The *Drosophila* Nuclear Lamina Protein YA Binds to DNA and Histone H2B with Four Domains

Jing Yu* and Mariana F. Wolfner[†]

Department of Molecular Biology and Genetics, Cornell University, Ithaca, New York 14853-2703

Submitted July 5, 2001; Revised November 16, 2001; Accepted November 16, 2001

Monitoring Editor: Tim Stearns

Dramatic changes occur in nuclear organization and function during the critical developmental transition from meiosis to mitosis. The *Drosophila* nuclear lamina protein YA binds to chromatin and is uniquely required for this transition. In this study, we dissected YA's binding to chromatin. We found that YA can bind to chromatin directly and specifically. It binds to DNA but not RNA, with a preference for double-stranded DNA (linear or supercoiled) over single-stranded DNA. It also binds to histone H2B. YA's binding to DNA and histone H2B is mediated by four domains distributed along the length of the YA molecule. A model for YA function at the end of *Drosophila* female meiosis is proposed.

INTRODUCTION

Upon fertilization, there is a transition between meiosis and mitosis that involves a number of reorganizations of the structure of the nucleus and its contents. For example, in most animals meiotic progression is reinitiated upon fertilization/egg activation (for reviews, see Schultz and Kopf, 1995; Page and Orr-Weaver, 1997), and the condensed chromosome sets complete their divisions. The chromosomes of one of the female haploid meiotic products then decondense as it becomes a pronucleus. The sperm nucleus decondenses in a stepwise process (see Cameron and Poccia, 1994; Collas and Poccia, 1995, 1998; Cothren and Poccia, 1993; Longo *et al.*, 1994; Poccia and Collas, 1995, 1997; Wright, 1999 for reviews; Yamashita *et al.*, 1990). First, it loses its nuclear envelope (if it has one). Then its chromatin decondenses as factors such as nucleoplasmin (*Xenopus*, Philpott *et al.*, 1991; molluscs, Rice *et al.*, 1995; *Drosophila*, Ito *et al.*, 1996b; mice, Maeda *et al.*, 1998; salmon, Iwata *et al.*, 1999) aid in replacing sperm histones/protamines with somatic histones (Philpott and Leno, 1992; reviewed by Laskey *et al.*, 1993). The sperm nucleus then acquires a new nuclear envelope largely from maternal components to form the male pronucleus (Longo, 1985; Stricker *et al.*, 1989; Poccia and Collas, 1996; Liu *et al.*, 1997) and then completes its chromatin decondensation in a membrane-dependent manner (Lohka and Masui, 1984; Collas and Poccia, 1995; Poccia and Collas, 1996, 1997).

These changes in nuclear condensation state appear to be necessary for progression of zygotic development. In *Xenopus*, nucleoplasmin is required for sperm DNA to be replication-competent (Gillespie and Blow, 2000). In rhesus monkey zygotes, DNA replication will not initiate until all the sperm chromatin has decondensed, and improper chromosome decondensation in either the female or the male pronucleus causes cell cycle arrest at the interphase of the first mitosis (Hewitson *et al.*, 1996, 1999). In *Drosophila*, a condensed sperm nucleus is unable to participate in development (*snky*, Fitch and Wakimoto, 1998; *ssm*, Loppin *et al.*, 2000; *ms(3)K81*, Fuyama, 1986; Yasuda *et al.*, 1995; *mh*, Gans *et al.*, 1975; Zalokar *et al.*, 1975; Santamaria and Gans, 1980; Santamaria, 1983; Edgar *et al.*, 1986; Loppin *et al.*, 2001). Mutations in the *fs(1)Ya* (*Ya*) gene that result in abnormal chromatin condensation and postmeiotic association of pronuclei (Liu *et al.*, 1995; Lopez, 1996) arrest development during the transition from meiosis to mitosis (Lin and Wolfner, 1991; Liu *et al.*, 1995; Lopez, 1996).

A few chromatin decondensation factors have been identified that function in this critical cellular process. In *Xenopus*, nucleoplasmin functions at the first step of sperm chromatin decondensation. Nucleoplasmin has been suggested to also function in sperm chromatin decondensation in other organisms (*Mytilus*, Rice *et al.*, 1995; *Drosophila*, Ito *et al.*, 1996b; mice, Maeda *et al.*, 1998; salmon, Iwata *et al.*, 1999). The nuclear envelope also plays a role in sperm chromatin decondensation. In sea urchins, nuclear swelling requires the nuclear lamina (Lohka and Masui, 1984; Collas and Poccia, 1995). In *Drosophila*, decondensation proteins purified from early embryos include dNAP-1 (*Drosophila* nucleosome assembly protein 1), dNLP (*Drosophila* nucleoplasmin-like protein), CRP1, P22, and DF 31 (Kawasaki *et al.*, 1994; Crevel and Cotterill, 1995; Ito *et al.*, 1996a, 1996b; Crevel *et al.*, 1997). They may function through their binding to chromatin (Crevel *et al.*, 1997), especially to core histones (Crevel

Article published online ahead of print. Mol. Biol. Cell 10.1091/mbc.01-07-0336. Article and publication date are at www.molbiol-cell.org/cgi/doi/10.1091/mbc.01-07-0336.

* Corresponding author. E-mail address: mfw5@cornell.edu.

[†] Present address: Department of Molecular and Cellular Biology, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138.

and Cotterill, 1995; Ito *et al.*, 1996b). Some of these proteins have been shown to decondense sperm chromatin in vitro (P22, Kawasaki *et al.*, 1994; DF 31, Crevel and Cotterill, 1995; dNAP-1, Ito *et al.*, 1996b; CRP1, Crevel *et al.*, 1997), although their exact roles during pronuclear formation and mitosis in vivo are not known.

The essential, maternally provided *Drosophila* nuclear lamina protein YA appears to be involved in regulation of chromosome condensation state at the end of meiosis. YA, which is in the nuclear lamina of fertilized eggs, is required only for the transition from female meiosis to embryo mitosis (Lin and Wolfner, 1991; Liu *et al.*, 1995). Oogenesis and meiosis, including chromosome segregation, in eggs from *Ya*-deficient females ("*Ya*² eggs" for simplicity in the rest of this text) progress normally (Lin and Wolfner, 1991; Liu *et al.*, 1995; Lopez, 1996; Berman, 2000). At the end of meiosis, a nuclear envelope forms around each of the four female meiotic products, and if the egg is fertilized, a functional nuclear envelope with a nuclear lamina also forms around the male pronucleus (Liu *et al.*, 1997). However, the DNA condensation state of all the haploid nuclei in *Ya*² zygotes is abnormal at the end of meiosis (Liu *et al.*, 1995). In wild-type *Drosophila* fertilized eggs, all four female meiotic products and the male pronucleus decondense their chromatin. Then presumably after DNA replication, the male and female pronuclei condense their DNA, initiate the first mitotic division (the gonameric division) and associate, and the three polar body nuclei also condense their chromatin and associate (Sonnenblick, 1950; Callaini and Riparbelli, 1996). In fertilized *Ya*² eggs, nuclei are of different chromatin condensation states, and they associate randomly (Liu *et al.*, 1995; Lopez, 1996). Mitosis never occurs, and the embryos arrest at the pronuclear stage (Lin and Wolfner, 1991). The phenotypes of *Ya*² eggs suggest that YA may play a role in modulating chromosome condensation state at the end of meiosis. Consistent with this, YA protein in embryo extracts binds to decondensed sperm chromatin in vitro, and YA binds to polytene chromosomes when ectopically expressed (Lopez and Wolfner, 1997). It was not known whether YA binds to chromatin directly and what interactions mediate this binding. To help understand how chromatin condensation state is regulated at the end of *Drosophila* female meiosis and YA's roles in this process, we dissected YA's binding to chromosomes. We show that YA can bind directly to chromosomes through interactions with DNA and histone H2B. This binding involves four chromatin-binding domains in YA, all of which bind to both DNA and histone H2B.

