INTRODUCTION

Cyclin A or B forms a complex with cyclin-dependent kinase 1 (Cdk1, or Cdc2), which is required for entry into mitosis (reviewed by Murray and Hunt, 1993; Nigg, 1995). In eukaryotes, Cdk1 activity regulates both nuclear and cytoskeletal events. For example, its activity is required for nuclear envelope breakdown, chromosome condensation (reviewed by Nigg, 1993), and the transition of astral microtubules into mitotic spindles (reviewed by Karsenti, 1991; Murray and Hunt, 1993). While much is known about nuclear cell-cycle progression, less is known about cytoskeleton regulation with respect to the cell cycle. Microtubules polymerize and depolymerize to form structures required for cell-cycle progression (reviewed by Desai and Mitchison, 1997). Mitotic cyclins are key regulators in the initiation of mitosis and depolymerization of microtubules (reviewed by McNally, 1996). Long, stable astral microtubules become shorter and more dynamic, and organize into a mitotic spindle which aligns chromosomes on the metaphase plate.

Exit from metaphase requires degradation of the mitotic cyclins (reviewed by Hunt, 1991). Blocking degradation of cyclins A, B or B3 causes metaphase, early anaphase or late anaphase arrest, respectively (Sigrist et al., 1995; Su et al., 1998). For example, degradation of cyclin B, which localizes to the metaphase spindle (Huang and Raff, 1999), is required for anaphase movements (Su et al., 1998). Since these mitotic cyclins all bind to Cdk1, this suggests they are functionally related, but how is functional specificity achieved? In several organisms including starfish, humans and Drosophila, cyclins A and B localize differently within the cell (Jackman et al., 1995; Lehner and O’Farrell, 1989; Pines and Hunter, 1994; Gallant and Nigg, 1992; Ookata et al., 1992; Maldonado-Codina and Glover, 1992). Differences in cyclin localization could account for their phase-specific functions. For example, cyclin B-Cdk1 activity regulates microtubule dynamics in Xenopus egg extracts (Verde et al., 1990, 1992). The microtubule associated proteins (MAPs) are direct targets of this kinase. Phosphorylated forms of the MAPs inhibit stabilization of microtubules (Ookata et al., 1995).

To test whether cyclin B-Cdk1 regulates microtubule stability in vivo, we varied the amount of cyclin B in the Drosophila embryo. Our analyses focused on cycles 1-8, for several reasons. Firstly, the early cycles are composed only of S and M phases and cell-cycle regulation depends on cyclin-Cdk1 activity (Edgar et al., 1994). Secondly, these first eight cycles are equal in their duration (about 9 minutes) and occur in a syncytium (Zalokar and Erk, 1976; Foe and Alberts, 1983). Thirdly, these mitoses are maternally controlled (Edgar et al., 1986), which allows one to vary gene doses in the mother and test for a response in the syncytium. Finally, nuclei are located deep in the embryo, divide synchronously, and are surrounded by spheres of microtubules providing an exponential increase of nuclear and microtubule mass (Baker et al., 1993).

SUMMARY

In eukaryotes, mitotic cyclins localize differently in the cell and regulate different aspects of the cell cycle. We investigated the relationship between subcellular localization of cyclins A and B and their functions in syncytial preblastoderm Drosophila embryos. During early embryonic cycles, cyclin A was always concentrated in the nucleus and present at a low level in the cytoplasm. Cyclin B was predominantly cytoplasmic, and localized within nuclei only during late prophase. Also, cyclin B colocalized with metaphase but not anaphase spindle microtubules. We changed maternal gene doses of cyclins A and B to test their functions in preblastoderm embryos. We observed that increasing doses of cyclin B increased cyclin B-Cdk1 activity, which correlated with shorter microtubules and slower microtubule-dependent nuclear movements. This provides in vivo evidence that cyclin B-Cdk1 regulates microtubule dynamics. In addition, the overall duration of the early nuclear cycles was affected by cyclin A but not cyclin B levels. Taken together, our observations support the hypothesis that cyclin B regulates cytoskeletal changes while cyclin A regulates the nuclear cycles. Varying the relative levels of cyclins A and B uncoupled the cytoskeletal and nuclear events, so we speculate that a balance of cyclins is necessary for proper coordination during these embryonic cycles.

