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

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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) (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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present in the partially purified fraction. These and other data collectively suggest a model for chromatin assembly wherein nucleoplasmin and/or NAP-1 function as a carrier for H2A-H2B, N1/N2 and/or CAP-1 function as a carrier for H3-H4, and an additional factor(s) (in the dCAF-4 fraction) mediates the ATP-dependent assembly of nucleosomes by acting in conjunction with the histone chaperones.

In this study, we focus upon the further investigation of components in the dCAF-4 fraction, and describe the purification, cloning, and characterization of a second core histone-binding protein. The primary amino acid sequence of this protein resembles that of X. laevis nucleoplasmin, and it has therefore been termed dNLP, for Drosophila nucleoplasmin-like protein.

**EXPERIMENTAL PROCEDURES**

**Purification of dNLP—**All operations were performed at 4 °C. The S-190 chromatin assembly extract (28, 30) was sequentially subjected to chromatography on DEAE-Sepharose FF (Pharmacia Biotech Inc.), SP-Sepharose FF (Pharmacia), and Q-Sepharose FF (Pharmacia) resins to give the dCAF-4 fraction (29). To this fraction, 4 M ammonium sulfate (adjusted to pH 7 by the addition of NaOH) was added to a final concentration of 2.75 M. The mixture was incubated for 20 min and then subjected to centrifugation (Sorvall SS-34 rotor; 10,000 rpm for 10 min). The supernatant (containing the dNLP) was applied to a phenyl-Sepharose CL-4B (Pharmacia) column (column volume = 0.5 ml; column dimensions (diameter × length) = 0.5 × 2.5 cm; flow rate = 0.1 ml/min; fraction size = 0.5 ml). The column was washed with three volume columns of buffer B (10 mM Hepes (KOH), 1.5 mM MgCl₂, 10% glycerol, 10 mM β-glycerophosphate, 1 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride) containing 2.5 M ammonium sulfate, and protein was eluted with a linear gradient (over 10 column volumes) from 2.5 M to 0 M ammonium sulfate in buffer B. Fractions were analyzed by SDS-polyacrylamide gel electrophoresis, and the peak dNLP-containing fractions were pooled and then dialyzed against buffer R.

**Isolation of cDNA Encoding dNLP—**The purified dNLP was subjected to electrophoresis on a 12% SDS-polyacrylamide gel, and the protein was visualized by staining with Coomassie Brilliant Blue G. The protein was visualized by staining with Coomassie Brilliant Blue G.

**Purification of Recombinant dNLP and dNLP-S—**

**Analysis of Protein-Histone Interactions—**Polyclonal antibodies against purified recombinant dNLP were generated in rabbits. The immunoprecipitation of dNLP and dNLP-S from a crude Drosophila embryo extract was performed as described previously (28–30). The proteins were purified from Drosophila embryos (collected from 0 to 12 h after egg deposition) by using a nondenaturing procedure, as described by Bulger and Kadonaga (30). Recombinant dNAP-1 was purified as described elsewhere (21). DNA supercoiling and micrococcal nuclease digestion assays were carried out as described previously (28-30). All experiments were performed a minimum of two times (but typically, several times) to ensure reproducibility of the data.

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**Purification of Recombinant dNLP and dNLP-S—**A freshly transformed E. coli strain containing the plasmid pETdNLP-S was grown at 37 °C for 18 h in Luria-Bertani medium containing 100 μg/ml ampicillin. The cells were harvested by centrifugation (Sorvall SS-34 rotor; 10,000 rpm for 10 min). The supernatant was then subjected to 15% SDS-polyacrylamide gel electrophoresis and Staining with Coomassie Brilliant Blue G.
**Boehringer Mannheim** for 1 h at room temperature in buffer B; and rinsed with 1× PBS. The samples were then mounted in 50% glycerol and placed on a glass slide. The cells were observed by using a 100× oil immersion objective with a Nikon microscope.