MATERIALS AND METHODS

Constructs, Proteins, and *Escherichia coli* Expression of YA Fragments

The full-length *Ya* cDNA, *Ya* cDNAs with inactivating mutations in the first (C1), second (C2), or both zinc fingers (C1C2; Liu and Wolfner, 1998), and all *Ya* fragments were cloned in frame into either the pGEX-2T vector (Pharmacia, Piscataway, NJ) to make GST fusion proteins or into the pMAL-C2-HMK(R) vector (modified from pMAL-C2 [New England BioLabs, Beverly, MA] by Z. Li and M.L. Goldberg) to express fusion proteins with MBP (maltose binding protein) fused to phosphorylation target sites for HMK (heart muscle kinase; Blonar and Rutter, 1992); details of the clonings, including primers used, are available in Yu (2000).

Protein induction and purification were according to the NEB protein fusion and purification (pMAL) instruction manual (for MBP-HMK fusions) and the GST gene fusion system manual (Pharmacia; for GST fusions) with minor modifications. Fusion proteins were purified by column or batch purification with glutathione beads (Sigma, St. Louis, MO; for GST fusions) or amylose beads (New England BioLabs; for MBP-HMK fusions) to near homogeneity and dialyzed against TK buffer (50 mM Tris-HCl, pH 7.5, 70 mM KCl, 1 mM DTT, 2.5 mM benzamidine, 1 mM PMSF). The proteins were checked for concentration and size by SDS-PAGE and Western blotting with both anti-YA antibodies (affinity-purified guinea pig anti-full-length YA antibodies [see below] or affinity-purified rabbit anti-C-terminal YA antibodies (Lin and Wolfner, 1991; Lopez *et al.*, 1994) and monoclonal anti-GST antibodies (for GST fusion proteins; Sigma) or polyclonal anti-MBP antisera (for MBP-HMK fusion proteins, New England BioLabs). Proteins were checked again by SDS-PAGE immediately before the mitotic chromosome binding reactions. Like endogenous YA, MBP-HMK-YA protein can interact with embryonic YA (unpublished observations). MBP-HMK-YA can also be incorporated into the nuclear envelope of in vitro assembled nuclei in *Xenopus* egg extracts (M.F. Wolfner, unpublished observations), suggesting that it at least retains some YA functions.

Drosophila core histones, purified as in Bulger and Kadonaga (1994), were kindly provided by Dr. Lee Kraus. Calf thymus histone H1, H2A, and H2B were purchased from Roche Molecular Biochemicals (Indianapolis, IN). Salmon sperm DNA (Sigma) was deproteinated and resuspended in TK buffer. Plasmid DNA used for mitotic chromosome binding assays was purified with QIAfilter Plasmid Maxi kit (QIAGEN, Santa Clarita, CA). Linear plasmid DNAs were generated by *Eco*RI digestion. The linearized DNA and tRNA were deproteinated and dissolved in dH₂O. Single-stranded plasmid DNA was generated by denaturation of linear plasmid DNA at 95°C for 5 min.

Antibodies

Guinea pig anti-full-length YA antisera were produced by Covance Research Products (Denver, PA) from purified MBP-HMK-YA protein, and affinity-purified. The specificity of the antibodies was verified by Western blotting. Polyclonal rabbit anti-*Drosophila* core histone antibodies (Ito *et al.*, 1996a) were gifts from Dr. Lee Kraus.

Mitotic Chromosome Binding Assay

Mitotic chromosomes were isolated from Chinese hamster ovary (CHO) cells as described in Glass and Gerace (1990). The binding of mitotic chromosomes with fusion proteins containing YA or YA fragments was according to Goldberg *et al.* (1999) with a few modifications. Mitotic chromosomes were examined with an Olympus BX-50 microscope (Lake Success, NY) equipped with epifluorescence and a Pentamax camera (Princeton Instruments, Monmouth Junction, NJ). Data were processed with Metamorph software (Universal Imaging, West Chester, PA). All binding experiments were done at least twice; each time more than 10 chromosomes were examined. The results shown are representative. DNA competitors were added at 200 ng/ μ l unless otherwise noted. Histone competitors were added at 7 μ M for each histone. Spermine and spermidine competitors were added at 1000-fold molar excess. Polynucleosomes were purified from rat liver as described in Goldberg *et al.* (1999). Primary antibodies used for immunostaining were purified polyclonal rabbit anti-YA antibodies, polyclonal anti-MBP, or monoclonal mouse anti-GST antibody. Secondary antibodies used were rhodamine-conjugated anti-rabbit antibodies or rhodamine-conjugated anti-mouse antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA).

Solid-phase Chromatin Binding Assay

Purified MBP-HMK-YA and MBP-HMK were ³²P-labeled with heart muscle kinase according to Blonar and Rutter (1992) to a specific

of different sizes incubated with the same protein show comparable binding results (e.g., see Figure 8I for two apposed smaller chromosomes incubated with MBP-HMK). Bar: 10 μ m.

activity of $\sim 1.2 \times 10^6$ cpm/ μ g. Polynucleosomes of 8–30 nucleosomes were purified and bound to the solid phase as described in Goldberg *et al.* (1999). Radiolabeled MBP-HMK-YA or MBP-HMK protein (75 μ g/ml) was incubated with polynucleosomes, in the presence or absence of unlabeled MBP-HMK-YA or MBP-HMK competitor. Duplicate data points were taken and repeats of all assays yielded comparable results. To calculate apparent K_d , the data were expressed in linearizing plots for single-site competitive interactions (Hulme and Birdsall, 1992).

MBP Pull-down Assay

For MBP pull-down assays on DNA, 4 μ g MBP-HMK-YA or 32 μ g MBP-HMK in TK buffer was incubated with 1.2 μ g plasmid DNA in TK buffer at 4°C for 1 h. Amylose beads (20 μ l) were then added, and the mixture was incubated at 4°C for 4 h. The beads were harvested and washed with TK buffer containing 130 mM NaCl. DNA and proteins were eluted from the beads by incubation in SDS-PAGE sample buffer without β -mercaptoethanol at 65°C for 10 min. DNA and proteins were analyzed by agarose gel electrophoresis and Western blotting, respectively. For MBP pull-down assays on histones, 4 μ g of calf thymus histone H2A, H2B, or 4 μ g of purified *Drosophila* core histone mix (an equimolar mixture of all four core histones) was mixed with 4 μ g of MBP-HMK-YA or 32 μ g of MBP-HMK in the binding buffer (20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1 mM EDTA). The binding conditions were as above for MBP pull-down assays on DNA. For washes, beads from pull-down reactions on histone H2A or H2B were washed four times with the binding buffer, whereas beads from the pull-down reaction on core histone mix were washed three times with the binding buffer supplemented with 100 mM NaCl. The proteins bound to beads were eluted by boiling in SDS-PAGE sample buffer for 5 min.

