerase chain reaction.