**Assay for Decondensation of Xenopus Sperm Chromatin**—Demembranated Xenopus sperm nuclei (5 × 10^4 nuclei/ml) were prepared by the method of Lohka and Masui (31) and were the generous gift of Drs. H. Yan and J. Newport (University of California, San Diego). The demembranated sperm nuclei (final concentration of 5 × 10^3 nuclei/ml) were incubated with purified native dNLP, purified recombinant dNLP, or purified recombinant dNAP-1 (at a final concentration of 700 μg/ml for each protein) in a final volume of 10 μl in 50 mM Hepes (KOH), pH 7.6, buffer containing 75 mM potassium acetate, 0.5 mM spermidine, and 0.15 mM spermine. The samples were mixed gently, and at each time point, a 2-μl aliquot was removed and mixed with an equal volume of 50% glycerol in 1× PBS containing 10 μg/ml Hoechst 33258 and 1% formaldehyde. The slides were examined by using a Nikon microscope.

**RESULTS**

**Purification and Cloning of dNLP**—Further analysis of the dCAF-4 fraction (29), from which we had purified and cloned dNAP-1 (21), led to the identification of a second, less abundant protein with an apparent molecular mass of 22 kDa that appeared to bind to core histones. To investigate the function of this histone-binding protein in chromatin assembly, we purified the 22-kDa protein from the dCAF-4 fraction to greater than 90% homogeneity, generated and purified peptides, obtained partial amino acid sequence of the peptides, and isolated six independent cDNAs that encoded the protein (Fig. 1A).

Comparison of the predicted amino acid sequence of this histone-binding protein with those of known proteins (Fig. 1B) revealed a similarity (31% identity) to another core histone-binding protein, nucleoplasmin, which has been cloned from X. laevis (12, 13). Therefore, the 22-kDa protein from the dCAF-4 fraction was termed *Drosophila* nucleoplasmin-like protein (dNLP). Examination of dNLP, nucleoplasmin, and dNAP-1 reveals that all three factors have a C-terminal stretch of 24–28 amino acid residues that contains a high proportion (71–81%) of acidic residues (Fig. 1, B and C), which may be important for the binding of histones.

Among the six independent cDNAs that were obtained, there were two different types. Four of the cDNAs correspond to the protein that we have denoted as dNLP, while two of the cDNAs encode an N-terminally truncated version of dNLP that we have designated as dNLP-Short (dNLP-S). The calculated molecular mass of dNLP is 16,985 daltons (which is significantly less than its apparent molecular mass of 22 kDa; see, for instance, Fig. 2A), while the calculated mass of dNLP-S is 13,522 daltons (which has an apparent molecular mass of 18 kDa; Fig. 2A). Both of the independently isolated dNLP-S cDNAs differ from the dNLP cDNAs only upstream of nucleotide 112, as in the nomenclature depicted in Fig. 1A; thus, the dNLP-S cDNAs may have been generated from alternatively spliced mRNA species. Northern blot analysis with a probe that is complementary to both dNLP and dNLP-S mRNAs revealed an apparent single band at approximately 1.2 kilobases. Southern blot analysis indicated that there is a single gene encoding dNLP/dNLP-S, but unfortunately, we were not able to determine the cytogenetic location of the dNLP gene in *Drosophila*

**Fig. 1.** The 22-kDa core histone-binding protein in the dCAF-4 fraction is a *Drosophila* nucleoplasmin-like protein (dNLP). A, nucleotide sequence of a cDNA encoding dNLP. The predicted amino acid sequence is given in the single-letter amino acid code. The N-terminal three amino acid residues of dNLP-S are indicated in **bold type**. The amino acid sequences of peptides that were derived from purified native dNLP are **underlined**. The dNLP and dNLP-S cDNA sequences have been submitted to GenBank (accession numbers U59498 and U59497, respectively). B, alignment of the amino acid sequence of dNLP with that of nucleoplasmin from *X. laevis* (12, 13). The sequence alignment was compiled by using the PileUp program of the GCG sequence analysis package. The asterisks denote amino acid residues that are identical in both proteins. A stretch of acidic amino acid residues that is present in both proteins is indicated in **bold italic**. C, comparison of the distribution of acidic and basic amino acid residues in dNLP and dNAP-1.
lanes represent increasing concentrations, from left to right, of micrococcal nuclease used to digest the chromatin. The resulting DNA fragments were subjected to agarose gel electrophoresis and visualized by staining with ethidium bromide. The molecular mass markers (M) are the 123-base pair ladder (Life Technologies, Inc.).