RESULTS

YA Binds to Mitotic Chromosomes Directly In Vitro

To determine if YA binds directly to chromatin and to investigate the characteristics of this binding, we used a mitotic chromosome binding assay that had been previously used to characterize lamin-chromatin binding (Glass and Gerace, 1990; Glass *et al.*, 1993; Taniura *et al.*, 1995) including that of *Drosophila* lamin Dm α -chromatin binding (Goldberg *et al.*, 1999). Purified *E. coli*-made MBP-HMK-YA protein or the control MBP-HMK protein was incubated with CHO cell

Figure 1. YA binds to mitotic chromosomes directly. Purified MBP-HMK-YA (A–C) or MBP-HMK (D–F) was incubated with mitotic chromosomes. The chromosomes were stained with the DNA dye DAPI (A and D, and blue in C and F) and anti-MBP antisera (B and E, and red in C and F). MBP, maltose binding protein; HMK, phosphorylation sites for heart muscle kinase. The difference in staining between the chromosomes in A–C vs. the one in D–F is due to the difference in the ability of MBP-HMK-YA and MBP-HMK to bind to them; it is not due to their difference in size. Chromosomes

mitotic chromosomes (MBP-HMK stands for maltose binding protein fused to the phosphorylation target sites for heart muscle kinase; Blunar and Rutter, 1992). As shown in Figure 1, anti-MBP antisera stained chromosomes incubated with MBP-HMK-YA (Figure 1B, and red in 1C) but did not stain chromosomes incubated with MBP-HMK (Figure 1E, and red in 1F). Thus, signals seen on chromosomes incubated with MBP-HMK-YA reflect chromosome binding by the YA moiety of MBP-HMK-YA. More bound MBP-HMK-YA is detected at the surface of the mitotic chromosomes relative to the interior, similar to the apparently preferential staining of mitotic chromosome surfaces reported for human, rat, and *Drosophila* lamins (Glass and Gerace, 1990; Glass *et al.*, 1993; Goldberg *et al.*, 1999). This staining pattern could be due to the better accessibility of MBP-HMK-YA (and lamin) or antibodies to the surface of mitotic chromosomes, or it could reflect preferential binding of YA (and lamin) to the surface domain of mitotic chromosomes.

MBP-HMK-YA's binding to the mitotic chromosomes is not simply a nonspecific charge interaction. It was unaffected by the presence of 1000-fold molar excess of polycations such as spermine or spermidine (Figure 2, compare panels B and C with panel A), nor was it affected by a 8×10^6 -fold molar excess of BSA in the binding reaction (unpublished observations) or by a 25-fold molar excess of MBP-HMK protein (Figure 2D).

The binding of MBP-HMK-YA to mitotic chromosomes is mediated by YA's binding to nucleosomes, because an 18-fold excess of polynucleosome competitors greatly reduced MBP-HMK-YA's binding to mitotic chromosomes (Figure 2E). To measure the affinity of MBP-HMK-YA's binding to chromatin, we used the displacement assay described for lamin (Taniura *et al.*, 1995; Goldberg *et al.*, 1999). Radioactively labeled MBP-HMK-YA or MBP-HMK was incubated with polynucleosomes bound to the solid phase in the presence or absence of different concentrations of nonlabeled MBP-HMK-YA or MBP-HMK. MBP-HMK-YA bound to immobilized chromatin at significant levels, whereas MBP-HMK bound to chromatin poorly (6–10% of the amount of MBP-HMK-YA). With increasing amounts of unlabeled MBP-HMK-YA, less radioactivity is detected on immobi-

Figure 2. YA's binding to mitotic chromosomes is specific. Binding reactions of MBP-HMK-YA with mitotic chromosomes were carried out in the presence of 1000-fold molar excess of spermidine or spermine, 25-fold molar excess of MBP-HMK or 18-fold molar excess of polynucleosomes. Chromosomes were stained with DAPI (DNA) and anti-YA antibodies (Anti-YA). Bar: 10 μ m.

lized polynucleosomes (Figure 3A). Repeats of these experiments resulted in calculated K_d values between 1.1 and 2.4 μ M. An experiment that gave a K_d of 1.1 μ M is shown in Figure 3. The K_d of YA's binding to chromatin is similar to that of *Drosophila* lamin Dm₀ (~1 μ M; Goldberg *et al.*, 1999). They are both lower than that those reported for human lamin Dm₀ A/C and B (Taniura *et al.*, 1995). This is either because *Drosophila* nuclear lamina proteins have lower affinity for chromatin or because they bind more weakly than mammalian lamin to non-*Drosophila* (mammalian) chromatin, the standard substrate for this assay.

YA Binds to DNA

That MBP-HMK-YA's binding to mitotic chromosomes is competed by polynucleosomes suggests that YA binds to DNA and/or chromosomal proteins. To test whether YA binds to DNA, deproteinated salmon sperm DNA was

Figure 3. MBP-HMK-YA binds to immobilized chromatin with apparent K_d of 1.1 μ M. (A) 32 P-labeled MBP-HMK-YA was incubated with immobilized polynucleosomes in the presence of increasing concentration of unlabeled MBP-HMK-YA. Binding at each point was corrected for nonspecific binding by subtraction of values obtained with 2300 μ g/ml unlabeled MBP-HMK-YA. (B) Data for specific binding were analyzed as in Hulme and Birdsall (1992). RL is the amount of radioactive protein bound to chromatin at unlabeled protein concentration A. RL₀ is the amount of radioactive protein bound to chromatin in absence of unlabeled competitor. The slope given by this plot equals $-1/K_d$.

added to the mitotic chromosome binding reaction (Figure 4A). MBP-HMK-YA's binding to mitotic chromosomes was reduced by the presence of DNA in a dose-dependent manner, nearing background at 200 ng/ μ l salmon sperm DNA, the highest concentration tested (Figure 4A, panel D). The residual binding seen at the highest concentration of competitor could reflect incomplete competition by DNA or MBP-HMK-YA's binding to chromosomal proteins (see below). Addition of 200 ng/ μ l tRNA to the binding reaction had no effect on MBP-HMK-YA's binding to mitotic chromosomes (Figure 4A, panel E), suggesting that YA does not bind to RNA. In addition to the overall decrease in the amount of MBP-HMK-YA staining on chromosomes with increasing concentrations of added salmon sperm DNA, the staining became more punctate at higher competitor concentrations. We believe that the punctate staining is caused by MBP-HMK-YA aggregation on mitotic chromosomes, because YA can interact with itself directly (Liu and Wolfner, 1998, and our unpublished observations). Similar aggregation was reported for the binding of *Drosophila* lamin Dm₀ to mitotic chromosomes (Goldberg *et al.*, 1999). An alternative explanation for the appearance of punctate staining at high concentrations of added DNA would be that YA binds to some sequences with higher affinity, although no evidence of highly preferential binding sites was seen upon binding of ectopically expressed YA to polytene chromosomes (Lopez and Wolfner, 1997). Another possibility is that DNA in some regions of mitotic chromosomes is more accessible.

