Developmental and Cell Cycle Regulation of the *Drosophila* Histone Locus Body[□]

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Cyclin E/Cdk2 is necessary for replication-dependent histone mRNA biosynthesis, but how it controls this process in early development is unknown. We show that in *Drosophila* embryos the MPM-2 monoclonal antibody, raised against a phosphopeptide from human mitotic cells, detects Cyclin E/Cdk2-dependent nuclear foci that colocalize with nascent histone transcripts. These foci are coincident with the histone locus body (HLB), a Cajal body-like nuclear structure associated with the histone locus and enriched in histone pre-mRNA processing factors such as Lsm11, a core component of the U7 small nuclear ribonucleoprotein. Using MPM-2 and anti-Lsm11 antibodies, we demonstrate that the HLB is absent in the early embryo and occurs when zygotic histone transcription begins during nuclear cycle 11. Whereas the HLB is found in all cells after its formation, MPM-2 labels the HLB only in cells with active Cyclin E/Cdk2. MPM-2 and Lsm11 foci are present in embryos lacking the histone locus, and MPM-2 foci are present in *U7* mutants, which cannot correctly process histone pre-mRNA. These data indicate that MPM-2 recognizes a Cdk2-regulated protein that assembles into the HLB independently of histone mRNA biosynthesis. HLB foci are present in histone deletion embryos, although the MPM-2 foci are smaller, and some Lsm11 foci are not associated with MPM-2 foci, suggesting that the histone locus is important for HLB integrity.

INTRODUCTION

Cell cycle-regulated histone protein biosynthesis is controlled primarily through the regulation of histone mRNA abundance, which in cultured mammalian cells increases 35-fold at the G1–S transition (Breindl and Gallwitz, 1973; Borun *et al.*, 1975; Detke *et al.*, 1979; Parker and Fitschen, 1980; DeLisle *et al.*, 1983; Heintz *et al.*, 1983; Harris *et al.*, 1991). This rapid rise in mRNA is achieved by increases in the rate of transcription initiation and pre-mRNA processing as cells enter S phase, followed by rapid degradation of histone mRNA at the end of S phase (Marzluff and Duronio, 2002). How these various aspects of histone mRNA metabolism are linked to other events that drive progression through the cell cycle by regulating the activity of the cyclin-dependent kinases (Cdks) remains incompletely understood.

In animal cells Cyclin E/Cdk2 promotes the G1-to-S transition in part by phosphorylating proteins that mediate changes in gene expression associated with the onset of DNA replication (e.g., pRb; Du and Pogoriler, 2006). These include proteins that regulate histone expression. For

example, human NPAT and human HIRA are Cyclin E/Cdk2 substrates that act to stimulate and repress, respectively, histone gene transcription in cell culture experiments (Ma *et al.*, 2000; Zhao *et al.*, 2000; Hall *et al.*, 2001; Nelson *et al.*, 2002; Miele *et al.*, 2005). How the activity of such factors is modulated by Cyclin E/Cdk2 and integrated into cell cycle-regulated histone gene expression in vivo is not known.

Cyclin E/Cdk2 may also regulate features of histone mRNA biosynthesis other than transcription, such as pre-mRNA processing. Rather than being polyadenylated, histone mRNAs terminate in a conserved stem loop structure that regulates all aspects of replication-associated histone mRNA metabolism, including biosynthesis, translation, and stability (Marzluff, 2005). This unique mRNA 3' end is formed through a pre-mRNA processing reaction that cleaves the histone pre-mRNA four to five nucleotides after the stem loop, producing mature histone mRNA (Dominski and Marzluff, 1999; Marzluff, 2005). The cleavage endonuclease complex is recruited to histone pre-mRNA by Stem Loop Binding Protein (SLBP), which binds the stem loop in the 3' untranslated region (UTR), and U7 small nuclear ribonucleoprotein (snRNP), which binds a purine-rich sequence located downstream of the cleavage site.

In mammalian cells and *Xenopus* oocytes U7 snRNP localizes to Cajal bodies (CBs), which are subnuclear organelles involved in several aspects of RNA metabolism, including snRNP maturation (Kiss, 2004; Cioce and Lamond, 2005; Matera and Shpargel, 2006; Stanek and Neugebauer, 2006). Histone mRNA biosynthesis is thought to occur within or near a subset of Cajal bodies. Unlike U7 snRNP, which is found in all Cajal bodies (Frey and Matera, 1995), NPAT

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Abbreviations used: CB, Cajal body; HLB, histone locus body; SLBP, stem-loop binding protein.

localizes to the subset of Cajal bodies associated with histone genes (Ma *et al.*, 2000; Zhao *et al.*, 2000). Thus, Cyclin E/Cdk2 may regulate Cajal body function or the activity of proteins that act within Cajal bodies to regulate histone mRNA biosynthesis.

Here, we examine the connection between Cyclin E/Cdk2 activity and cell cycle-regulated histone mRNA biosynthesis in *Drosophila* embryos, which have provided fundamental insight into the regulation of the cell cycle and how this regulation is coordinated with development (Lee and Orr-Weaver, 2003; Swanhart *et al.*, 2005). *Drosophila* nuclei contain both Cajal bodies and a distinct nuclear body that is often observed in proximity to the Cajal body called the histone locus body (HLB) (Liu *et al.*, 2006). The HLB is associated with the histone genes, which are contained in a 5-kb sequence present in ~100 tandemly repeated copies, and it is enriched in U7 snRNP particles (Liu *et al.*, 2006). Cyclin E/Cdk2 activity is necessary for histone gene expression during embryogenesis (Lanzotti *et al.*, 2004), but how this occurs is not known. In this report, we demonstrate that the HLB contains a cell cycle-regulated, Cyclin E/Cdk2-dependent phospho-epitope recognized by the MPM-2 monoclonal antibody.

The MPM-2 antibody was generated using a mitotic HeLa cell extract and recognizes conserved cell cycle-dependent phospho-epitopes present in a variety of proteins across many species (Davis *et al.*, 1983). One epitope recognized by MPM-2 is a consensus Cdk phosphorylation site (Westendorf *et al.*, 1994). MPM-2 has been used extensively to study mitotic phospho-proteins in a variety of systems (Kuang *et al.*, 1989; Hirano and Mitchison, 1991; Yaffe *et al.*, 1997; Logarinho and Sunkel, 1998; do Carmo Avides *et al.*, 2001; Albert *et al.*, 2004; Lange *et al.*, 2005). In *Drosophila* ovarian cells, MPM-2 labels a spherical nuclear body whose cell cycle appearance is dependent on Cyclin E/Cdk2 activity (Calvi *et al.*, 1998). Here, we show that MPM-2 nuclear foci are coincident with the HLB, and we exploit this finding to characterize the connection between Cyclin E/Cdk2 activity, nuclear organization, and histone mRNA biosynthesis during early *Drosophila* development.

MATERIALS AND METHODS

Drosophila Stocks

*Slbp*¹⁵ (Sullivan *et al.*, 2001), *stg*⁴ (Edgar and O'Farrell, 1989), *cyclin E*^{AR95} (Knoblich *et al.*, 1994), *U7*¹⁴ (Godfrey *et al.*, 2006), *Df(3R)stg*^{AR2} (Lehman *et al.*, 1999), the histone locus deletion *Df(2L)Ds6* (Moore *et al.*, 1983), *cyclin E*^{hsp70} (Richardson *et al.*, 1995), *UAS:YFP-Lsm11* (Liu *et al.*, 2006), and *daGAL4* (Wodarz *et al.*, 1995) were all described previously. *cyclin E* mutant embryos were unambiguously identified using a *CyO P[wg-lacZ]* balancer chromosome. *w*¹¹¹⁸ flies were used as wild type control, except in Figure 6A where a sibling embryo of the *stg* mutant was used as control.

