Cyclins A and B Associate with Chromatin and the Polar Regions of Spindles, Respectively, and Do Not Undergo Complete Degradation at Anaphase in Syncytial *Drosophila* Embryos

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Abstract. Maternally contributed cyclin A and B proteins are initially distributed uniformly throughout the syncytial *Drosophila* embryo. As dividing nuclei migrate to the cortex of the embryo, the A and B cyclins become concentrated in surface layers extending to depths of ~30–40 μm and 5–10 μm, respectively. The initiation of nuclear envelope breakdown, spindle formation, and the initial congression of the centromeric regions of the chromosomes onto the metaphase plate all take place within the surface layer occupied by cyclin B on the apical side of the blastoderm nuclei. Cyclin B is seen mainly, but not exclusively, in the vicinity of microtubules throughout the mitotic cycle. It is most conspicuous around the centrosomes. Cyclin A is present at its highest concentrations throughout the cytoplasm during the interphase periods of the blastoderm cycles, although weak punctate staining can also be detected in the nucleus. It associates with the condensing chromosomes during prophase, segregates into daughter nuclei in association with chromosomes during anaphase, to redistribute into the cytoplasm after telophase. In contrast to the cycles following cellularization, neither cyclin is completely degraded upon the metaphase-anaphase transition.

THE entry into mitosis, controlled by a mechanism common to all eukaryotes, requires the activation of a mitotic kinase often referred to as cdc2-kinase, after the name of this gene in the fission yeast *Schizosaccharomyces pombe*. In *S. pombe*, *cdc2* requires the expression of *cdc25* to become activated, and is under the negative regulation of *weel*, which itself is negatively controlled by *niml* (see Nurse, 1990; and Pines and Hunter, 1990 for reviews). cdc2-kinase has its counterpart in vertebrates and was first identified as a component of maturation (or mitotic) promoting factor (MPF),[1] an activity required for the maturation of prophase-arrested frog oocytes, and which then oscillates in the mitotic divisions of fertilized eggs (Masui and Markert, 1971; Wasserman and Smith, 1978; Gerhart et al., 1984). Highly purified MPF from *Xenopus* comprises two proteins, a 32-kD cdc2-kinase and a 45-kD protein, shown to be a cyclin (Lohka et al., 1988; Gautier et al., 1990). Cyclins were first described in sea urchin eggs as proteins that undergo periodic degradation at the metaphase-anaphase transition of each cycle (Evans et al., 1983). Although two classes of mitotic cyclin, A and B, have been identified in many multicellular eukaryotes, as yet only a B-type cyclin has been found in fission yeast where it corresponds to the product of the gene *cdc13* (Booher and Beach, 1988; Goebl and Byers, 1988; Hagan et al., 1988; Soloman et al., 1988). There is also evidence from immunoprecipitation experiments and from the biochemical analysis of purified MPF that cdc2-kinase and B-type cyclins are associated in *S. pombe* (Moreno et al., 1989; Booher et al., 1989), starfish (Labbe et al., 1989), sea urchins (Meijer et al., 1989), clams (Draetta et al., 1989), and human cells (Pines and Hunter, 1989). *S. pombe cdc2*, and its counterpart *cdc28* of *Saccharomyces cerevisiae* (Beach et al., 1982), are not only needed at the G2-M transition but also at *start* in G1 phase for entry into S-phase. In *S. cerevisiae*, this requires that *cdc28* associate with a second set of "G1-cyclins" encoded by the genes CLN1, CLN2, and CLN3 (Hadwiger et al., 1989; Richardson et al., 1989; Wittenberg et al., 1990). G1-cyclins are as yet poorly characterized in other organisms.

The functional differences between the two mitotic cyclins has not been clarified. In mitotic cycles, cyclin A has been observed to be degraded ahead of cyclin B in several organisms. Moreover, arrest of the cell cycle in a metaphase-like state by drugs that destabilize microtubules, defines a point at which cyclin A has been degraded, and at which cyclin B continues to accumulate (Westendorf et al., 1989; Minshull et al., 1989; Whitfield et al., 1990). This temporal profile is reflected in the activation of the cdc2-kinase associated with the two types of cyclin in *Xenopus* eggs (Minshull et al., 1990).