The fact that MBP-HMK-YA's binding to mitotic chromosomes can be competed by salmon sperm DNA, but not by RNA, suggests that YA binds to DNA. To determine additional characteristics of this binding, we carried out a similar competition experiment, except using 88 ng/ μ l DNA of plasmid pGEX-2T, which has a more simple sequence composition than salmon sperm DNA (Figure 4B). Single-stranded plasmid DNA did not displace MBP-HMK-YA's binding to mitotic chromosomes as well as double-stranded DNA (compare Figure 4B, panel E, in which ubiquitous YA signals overlapped with the mitotic chromosome, with pan-

Figure 4. YA's binding to mitotic chromosomes can be competed by salmon sperm DNA, by different forms of plasmid DNA, but not by tRNA. The binding reactions of MBP-HMK-YA with mitotic chromosomes in Figure 4A were performed in the presence of deproteinized salmon sperm DNA at the indicated concentrations (4A, panels A–D) or 200 ng/μl yeast tRNA (4A, panel E). The mitotic chromosome bindings shown in Figure 4B were of 88 ng/μl salmon sperm DNA or plasmid DNA of the indicated forms. Mitotic chromosomes were stained with DAPI (DNA) and anti-YA antibodies (Anti-YA). Bar: 10 μm.

els B, C, and D), indicating that YA binds to double-stranded DNA better than to single-stranded DNA. Supercoiled and linear plasmid DNA were both able to displace MBP-HMK-YA's binding to mitotic chromosomes better than salmon sperm DNA (compare Figure 4B, panels C and D with panel B), suggesting that YA's binding to DNA may have some sequence preference. Confirming YA's ability to bind to both supercoiled and linear DNA, using an MBP pull-down assay we observed that double-stranded plasmid DNA of all three forms (supercoiled, linear, and open circle) was pulled down by amylose beads together with MBP-HMK-YA but not with MBP-HMK (Figure 5).

YA Binds to Histone H2B

To test whether YA also binds to chromosomal proteins, purified histone H1 (20 μM) or an equimolar mixture of purified *Drosophila* core histones (7 μM of each) was added to the mitotic chromosome binding reaction with MBP-HMK-YA. As shown in Figure 6, MBP-HMK-YA's binding to mitotic chromosomes was not affected by the presence of histone H1 (Figure 6, cf. A and B) but was greatly reduced in presence of core histones (Figure 6, cf. A and C). The residual binding in panel C may be from MBP-HMK-YA's binding to DNA or from incomplete competition. These results suggest that YA also binds to core histones.

To confirm YA's binding to core histones and to determine which core histone(s) binds to YA, we performed an MBP pull-down assay. An equimolar (1 μg each) mixture of all four core histones was incubated with either MBP-HMK-YA or MBP-HMK. Amylose beads were then added to pull down MBP fusion proteins. Only one core histone band was pulled down by the beads together with MBP-HMK-YA (Figure 7A, lane 3); it was not pulled down by beads in the presence of MBP-HMK (Figure 7, lane 4), indicating that this core histone binds to YA specifically. The SDS-polyacryl-

amide gel mobility of this band matched that of histone H2B or H2A. To test whether YA binds to histone H2B or H2A or both, we performed a mitotic chromosome binding assay with MBP-HMK-YA in the presence of either purified histone H2B or purified H2A (Figure 6, D and E). In the presence of purified histone H2B, MBP-HMK-YA's binding to mitotic chromosomes was greatly reduced (Figure 6D), but the presence of histone H2A did not interfere with MBP-HMK-YA's binding to mitotic chromosomes (Figure 6E). To confirm that histone H2B but not H2A binds to YA, we performed MBP pull-down assays with purified histone H2B or H2A. As shown in Figure 7B, histone H2B was pulled down by amylose beads together with MBP-HMK-YA but not with MBP-HMK, confirming that histone H2B binds to YA. In contrast, MBP pull-downs with purified histone H2A showed that H2A did not bind to MBP-HMK-YA (Figure 7C).

Four Domains in YA Bind to DNA and Histone H2B

To define the domains of YA that mediate YA's binding to mitotic chromosomes, mitotic chromosome binding assays were performed with YA fragments as GST or MBP-HMK fusion proteins, with GST or MBP-HMK controls, as in Figure 1; representative examples are shown in Figure 8, A–I. Their binding to DNA or histone H2B was also tested separately, by competition assays as in Figures 4 and 6; representative examples of each are shown in Figure 8, J–M. GST alone did not bind to mitotic chromosomes (Figure 8E). As summarized in Figure 9A, four separable domains in YA were shown to bind to mitotic chromosomes. These minimal binding regions are: aa1–117 (domain A in Figure 9A; Figure 8A), which contains two C2H2-type zinc fingers and a half zinc finger similar to Krox-20 (Chavrier *et al.*, 1990); aa270–396 (domain B in Figure 9A; Figure 8F), which contains the Q-rich opa region and part of the Ser/Thr rich region; aa

Figure 5. DNA is pulled down with MBP-HMK-YA in a MBP pull-down assay. MBP-HMK-YA (4 μ g; Tag-YA) or MBP-HMK (32 μ g; Tag) was incubated with 1.2 μ g plasmid pGEX-2T DNA and then pulled-down with amylose beads. The DNA was separated on a 1% agarose gel. Arrows point to DNA bands corresponding to (from highest to lowest) supercoiled, circular, and linear forms of plasmid DNA. Sup., Supernatant.

397–472 (domain C in Figure 9A; Figure 8G), which contains the rest of the Ser/Thr rich region; and aa 506–696 (domain D in Figure 9; Figure 8D), which contains the SPKK potential DNA-binding motif and is highly positively charged (Lin and Wolfner, 1991; Liu and Wolfner, 1998). The binding of each of the regions to mitotic chromosomes was competed by DNA (as in Figure 4A, panel D) and by histone H2B (as in Figure 6D). These data, summarized in Figure 9B, suggesting that each of the domains binds to both DNA and histone H2B.

YA's Q-rich region is not required for chromosome binding, as fragment dQ aa 230–396 from which the Q-rich region was deleted (Liu and Wolfner, 1998) can still bind to chromosomes (Figure 8H).