Immunostaining and In Situ Hybridization

Embryos were dechorionated, fixed in a 1:1 mixture of 5% formaldehyde/heptane for 25 min or 20% formaldehyde/heptane for 10 min, and incubated with primary and secondary antibodies each for 1 h at 25°C or overnight at 4°C. Yellow fluorescent protein (YFP)-Lsm11 embryos were fixed in a 1:1 mixture of 4% formaldehyde/heptane for 20 min. Fat bodies were dissected in Schneider's media, fixed in 5% formaldehyde for 25 min, permeabilized with 0.3% Triton X-100 (Acros Organics, Fairlawn, NJ) for 45 min, blocked with 1% bovine serum albumin, and incubated with primary antibodies overnight at 4°C and with secondary antibodies for 1 h at 25°C. The following primary antibodies were used: monoclonal mouse anti-Ser/Thr-Pro-MPM-2 (1:1000; Upstate Biotechnology, Lake Placid, NY), monoclonal mouse anti-phospho-histone H3 (Ser10) (1:1000; Upstate Biotechnology), polyclonal rabbit anti-phospho-histone H3 (Ser10) (1:1000; Upstate Biotechnology), polyclonal rabbit anti-phospho-tyrosine (1:100; Upstate Biotechnology), chicken anti-green fluorescent protein (GFP) (1:2000; Upstate Biotechnology), monoclonal rat anti-phospho-tyrosine (1:100; R&D Systems, Minneapolis, MN), and chicken anti-β-gal (1:1000; ProSci, Poway, CA); rabbit anti-GFP (1:2000; Abcam, Cambridge, MA); and affinity-purified polyclonal rabbit anti-Lsm11 (1:1000; gift from Joe Gall, Department of Embryology, Carnegie Institution, Baltimore, MD; Liu *et al.*, 2006). Embryos that were hybrid-

ized with H3/H4 DNA probes, *cycE*^{AR95}, *hsp70::cycE*, and its control embryos were incubated overnight with anti-Lsm11 antibodies at 37°C. The following secondary antibodies were used: goat anti-mouse IgG labeled with Oregon Green 488 (Invitrogen, Carlsbad, CA), Cy3 or Cy5 (Jackson ImmunoResearch Laboratories, West Grove, PA); goat anti-rabbit IgG labeled with rhodamine red (Invitrogen), Cy2 (Jackson ImmunoResearch Laboratories), or Cy5 (Abcam); goat anti-rat IgG labeled with Cy3 (Jackson ImmunoResearch Laboratories); donkey anti-rat Cy5 (Jackson ImmunoResearch Laboratories); and donkey anti-chicken IgY labeled with Cy2, Cy3, or Cy5 (all from Jackson ImmunoResearch Laboratories). DNA was detected by staining embryos with 4,6-diamidino-2-phenylindole (DAPI) (1:2000 of 1 mg/ml stock; Dako North America, Carpinteria, CA) for 30 s.

Histone H3 transcripts were detected by fluorescent in situ hybridization by using digoxigenin-labeled H3 coding or H3-ds probes as described previously (Lanzotti *et al.*, 2002). Hybrids were detected using the fluorescein tyramide signal amplification fluorescence system (PerkinElmer Life and Analytical Sciences, Boston, MA).

The histone locus was detected by fluorescent in situ hybridization with a biotin-labeled DNA probe (125 pg/μl) as described previously (Dernburg and Sedat, 1998). The probe was derived from a clone containing both the H3 and H4 genes (Lanzotti *et al.*, 2002) that was digested with MaeIII, RsaI, MseI, and HaeIII to generate fragments of an average length of 50 base pairs. DNA fragments were biotin labeled using the BrightStar psoralen-biotin labeling kit (Ambion, Austin, TX). Before hybridization, embryos were stained with MPM-2 and anti-Lsm11 antibodies by using methods described above. Biotinylated probe was detected using the cyanine 5 tyramide signal amplification fluorescence system (PerkinElmer Life and Analytical Sciences).

Cultured Cell Immunostaining and RNA Interference (RNAi)

Dmel-2 cells were grown in Sf-900 II SFM serum-free media by using standard techniques. Double-stranded (ds)RNAs were made by in vitro transcription by using a polymerase chain reaction (PCR) product as template and T7 polymerase. The following primer pairs were used to amplify *Lsm11* and *PTB* (control), respectively: 5'-CGTAATACGACTCATATAGTGAATCGAGGGACCGGAAAAC-3', 5'-GGTAATACGACTCATATAGCAA CAGTTCACCCCTGCACACTGCC-3', and 5'-GGTAATACGACTCATATAGTGAATCGAGGGACCGGAAAAC-3', 5'-GGTAATACGACTCATATAGTGAATCGAGGGACCGGAAAAC-3'. Cells (2 × 10⁶) were plated in six-well plates and treated with 10 μg of dsRNA daily for 5 d, and they were split 1:1 on days 3 and 5. Knockdown was confirmed by Western blot (data not shown). Cells were fixed directly to coverslips in 10% formaldehyde for 10 min, extracted using 0.1% Triton X-100 for 15 min, and blocked with 5% normal goat serum in phosphate-buffered saline/Tween 20 for 20 min. The same antibodies and incubation times used to stain embryos were used to stain cells.

Microscopy

Confocal images were taken at a zoom of 1.0–2.0 with a 63× (numerical aperture 1.40) Plan Apochromat objective on a Zeiss 510 laser scanning confocal microscope using the LSM data acquisition software (Carl Zeiss, Jena, Germany). YFP-Lsm11 embryo images were acquired on a Zeiss 410 laser scanning confocal microscope (Carl Zeiss). Image false coloring and contrast was adjusted using Photoshop (Adobe Systems, Mountain View, CA).

Measurement of MPM-2 Focus Size

MetaMorph software (Molecular Devices, Sunnyvale, CA) was used to characterize HLB structure from confocal images. Measurements were made using a 4-μm-deep compilation of confocal images from the tip of the extended germ band in three embryos per genotype. Both the length and width of MPM-2 foci were measured using the linescan function of MetaMorph. Two perpendicular, 1-pixel-thick lines from 10 to 30 pixels in length were drawn across the nuclear MPM-2 focus in cells that contained only one MPM-2 focus. The lines were long enough to include pixels that were outside of the MPM-2 focus. The average background fluorescence was calculated by taking the mean of six randomly chosen pixels from each linescan that were outside of the MPM-2 focus. The half-maximum value for each linescan was determined by taking 50% of the difference of the peak of fluorescence in the MPM-2 focus and average background fluorescence. The number of pixels whose fluorescence fell within or equaled the half-max was summed and multiplied by a conversion factor of 0.12 μm/pixel, which was obtained from the LSM Image Browser software (Carl Zeiss). The sum of the length and width measurements in micrometers was calculated for 75 cells from wild-type and 75 cells from *Df(2L)Ds6* homozygous mutant embryos. Data are reported as the average of these sums ±SD. A p value was obtained by conducting a Student's *t* test with two tails and unequal variance. The SE was ±0.043 for wild-type and ±0.031 for *Df(2L)Ds6*.

RESULTS

MPM-2 Labels the Histone Locus Body

MPM-2 labels a discrete spherical body in the nuclei of polyploid cells of the salivary gland and ovary (Millar *et al.*, 1987; Calvi *et al.*, 1998). To determine whether this MPM-2

body preferentially accumulates at a particular genomic locus, polytene chromosomes from squashed and whole mount salivary glands of third instars were stained with MPM-2 antibodies (Figure 1, A–A'; data not shown). MPM-2 strongly labels a single locus near the chromocenter on the left arm of chromosome 2. Interpretation of the cytogenetic

Figure 1. MPM-2 labels the histone locus body. (A and A') Squashed polytene chromosomes from CyO/+ third instar salivary glands were stained with MPM-2 (A; green in A') and the DNA binding dye DAPI (A'). (B–B'') *w¹¹¹⁸* syncytial blastoderm (cycle 13) embryos were stained with MPM-2 (B; green in B''), α -Lsm11 (B'; red in B''), and hybridized with an H3/H4 gene probe (B''; magenta in B''). (C–C'') Germ band extended *w¹¹¹⁸* embryos were stained with α -Lsm11 (C; red in C''), MPM-2 (C'; green in C''), and α -phospho-tyrosine (P-Tyr; blue in C'') to visualize cell boundaries. Arrows and arrowheads in B and C panels indicate nuclei with one or two HLB, respectively. (D–D'') stage 12 *w¹¹¹⁸* embryo stained with MPM-2 (green in D''), α -Lsm11 (red in D''), and α -P-Tyr (blue in D'). Arrows indicate an *S₁₇* cell containing an MPM-2 focus that colocalizes with Lsm11. White arrowheads indicate a *G₁₁₇* cell lacking MPM-2 foci but containing Lsm11 in the HLB. Yellow arrowheads indicate amnioserosal cells, which have permanently exited the cell cycle in *G₂₁₄*. Anterior is to the top and ventral to the left. Bars, 20 μ m.

banding pattern of these chromosomes indicated that this label is near or coincident with the chromosomal location of the histone gene cluster (data not shown). Cyclin E/Cdk2 activity is required for MPM-2 labeling of this subnuclear body, and for histone gene expression (Calvi *et al.*, 1998; Lanzotti *et al.*, 2004). We therefore hypothesized that MPM-2 recognizes a phospho-protein(s) that localizes to the histone locus body.