Key words: Cyclin A and B, Cytoskeleton dynamics, Microtubule, Drosophila, Embryonic cell cycle, Cyclin A and B dose

INTRODUCTION

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We investigated the relationship between increase in microtubule mass (Baker et al., 1993) and the parallel decline in cyclin B during early embryogenesis (Edgar et al., 1994). Here we report the first in vivo evidence of microtubule regulation by cyclin B-Cdk1 in a dose-dependent manner. Increased levels of cyclin B in embryos resulted in increased cyclin B-Cdk1 activity and decreased microtubule volume. Conversely, decreased cyclin B led to lower kinase activity and longer, more stable microtubules. In addition, we tested whether cyclin localization correlated with the regulation of specific cell-cycle events. Our results suggest that location does confer functional specificity. We found that cyclin A primarily regulated progression of the nuclear cycle, and cyclin B the dynamic changes of the cytoskeleton. Our observations expose an additional level of cell-cycle complexity, because changes in the cytoplasm must be coordinated with nuclear events.

MATERIALS AND METHODS

Stocks

Control data are from wild-type 'Sevelen' flies. Stocks with decreased or increased cyclin B were obtained from C. Lehner (Jacobs et al., 1998). Embryos are referred to by their maternal cyclin B dose, i.e. a '1-cyclin B' embryo was the offspring of a female with only one cyclin B gene copy, a '2-cyclin B' embryo was a wild-type embryo, and a '4-cyclin B' embryo was from a female with two additional gene copies of cyclin B. Flies with reduced cyclin A were heterozygous for the neo124 allele (Cooley et al., 1988; Lehner and O'Farrell, 1989).

Embryo collection and video analysis

Embryos were collected for 10 minutes after precollection, dechorionated and mounted on slides according to Schubiger and Edgar (1994). Embryos were raised at room temperature (approx. 21°C). Timing of embryogenesis and velocity was determined by time-lapse video, using a Sony CCD camera on a compound scope with Nomarski optics and a 16x objective. A Mitsubishi time-lapse cassette recorder and a Boeckeler IMG-100 image enhancer were used.

The duration of cycles from cycle 2 until gastrulation and the velocity of migrating energids (nuclei and surrounding cytoplasm) were calculated from video recordings. Dynamic changes in the structure of energids allowed us to define cell-cycle phases and cleavage cycles (Baker et al., 1993). The speed of 1-7 energids in each embryo was measured. When comparing the velocities in embryos with different cyclin B doses, we used the mean value for each embryo. Then the isotonic regression test, a type of ordered heterogeneity test, was used to test whether an increase in cyclin B amount is significantly associated with a slower movement (Gaines and Rice, 1990).

Fixation and antibody staining

Ovaries were dissected and fixed according to Gillespie and Berg (1995). They were stained for either DNA or microtubules and all antibodies were diluted in PBS/Saponin (0.05%). We used a rhodamine-conjugated monoclonal histone antibody (Chemotub, 1:300-500) to stain nuclei (Baker et al., 1993). For microtubule staining we used β-tubulin antibody (Amersham, 1:800) with goat anti-mouse BODIPY (Molecular Probes, 1:200) as a secondary antibody. Ovaries were dehydrated with methanol and mounted in Murray mounting medium (MMM, 1:2 benzyl benzoate/benzyl alcohol).

To analyze mitotic index, embryos were dechorionated in 100% bleach, rinsed with distilled water and embryo wash (0.5% NaCl, 0.03% Triton X-100), and then fixed in 37% formaldehyde under heptane (Theurkauf, 1992). Embryos were devitellinized with cold methanol, stained with histone antibody in PBS/Triton X-100 (0.1%) and mounted in MMB.

For microtubule analysis (Table 2, Figs 3, 4 and 5) embryos were fixed in 20% formaldehyde diluted with PBS/Triton X-100 (0.1%). To compare differences in microtubule configuration, we handled embryos of different genotypes uniformly, at the same time. We used the same solutions throughout dechorionation, fixation, staining, dehydration and mounting. The tubulin staining protocol was the same as for ovaries with the following exception: before double staining with rhodamine-conjugated histone, embryos were first incubated with 20% normal mouse serum (Sigma). Embryos were mounted with DPX mounting medium (Fluka Chemical Corp.).

Embryos used for cyclin A localization (Fig. 6C-G) were also fixed in buffered 20% formaldehyde. Embryos prepared for cyclin B localization (Fig. 6A,B) were fixed in buffered methanol according to Huang and Raff (1999). Goat anti-mouse Texas Red was used as a secondary for the tubulin primary. Cyclins A and B were identified using Rb 270 and 271 antibodies (Whitfield et al., 1990).