Both zinc fingers are important for YA's binding to mitotic chromosomes, because mutation of two cysteines in either zinc finger (C1 or C2; Liu and Wolfner, 1998) greatly decreased fragment aa 1–117's binding to mitotic chromosomes (Figure 8, B and C). As the binding to mitotic chromosomes of aa 1–117 mutant in just one zinc finger can still be competed by DNA competitors (Figure 8, J and K), both zinc fingers bind to DNA. Binding of C1 aa1–117 (mutant in zinc finger 1 but with normal zinc finger 2) to mitotic chro-

Figure 6. YA's binding to mitotic chromosomes can be competed by histone H2B. Binding reactions of MBP-HMK-YA with mitotic chromosomes were performed in the presence of 20 μ M *Drosophila* histone H1 (B), equi-molar mix of *Drosophila* core histones (7 μ M each, C), 7 μ M of histone H2B (D), or 7 μ M of histone H2A (E). Mitotic chromosomes were stained with DAPI (DNA) and anti-MBP antibodies (Anti-MBP). Bar: 10 μ m.

mosomes was not competed by histone H2B (Figure 8L). Binding of C2 aa1–117 (mutant in zinc finger 2 but with normal zinc finger 1) to mitotic chromosomes was competed by histone H2B (Figure 8M). These data suggest that zinc finger 1 but not zinc finger 2 binds to histone H2B. Although the zinc finger is thought mainly to mediate protein-DNA binding, it has been found to be also involved in protein-protein interactions (for a recent review, see Leon and Roth, 2000); this appears to be the case for zinc finger 1 in YA.

The binding of these four domains to mitotic chromosomes is likely to be specific, because MBP-HMK (Figure 8I), GST (Figure 8E), or many smaller YA fragments did not bind to mitotic chromosomes under the same conditions.

Figure 7. YA binds to histone H2B. MBP-HMK-YA (4 μ g; Tag-YA) or MBP-HMK(32 μ g; Tag) was incubated with 4 μ g of a mixture of core histone (A), 4 μ g histone H2B (B), or 4 μ g histone H2A (C). The mixture was then incubated with amylose beads. Proteins bound to the beads were analyzed by SDS-PAGE and Western blotting with polyclonal anti-core histone antibodies (Ito *et al.*, 1996a).

DISCUSSION

YA Binds to DNA and Histone H2B

Chromosome condensation state is important for nuclear functions such as DNA replication, transcription, and chromosome segregation (for reviews, see Koshland and Strunnikov, 1996; Wolffe, 1996; Qumsiyeh, 1999) and is an active process that requires chromosome decondensation and condensation factors. The YA phenotype (Liu *et al.*, 1995; Berman, 2000) suggests that YA may be essential to attain the proper chromatin condensation state at the end of female meiosis in *Drosophila* and is most consistent with YA's action being to decondense the chromatin following meiosis.

YA is normally found at the nuclear periphery as well as throughout the nucleoplasm (Lin and Wolfner, 1991; Lopez *et al.*, 1994). YA had previously been shown to bind to decondensed sperm chromatin in *Xenopus* egg extracts and to polytene chromosomes when ectopically expressed (Lopez and Wolfner, 1997), but the nature and mediators of its chromatin binding were not known. Here, we showed that YA can bind directly to chromatin, with an affinity ($K_d = 1.1 \mu$ M) similar to that with which *Drosophila* lamin Dm α_0 binds chromatin (Goldberg *et al.*, 1999). YA binds to chromatin through its interaction with DNA and histone H2B. YA prefers double-stranded DNA to single-stranded and can bind DNA of different superhelicity states.

All chromatin decondensation factors tested thus far have been shown to bind to core histones (nucleoplasmin, Dilworth *et al.*, 1987; Kleinschmidt *et al.*, 1990; Philpott and Leno, 1992; DF 31, Crevel and Cotterill, 1995; Ito *et al.*, 1996b; dNAP-1, Ito *et al.*, 1996a), suggesting that binding to core

histones may be a general way to regulate chromatin decondensation. However, how binding to core histones affects chromosome decondensation is not fully understood and may benefit from the sort of in vivo analysis made possible by YA mutants. Studies of the role of histones in chromosome condensation have mainly focused on histone H1 and H3 (for recent reviews, see Koshland and Strunnikov, 1996; Hirano, 2000). However, there is also evidence for a role of histone H2B in this process. *Trypanosoma cruzi*, whose chromatin contains a unique variant of histone H2B, retains its chromatin in an unusual decondensed state throughout the entire cell cycle (Toro *et al.*, 1993). In addition, in the slime mold *Physarum polycephalum*, histones H2A and H2B are ubiquitinated from anaphase to prophase and are deubiquitinated during metaphase, suggesting that ubiquitination is an early step in chromosome decondensation and deubiquitination is a late step in chromosome condensation (Mueller *et al.*, 1985). The binding of YA to histone H2B may be another case of the involvement or modulation of histone H2B in chromosome condensation state, in this case at a specific developmental time.

Incubation of MBP-HMK-YA with mitotic chromosomes did not visibly alter the condensation state of those chromosomes, suggesting that YA is not sufficient for chromatin decondensation per se. It is possible that certain histone modifications such as ubiquitination at the end of meiosis and/or other factors such as the nuclear envelope might be needed for YA to participate in modifying chromatin structure. For example, the nuclear envelope is important for sperm chromatin decondensation (Lohka and Masui, 1984; Collas and Poccia, 1995; Poccia and Collas, 1996, 1997). The C-terminal fragment of YA, aa 506–696, contains both a chromosome-binding domain (this study) and a lamin-binding domain (Goldberg *et al.*, 1998; Rajagopal, Fan, Garfinkel, Mani, and Wolfner, unpublished results). It is possible that YA's binding to lamin and chromatin with overlapping domains brings chromatin close to the nuclear envelope and hence facilitates chromosome decondensation.

Why Might YA's Binding to Chromatin Be Important in Development?

At the end of female meiosis in wild-type *Drosophila* embryos, chromatin begins to decondense in telophase II. It then enters an interphase-like state; at this time YA is first seen in nuclei (Yu *et al.*, 1999). Chromatin then recondenses and starts the first (gonomeric) mitosis (Callaini and Riparbelli, 1996). The sperm's nucleus decondenses during this time, losing its paternal investments (Liu *et al.*, 1997) and becoming spherical. Eggs and embryos produced by mothers lacking Ya function arrest development immediately after meiosis (Lopez, 1996; Berman, 2000; Lopez, Berman, Yu, Dernburg and Wolfner, unpublished results). Their chromatin is abnormally condensed, and nuclei within YA-deficient eggs show lack of coordination in condensation state (Liu *et al.*, 1995). Fertilized eggs lacking YA do convert the sperm nucleus to a male pronucleus but it, and the abnormally condensed nuclei resulting from female meiosis, fail to associate correctly or to initiate the first embryonic cell cycle. The observations reported here, that YA protein binds to DNA and histone H2B and that the YA regions responsible for this binding correlate with those required for YA's function, lead to the model that YA's binding to DNA and

Figure 8. Representative YA fragments' binding to mitotic chromosomes in the presence or absence of salmon sperm DNA (DNA) or histone H2B (H2B) competitors. Purified YA fragments produced as GST or MBP-HMK fusion proteins were incubated with mitotic chromosomes. The chromosomes were stained with DAPI (DNA) and anti-GST antibodies (for GST fusion proteins, panels A–D and J–M; YA) or anti-MBP antisera (for MBP-HMK fusion proteins, panels F–H; YA). GST (panel E) or MBP-HMK (panel I) proteins were incubated with mitotic chromosomes as negative controls. Two chromosomes close to each other are shown in panel I. C1, C2: site-directed mutants in the zinc-fingers, with the cysteines in the first or the second zinc finger, respectively, mutated to alanines (Liu and Wolfner, 1998). dQ, a construct from which the glutamine-rich region was deleted. Bar: 10 μ m.