Assembly of Nucleosomal Arrays with dNLP—Next, we tested the ability of dNLP and dNLP-S to function in the ATP-facilitated assembly of nucleosomal arrays in conjunction with the assembly factor(s) in the dCAF-1 fraction. First, we purified native dNLP, recombinant E. coli-synthesized dNLP, and recombinant E. coli-synthesized dNLP-S to greater than 90% homogeneity (Fig. 2A). (The preparation of native dNLP did not contain any detectable amount of dNLP-S.) Then, we compared the relative activities of native dNLP, recombinant dNLP, recombinant dNLP-S, and purified recombinant dNAP-1 (synthesized in E. coli; Ref. 21) in assembly reactions that contained dCAF-1 fraction, DNA, purified core histones, and ATP. With the DNA supercoiling assay (Fig. 2B), dNAP-1 exhibited the highest assembly activity, whereas native and recombinant dNLP possessed assembly activity that was consistently lower than that of dNAP-1. In contrast, dNLP-S was inactive. We further characterized the assembly of chromatin with dNLP by using DNA supercoiling and micrococcal nuclease digestion assays. As depicted in Fig. 2 (C and D), the efficient assembly of nucleosomal arrays requires dNLP, dCAF-1 fraction, and ATP. In addition, when both dNAP-1 and dNLP were used simultaneously, there was no apparent synergism between the factors (data not shown). Therefore, both dNAP-1 and dNLP are able to function with the dCAF-1 fraction in the ATP-facilitated assembly of nucleosomal arrays, although dNAP-1 appears to mediate chromatin assembly more efficiently than dNLP.

**Binding of dNLP and dNLP-S to Core Histones**—Because dNLP was initially identified as a histone-binding protein in the dCAF-4 fraction, we examined the binding of dNLP and dNLP-S to core histones. First, we tested whether core histones will co-immunoprecipitate with dNLP or dNLP-S when either protein is combined with core histones in an *in vitro* binding assay. In this experiment, native dNLP, recombinant dNLP, or recombinant dNLP-S were combined with purified core histones at a 10:1 molar ratio of protein:octamer (which is identical to a 1.25:1 ratio of protein:histone, at which dNLP functions in chromatin assembly; Fig. 2B), and then the dNLP species were immunoprecipitated with polyclonal antibodies that recognize both dNLP and dNLP-S (or with preimmune serum, as a control). As shown in Fig. 3A, all four core histones co-immunoprecipitated in the presence, but not in the absence of either dNLP or dNLP-S. It thus appears that both dNLP and dNLP-S can bind to the core histones.

We then investigated whether core histones will co-immunoprecipitate with dNLP from a whole *Drosophila* embryo extract. A crude extract (prepared from embryos collected from 0 to 2 h after egg deposition) was therefore subjected to immunoprecipitation with polyclonal antibodies that recognize dNLP (or with preimmune serum as a control). Because it is known that the core histones H2A-H2B will co-immunoprecipitate with dNAP-1 from a whole embryo extract (21), we had also used antibodies against dNAP-1 as a positive control. The resulting immunoprecipitates were subjected to Western blot analysis with antibodies that recognize H2A, H2B, H3 (but not H4), dNLP, and dNAP-1. As seen in Fig. 3B, there was no detectable co-immunoprecipitation of core histones with dNLP, while under the same conditions, the core histones H2A-H2B co-immunoprecipitated with dNAP-1, as observed previously (21). Moreover, we had performed additional experiments with embryos at later stages of development, and did not observe any co-immunoprecipitation of histones with dNLP (data not shown). Hence, as reported previously for *X. laevis* nucleoplasmin-like proteins (Refs. 32 and 33, but see also Refs. 15 and 17), there is no detectable co-immunoprecipitation of core histones with dNLP in *Drosophila* embryos. It is, in addition, unlikely that the antibodies against dNLP inhibit binding of core histones to dNLP because the histones are able to co-immunoprecipitate with dNLP when the purified proteins are combined (Fig. 3A). These results therefore suggest that the majority of dNLP in embryos is not stably associated with core histones.