Confocal microscopy

A BioRad MRC-600 confocal microscope was used to generate images with either a Plan Apochromatic 60x oil-immersion lens or a 20x objective. A Kalman-averaging filter was used to reduce background. For quantitative analysis of microtubule asters, confocal work was done at the same time with identical settings for gain, black level and aperture opening. We processed and analyzed the material at the same time to prevent differences in confocal laser intensity. To generate a constant denominator for quantification of microtubule asters we selected embryos with equal background levels of cortical tubulin, as well as an equal background from the histone stain.

For quantification of microtubule asters (Table 2), we selected embryos in interphase of cycle 6. At this stage the nuclei are equally spaced within the embryo and the microtubules form a complete network, connecting the 32 nuclei. To precisely stage embryos in the same phase of interphase, only embryos with nuclei of the same diameter and with no observed chromosome condensation were used. The microtubule configurations were defined from longitudinal optical sections of the first ten nuclei encountered in a Z-series (1 μm intervals) at 60X. Volumes were quantified from these sections in NIH image without prior processing. In each section the area of the microtubule aster was outlined and the volume of an aster was calculated as the sum of the microtubule areas of the Z-series.

To illustrate wild-type microtubule configurations (Fig. 3) and differences in microtubule configurations among embryos with different cyclin B levels (Figs 4, 5), we present representative images of embryos. Embryos used to make these images were collected, fixed, stained and mounted in parallel. We adjusted the confocal microscope and Photoshop settings to equalize black levels and to get proper contrast among embryos.

Western and histone H1 kinase assay

The amount of cyclin B in embryos was estimated by western blotting as described by Edgar et al. (1994) with the following modifications: the phosphatase inhibitor Na3VO4 was not used and proteins were analyzed using ECL-western blotting protocol (Amersham) after 12% SDS-polyacrylamide gel electrophoresis (PAGE). Antibodies were used at the following dilutions: cyclin B (a gift from P. H. O’Farrell; 1:20), actin (Sigma; 1:100), HRP-conjugated anti-mouse (Amersham; 1:500). ECL film was preflashed to improve linearity of exposure, scanned and quantified using the gel-imaging software in NIH image. The amount of cyclin B was estimated by calculating the ratio of band intensities (OD) of cyclin B to actin control. These might be a rough estimate because we did not define the amount of protein by using a standard curve; rather, we used a densitometric ratio in which the control actin likely saturated the film.
Histone H1 kinase assay was also performed as described by Edgar et al. (1994) with the following modifications: for the lysate of each sample, five embryos in cycle 4-6 were crushed with 48 μl extraction buffer and then incubated with 2.5 μl of either anti-cyclin A or B rabbit serum (Rb 270 and 271, respectively; a gift from W. G. F. Whitfield), 30 μl of protein A-Sepharose beads (Amersham Pharmacia Biotech.) in 30 μl of extraction buffer were added and incubated for 1 hour at 4°C. Then the beads were rinsed four times and assayed for histone H1 kinase activity. After 20 minutes of incubation with 30 μl kinase assay buffer at room temperature, 7 μl of 6x SDS sample buffer was added, boiled and separated by 12% SDS-PAGE. Proteins were then transferred to Immobilon-P membrane (Millipore). A Phosphoimager (Molecular Dynamics) was used to quantify levels of the radio-labeled histone H1.

RESULTS

Cdk1 activity correlates with cyclin B levels

Western analysis was used to compare relative levels of cyclin B protein. For this, embryos with 0-, 1-, 2- and 4-cyclin B were fixed during cycles 1-2. As shown in Fig. 1A, cyclin B levels corresponded to maternal gene dosage. Embryos with fewer copies of maternal cyclin B had less cyclin B protein, and the addition of cyclin B increased its protein levels (ANOVA test, \( P=0.008 \)). We repeated the western blot with a dilution series for 1-, 2- and 4-cyclin B embryos and found again that maternal gene copy number correlated with the amount of the cyclin B protein, regardless of whether we used 5, 4 or 3 embryos (Fig. 1B). The dilution series confirmed that increasing maternal cyclin B gene copies increased cyclin B protein levels in the embryos.

To test whether different cyclin B levels correspond to Cdk1 activities, we immunoprecipitated cyclin B-Cdk1 complex from 1, 2 and 4-cyclin B embryos (cycles 4-6) using anti-cyclin B antiserum, and then performed histone H1 kinase assay. We found that 1-cyclin B had the lowest Cdk1 activity, 2-cyclin B intermediate, and 4-cyclin B embryos the highest (Fig. 1C). To test if increasing cyclin B reduces the amount of cyclin A-Cdk1 complexes, we performed the same assay with anti-cyclin A antiserum. We found no difference (data not shown). This indicates that cyclin B does not compete with cyclin A in binding with Cdk1. Furthermore, these observations support that Cdk1 is not limiting, consistent with observations made by Edgar et al. (1994). Therefore, varying maternal cyclin B doses changes Cdk1 activity in embryos.