A.

B.

Figure 9. (A) Four regions in YA bind to chromosomes. Purified YA fragments as GST or MBP-HMK fusion were used for mitotic chromosome binding assays. Horizontal lines show the YA regions tested for binding. (A) Four regions, aa 1–117 (domain A), aa 270–396 (domain B), aa 397–472 (domain C), and aa 506–696 (domain D) bind to chromosomes. (B) The binding of all four regions can be competed by salmon sperm DNA and histone H2B. Q-rich: glutamine-rich region. S/T rich: Ser/Thr rich region. C1, C2, C1C2: site-directed mutants in the zinc-fingers, with the cysteines in the first, the second or both zinc fingers mutated ("X") to alanines (Lin and Wolfner, 1991; Liu and Wolfner, 1998). dQ, a construct from which the Q-rich region was deleted. +: strong binding. +/-: very weak (close to background level of binding), -: background level of binding.

histone H2B may regulate the condensation state of nuclei at the time of fertilization.

Proper chromosome condensation state appears to be critical for making the transition from meiosis to mitosis. In rhesus monkey zygotes, Hewitson *et al.* (1999) proposed a checkpoint that monitors pronuclear chromosome condensation state and must be passed to allow the onset of DNA replication for the zygote's first mitosis. If the male or female pronucleus has a chromosome condensation defect, development is arrested at the pronuclear stage (Hewitson *et al.*, 1996). If chromatin decondensation is simply delayed in either the female or the male pronucleus of rhesus monkey zygotes, initiation of DNA replication is similarly delayed in both pronuclei, until the chromatin has decondensed (Hewitson *et al.*, 1999). This suggests that a G1/S transition checkpoint may monitor chromatin condensation state at the pronuclear stage in rhesus monkey zygotes. Inability to properly decondense the chromatin or to sense that this had occurred would thus arrest development after meiosis but before the embryo initiates mitosis.

The *Ya* null mutant phenotype suggests that YA's activity might be necessary to pass an analogous checkpoint in *Drosophila* development (Lopez, Berman, Yu, Dernburg, and Wolfner, unpublished results). *Ya* embryos arrest development at the pronuclear stage (Lin and Wolfner, 1991) with nuclei that show abnormal chromatin condensation (Liu *et al.*, 1995). *Ya*'s epistasis to mutations such as *gnu* (Liu *et al.*, 1997) that affect S/M coordination and result in multiple rounds of DNA replication (Freeman *et al.*, 1986; Freeman and Glover, 1987; Elfring *et al.*, 1997) suggests that arrest of *Ya* embryos occurs before the initiation of DNA replication. This phenotype is analogous to that of the rhesus monkey zygotes described above. There is, however, one difference between the trigger for this potential checkpoint in *Drosophila* vs. rhesus monkeys. *Drosophila* mutations that affect male pronuclear chromatin condensation state only, such as *maternal haploid*, *ms(3)K81*, and *sésame*, do not trigger arrest of initiation of mitosis by the female pronucleus (Zalokar *et al.*, 1975; Yasuda *et al.*, 1995; Loppin *et al.*, 2000) in contrast to the G1/S block in rhesus monkey zygotes with abnormal condensation of the male pronucleus. (The converse type of mutation [leaving the female pronucleus highly condensed but allowing mitosis by the male pronucleus] has never been reported) This suggests that, in *Drosophila*, sensing the condensation state of the female pronucleus may be sufficient to determine whether the checkpoint can be passed. If the male pronucleus does not decondense sufficiently, this is not grounds for aborting the first cell cycle. The fact that some insects can develop into viable fertile haploids might have resulted in less stricture on the male pronucleus' structure to "trip" the early development checkpoint.

In summary, we have shown here that the nuclear lamina protein YA binds to chromatin via interactions with DNA and with histone H2B; YA's interaction with chromatin has a similar K_d to lamin-chromatin interaction. The YA regions that bind to DNA and histone H2B correlate with regions required for YA function. Taken together with phenotypic data, these data suggest that YA's binding to DNA and histone H2B act to mediate proper chromosome condensation state during the transition from meiosis to mitosis.

ACKNOWLEDGMENTS

The authors thank Drs. K. Kemphues, R. Cerione, J. Lis, L. Kraus, J. Liu, and S. Mani for helpful suggestions and for valuable comments on the manuscript; L. Kraus for *Drosophila* core histones and anti-*Drosophila* core histone antibodies; R. Rajagopal for the YA C-terminal deletion constructs; and Drs. Y. Gruenbaum and M. Goldberg for protocols. The work was funded by National Institutes of Health grant GM44659 to M.F.W.

REFERENCES

- Berman, C.L. (2000). The Role of YA Protein in *Drosophila* Female Meiosis [M.S. Thesis]. Ithaca, NY: Cornell University.
- Blonar, M.A., and Rutter, W.J. (1992). Interaction cloning: identification of a helix-loop-helix zipper protein that interacts with c-Fos. *Science* 256, 1014–1018.
- Bulger, M., and Kadonaga, J.T. (1994). Biochemical reconstitution of chromatin with physiological nucleosome spacing. *Meth. Molec. Genet.* 5, 241–262.
- Callaini, G., and Riparbelli, M.G. (1996). Fertilization in *Drosophila melanogaster*: centrosome inheritance and organization of the first mitotic spindle. *Dev. Biol.* 176, 199–208.
- Cameron, L.A., and Poccia, D.L. (1994). In vitro development of the sea urchin male pronucleus. *Dev. Biol.* 162, 568–578.
- Chavrier, P., Vesque, C., Galliot, B., Vigneron, M., Dolle, P., Duboule, D., and Charnay, P. (1990). The segment-specific gene *Krox-20* encodes a transcription factor with binding sites in the promoter region of the *Hox-1.4* gene. *EMBO J.* 9, 1209–1218.
- Collas, P., and Poccia, D. (1995). Formation of the sea urchin male pronucleus *in vitro*: membrane-independent chromatin decondensation and nuclear envelope-dependent nuclear swelling. *Mol. Rep. Dev.* 42, 106–113.
- Collas, P., and Poccia, D. (1998). Remodeling the sperm nucleus into a male pronucleus at fertilization. *Theriogenology* 49, 67–81.
- Cothren, C.C., and Poccia, D.L. (1993). Two steps required for male pronucleus formation in the sea urchin egg. *Exp. Cell Res.* 205, 126–133.
- Crevel, G., and Cotterill, S. (1995). DF 31, a sperm decondensation factor from *Drosophila melanogaster*: purification and characterization. *EMBO J.* 14, 1711–1717.
- Crevel, G., Huikeshoven, H., Cotterill, S., Simon, M., Wall, J., Philpott, A., Laskey, R.A., McConnell, M., Fisher, P.A., and Berrios, M. (1997). Molecular and cellular characterization of CRP1, a *Drosophila* chromatin decondensation protein. *J. Struct. Biol.* 118, 9–22.
- Dilworth, S.M., Black, S.J., and Laskey, R.A. (1987). Two complexes that contain histones are required for nucleosome assembly in vitro: role of nucleoplasmin and N1 in *Xenopus* egg extracts. *Cell* 51, 1009–1018.
- Edgar, B.A., Kiehle, C.P., and Schubiger, G. (1986). Cell cycle control by the nucleo-cytoplasmic ratio in early *Drosophila* development. *Cell* 44, 365–372.
- Elfring, L.K., Axton, J.Y., Fenger, D.D., Page, A.W., Carminati, J.L., and Orr-Weaver, T.L. (1997). *Drosophila* PLUTONIUM protein is a specialized cell cycle regulator required at the onset of embryogenesis. *Mol. Biol. Cell* 8, 583–593.
- Fitch, K.R., and Wakimoto, B.T. (1998). The paternal effect gene *ms(3)sneaky* is required for sperm activation and the initiation of embryogenesis in *Drosophila melanogaster*. *Dev. Biol.* 197, 270–282.
- Freeman, M., and Glover, D.M. (1987). The *gnu* mutation of *Drosophila* causes inappropriate DNA synthesis in unfertilized and fertilized eggs. *Genes Dev.* 1, 924–930.