To test this hypothesis, we used confocal microscopy to analyze embryos labeled with both MPM-2 and anti-Lsm11 antibodies. Liu *et al.* (2006) originally visualized the HLB primarily by detecting Lsm11, an Sm-like protein that is a core component of the U7 snRNP. *Drosophila* embryos undergo a stereotyped cell cycle program that is characterized by distinct modes of cell cycle progression that occur at successive stages of embryogenesis. During the first 2 h of embryogenesis, maternally contributed factors drive 13 rapid and synchronous nuclear cycles, consisting only of S-M phases, in a syncytial cytoplasm. At the end of this period (i.e., nuclear cycle 13), MPM-2 labels both the nucleoplasm and distinct foci within the nucleus that colocalize with Lsm11 foci (Figure 1, B-B'). MPM-2 foci also colocalize with the histone locus, which was detected by fluorescence in situ hybridization by using a labeled DNA probe containing the H3 and H4 genes (Figure 1, B''-B'''). These data indicate that an MPM-2 epitope is associated with the HLB. By Western blot analysis, MPM-2 is known to react with at least a dozen targets in *Drosophila* (Logarinho and Sunkel, 1998; Lange *et al.*, 2005) (Leslie and Duronio, unpublished data), and thus the nucleoplasmic staining may not be the same protein that concentrates in the HLB.

Cellularization takes place during cycle 14, when after the completion of S phase the nuclei pause for the first time in G2. Then, as gastrulation ensues, groups of cells in different regions of the embryo enter mitosis 14 together. These groups of cells, called "mitotic domains," enter into mitosis 14 at different times, generating a reproducible and well described pattern of mitosis (Foe, 1989). Entry into mitosis 14 requires zygotic transcription of the *string^{cdc25}* (*stg*) gene, which encodes a Cdc25-type phosphatase that stimulates mitotic Cdk1 activity by removing inhibitory Y15 phosphorylation from Cdc2 (Edgar and O'Farrell, 1989). Cycles 15 and 16 are also regulated at the G2-M transition by developmentally controlled pulses of *stg* transcription, and still lack G1 phase as in the early syncytial cycles (Edgar and O'Farrell, 1990; Edgar *et al.*, 1994; Lehman *et al.*, 1999). MPM-2 and Lsm11 nuclear foci colocalize and are continuously present during interphase of these "postblastoderm" cell cycles (Figure 1, C-C'). MPM-2 foci are continuously present most likely because Cyclin E/Cdk2 activity is also ubiquitous at this time, including during G2 phase (Sauer *et al.*, 1995).

G1 phase first occurs during cycle 17, and subsequent entry into S phase in all cell types requires zygotic expression of *cyclin E* (Knoblich *et al.*, 1994). Consistent with previous observations that MPM-2 foci are Cyclin E dependent, MPM-2 only labels replicating cells where Cyclin E/Cdk2 is active (Figure 1, D-D', arrow). Cells that have exited the cell cycle, such as those in the amnioserosa (Figure 1, D-D', yellow arrowhead) and epidermal cells arrested in G1 (Figure 1, D-D', white arrowhead), do not contain MPM-2 foci. In contrast, Lsm11 foci can be detected in all cells (Figure 1, arrows). Thus, the HLB is present ubiquitously, as described previously for postembryonic stages of development (Liu *et al.*, 2006), but the MPM-2 epitope occurs at the HLB only in cells that contain active Cyclin E/Cdk2.

One obvious feature of Lsm11 and MPM-2 staining of blastoderm embryos is that each nucleus contains either one or two foci (Figure 1, B-B'', C-C'', arrows and arrowheads, respectively). Homologous chromosomes are often paired in *Drosophila* cells, and we therefore hypothesized that one and two foci results from paired and unpaired homologous chromosomes, respectively. In the early embryo, the pairing of homologous chromosomes is dynamic, such that the frequency of pairing at any particular locus increases as the embryo ages (Fung *et al.*, 1998). We counted the number of cells with one or two MPM-2 foci in blastoderm embryos during interphase of cell cycle 14. At this stage, 71% of cells contained one MPM-2 focus and 29% contained two MPM-2 foci ($n = 293$). Fung *et al.* (1998) reported a pairing frequency for the histone locus of 71 and 84% at 2.5 and 4 h of development, respectively, which encompasses the cycle 14 cells we analyzed (Fung *et al.*, 1998). By the time of germ band retraction during cycle 17, most cells contain a single focus (Figure 1D). These data are consistent with the HLB specifically associating with the histone locus (Figure 1B''') (Liu *et al.*, 2006). Also in support of this interpretation is the observation that polyploid nurse cells in the ovary have partially unpaired sister chromatids at the histone locus and contain multiple MPM-2 foci (data not shown) (Hammond and Laird, 1985; Calvi *et al.*, 1998). Together, these results indicate that MPM-2 recognizes an epitope at the histone locus that is a component of the Lsm11-containing HLB.

Embryonic MPM-2 Foci Depend on Cyclin E/Cdk2 Activity

If the embryonic MPM-2 foci are related to the previously described MPM-2 foci in follicle cells, then their presence should depend on Cyclin E activity. To test this, we characterized MPM-2 staining in stage 13 (cycle 17 in the epidermis) *cyclin E* mutant embryos relative to controls. Wild-type embryos at this developmental stage contain proliferating diploid cells in the central and peripheral nervous systems as well as endoreduplicating cells in various tissues (e.g., midgut, salivary gland, and posterior spiracles). *cyclin E* mutant embryos develop normally until this stage because of maternal deposition of Cyclin E, and then they arrest in G1 of cycle 17. Consequently, DNA synthesis in both proliferating neuronal cells and endoreduplicating cells is severely compromised in *cyclin E* mutants (Knoblich *et al.*, 1994). We focused on endoreduplicating cells in the posterior spiracle primordium, because they are near the surface and relatively easy to image. In control embryos these cells contain robust MPM-2 foci (Figure 2, A-A', arrow). In contrast, the posterior spiracle cells of stage matched *cyclin E* mutants lack detectable MPM-2 foci (Figure 2, B-B', arrow), whereas they still contain Lsm11 foci (Figure 2, C-C', arrow). These data indicate that Cyclin E is not required for maintenance of the HLB, but rather for the appearance of an MPM-2 epitope at the HLB. We also performed the reciprocal experiment of Cyclin E overexpression. Epidermal cells arrest in G1 of cycle 17, and they contain Lsm11 foci but not MPM-2 foci (Figure 2D). Ubiquitous expression of Cyclin E with a heat-inducible promoter (*hsp70::cyclin E*) drives these G1₁₇ cells into S phase (Knoblich *et al.*, 1994; Duronio and O'Farrell, 1995). This treatment also results in the appearance throughout the epidermis of MPM-2 foci that colocalize with Lsm11 foci (Figure 2E, arrowheads). We conclude from these data that Cyclin E/Cdk2 activity in the embryo is both necessary and sufficient to produce nuclear MPM-2 foci that colocalize with the HLB. Because histone gene expression is lost in *cyclin E* mutant embryos when they undergo cell cycle arrest in G1 of cycle 17, we hypothesize that MPM-2 recog-

Figure 2. Embryonic MPM-2 foci depend on Cyclin E activity. (A–C) Images of the posterior spiracles of germ band retracted (stage 13) embryos. (A) *w¹¹¹⁸* embryos stained with MPM-2 (A; green in A') and α -P-Tyr (blue in A'). (B and C) *cycE^{AR95}* homozygous mutant embryos stained with MPM-2 (B; green in B') or α -Lsm11 (C; red in C') and α -P-Tyr (blue in B' and C'). Arrows indicate a posterior spiracle cell. *w¹¹¹⁸* control (D) or *hsp70::cyclin E* transgenic (E) embryos were given a 37°C heat shock for 30 min before fixation and then stained with MPM-2 (green), α -Lsm11 (red), and α -P-Tyr (blue). Epidermal cells of the first thoracic segment are shown. Arrowheads indicate the HLB. Anterior is to the top. Bar, 20 μ m.

nizes a Cyclin E/Cdk2 substrate that contributes to histone mRNA biosynthesis. We therefore characterized embryonic MPM-2 foci in more detail.