To characterize further the binding of dNLP to core histones,
we carried out glycerol gradient sedimentation analyses (Fig. 4). In these experiments, we were particularly interested in the relative affinities of the binding of dNLP, dNLP-S, and dNAP-1 to the core histones. For instance, when dNLP-S was incubated with core histones for 30 min (at a protein:histone ratio of 1:25:1) and then combined with dNLP (at a dNLP-S:dNLP ratio of 1:1) and subjected to glycerol gradient sedimentation, the core histones were associated with the dNLP and not, to any detectable extent, with the dNLP-S (Fig. 4E). Hence, it appears that dNLP binds to histones with higher affinity than dNLP-S. We then performed a similar experiment with dNLP and dNAP-1 and found that dNAP-1 binds to histones with a higher affinity than dNLP (Fig. 4F). (Note that these experiments were performed with an excess of histone-binding proteins relative to core histones. At a higher core histone:histone-binding protein ratio, both dNAP-1 and dNLP were bound to the histones.) Thus, these data suggest that the relative affinity of the proteins for core histones is as follows: dNAP-1 > dNLP > dNLP-S. Moreover, the results shown in Fig. 4F, in which the core histones were associated with dNAP-1 in a mixture of dNAP-1 and dNLP, are consistent with the co-immunoprecipitation studies with the embryo extracts (Fig. 3B), wherein the core histones were bound to dNAP-1 but not to dNLP.

The glycerol gradient sedimentation analysis also revealed that dNLP-S appears to form a high molecular mass aggregate with core histones (Fig. 4D), which is in contrast to the behavior of dNLP, which forms a soluble histone complex (Fig. 4E).

These results suggest that the co-immunoprecipitation of core histones with dNLP-S, as observed above (Fig. 3A), might be due to nonspecific interaction of the histones with dNLP-S. Thus, although dNLP-S differs from dNLP only by deletion of 31 amino acid residues at the N terminus and retains the majority of dNLP that is related to nucleoplasmin (Fig. 1, A and B), the ability of dNLP-S to interact with core histones and to function in chromatin assembly is distinct from that of dNLP. dNAP-1, but Not dNLP, Can Mediate Decondensation of Sperm Chromatin—Xenopus nucleoplasmin has been shown to mediate the decondensation of sperm chromatin (34), by a mechanism that appears to involve the loss of two sperm-specific proteins and the incorporation of H2A and H2B into the chromatin (36). In addition, two Drosophila proteins, termed...
p22 (37) and DF 31 (38), were also observed to decondense *Xenopus* sperm chromatin. Both p22 and DF 31 exhibit some characteristics, such as heat stability and sperm decondensation activity, that are similar to those of nucleoplasmin. Moreover, because p22 (37) and dNLP (this study) migrate on SDS-polyacrylamide gels with the same apparent molecular mass, it is possible that these proteins are related.

We therefore examined the ability of dNLP and, for comparison, dNAP-1 to mediate decondensation of demembranated *Xenopus* sperm chromatin (Fig. 5). In contrast to the behavior of nucleoplasmin or p22, neither native dNLP nor recombinant dNLP was able to promote the decondensation of sperm chromatin under various conditions and concentrations (Fig. 5, panels A–F; data not shown). However, dNAP-1 was highly active for sperm chromatin decondensation (Fig. 5, panels G–L). These results indicate that dNAP-1, but not dNLP, is able to mediate the decondensation of *Xenopus* sperm chromatin. The degree to which NAP-1 participates in sperm chromatin decondensation in vivo remains to be determined, but the involvement of NAP-1 in this process should at least be considered. Furthermore, it should be noted that the activity of either native or recombinant dNLP is not identical to that of p22, and therefore, either the proteins are not related or some aspect of the preparation of p22 (relative to that of dNLP) renders the protein active for sperm chromatin decondensation.

**Subcellular Localization and Presence of dNLP throughout Development**—We have also examined the subcellular localization of dNLP. dNLP is located in the nucleus of *Drosophila* Schneider cultured cells (Fig. 6) and *Drosophila* embryos (data not shown). By comparison, in *Drosophila* embryos, dNAP-1 appears to be predominantly cytoplasmic during G2 phase and in the nucleus and cytoplasm during S phase (21). Moreover, we did not observe nuclear localization of dNLP, as has been observed with the nucleoplasmin-related NO38 protein (39–41). Thus, dNLP appears to be exclusively localized to the nucleus, as is *Xenopus* nucleoplasmin (32, 33, 42).