Developmental consequences of cyclin B levels

We observed that the egg-laying rates were reduced by 30-40% in 1- and 4-cyclin B lines. 2-cyclin B embryos had a hatching rate of 96% (\( n=354 \)), while 1- and 4-cyclin B embryos had reduced hatching rates of 89% (\( n=626 \)) and 60% (\( n=350 \)), respectively. In oocytes, nurse cells and follicle cells in 1-, 2- and 4-cyclin B lines, microtubule organization and cell morphology appeared normal (data not shown). Although altered doses of cyclin B affected embryonic survival, the majority of embryos appear healthy and viable. We think reduced hatching rates in these lines is due to errors in mitosis caused by altered cyclin B levels instead of oogenesis defects.

To amplify the phenotypes of 1- and 4-cyclin B embryos, we analyzed 0- and 10-cyclin B embryos. The nuclear and microtubule morphology of the oocytes, nurse cells and follicle cells from 10-cyclin B females were not different from 2-cyclin B females. However, 10-cyclin B eggs were slightly longer, egg-laying was reduced by 90% from the wild-type rate, and fewer than 3% of the embryos hatched. Ovaries from 0-cyclin B females were approximately one quarter the size of normal ovaries. Within egg chambers the number of nurse cells was reduced from the normal 15 down to 5-11. Also, the follicle cells were small and round instead of columnar, and yolk uptake was reduced or absent. In 0-cyclin B females approximately 10% of the egg chambers matured and the egg-laying rate was severely reduced. 0-cyclin B eggs were 10% shorter than wild type, had fragile chorions with frequently fused appendages, and the embryos never formed a cellular blastoderm. Therefore, the phenotypes observed in 0- and 10-cyclin B embryos are due to altered cyclin B levels as well as to defective oogenesis.

Varying cyclin B affects early embryonic cell-cycle events

Axial expansion occurs during cycles 4-6 when nuclei move along the anterior/posterior axis of the embryo. In cycles 7-10, nuclei migrate to the embryonic cortex (Zalokar and Erk, 1976; Foe and Alberts, 1983; Baker et al., 1993). We investigated the
phenotypic consequences of varied cyclin B levels on these processes. During axial expansion, migrating nuclei undergo cell-cycle phase-specific movements: migration begins in late interphase, ceases in metaphase, and retracts slightly in anaphase and telophase (Baker et al., 1993; von Dassow and Schubiger, 1994). We used phase-specific movements to define the cell-cycle length in 1-, 2- and 4-cyclin B embryos using time-lapse video microscopy. Cyclin B levels did not significantly influence the overall duration of preblastoderm cell cycles (Fig. 2, cycles 2-8) but did affect the velocity of nuclear migration. Nuclei in 1-cyclin B embryos migrated faster than those of wild-type embryos, while nuclei in 4-cyclin B embryos migrated slower than wild type. We observed these differences during both axial expansion and cortical migration (Table 1).

We also measured the timing of somatic bud formation as an indicator of nuclear velocity (Fig. 2, triangles). Wild-type (2-cyclin B) embryos formed somatic buds when nuclei reached the periphery during cycle 10. In video analysis, 1-cyclin B embryos formed somatic buds early: 20% during cycle 8 and 80% during cycle 9. 58% of 4-cyclin B embryos formed somatic buds at cycle 10 and 33% during cycle 11. The nuclei in 4-cyclin B embryos were often unevenly dispersed at the surface and frequently reached one or both poles late. In one of twelve 4-cyclin B embryos, nuclei did not reach the cortex until cycle 13. After the nuclei reached the periphery, this embryo completed six additional 9.5-minute blastoderm cycles and never cellularized. The continuation of multiple rapid cycles is also observed in 10-cyclin B embryos (data not shown). This resembles the phenotype of grapes mutants in which Cdk1 is not inactivated and the cell cycle does not slow down during the blastoderm cycles (Sibon et al., 1997). 1-cyclin B embryos that formed somatic buds early still cellularized after the 13th mitosis by completing five, rather than four, blastoderm cycles. Furthermore, if nuclei arrived one cycle late (4-cyclin B embryos), only three blastoderm cycles were observed and embryos cellularized at the correct nuclear density and cell cycle.