MPM-2 Foci Colocalize with Nascent Histone Transcripts

If MPM-2 recognizes an HLB phospho-protein involved in histone gene expression, then it should label sites of histone mRNA biosynthesis. We previously developed a cytological assay that detects nascent, chromatin-associated histone mRNA transcripts in intact *Slbp* mutant embryos (Lanzotti *et al.*, 2002). *Slbp* mutants are defective in normal histone pre-mRNA processing, and accumulate inappropriately long, polyadenylated histone mRNAs (Sullivan *et al.*, 2001). These aberrant mRNAs result from the use of cryptic polyadenylation signals located downstream of the normal pre-mRNA processing site in each of the five replication-associated histone genes (Lanzotti *et al.*, 2002). Because the 3' UTR of these polyadenylated histone mRNAs contains sequences not found in the wild-type mRNA, we were able to develop a probe (H3-ds) that specifically recognizes the polyadenylated form of histone H3 mRNA. Cryptic polyadenylation is less efficient than the normal 3'-end processing, causing nascent pre-mRNA to accumulate on the chromatin template in *Slbp* mutants. These nascent transcripts can be visualized as a nuclear focus by fluorescent whole mount in situ hybridization with the H3-ds probe (Figure 3, A and B)

(Lanzotti *et al.*, 2002, 2004). In addition, nascent histone transcripts occur soon after zygotic transcription of the histone genes begins, because there is no maternal SLBP in the early embryo. We previously showed that nascent H3 transcripts arise in very late G2₁₄ immediately preceding each mitosis and persist into S phase of cycle 15 (Lanzotti *et al.*, 2004). Fluorescent in situ hybridization of *Slbp* mutant embryos with the H3-ds probe in conjunction with MPM-2 staining showed that MPM-2 foci colocalize with these nascent H3 transcripts (Figure 3, A and B, arrows). Thus, MPM-2 foci colocalize with sites of active histone mRNA biosynthesis in embryonic cells. MPM-2 also stains foci in cells in early G2₁₄ that lack nascent H3 transcripts but that are known to contain active Cyclin E/Cdk2 (Figure 3, A and C, arrowhead). This suggests that MPM-2 foci can be present without ongoing active histone gene transcription (see below; Figure 6).

The HLB Disassembles during Mitosis

Components of the mammalian Cajal body such as NPAT disassemble at the metaphase–anaphase transition and reassemble in the following interphase (Ma *et al.*, 2000). To determine whether HLBs behave similarly, embryos were stained with MPM-2 or anti-Lsm11 antibodies and anti-phospho-histone H3 antibody (PH3) to mark mitotic cells. MPM-2 staining was examined in the early mitotic domains

Figure 3. MPM-2 foci colocalize with the histone locus body in replicating cells. (A) An *Slbp*¹⁵ homozygous mutant postblastoderm embryo was stained with α -P-Tyr (left in blue), MPM-2 (green in merge), and hybridized with a fluorescent probe that recognizes misprocessed, polyadenylated H3 mRNA as nascent transcripts in the nucleus (H3-ds; left in red). Arrows indicate a cell in S₁₅, which contains an MPM-2 focus that colocalizes with nascent H3 transcripts. Arrowheads indicate an MPM-2 focus in a G₂₁₄ cell that lacks nascent H3 transcripts. Anterior is to the top and ventral to the right. Bar, 20 μ m. (B) S₁₅ cell marked by arrows in A. Bar, 2 μ m. (C) G₂₁₄ cell marked by arrowheads in A. Bar, 2 μ m.

of cell cycle 14 in gastrulating embryos (Figure 4). Because the groups of cells making up a mitotic domain do not enter mitosis synchronously, we could examine all stages of mitosis within a single domain. The nuclear MPM-2 foci present in G₂₁₄ persist into early mitosis, and all M₁₄ prophase cells examined contained MPM-2 foci associated with condensing chromosomes ($n = 125$; Figure 4A, arrow). In contrast, only 77% of metaphase cells ($n = 106$; Figure 4A, arrowhead) and none of the anaphase cells ($n = 102$; Figure 4A, yellow arrowhead) contain detectable chromosome-associated MPM-2 foci. At the following interphase, the nuclear MPM-2 foci reappear. In syncytial blastoderm embryos where progression through mitosis occurs synchronously, we also failed to detect MPM-2 foci during anaphase and some metaphase embryos (Supplemental Figure 1). These data indicate that the MPM-2 phospho-epitope begins to decline during metaphase and becomes undetectable by anaphase, either because the HLB disassembles or because the protein containing the MPM-2 epitope is destroyed or dephosphorylated. A similar analysis was performed in cycle 14 embryos with Lsm11 antibodies. As with MPM-2 foci, Lsm11 foci are present in prophase, become undetectable by anaphase, and return in interphase (Figure 4B). These data suggest that components of the HLB disassemble during mitosis and are similar to what has been reported previously for NPAT in fibroblast cell lines (Ma *et al.*, 2000; Zhao *et al.*, 2000). *Drosophila* centrosomes have been reported to contain proteins with phospho-epitopes that are recognized by MPM-2 during mitosis (Logarinho and Sunkel, 1998; do Carmo Avides *et al.*, 2001; Lange *et al.*, 2005). With the

fixation conditions we used, MPM-2 staining increases throughout the cell during mitosis, but we did not observe specific staining of centrosomes.

MPM-2 Foci Assemble in the Absence of the Histone Pre-mRNA Processing Factors SLBP and U7 snRNP

Our analysis thus far suggests that MPM-2 recognizes a Cyclin E/Cdk2 substrate found in the HLB that could be involved in histone mRNA biosynthesis. One possibility is that one or more components of the histone pre-mRNA processing machinery are required for the formation of MPM-2 foci. To test this, we stained *Slbp* mutants with MPM2 antibodies. As shown above (Figure 3), MPM-2 foci are observed in *Slbp* mutant embryos, which are deficient in SLBP because there is no maternal store of SLBP protein (Lanzotti *et al.*, 2002). This indicates that SLBP does not contain the primary MPM-2 epitope in the HLB and suggests that components of the HLB assemble independently of SLBP. Consistent with this, Lsm11 foci are readily detected in *Slbp* mutant embryos, and they colocalize with sites of nascent histone H3 transcription (data not shown).

To test whether the U7snRNP is required for either MPM-2 foci or the HLB to assemble, we stained tissues from U7 snRNA mutant third instars. Because of maternal loading of U7 snRNA, the third instar is the earliest time during development when U7 snRNA is depleted and the U7 mutant phenotype occurs (Godfrey *et al.*, 2006). We confirmed the disruption of the U7 snRNP by assaying for Lsm11, a U7 snRNP-specific protein that no longer accumulates in the HLB in U7 mutant cells (Figure 5, B and D). MPM-2 foci form in U7 mutant salivary gland-associated fat bodies and eye discs (Figure 5, A and C), as they do in *SLBP* mutant embryos. Similarly, the MPM-2 antigen, but not Lsm11, localizes to the HLB in U7 mutant follicle cells in the adult ovary (Gall, personal communication). Therefore, the HLB with an associated protein recognized by MPM-2 assembles independently of the U7 snRNP. To confirm this result, we performed RNAi knockdown of Lsm11 in *Dmel*-2 cells, and we observed that MPM-2 staining was unperturbed after *Lsm11* dsRNA treatment, although the Lsm11 foci were no longer detectable (Supplemental Figure 2). In sum, our results indicate that the MPM-2 phospho-epitope and Lsm11 are detected in the HLB whether or not SLBP is present in the cell. Unlike Lsm11, however, the detection of the MPM-2 phospho-epitope in the HLB does not depend on U7 snRNA, suggesting that at least a partial HLB can form independently of an intact U7 snRNP complex.