Finally, we determined the amount of dNLP and dNAP-1 proteins that were present throughout *Drosophila* development (Fig. 7). Both dNLP and dNAP-1 were at the highest levels in early embryos and remained at reduced, but significant levels throughout development. This property of dNLP and dNAP-1 is different from that seen with *Xenopus* nucleoplasmin, which is present in embryos to the feeding tadpole stage, but then decreases to undetectable levels in advanced tadpoles and adults (12, 43, 44). The presence of dNLP and dNAP-1 at their highest levels in early embryos and at lesser but detectable amounts during later stages of development are consistent with a function, such as chromatin assembly/disassembly or condensation/decondensation (as in mitosis), that would be needed throughout the life cycle, but at especially high levels in the early embryo during which every cycle of DNA replication and nuclear division is completed approximately every 10 min.

**DISCUSSION**

We have described the purification, cloning, and characterization of a nucleoplasmin-like protein from *D. melanogaster*, termed dNLP, which is a nuclear protein (Fig. 6) that is present throughout development (Fig. 7). dNLP binds to core histones in *vitro* (Figs. 3A and 4E) and functions in the ATP-facilitated assembly of nucleosomal arrays in conjunction with an activity (or activities) in a partially purified dCAF-1 fraction (Fig. 2). The properties of dNLP resemble those of nucleoplasmin as well as those of two *Drosophila* proteins named p22 (37) and DF 31 (38), with the major exception that dNLP, unlike the other three proteins, is not able to promote the decondensation of sperm chromatin (Fig. 5). These and other data suggest that dNLP may participate in parallel with other histone-binding proteins such as dNAP-1 in the assembly of chromatin.

**dNLP-S**—Two independently isolated cDNA clones encode an N-terminally truncated version of dNLP, designated as dNLP-S, which is identical to dNLP except that it is lacking amino acid residues 2–32. However, dNLP-S is not present in our purified preparations of native dNLP (Fig. 2A), is not detectable by Western blot analysis (Fig. 7), and is inactive for chromatin assembly (Fig. 2B). dNLP-S is able to bind to core histones in *vitro* in a co-immunoprecipitation assay (Fig. 3A), but it appears to form a high molecular mass aggregate with the core histones, as analyzed by glycerol gradient sedimentation.
protein A. The lower panel with immunoaffinity-purified anti-dNLP-1 and 125I-labeled protein A. dNLP, as detected with affinity-purified anti-dNLP and 125I-labeled sis and Western blot analysis. The was normalized to the amount of an

lanes, the value obtained for each test sample (either dNLP or dNAP-1) were determined by using a PhosphorImager (Molecular Dynamics). To correct for variation in the total protein concentration in each of the

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[50x319]tion (Fig. 4  

Drosophila

(9, 42) and DF 31 (38) are among the predominant thermo-

stable proteins in Xenopus eggs and oocytes and Drosophila embryos, respectively. Thus, although dNLP is similar to nucleoplasmin at the level of their primary amino acid sequences (Fig. 1B), it is possible that DF 31 may resemble nucleoplasmin more closely than dNLP.

Because of the similar apparent molecular masses of dNLP and p22, it is tempting to suggest that the dNLP cDNA encodes p22, but this postulate is inconsistent with the inability of either native or recombinant dNLP to mediate sperm chromatin decondensation, which is the characteristic biochemical activity of p22 (37). To attempt to clarify this issue, we had subjected dNLP as well as dNLP-containing fractions to Western blot analysis with antibodies that were generated against Drosophila p22 (kindly provided by Dr. P. Fisher, SUNY Stony Brook), but the results were inconclusive (data not shown). Thus, the available data do not suggest a relation between dNLP and p22, although this point would be resolved if the gene(s) encoding p22 were cloned. It should be further noted that the methodology for the purification of dNLP is distinct from that for the purification of p22, and hence, if these proteins are in fact identical, differences in the properties of dNLP and p22 may also be due to some aspect of the treatment or the purity of the proteins.