**Cyclin B alters microtubule morphology in vivo**

The microtubule network increases in volume as the number of asters doubles with every cycle. Baker et al. (1993) and von Dassow and Schubiger (1994) recognized the significance of the cytoskeleton in nuclear migration and the requirement for

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**Fig. 2.** Cyclin B dose affects the timing of nuclear migration to the cortex and initiation of slower blastoderm cycles. Lengths of cycles 2-13 in embryos with increased or decreased levels of cyclin B are shown. Time analysis shows little difference in the duration of cycles 2-8 among 1-, 2- or 4-cyclin B embryos. Triangles indicate the cycle(s) at which nuclei are visible reaching the periphery of the embryo and forming somatic buds. Triangles also indicate the beginning of the blastoderm cycles. In 1-cyclin B and 4-cyclin B embryos more than one triangle is found, indicating that somatic bud formation was observed at different cell cycles.

**Fig. 3.** Cell-cycle phase-specific changes of microtubule (green) and DNA configuration (red) in cycle 6 wild-type embryos. Images are from embryos in interphase (I), prophase (P), metaphase (M), anaphase (A) and telophase (T), and are projections of six 1 µm optical sections. Scale bar, 15 µm.

**Fig. 4.** Cyclin B dose affects microtubule length. Microtubule (green) and DNA (red) configurations are shown in 1-, 2- or 4-cyclin B embryos fixed during cycle 6. Below the color merges are the corresponding tubulin images. The upper panels are images of embryos in interphase. The lower panels are images of embryos in metaphase. Images are projections of six 1 µm optical sections. Scale bar, 15 µm.
microfilament breakdown during axial expansion. They proposed that microtubules regulate the stability of the microfilaments and direct their breakdown, thus controlling the movement of the nuclei. They also showed that the network of astral microtubules pushes nuclei to the cortex of the embryo. These movements occur periodically and correlate with dynamic changes of the network. As shown in Fig. 3, microtubules undergo dramatic phase-specific morphological changes during cycles 4-7. Interphase astral microtubules radiate from centrosomes and form a network, which generates even spacing among nuclei. This network breaks down in prophase and microtubules reorganize into mitotic spindles in metaphase. In anaphase the microtubules bring chromatids to the poles and the mitotic spindle breaks down; a midbody remains between the separating chromatids. Finally, microtubules reform asters as division is completed in telophase, and the midbody persists through interphase (von Dassow and Schubiger, 1994).

Cyclin B has been shown to regulate microtubule dynamics in *Xenopus* extracts (Verde et al., 1990, 1992), and cyclin B localizes to microtubules in blastoderm stage *Drosophila* embryos (Huang and Raff, 1999). We have shown earlier that microtubular dynamics affects nuclear migration in the preblastoderm embryos (Baker et al., 1993). Here we tested whether cyclin B levels affect axial expansion and cortical migration during preblastoderm cycles. To do this, we calculated the volume of interphase asters and observed microtubule configurations in 1-, 2- and 4-cyclin B embryos. As shown in Table 2, astral microtubule volume was inversely proportional to cyclin B dose. Confocal images revealed a similar correlation between microtubule length and cyclin B dose during interphase and metaphase (Fig. 4). In interphase, microtubules were longer in 1- and 2-cyclin B embryos compared to those of 4-cyclin B embryos (Fig. 4, interphase).

**Fig. 5.** Images of microtubules (green) and DNA (red) from embryos with 0, 2 or 10 copies of cyclin B. The inverse relationship between microtubule stability and cyclin B dose is maintained in the 0- and 10-cyclin B embryos. Note the close association of the two interphase nuclei in 10-cyclin B embryos. Embryos were fixed approximately during cycle 8 and images are projections of three 1 μm optical sections. Scale bar, 15 μm.

**Fig. 6.** Differences in cyclin B and A localization in wild-type embryos during cycles 6, 12 and mitosis 14. Cyclin B is predominantly cytoplasmic in wild-type embryos during cycle 6 (A,B). Cyclin B (green) is observed on metaphase spindle microtubules (red) (A) but gone from the anaphase spindle microtubules (B). Cyclin B is not present in interphase nuclei (data not shown). (C-G) Cyclin A (green) and DNA configuration (red). Cyclin A persists in the nucleus during all phases of cycle 6; interphase (C), metaphase (E) and anaphase (F) are shown. At cycle 6 more cyclin A is in interphase nuclei (C) than at cycle 12 (D). The colocalization of cyclin A with chromosomes during metaphase and anaphase (E,F) of cycle 6 is contrasted with the absence in late metaphase and anaphase of mitosis 14 (G). Scale bars, 10 μm.
During prophase in 1-cyclin B embryos, asters did not break down as completely as they did in 4-cyclin B embryos (data not shown). During metaphase, mitotic spindles appeared larger in 1-cyclin B embryos and smaller in 4-cyclin B embryos, compared to 2-cyclin B embryos (Fig. 4, metaphase). In addition, metaphase asters were longer and were clearly visible in 1-cyclin B compared to those in 2-cyclin B, but they were not detectable in 4-cyclin B embryos. During anaphase and telophase, larger microtubule asters were observed in 1-cyclin B embryos compared to those in 2- and 4-cyclin B embryos (data not shown).