Embryonic MPM-2 Foci Do Not Require String^{cdc25} or Histone Transcription

Cyclin E/Cdk2 phosphorylation of the protein containing the MPM-2 epitope may regulate, or even result from, the transcription of histone genes. To test this, we examined MPM-2 staining in *string* mutant embryos. In late G₂₁₄, histone gene transcription occurs in a pattern that precisely correlates with the mitotic domain pattern, and this transcription is lost in *string* mutants, which arrest in G₂₁₄ after destruction of maternal *string* mRNA and protein (Figure 6, A and B) (Lanzotti *et al.*, 2004). MPM-2 foci can be readily detected in *string* *Slbp* null mutant embryos that lack nascent histone transcripts (Figure 6, A and C). This result indicates that formation of MPM-2 foci does not require histone gene transcription or the function of mitotic Cdk2s activated by *string*^{cdc25}. Consistent with this interpretation, MPM-2 foci are present in all cells throughout interphase 14 (i.e., both S phase and G2 when Cyclin E/Cdk2 is active) at times when neither *string*^{cdc25} is active nor histone transcription occurs

Figure 4. MPM-2 and Lsm11 foci are cell cycle dependent. Postblastoderm *w¹¹¹⁸* embryos in cell cycle 14 were stained with α -P-Tyr (blue), α -PH3 (red), and MPM-2 (A) or α -Lsm11 (B) (middle; green in merge). Arrows indicate prophase cells, arrowheads indicate metaphase cells, and yellow arrowheads indicate anaphase cells. Bar, 20 μ m. Single prophase, metaphase, and anaphase cells are shown in A' and B', A'' and B'', and A''' and B''', respectively. Bars, 2 μ m. Mitotic domain 5 is shown in A, and mitotic domain 1 is shown in B. Note that the rat α -P-Tyr used here recognizes an antigen at the HLB and the mouse α -PH3 used in B stains interphase cells weakly.

(Figure 3C). These data indicate that the formation of MPM-2 foci occurs independently of string^{cdc25} (and thus by inference Cdk1 activity) and histone transcription.

MPM-2 Foci Occur Coincidentally with Activation of Zygotic Histone Gene Expression

Most zygotic gene expression begins at the blastoderm stage during cycle 14 (Edgar and Schubiger, 1986). Histone genes are an exception, and become transcriptionally active precisely in nuclear cycle 11 (Edgar and Schubiger, 1986). If the protein containing the MPM-2 epitope regulates some aspect(s) of histone mRNA biosynthesis, then we expect MPM-2 foci to be present once zygotic expression of the histone genes has begun. To determine whether there is a relationship between the onset of zygotic histone transcription, the assembly of the HLB, and the appearance of the MPM-2 epitope at the HLB, we carefully analyzed syncytial blastoderm embryos stained with MPM-2 and anti-Lsm11 antibodies. Nuclear density was used to accurately stage the embryos with respect to each nuclear cycle. Uniform MPM-2 staining throughout the nucleus was detectable in all syncytial blastoderm cycles, perhaps because maternal Cyclin E/Cdk2 is present throughout the early embryo. MPM-2 foci occur for the first time precisely during nuclear cycle 11, concomitantly with the onset of zygotic histone gene tran-

scription (Figure 7). Similarly, nuclear Lsm11 staining is undetectable before cycle 11, and Lsm11 foci first occur during nuclear cycle 11 and colocalize with MPM-2 foci (Figure 7). The appearance of Lsm11 foci in cycle 11 is not the result of new, zygotic synthesis, because Lsm11 protein is present in 0- to 30-min embryos (data not shown), before the onset of zygotic gene expression. In addition, maternal expression of a YFP-Lsm11 fusion protein in da-GAL4/UAS-YFP-Lsm11 females results in appearance of YFP-Lsm11 foci in embryonic cycle 11 (Supplemental Figure 3). These data indicate that the onset of zygotic histone gene transcription and formation of the MPM-2-positive HLB during early development are temporally and spatially coincident.

MPM-2 Foci Form Independently of the Histone Locus

The coincidence in developmental timing of the onset of zygotic histone transcription and formation of the HLB suggests two possibilities. Histone gene transcription may directly result in the nucleation of the HLB at the histone locus, or de novo assembly of the HLB may trigger the initiation of histone transcription. To attempt to distinguish between these possibilities, we stained embryos containing a homozygous deletion of the entire histone locus [*Df(2L)Ds6*] with MPM-2 and anti-Lsm11 antibodies. We hypothesized that chromatin at or near the histone locus is necessary for

Figure 5. MPM-2 foci form independently of the U7 snRNP. Fat bodies (A and B) and eye discs (C and D) dissected from *w¹¹¹⁸* and *U7¹⁴* homozygous mutant third instars were stained with MPM-2 (left; green in merge) and α -Lsm11 (middle; red in merge). Fat bodies were also stained with DAPI (blue in merge). Arrows and arrowheads indicate nuclei with and without MPM-2 foci, respectively. Note that in wild type Lsm11 foci are present in cells without MPM-2 (arrowhead in C) and that Lsm11 foci are absent in the *U7* mutant cells. Bars, 20 μ m.

the assembly of the HLB, as it might function as a scaffold on which the HLB is assembled to carry out histone mRNA biosynthesis. Surprisingly, both MPM-2 and Lsm11 foci are detected in *Df(2L)Ds6* homozygous embryos lacking the histone genes (Figure 8, arrows), which were distinguished from siblings containing histone genes by in situ hybridization of histone H3 mRNA (Figure 8, left). Moreover, the distribution of nuclei with one versus two MPM-2 foci is similar between wild-type and *Df(2L)Ds6* embryos, such that the percentage of cells containing one MPM-2 focus in wild type and *Df(2L)Ds6* embryos is 89 and 84%, respectively, and the percentage of cells containing two MPM-2 foci in wild-type and *Df(2L)Ds6* embryos is 11 and 7.8%, respectively. This result indicates that the histone locus, and therefore histone transcription, is not required for the initial nucleation of the HLB. This is consistent with our analysis of *string* mutant embryos, which also indicated that histone transcription is not required to maintain MPM-2 foci.

Although HLBs form in the absence of the histone locus, we observed differences in MPM-2 and Lsm11 staining between wild-type and *Df(2L)Ds6* embryos. First, the lack of the histone gene cluster altered the high incidence of MPM-2 and Lsm11 colocalization (Table 1). Using fluorescent images, nuclei were assigned to a category based on the type of MPM-2 and Lsm11 foci present in the nucleus of

stage 11 wild-type or *Df(2L)Ds6* embryos. Whereas in wild-type embryos only 5% of interphase nuclei contained Lsm11 foci that did not colocalize with MPM-2, in *Df(2L)Ds6* mutants the proportion of this class of nuclei increased to ~35% (Table 1). In addition, 8% of the nuclei contained more than one Lsm11 focus that did not colocalize with MPM-2 foci (Table 1). These data indicate that full association of the U7 snRNP and the protein containing the MPM-2 epitope requires the histone locus. In addition, the HLBs in *Df(2L)Ds6* embryos are smaller than in controls as revealed by measuring the size (see *Materials and Methods*) of the MPM-2 nuclear focus in 75 cells from two stage-matched, stage 11 embryos. The average size (defined as length plus width) of the MPM-2 focus in control and *Df(2L)Ds6* embryos is 1.48 ± 0.37 and 1.06 ± 0.27 μ m, respectively ($p < 0.001$). Thus, although the histone locus is not absolutely essential for the formation of the HLB, as assessed by colocalization of MPM-2 and Lsm11 foci, full, stable assembly of the HLB requires the histone locus.