- Freeman, M., Nusslein-Volhard, C., and Glover, D.M. (1986). The dissociation of nuclear and centrosomal division in *gnu*, a mutation causing giant nuclei in *Drosophila*. *Cell* 46, 457–468.
- Fuyama, Y. (1986). Genetics of parthenogenesis in *Drosophila melanogaster*. II. Characterization of a gynogenetically reproducing strain. *Genetics* 114, 495–510.
- Gans, M., Audit, C., and Masson, M. (1975). Isolation and characterization of sex-linked female sterile mutants in *Drosophila melanogaster*. *Genetics* 81, 683–704.
- Gillespie, P.J., and Blow, J.J. (2000). Nucleoplasmin-mediated chromatin remodelling is required for *Xenopus* sperm nuclei to become licensed for DNA replication. *Nucleic Acids Res.* 28, 472–480.
- Glass, C.A., Glass, J.R., Taniura, H., Hasel, K.W., Blevitt, J.M., and Gerace, L. (1993). The alpha-helical rod domain of human lamins A and C contains a chromatin binding site. *EMBO J.* 12, 4413–4424.
- Glass, J.R., and Gerace, L. (1990). Lamins A and C bind and assemble at the surface of mitotic chromosomes. *J. Cell Biol.* 111, 1047–1058.
- Goldberg, M., Harel, A., Brandeis, M., Rechsteiner, T., Richmond, T.J., Weiss, A.M., and Gruenbaum, Y. (1999). The tail domain of lamin Dm0 binds histones H2A and H2B. *Proc. Natl. Acad. Sci. USA* 96, 2852–2857.
- Goldberg, M., Lu, H., Stuurman, N., Ashery-Padan, R., Weiss, A.M., Yu, J., Bhattacharyya, D., Fisher, P.A., Gruenbaum, Y., and Wolfner, M.F. (1998). Interactions among *Drosophila* nuclear envelope proteins lamin, otefin, and YA. *Mol. Cell. Biol.* 18, 4315–4323.
- Hewitson, L., Dominko, T., Takahashi, D., Martinovich, C., Ramalho-Santos, J., Sutovsky, P., Fanton, J., Jacob, D., Monteith, D., Neuringer, M., Battaglia, D., Simerly, C., and Schatten, G. (1999). Unique checkpoints during the first cell cycle of fertilization after intracytoplasmic sperm injection in rhesus monkeys. *Nat. Med.* 5, 431–433.
- Hewitson, L.C., Simerly, C.R., Tengowski, M.W., Sutovsky, P., Navara, C.S., Haavisto, A.J., and Schatten, G. (1996). Microtubule and chromatin configurations during rhesus intracytoplasmic sperm injection: successes and failures. *Biol. Reprod.* 55, 271–280.
- Hirano, T. (2000). Chromosome cohesion, condensation, and separation. *Annu. Rev. Biochem.* 69, 115–144.
- Hulme, E.C., and Birdsall, N.J.M. (1992). Receptor Ligand Interactions: A Practical Approach. New York: Oxford University Press.
- Ito, T., Bulger, M., Kobayashi, R., and Kadonaga, J.T. (1996a). *Drosophila* NAP-1 is a core histone chaperone that functions in ATP-facilitated assembly of regularly spaced nucleosomal arrays. *Mol. Cell. Biol.* 16, 3112–3124.
- Ito, T., Tyler, J.K., Bulger, M., Kobayashi, R., and Kadonaga, J.T. (1996b). ATP-facilitated chromatin assembly with a nucleoplasmin-like protein from *Drosophila melanogaster*. *J. Biol. Chem.* 271, 25041–25048.
- Iwata, K., Hozumi, K., Iihara, A., Nomizu, M., Sakairi, N., and Nishi, N. (1999). Mechanism of salmon sperm decondensation by nucleoplasmin. *Int. J. Biol. Macromol.* 26, 95–101.
- Kawasaki, K., Philpott, A., Avilion, A.A., Berrios, M., and Fisher, P.A. (1994). Chromatin decondensation in *Drosophila* embryo extracts. *J. Biol. Chem.* 269, 10169–10176.
- Kleinschmidt, J.A., Seiter, A. and Zentgraf, H. (1990). Nucleosome assembly in vitro: separate histone transfer and synergistic interaction of native histone complexes purified from nuclei of *Xenopus laevis* oocytes. *EMBO J.* 9, 1309–1318.
- Koshland, D., and Strunnikov, A. (1996). Mitotic chromosome condensation. *Annu. Rev. Cell Dev. Biol.* 12, 305–333.
- Laskey, R.A., Mills, A.D., Philpott, A., Leno, G.H., Dilworth, S.M., and Dingwall, C. (1993). The role of nucleoplasmin in chromatin assembly and disassembly. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 339, 263–269.
- Leon, O., and Roth, M. (2000). Zinc fingers: DNA binding and protein-protein interactions. *Biol. Res.* 33, 21–30.
- Lin, H., and Wolfner, M.F. (1991). The *Drosophila* maternal-effect gene *fs(1)Ya* encodes a cell cycle-dependent nuclear envelope component required for embryonic mitosis. *Cell* 64, 49–62.
- Liu, J., Song, K., and Wolfner, M.F. (1995). Mutational analyses of *fs(1)Ya*, an essential, developmentally regulated, nuclear envelope protein in *Drosophila*. *Genetics* 141, 1473–1481.
- Liu, J., Song, K., and Wolfner, M.F. (1997). Formation of the male pronuclear lamina in *Drosophila*. *Dev. Biol.* 184, 187–196.
- Liu, J., and Wolfner, M.F. (1998). Functional dissection of YA, an essential, developmentally regulated nuclear lamina protein in *Drosophila melanogaster*. *Mol. Cell. Biol.* 18, 188–197.
- Lohka, M.J., and Masui, Y. (1984). Roles of cytosol and cytoplasmic particles in nuclear envelope assembly and sperm pronuclear formation in cell-free preparations from amphibian cells. *J. Cell Biol.* 98, 1222–1230.
- Longo, F.J. (1985). Pronuclear events during fertilization. In: *Biology of Fertilization*, Vol. 3, ed. C. B. Metz and C. B. Monroy, New York: Academic Press, 251–293.
- Longo, F.J., Mathews, L., and Palazzo, R.E. (1994). Sperm nuclear transformations in cytoplasmic extracts from surf clam (*Spisula solidissima*) oocytes. *Dev. Biol.* 162, 245–258.
- Lopez, J., Song, K., Hirshfeld, A., Lin, H., and Wolfner, M.F. (1994). The *Drosophila fs(1)Ya* protein, which is needed for the first mitotic division, is in the nuclear lamina and in the envelopes of cleavage nuclei, pronuclei and nonmitotic nuclei. *Dev. Biol.* 163, 202–211.
- Lopez, J.M. (1996). Functional studies of “YA,” a developmentally regulated nuclear lamina protein that is essential for *Drosophila* embryogenesis [Ph.D. Thesis]; Ithaca, NY: Cornell University.
- Lopez, J.M., and Wolfner, M.F. (1997). The developmentally regulated *Drosophila* embryonic nuclear lamina protein ‘Young Arrest’ (*fs(1)Ya*) is capable of associating with chromatin. *J. Cell Sci.* 110, 643–651.
- Loppin, B., Berger, F. and Couble, P. (2001). Paternal chromosome incorporation into the zygote nucleus is controlled by *maternal haploid* in *Drosophila*. *Dev. Biol.* 231, 383–396.
- Loppin, B., Docquier, M., Bonneton, F., and Couble, P. (2000). The maternal effect mutation *sesame* affects the formation of the male pronucleus in *Drosophila melanogaster*. *Dev. Biol.* 222, 392–404.
- Maeda, Y., Yanagimachi, H., Tateno, H., Usui, N., and Yanagimachi, R. (1998). Decondensation of the mouse sperm nucleus within the interphase nucleus. *Zygote* 6, 39–45.
- Mueller, R.D., Yasuda, H., Hatch, C.L., Bonner, W.M., and Bradbury, E.M. (1985). Identification of ubiquitinated histones 2A and 2B in *Physarum polycephalum*. Disappearance of these proteins at metaphase and reappearance at anaphase. *J. Biol. Chem.* 260, 5147–5153.
- Page, A.W., and Orr-Weaver, T.L. (1997). Stopping and starting the meiotic cell cycle. *Curr. Opin. Genet. Dev.* 7, 23–31.
- Philpott, A., and Leno, G.H. (1992). Nucleoplasmin remodels sperm chromatin in *Xenopus* egg extracts. *Cell* 69, 759–767.
- Philpott, A., Leno, G.H., and Laskey, R.A. (1991). Sperm decondensation in *Xenopus* egg cytoplasm is mediated by nucleoplasmin. *Cell* 65, 569–578.
- Poccia, D., and Collas, P. (1996). Transforming sperm nuclei into male pronuclei *in vivo* and *in vitro*. In: *Current Topics in Develop-*