To amplify the effects of cyclin B on microtubule morphology, we analyzed 0- and 10-cyclin B embryos. In 0-cyclin B embryos, microtubules were often elongated and organized into thick bundles throughout the embryo (Fig. 5). Astral microtubules were often observed without nuclei and centrosomes appeared to divide and migrate without associated nuclei. In 10-cyclin B embryos, microtubules were severely reduced in all phases of the cycle and nuclei remained close together (Fig. 5). Microtubule morphology in 0- and 10-cyclin B embryos was more severely altered than that of 1- and 4-cyclin B embryos. Thus cyclin B levels correlate with microtubule abundance in different cycle phases.

Cyclins A and B are differentially localized during early cycles

To analyze the subcellular localization of cyclins, microtubules and DNA, we stained cycle 4-6 embryos with a cyclin antibody, tubulin and histone antibodies (Fig. 6). Our immunocytochemical analysis detected cyclins A and B throughout the embryo during all phases of cycles 4-6 (Fig. 6 and data not shown), which was consistent with western analysis by Edgar et al. (1994). Cyclin B localization was similar to that reported by Huang and Raff (1999) during cycles 10-12. During interphase and early prophase, cyclin B was found in the cytoplasm, concentrated near the centrosomes and astral microtubules. However, in late prophase, we also observed cyclin B in nuclei (data not shown). Cyclin B was on the early metaphase nuclei (Fig. 6A), absent from the anaphase spindle but present on the asters (Fig. 6B).

Cyclin A localization during cycles 4-6 differed from the later blastoderm cycles. During interphase of cycles 4-6 we observed strong cyclin A staining in the nuclei and only weak staining throughout the cytoplasm (Fig. 6C). In contrast, during cycle 12 cyclin A was strong in the cytoplasm and weakly punctuated in the late interphase-prophase nucleus (Fig. 6D; Maldonado-Codina and Glover, 1992). During mitosis of cycle 6, cyclin A was strongly associated with the chromosomes and persisted through anaphase (Fig. 6E,F). This differs from localization during mitosis 14 when cyclin A levels increase in the nuclei during prophase, reach high levels in metaphase, degrade in late metaphase, and are completely absent in anaphase (Fig. 6G; Lehner and O’Farrell, 1989).

Therefore, cyclins A and B are present throughout the preblastoderm cycles but are compartmentalized differently, which suggests they have different targets.

Cyclin A affects the duration of early nuclear cycles

Because cyclin A localized in nuclei, we tested whether reducing cyclin A affected cell-cycle duration. Decreased cyclin A correlated with a significant increase in cell-cycle time during cycles 4-6 ($P=0.000049$; Fig. 7A), which was independent of cyclin B levels. Edgar et al. (1994) observed a slight increase in the length of time from egg deposition to cycle 10 in 1-cyclin A embryos. In contrast, increased cyclin B did not have a linear effect on cycle time (Fig. 7B). These observations show that overall cycle length is sensitive to cyclin A levels. A minor effect of cyclin B cannot be excluded but it cannot be statistically documented.

### Table 1. Velocity of nuclei in cycles 4-6 and 7-10

<table>
<thead>
<tr>
<th>Cyclin B dose</th>
<th>n*</th>
<th>Axial expansion (cycles 4-6)</th>
<th></th>
<th>Cortical migration (cycles 7-10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Velocity (μm/minute) s.d.</td>
<td></td>
<td>Velocity (μm/minute) s.d.</td>
</tr>
<tr>
<td>1-cyc B</td>
<td>9</td>
<td>19.6 3.1</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>2-cyc B</td>
<td>10</td>
<td>14.5 4.0</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>4-cyc B</td>
<td>10</td>
<td>13.0 2.4</td>
<td></td>
<td>8</td>
</tr>
</tbody>
</table>

*Significance levels are based on isotonic regression tests (Gaines and Rice, 1990), which test for an ordered association between cyclin B dose and measured variables.