DISCUSSION

Using the MPM-2 monoclonal antibody as a tool, we have presented evidence that a Cyclin E/Cdk2 substrate localizes to the HLB, a recently described *Drosophila* nuclear organelle

Figure 6. MPM-2 foci do not depend on *string* or *Slbp*. All embryos were stained with α -P-Tyr (blue), α -PH3 (red), and MPM-2 (middle, green in merge). (A) Stage-matched sibling control. (B) *stg*⁴ homozygous mutant. (C) *stg Slbp* double [*Df(3R)stg^{AR2}*] homozygous mutant. Lack of PH3 staining was used to distinguish *stg* mutants from siblings. Anterior is to the top and ventral to the right. Bar, 20 μ m.

likely to be directly involved in histone mRNA biosynthesis (Liu *et al.*, 2006). MPM-2 foci colocalize with the histone locus, nascent histone transcripts, and the U7 snRNP-specific protein Lsm11. We propose that the MPM-2 antibody recognizes an activator of histone mRNA biosynthesis that either stimulates histone transcription and/or pre-mRNA processing during S phase.

The identity of the protein containing the MPM-2 epitope is unknown, and there are few, if any, known *Drosophila* proteins with the properties one would predict for an MPM-2 target involved in histone mRNA biosynthesis. In contrast, the human NPAT protein has several of these properties and provides an example of the type of protein that may be recognized by MPM-2. NPAT is a Cyclin E/Cdk2 substrate that functions as a general activator of transcription of the replication-dependent histone genes that localizes to those Cajal bodies that are associated with histone loci during S phase (Zhao *et al.*, 1998, 2000; Ma *et al.*, 2000; Wei *et al.*, 2003; Zheng *et al.*, 2003; Miele *et al.*, 2005). The NH₂ terminus of NPAT contains a LisH domain that is necessary for H4 transcription (Wei *et al.*, 2003). Although there are 15 predicted LisH-containing proteins in *Drosophila*, no orthologue of NPAT has yet been identified. Coilin, which functions to maintain the integrity of the Cajal body, can be phosphorylated by Cyclin E/Cdk2 in vitro (Liu *et al.*, 2000; Hebert *et al.*, 2001; Tucker *et al.*, 2001). However, MPM-2 does not recognize Cajal bodies in HeLa cells (White and Duronio, unpublished data), and the *Drosophila* Cajal body in *Drosophila* cells is distinct from the HLB (Liu *et al.*, 2006). The orthologue of coilin has also not been reported in *Drosophila*.

HLB Behavior during Early *Drosophila* Development

Our cytological analysis of MPM-2 staining of the HLB during early *Drosophila* embryogenesis indicates that the

Figure 7. The histone locus body forms for the first time during nuclear cycle 11. *w¹¹¹⁸* syncytial blastoderm embryos were stained with MPM-2 (left; green in merge), α -Lsm11 (middle; red in merge), and α -P-Tyr (right; in blue). Interphase of nuclear cycles 9–14 are shown, as indicated on the left. Bar, 10 μ m.

HLB is a dynamic, cell cycle-regulated structure. As detected by MPM-2 and anti-Lsm11 staining, the HLB assembles for the first time in development during S phase of nuclear cycle 11 in the early syncytial embryo. HLB nucleation occurs during the exact same cycle that zygotic histone gene transcription begins. In frog oocytes, increasing U7 snRNA expression can induce formation of Cajal body like structures (Tuma and Roth, 1999), suggesting that in vertebrate cells U7 snRNP or nascent histone transcripts may trigger formation of Cajal bodies associated with the histone locus. Interestingly, however, HLB assembly does not require U7 snRNA or the histone locus, and therefore occurs independently of histone gene expression. We thus favor a model in which developmentally controlled HLB formation is essential for the onset of zygotic histone gene transcription.

Figure 8. Aberrant histone locus bodies form in the absence of the histone locus. Postblastoderm embryos hybridized with an H3 RNA probe (magenta) were stained with MPM-2 (green), α -Lsm11 (red), and DAPI (blue). *Df(2L)Ds6* homozygous mutant embryos that lack the histone gene cluster (B) were distinguished from control siblings (A) by the lack of zygotic histone H3 mRNA. Arrows indicate foci of MPM-2 and α -Lsm11 that colocalize. Arrowheads indicate cells containing a focus of colocalizing MPM-2 and α -Lsm11 as well as a focus of just α -Lsm11. Anterior is to the top. Bar, 20 μ m.

Table 1. Characterization of the HLB in histone deletion embryos

	1 ML ^a	2 ML	1 ML + 1 L	2 L	1 L	1 ML + 2 Ls	2 M	Other
<i>w¹¹¹⁸</i> (n = 191)	81.2	11.0	5.2	0	0.5	0	0	2.1
<i>Ds6</i> (n = 192)	54.7	4.2	20.3	3.1	3.6	7.8	0.5	5.7

^a ML indicates a focus of colocalized MPM-2 and Lsm11 staining, L indicates a focus containing only Lsm11, and M indicates a focus containing only MPM-2. "Other" refers to individual patterns of staining that are not represented in the indicated categories and that each comprise <0.5% of cells.

Although the HLB is capable of forming independently of the histone locus, the histone locus contributes to the structural integrity of the HLB. In histone deletion embryos, HLBs are smaller and some interphase nuclei contain Lsm11 foci that do not colocalize with MPM-2. The size of the nucleolus is determined by the amount of ribosomal gene transcription (Hernandez-Verdun, 2006). Thus, the size and overall composition of the HLB may be similarly dependent on transcription of the histone genes. Consistent with this, MPM-2 and Lsm11 foci are present at maximal size during prophase of cycle 14, just after the initiation of histone transcription in late G2₁₄. Nascent histone transcripts are likely aborted during mitosis (Shermoen and O'Farrell, 1991), which correlates with the loss of MPM-2 and Lsm11 staining we observe in anaphase. Alternatively, reduced HLB size may be a secondary consequence of cell cycle arrest, resulting from lack of histone biosynthesis (Smith *et al.*, 1993).

In wild-type embryos, we observe either one or two MPM-2 or Lsm11 foci per cell at frequencies similar to the known pairing frequencies of the histone loci on homologous second chromosomes (Fung *et al.*, 1998). This is also consistent with the association of the HLB with histone genes (Liu *et al.*, 2006). Surprisingly, we often detect one or two MPM-2 foci in histone deletion embryos, suggesting that HLB number is not dictated solely by homologous pairing at the histone locus. HLB components may associate with another chromosomal locus in the absence of the histone genes. Alternatively, the de novo formation of one or two HLBs may actually drive homologous pairing at the histone locus.

Cell Cycle Regulation of the HLB

Histone mRNA is greatly depleted in *cyclin E* mutant embryos (Lanzotti *et al.*, 2004). Because *cyclin E* mutant

embryos arrest in G1 phase, it is difficult to know this observation is indicative of a direct involvement of Cyclin E/Cdk2 in histone gene expression, or arises as a secondary consequence of cell cycle arrest. Because interphase MPM-2 foci are coincident with the HLB, are present in cells where Cyclin E/Cdk2 is active, and they are absent in cells that lack Cyclin E/Cdk2 activity, we favor the interpretation that Cyclin E/Cdk2 phosphorylates a protein directly involved in histone mRNA biosynthesis. How Cyclin E/Cdk2 participates in this process is not known, but it is not required for recruitment of U7 snRNP to the HLB, because Lsm11 remains a stable component of the HLB in wild-type cells arrested in G1 and in cells of *cyclin E* mutant embryos.