- mental Biology, vol. 34, ed. R. A. Pederson and G. Schatten, San Diego: Academic Press, 25–88.
- Poccia, D., and Collas, P. (1997). Nuclear envelope dynamics during male pronuclear development. *Dev. Growth Differ.* 39, 541–550.
- Qumsiyeh, M.B. (1999). Structure and function of the nucleus: anatomy and physiology of chromatin. *Cell Mol. Life Sci.* 55, 1129–1140.
- Rice, P., Garduno, R., Itoh, T., Katagiri, C., and Ausio, J. (1995). Nucleoplasmin-mediated decondensation of *Mytilus* sperm chromatin: identification and partial characterization of a nucleoplasmin-like protein with sperm-nuclei decondensing activity in *Mytilus californianus*. *Biochemistry* 34, 7563–7568.
- Santamaria, P. (1983). Analysis of haploid mosaics in *Drosophila*. *Dev. Biol.* 96, 285–295.
- Santamaria, P., and Gans, M. (1980). Chimeras of *Drosophila melanogaster* obtained by injection of haploid nuclei. *Nature* 287, 143–144.
- Schultz, R.M., and Kopf, G.S. (1995). Molecular basis of mammalian egg activation. *Curr. Top. Dev. Biol.* 30, 21–62.
- Sonnenblick, B.D. (1950). The early embryology of *Drosophila melanogaster*. In: *The Biology of Drosophila*, ed. M. Demerec, New York: John Wiley & Sons, 62–167.
- Stricker, S., Prather, R., Simerly, C., Schatten, H., and Schatten, G. (1989). Nuclear architecture changes during fertilization and development. In: *The Cell Biology of Fertilization*, ed. H. Schatten and G. Schatten, New York: Academic Press, 225–250.
- Taniura, H., Glass, C., and Gerace, L. (1995). A chromatin binding site in the tail domain of nuclear lamins that interacts with core histones. *J. Cell Biol.* 131, 33–44.
- Toro, G.C., Wernstedt, C., Hellman, U., and Galanti, N. (1993). Presence of histone H2B in *Trypanosoma cruzi* chromatin. *Biol. Res.* 26, 41–46.
- Wolffe, A.P. (1996). Chromatin and gene regulation at the onset of embryonic development. *Reprod. Nutr. Dev.* 36, 581–606.
- Wright, S.J. (1999). Sperm nuclear activation during fertilization. *Curr. Top. Dev. Biol.* 46, 133–178.
- Yamashita, M., Onozato, H., Nakanishi, T., and Nagahama, Y. (1990). Breakdown of the sperm nuclear envelope is a prerequisite for male pronucleus formation: direct evidence from the gynogenetic crucian carp *Carassius auratus langsdorffii*. *Dev. Biol.* 137, 155–160.
- Yasuda, G.K., Schubiger, G., and Wakimoto, B.T. (1995). Genetic characterization of *ms(3)K81*, a paternal effect gene of *Drosophila melanogaster*. *Genetics* 140, 219–229.
- Yu, J. (2000). *Drosophila* egg activation, and regulation and function of an essential, maternally-provided, nuclear protein YA [Ph.D. Thesis]. Ithaca, NY: Cornell University.
- Yu, J., Liu, J., Song, K., Turner, S.G., and Wolfner, M.F. (1999). Nuclear entry of the *Drosophila melanogaster* nuclear lamina protein YA correlates with developmentally regulated changes in its phosphorylation state. *Dev. Biol.* 210, 124–134.
- Zalokar, M., Audit, C., and Erk, I. (1975). Developmental defects of female-sterile mutants of *Drosophila melanogaster*. *Dev. Biol.* 47, 419–432.