### Table 2. Average volume of interphase microtubule asters

<table>
<thead>
<tr>
<th>Cyclin B dose</th>
<th>n*</th>
<th>Mean volume (μm³) s.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-cyc B</td>
<td>5</td>
<td>5093 894</td>
</tr>
<tr>
<td>2-cyc B</td>
<td>6</td>
<td>3389 651</td>
</tr>
<tr>
<td>4-cyc B</td>
<td>5</td>
<td>3074 633</td>
</tr>
</tbody>
</table>

For details on uniform handling and imaging see Materials and methods.

### Table 3. Percentage of time spent in each cell-cycle phase during cycles 2-8

<table>
<thead>
<tr>
<th>Cyclin B dose</th>
<th>Cell cycle phase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>1-cyc B</td>
<td>250</td>
</tr>
<tr>
<td>2-cyc B</td>
<td>436</td>
</tr>
<tr>
<td>4-cyc B</td>
<td>127</td>
</tr>
</tbody>
</table>

1-cyc B versus 4-cyc B; Chi-square test result: interphase $P<0.025$, metaphase $P<0.005$. I, interphase; P, prophase; M, metaphase; A, anaphase; T, telophase.
mitotic index was not different from controls (data not shown). We conclude that cyclin B dose affects metaphase and interphase duration while cyclin A regulates the overall cycle duration.

Cytoskeletal dynamics must be coordinated with the nuclear cycle during the metaphase-anaphase transition. If cyclin B predominantly affects cytoskeletal dynamics but not the overall cycle duration, then coordination of cytoskeletal and nuclear events in 0- and 10-cyclin B embryos should be more severely disrupted. We noticed that synchrony of early divisions was frequently lost in 0- and 10-cyclin B embryos. For example, within a single embryo we observed nuclei in all cell-cycle phases. Nuclei in 0-cyclin B embryos never completed more than four divisions and were of increased or decreased ploidy, indicating lack of cytoskeletal-nuclear coordination. While few nuclei reached the periphery, isolated microtubule asters and centrosomes completed migration (Fig. 5 and data not shown). We also observed that centrosomes duplicated in 0-cyclin B embryos and migrated without nuclei (data not shown). 10-cyclin B embryos also had too few nuclei, many of which were aneuploid. 27% of 10-cyclin B embryos were delayed (and presumably arrested) in metaphase after 3-8 nuclear divisions. Asynchronous division, aneuploidy and metaphase delay (or arrest) all indicate uncoordinated cytoskeletal and nuclear events. Therefore, by changing the relative amounts of mitotic cyclins A and B, we disrupt this coordination.

**DISCUSSION**

**Cyclin B regulates microtubule dynamics**

Our results offer the first in vivo documentation of a dose-dependent effect of cyclin B on microtubule morphology and function. We show that cyclin B but not Cdk1 limits the kinase activity, and cyclin B levels affect microtubule dynamics. These results are consistent with the model that cyclin B-Cdk1 indirectly regulates microtubule stability via microtubule associated protein 4 (MAP4). Unphosphorylated MAP4 binds and stabilizes microtubules in HeLa cells and was shown to be a target of cyclin B-Cdk1 both in vitro and in vivo (Ookata et al., 1995, 1997). Does cyclin A-Cdk1 also regulate microtubule dynamics in *Drosophila* embryo? This is unlikely because cyclin B, but not cyclin A, immunoprecipitates with microtubules in *Drosophila* embryos (Huang and Raff, 1999 and personal communication). Also, Verde et al. (1992) observed that cyclin B-Cdk1 induces dramatic shortening of astral microtubule in *Xenopus* egg extracts while cyclin A-Cdk1 does not.

Altered cyclin B levels affect nuclear movements during early embryogenesis and we propose that these defects were due to microtubule stability, since polymerizing microtubules generate a pushing force on nuclei (Inoué and Salmon, 1995). Important clues to the role of microtubules during syncytial development come from analysis of *Sciara* coprophila and *Drosophila* embryogenesis. Unfertilized *Sciara* embryos lack centrosomes and astral microtubules but still go through a few nuclear divisions. In these embryos, nuclear spacing is greatly reduced and nuclei often clump together in polypliod masses (de Saint Phalle and Sullivan, 1998). In *Drosophila*, astral microtubules maintain spacing between nuclei and also control nuclear migration during preblastoderm development (Baker et
al., 1993). We find that lowering cyclin B causes longer microtubules, thereby increasing the velocity of nuclear migration. Conversely, increased cyclin B levels result in microtubule destabilization and slower nuclear movement.