During the early embryonic cell cycles that have constitutive Cyclin E/Cdk2 activity, MPM-2 foci disappear as cells progress through mitosis and are undetectable by anaphase. Focal Lsm11 staining is also lost during mitosis, suggesting the disassembly of HLB components into the cytoplasm subsequent to nuclear envelope breakdown rather than simply dephosphorylation or destruction of the MPM-2 target(s). The HLB rapidly reforms during the subsequent interphase. These behaviors are similar to the behavior of NPAT foci during mitosis in cultured mammalian fibroblasts (Ma *et al.*, 2000; Zhao *et al.*, 2000). The nucleolus also disassembles during mitosis (Hernandez-Verdun, 2006), suggesting that dynamic disassembly/reassembly of specific nuclear compartments involved in gene expression is a general feature of nuclear behavior during metazoan mitosis.

Summary

The HLB is a dynamic structure capable of receiving input from both the histone locus and the cell cycle. Our analysis raises many questions, including how HLB assembly occurs at a specific time in a syncytial cytoplasm from abundant

maternal components, even in the absence of a histone locus template, and how Cyclin E/Cdk2 regulates HLB function and histone mRNA biosynthesis. Determining the identity of the HLB protein(s) recognized by MPM-2 antigen will help answer such questions, and it will provide a useful tool to examine how the regulation of such a fundamental process as histone mRNA biosynthesis is modulated by developmental programs.

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REFERENCES

- Albert, A. L., Lavoie, S. B., and Vincent, M. (2004). Multisite phosphorylation of Pin1-associated mitotic phosphoproteins revealed by monoclonal antibodies MPM-2 and CC-3. *BMC Cell Biol.* 5, 22.
- Borun, T. W., Gabrielli, F., Ajiro, K., Zweidler, A., and Baglioni, C. (1975). Further evidence of transcriptional and translational control of histone messenger RNA during the HeLa S3 cycle. *Cell* 4, 59–67.
- Breindl, M., and Gallwitz, D. (1973). Identification of histone messenger RNA from HeLa cells. Appearance of histone mRNA in the cytoplasm and its translation in a rabbit-reticulocyte cell-free system. *Eur. J. Biochem.* 32, 381–391.
- Calvi, B. R., Lilly, M. A., and Spradling, A. C. (1998). Cell cycle control of chorion gene amplification. *Genes Dev.* 12, 734–744.
- Cioce, M., and Lamond, A. I. (2005). Cajal bodies: a long history of discovery. *Annu. Rev. Cell Dev. Biol.* 21, 105–131.
- Davis, F. M., Tsao, T. Y., Fowler, S. K., and Rao, P. N. (1983). Monoclonal antibodies to mitotic cells. *Proc. Natl. Acad. Sci. USA* 80, 2926–2930.
- DeLisle, A. J., Graves, R. A., Marzluff, W. F., and Johnson, L. F. (1983). Regulation of histone mRNA production and stability in serum-stimulated mouse 3T6 fibroblasts. *Mol. Cell. Biol.* 3, 1920–1929.
- Dernburg, A. F., and Sedat, J. W. (1998). Mapping three-dimensional chromosome architecture in situ. *Methods Cell Biol.* 53, 187–233.
- Detke, S., Lichtler, A., Phillips, I., Stein, J., and Stein, G. (1979). Reassessment of histone gene expression during cell cycle in human cells by using homologous H4 histone cDNA. *Proc. Natl. Acad. Sci. USA* 76, 4995–4999.
- do Carmo Avides, M., Tavares, A., and Glover, D. M. (2001). Polo kinase and Asp are needed to promote the mitotic organizing activity of centrosomes. *Nat. Cell Biol.* 3, 421–424.
- Dominski, Z., and Marzluff, W. F. (1999). Formation of the 3' end of histone mRNA. *Gene* 239, 1–14.
- Du, W., and Pogoriler, J. (2006). Retinoblastoma family genes. *Oncogene* 25, 5190–5200.
- Duronio, R. J., and O'Farrell, P. H. (1995). Developmental control of the G1 to S transition in *Drosophila*: cyclin E is a limiting downstream target of E2F. *Genes Dev.* 9, 1456–1468.
- Edgar, B. A., and O'Farrell, P. H. (1989). Genetic control of cell division patterns in the *Drosophila* embryo. *Cell* 57, 177–187.
- Edgar, B. A., and O'Farrell, P. H. (1990). The three postblastoderm cell cycles of *Drosophila* embryogenesis are regulated in G2 by string. *Cell* 62, 469–480.
- Edgar, B. A., Lehman, D. A., and O'Farrell, P. H. (1994). Transcriptional regulation of string (cdc25): a link between developmental programming and the cell cycle. *Development* 120, 3131–3143.
- Edgar, B. A., and Schubiger, G. (1986). Parameters controlling transcriptional activation during early *Drosophila* development. *Cell* 44, 871–877.
- Foe, V. E. (1989). Mitotic domains reveal early commitment of cells in *Drosophila* embryos. *Development* 107, 1–22.
- Frey, M. R., and Matera, A. G. (1995). Coiled bodies contain U7 small nuclear RNA and associate with specific DNA sequences in interphase human cells. *Proc. Natl. Acad. Sci. USA* 92, 5915–5919.
- Fung, J. C., Marshall, W. F., Dernburg, A., Agard, D. A., and Sedat, J. W. (1998). Homologous chromosome pairing in *Drosophila melanogaster* proceeds through multiple independent initiations. *J. Cell Biol.* 141, 5–20.
- Godfrey, A. C., Kupsco, J. M., Burch, B. D., Zimmerman, R. M., Dominski, Z., Marzluff, W. F., and Duronio, R. J. (2006). U7 snRNA mutations in *Drosophila* block histone pre-mRNA processing and disrupt oogenesis. *RNA* 12, 396–409.
- Hall, C., Nelson, D. M., Ye, X., Baker, K., DeCaprio, J. A., Seeholzer, S., Lipinski, M., and Adams, P. D. (2001). HIRA, the human homologue of yeast Hir1p and Hir2p, is a novel cyclin-cdk2 substrate whose expression blocks S-phase progression. *Mol. Cell. Biol.* 21, 1854–1865.
- Hammond, M. P., and Laird, C. D. (1985). Chromosome structure and DNA replication in nurse and follicle cells of *Drosophila melanogaster*. *Chromosoma* 91, 267–278.
- Harris, M. E., Bohni, R., Schneiderman, M. H., Ramamurthy, L., Schumperli, D., and Marzluff, W. F. (1991). Regulation of histone mRNA in the unperturbed cell cycle: evidence suggesting control at two posttranscriptional steps. *Mol. Cell. Biol.* 11, 2416–2424.
- Hebert, M. D., Szymczyk, P. W., Shpargel, K. B., and Matera, A. G. (2001). Coilin forms the bridge between Cajal bodies and SMN, the spinal muscular atrophy protein. *Genes Dev.* 15, 2720–2729.
- Heintz, N., Sive, H. L., and Roeder, R. G. (1983). Regulation of human histone gene expression: kinetics of accumulation and changes in the rate of synthesis and in the half-lives of individual histone mRNAs during the HeLa cell cycle. *Mol. Cell. Biol.* 3, 539–550.
- Hernandez-Verdun, D. (2006). The nucleolus: a model for the organization of nuclear functions. *Histochem. Cell Biol.* 126, 135–148.
- Hirano, T., and Mitchison, T. J. (1991). Cell cycle control of higher-order chromatin assembly around naked DNA in vitro. *J. Cell Biol.* 115, 1479–1489.
- Kiss, T. (2004). Biogenesis of small nuclear RNPs. *J. Cell Sci.* 117, 5949–5951.
- Knoblich, J. A., Sauer, K., Jones, L., Richardson, H., Saint, R., and Lehner, C. F. (1994). Cyclin E controls S phase progression and its down-regulation during *Drosophila* embryogenesis is required for the arrest of cell proliferation. *Cell* 77, 107–120.
- Kuang, J., Zhao, J., Wright, D. A., Saunders, G. F., and Rao, P. N. (1989). Mitosis-specific monoclonal antibody MPM-2 inhibits *Xenopus* oocyte maturation and depletes maturation-promoting activity. *Proc. Natl. Acad. Sci. USA* 86, 4982–4986.
- Lange, B. M., Kirfel, G., Gestmann, I., Herzog, V., and Gonzalez, C. (2005). Structure and microtubule-nucleation activity of isolated *Drosophila* embryo centrosomes characterized by whole mount scanning and transmission electron microscopy. *Histochem. Cell Biol.* 124, 325–334.
- Lanzotti, D. J., Kaygun, H., Yang, X., Duronio, R. J., and Marzluff, W. F. (2002). Developmental control of histone mRNA and dSLBP synthesis during *Drosophila* embryogenesis and the role of dSLBP in histone mRNA 3' end processing in vivo. *Mol. Cell. Biol.* 22, 2267–2282.
- Lanzotti, D. J., Kupsco, J. M., Marzluff, W. F., and Duronio, R. J. (2004). string(cdc25) and cyclin E are required for patterned histone expression at different stages of *Drosophila* embryonic development. *Dev. Biol.* 274, 82–93.
- Lee, L. A., and Orr-Weaver, T. L. (2003). Regulation of cell cycles in *Drosophila* development: intrinsic and extrinsic cues. *Annu. Rev. Genet.* 37, 545–578.
- Lehman, D. A., Patterson, B., Johnston, L. A., Balzer, T., Britton, J. S., Saint, R., and Edgar, B. A. (1999). Cis-regulatory elements of the mitotic regulator, string/Cdc25. *Development* 126, 1793–1803.
- Liu, J., Hebert, M. D., Ye, Y., Templeton, D. J., Kung, H., and Matera, A. G. (2000). Cell cycle-dependent localization of the CDK2-cyclin E complex in Cajal (coiled) bodies. *J. Cell Sci.* 113, 1543–1552.
- Liu, J. L., Murphy, C., Buszczak, M., Clatterbuck, S., Goodman, R., and Gall, J. G. (2006). The *Drosophila melanogaster* Cajal body. *J. Cell Biol.* 172, 875–884.
- Logarinho, E., and Sunkel, C. E. (1998). The *Drosophila* POLO kinase localises to multiple compartments of the mitotic apparatus and is required for the phosphorylation of MPM2 reactive epitopes. *J. Cell Sci.* 111, 2897–2909.
- Ma, T., Van Tine, B. A., Wei, Y., Garrett, M. D., Nelson, D., Adams, P. D., Wang, J., Qin, J., Chow, L. T., and Harper, J. W. (2000). Cell cycle-regulated phosphorylation of p220(NPAT) by cyclin E/Cdk2 in Cajal bodies promotes histone gene transcription. *Genes Dev.* 14, 2298–2313.
- Marzluff, W. F. (2005). Metazoan replication-dependent histone mRNAs: a distinct set of RNA polymerase II transcripts. *Curr. Opin. Cell Biol.* 17, 274–280.
- Marzluff, W. F., and Duronio, R. J. (2002). Histone mRNA expression: multiple levels of cell cycle regulation and important developmental consequences. *Curr. Opin. Cell Biol.* 14, 692–699.
- Matera, A. G., and Shpargel, K. B. (2006). Pumping RNA: nuclear bodybuilding along the RNP pipeline. *Curr. Opin. Cell Biol.* 18, 317–324.