dNLP Versus dNAP-1—dNAP-1 binds to core histones with higher affinity than dNLP. For instance, when core histones were incubated with dNLP (for 30 min at a dNLP:histone ratio of 1.25:1; under these conditions, the majority of the histones bind to dNLP; see Fig. 4E) and then dNAP-1 was added to the dNLP-histone complexes (at a dNAP-1:dNLP ratio of 1:1), the histones transferred from the dNLP to the dNAP-1 (Fig. 4F). Thus, when dNLP and dNAP-1 are present in excess relative to the core histones, the histones preferentially bind to dNAP-1, even if they are prebound to the dNLP. This preferential binding of histones to dNAP-1 relative to dNLP might also explain why core histones co-immunoprecipitate from a whole embryo extract with dNAP-1, but not with dNLP (Fig. 3B). Moreover, when compared directly, dNAP-1 was more active than dNLP for chromatin assembly (Fig. 2B) as well as for sperm chromatin decondensation (Fig. 5). It seems likely that the greater activity of dNAP-1 relative to dNLP is due to the higher affinity of dNAP-1 for binding to core histones (and, presumably, sperm-specific proteins/protamines) relative to dNLP. Given the complexity of these processes in vivo, however, it is not possible to predict whether the higher affinity of dNAP-1 for histones relative to dNLP necessarily reflects a more significant role for dNAP-1 in chromatin assembly relative to dNLP.

Because of the common ability of both dNLP and dNAP-1 to participate in the ATP-facilitated assembly of nucleosomal arrays, it is reasonable to consider that there is some redundancy in the function of these factors. Consistent with this hypothesis, there is another NAP-1-related protein termed SET in Drosophila as well as in humans (46, 47). Moreover, a search of the yeast genome data base revealed a putative NAP-1/SET-related protein in Saccharomyces cerevisiae, designated “ORF YNL246w” (GenBank accession number Z71522 (1996)), which exhibits 21% amino acid identity with Drosophila SET (data not shown). (On the other hand, a search of the S. cerevisiae genome data base did not reveal any apparent homologues of dNLP or Xenopus nucleoplasmin in yeast (data not shown), which is consistent with the notion that these proteins have an important function in preblastoderm embryos (a stage in development for which there is no yeast counterpart.) This NAP-1/SET-related ORF YNL246w protein may compensate for the absence of NAP-1 and thus be responsible, at least in part, for the observation that NAP-1 is not essential for viability of S. cerevisiae (46).

**Fig. 7.** Western blot analysis of dNLP and dNAP-1 at different stages of Drosophila development. Equivalent amounts of total protein extracts (25 µg) derived from Drosophila at the indicated stages of development were subjected to SDS-polyacrylamide gel electrophoresis and Western blot analysis. The upper panel shows the presence of dNLP, as detected with affinity-purified anti-dNLP and 125I-labeled protein A. The lower panel reveals the presence of dNAP-1, as detected with immunoaﬃnity-purified anti-dNAP-1 and 125I-labeled protein A. The relative protein levels (with 0–3-h embryos as the reference = 1.0) were determined by using a PhosphorImager (Molecular Dynamics). To correct for variation in the total protein concentration in each of the lanes, the value obtained for each test sample (either dNLP or dNAP-1) was normalized to the amount of an α-tubulin internal control that was quantitated in a parallel analysis.
In addition, we have found that dNLP and dNAP-1 exhibit distinct subcellular localization in *Drosophila* cells, i.e. that dNLP is present only in the nucleus (Fig. 6), while dNAP-1 is predominantly cytoplasmic during G$_2$ phase and both nuclear and cytoplasmic during S phase (21). It is thus possible that the relative degree to which dNLP or dNAP-1 (as well as perhaps SET) participates in chromatin assembly may vary with the phase of the cell cycle. Therefore, the available data suggest that dNLP and dNAP-1 may function by parallel and/or complementary mechanisms in the assembly of chromatin in *vivo*.

**Conclusions**—We are in the early stages in the analysis of the mechanism of chromatin assembly. For instance, there are assembly factors that have yet to be purified and characterized, such as the ATP-utilizing activity in the dCAF-1 fraction. In addition, the specific functions of known factors, such as NAP-1, dNLP, nucleoplasmin, N1, and CAF-1, remain to be determined. The purification of these factors and the isolation of the genes encoding the proteins are, however, important steps toward addressing these issues. It is our hope that the further analysis of the factors that participate in the assembly of chromatin will provide new and significant insight into a fascinating and important biological process.

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