**Subcellular localization of cyclins A and B correlates with their functions**

Although both cyclins A and B bind with Cdk1, cyclin A but not B null mutants are homozygous lethal, which indicates that they have distinct functions. Since the cyclins do not oscillate globally during cycles 1-8 (Edgar et al., 1994), their distinct functions may be due to differential subcellular localization. Indeed, during cycles 4-6, we found cyclin A predominantly localized in the nucleus, while cyclin B was cytoplasmic except during late prophase. Maldonado-Codina and Glover (1992) described similar cyclin localization in cycle 12, except that cyclin A was weak and punctate in interphase and early prophase nuclei compared to the strong nuclear cyclin A we observed throughout cycle 6 (Fig. 6C). We observed the disappearance of cyclin B from the late metaphase spindle during cycle 6 (Fig. 6A,B). Recently, Huang and Raff (1999) reported cyclin B in prophase nuclei and associated with metaphase spindles, but it disappears from late metaphase spindles in blastoderm-stage embryos.

By reducing the amount of cyclin A in embryos, we increased the overall duration of the cell cycle. Cyclin B levels had no significant effect on this parameter. However, more cyclin B correlated with a longer metaphase and shorter interphase. We propose that more cyclin B caused the metaphase delay by destabilizing microtubules, thus prolonging the time required to form a functional spindle. The effect of cyclin B levels on metaphase duration is also apparent during normal development. In wild-type embryos, global cyclin B levels generally decline during preblastoderm development (Edgar et al., 1994), and we have observed that metaphase at cycle 6 is longer than at cycle 8 (J.-Y. Ji and G. Schubiger, unpublished observations). Our results indicate that metaphase at cycle 6 is longer than at cycle 8 (J.-Y. Ji and G. Schubiger, unpublished observations). We propose that more cyclin B caused the metaphase spindles, but it disappears from late metaphase spindles in blastoderm-stage embryos.

Localized degradation of mitotic cyclins

 Destruction of microtubules prevents degradation of cyclin B, but not cyclin A (Edgar et al., 1994), implying that they have distinct degradation mechanisms. More studies are needed to better understand local control of cyclin A activity during the early cycles. We report that cyclin A controls the nuclear cycle and is continuously present in the nucleus. We face a conundrum, because we observed cyclin A on anaphase chromosomes, yet cyclin A has to be destroyed to progress into anaphase (Su et al., 1998). Cyclin A-Cdk1 activity might locally modify its target proteins, which regulate chromosome behavior.

The mechanism of cyclin B’s spatial regulation is better understood. Cyclin B is degraded from the mitotic spindle during late metaphase (Huang and Raff, 1999). Blocking this degradation by injection of non-degradable cyclin B (without ubiquitin-dependent destruction box) causes anaphase arrest (Su et al., 1998). Accordingly, local removal or local ubiquitin-dependent degradation of mitotic spindle-associated cyclin B is required for progression to anaphase from metaphase (Huang and Raff, 1999). These observations indicate that metaphase is extended in 4-cyclin B embryos, because locally more cyclin B will have to be degraded prior to anaphase. Conversely, metaphase is shorter in 1-cyclin B embryos because less cyclin B must be degraded. Is the longer metaphase time in 4-cyclin B embryos solely the result of needing a longer time to degrade more cyclin B? We believe that increased cyclin B levels are also affecting the formation of a functional spindle, for two reasons. First, as shown in Table 3, varying doses of cyclin B correlate with changes of metaphase duration but no such trend is observed with respect to the anaphase duration. Second, the application of non-degradable cyclin B did not produce a metaphase arrest (Su et al., 1998), which indicates that metaphase duration is not affected.

In many organisms, cyclin-Cdk1 activity is regulated through periodic synthesis and degradation of the mitotic cyclins via the ubiquitination pathway (Glotzer et al., 1991). However, in *Drosophila* embryos, Cdk1 protein levels are constant and cyclin B is not completely degraded during cycles 1-8 (Edgar et al., 1994). Because cyclin B is not found on the anaphase spindle, this indicates localized degradation. Therefore, we assume that progression of these embryonic cell cycles depends on local rather than global oscillation of kinase activity, and microtubules play a critical role in this process. While cyclin B levels generally decline in preblastoderm cycles, microtubule mass steadily increases. This is consistent with the idea that microtubules titrate cyclin B as suggested by Edgar et al. (1994). By varying maternal doses of cyclin B, we show that cyclin B also titrates microtubules. Combined with the findings of Edgar et al. (1994), our results suggest a mutually antagonistic relationship between cyclin B and the microtubules.

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