- Miele, A. *et al.* (2005). HiNF-P directly links the cyclin E/CDK2/p220NPAT pathway to histone H4 gene regulation at the G1/S phase cell cycle transition. *Mol. Cell. Biol.* 25, 6140–6153.
- Millar, S. E., Freeman, M., and Glover, D. M. (1987). The distribution of a 'mitosis-specific' antigen during *Drosophila* development. *J. Cell Sci.* 87, 95–104.
- Moore, G., Sinclair, D., and T. Grigliatti. (1983). Histone gene multiplicity and position effect variegation in *Drosophila melanogaster*. *Genetics* 105, 327–344.
- Nelson, D. M., Ye, X., Hall, C., Santos, H., Ma, T., Kao, G. D., Yen, T. J., Harper, J. W., and Adams, P. D. (2002). Coupling of DNA synthesis and histone synthesis in S phase independent of cyclin/cdk2 activity. *Mol. Cell. Biol.* 22, 7459–7472.
- Parker, I., and Fitschen, W. (1980). Histone mRNA metabolism during the mouse fibroblast cell cycle. *Cell Differ.* 9, 23–30.
- Richardson, H., O'Keefe, L. V., Marty, T., and Saint, R. (1995). Ectopic cyclin E expression induces premature entry into S phase and disrupts pattern formation in the *Drosophila* eye imaginal disc. *Development* 121, 3371–3379.
- Sauer, K., Knoblich, J. A., Richardson, H., and Lehner, C. F. (1995). Distinct modes of cyclin E/cdc2c kinase regulation and S-phase control in mitotic and endoreduplication cycles of *Drosophila* embryogenesis. *Genes Dev.* 9, 1327–1339.
- Shermoen, A. W., and O'Farrell, P. H. (1991). Progression of the cell cycle through mitosis leads to abortion of nascent transcripts. *Cell* 67, 303–310.
- Smith, A. V., King, J. A., and Orr-Weaver, T. L. (1993). Identification of genomic regions required for DNA replication during *Drosophila* embryogenesis. *Genetics* 135, 817–829.
- Stanek, D., and Neugebauer, K. M. (2006). The Cajal body: a meeting place for spliceosomal snRNPs in the nuclear maze. *Chromosoma* 115, 343–354.
- Sullivan, E., Santiago, C., Parker, E. D., Dominski, Z., Yang, X., Lanzotti, D. J., Ingledue, T. C., Marzluff, W. F., and Duronio, R. J. (2001). *Drosophila* stem loop binding protein coordinates accumulation of mature histone mRNA with cell cycle progression. *Genes Dev.* 15, 173–187.
- Swanhart, L., Kupsco, J., and Duronio, R. J. (2005). Developmental control of growth and cell cycle progression in *Drosophila*. *Methods Mol. Biol.* 296, 69–94.
- Tucker, K. E., Berciano, M. T., Jacobs, E. Y., LePage, D. F., Shpargel, K. B., Rossire, J. J., Chan, E. K., Lafarga, M., Conlon, R. A., and Matera, A. G. (2001). Residual Cajal bodies in coilin knockout mice fail to recruit Sm snRNPs and SMN, the spinal muscular atrophy gene product. *J. Cell Biol.* 154, 293–307.
- Tuma, R. S., and Roth, M. B. (1999). Induction of coiled body-like structures in *Xenopus* oocytes by U7 snRNA. *Chromosoma* 108, 337–344.
- Wei, Y., Jin, J., and Harper, J. W. (2003). The cyclin E/Cdk2 substrate and Cajal body component p220(NPAT) activates histone transcription through a novel LisH-like domain. *Mol. Cell. Biol.* 23, 3669–3680.
- Westendorf, J. M., Rao, P. N., and Gerace, L. (1994). Cloning of cDNAs for M-phase phosphoproteins recognized by the MPM2 monoclonal antibody and determination of the phosphorylated epitope. *Proc. Natl. Acad. Sci. USA* 91, 714–718.
- Wodarz, A., Hinz, U., Engelbert, M., and Knust, E. (1995). Expression of crumbs confers apical character on plasma membrane domains of ectodermal epithelia of *Drosophila*. *Cell* 82, 67–76.
- Yaffe, M. B. *et al.* (1997). Sequence-specific and phosphorylation-dependent proline isomerization: a potential mitotic regulatory mechanism. *Science* 278, 1957–1960.
- Zhao, J., Dynlacht, B., Imai, T., Hori, T., and Harlow, E. (1998). Expression of NPAT, a novel substrate of cyclin E-CDK2, promotes S-phase entry. *Genes Dev.* 12, 456–461.
- Zhao, J., Kennedy, B. K., Lawrence, B. D., Barbie, D. A., Matera, A. G., Fletcher, J. A., and Harlow, E. (2000). NPAT links cyclin E-Cdk2 to the regulation of replication-dependent histone gene transcription. *Genes Dev.* 14, 2283–2297.
- Zheng, L., Roeder, R. G., and Luo, Y. (2003). S phase activation of the histone H2B promoter by OCA-S, a coactivator complex that contains GAPDH as a key component. *Cell* 114, 255–266.