Cyclin A was first identified in *Drosophila* by Lehner and O'Farrell (1989) who demonstrated its zygotic expression was essential for cell division. Maternal transcripts for both

1. *Abbreviation used in this paper*: MPF, maturation promoting factor.

cyclins are abundantly distributed throughout the syncytial embryo during the initial mitotic cycles, but in addition there is a particularly high concentration of cyclin B transcripts at the posterior pole (Whitfield et al., 1989; Lehner and O'Farrell, 1990; Raff et al., 1990). These polar transcripts become incorporated into pole cells, the precursors of the germ-line, although the significance of this is not clear. The embryo is a syncytium for the first 13 divisions which occur at ~10-min intervals, and which consist of rapidly alternating M and S-phases (Foe and Alberts, 1983). A G2 phase first appears in the 14th cycle after the cellularization of individual somatic nuclei. Mitosis then occurs within a series of spatially and temporally regulated domains (Foe, 1989). These divisions are under the control of *string*, the *Drosophila cdc25* homologue, whose transcription precedes mitosis by ~25 min in each of the domains (Edgar and O'Farrell, 1989). Ectopic expression of *string* throughout the embryo under the control of the major heat shock gene promoter results in inappropriate entry into mitosis (Edgar and O'Farrell, 1990). Although the cyclical degradation of the two cyclins can be seen after cellularization, this does not appear to occur within the syncytial embryo (Lehner and O'Farrell, 1989, 1990; Whitfield et al., 1990). In this paper, we carefully examine the behavior of the two cyclins in these syncytial mitoses, and in so doing have discovered that the two molecules differ in their subcellular distribution. We discuss these differences in relation to other aspects of cyclin behavior and in relation to their possible role as targeting subunits for cdc2-kinase.

Materials and Methods

Fixation and Preparation of Embryos from Immunostaining

Embryos were fixed and prepared for staining as described by Whitfield et al. (1990) with the following modifications. Embryos were dechorionated by soaking in undiluted hypochlorite bleach for 4 min, and then thoroughly washed in tap water. Embryos were then added to a glass vial containing 5 ml of heptane and 1 ml of 100 mM potassium phosphate, pH 6.8, 450 mM KCl, 150 mM NaCl (buffer B), to which was added 5 μl 1 mM taxol in DMSO (Karr and Alberts, 1986). The embryos were shaken for 30 s after which was added 1 ml of freshly prepared 8% paraformaldehyde in buffer B. The embryos were left to fix for 1 h with gentle shaking. Devitellinisation was carried out as described by Whitfield et al. (1990).

Immunostaining

Immunostaining of cyclins A and B was carried out as previously described using rabbit anti-*Drosophila* cyclin A and B antibodies (Whitfield et al., 1990), and also with mouse anticyclin antisera raised against the same fusion proteins, made and kindly supplied by W. Whitfield in our laboratory. The rat monoclonal YL1/2 was obtained from Sera Lab (Crawley Down, RH10 4FF, U.K.) and diluted 1/10 before use. Centrosomes were stained with the rabbit antibody RB188 (Whitfield et al., 1988). Secondary antibodies were obtained from Jackson Immunoresearch Laboratories Inc. (Avondale, PA) and diluted 1/500 before use. None of the immunostaining patterns which we describe and attribute to the specificity of the primary antibody could be seen when these secondary antibodies were used alone.

Results

Cyclins A and B Occupy Different Subcellular Regions as Embryogenesis Proceeds

We have previously shown that maternal transcripts of the cyclin A and B genes are initially distributed throughout the syncytial embryo, but become localized to its surface together with the majority of nuclei at blastoderm (Whitfield et al., 1989; Raff et al., 1990). During the blastoderm division cycles 12 and 13, the cyclin A transcripts come to occupy a surface layer 30–40 μm deep coincident with the layer of cortical cytoplasm. The cyclin B transcripts occupy a thinner layer ~5 μm deep on the apical side of the cortical nuclei. In addition, a proportion of the maternal cyclin B transcripts are deposited in polar granules during oogenesis and become incorporated into pole cells as embryogenesis proceeds (Whitfield et al., 1989; Lehner and O'Farrell, 1990; Raff et al., 1990). We sought to determine whether the spatial distribution of mRNA was reflected by the localization of the two cyclin proteins in the syncytial embryo, and so stained embryos for indirect immunofluorescence using the polyclonal antisera that we have previously described (Whitfield et al., 1990). We were unable to observe any significant concentration of cyclin B protein at the posterior pole either before or after pole cell formation. Otherwise, the distribution of the two cyclin proteins followed the same pattern as the maternal transcripts. Initially the two proteins are present uniformly throughout the embryo (data not shown). Cyclin A becomes more concentrated at the cortex during blastoderm in a thicker layer than cyclin B, but with some low levels of both proteins remaining within the interior of the embryo (Fig. 1).

The Spatial Organization of the Mitotic Apparatus in the Syncytial Blastoderm

As we wished to know whether the distribution of the two cyclin molecules might reflect their targeting to regions that behave differently in mitosis, we first undertook to examine the spatial events of mitotic cycles 11–13 which take place at the cortex of the embryo. It has been previously noticed that the chromosomes of these blastoderm nuclei are oriented with their centromeres at the apical (outer) side of the nucleus (Foe and Alberts, 1985), a feature that is most readily observable in late interphase and prophase. Serial optical sectioning of nuclei at these mitotic stages has recently been described by Hiraoka et al. (1990). We have made similar observations that are in full agreement with their finding that the centromeric regions are the first to congress onto the metaphase plate, and that dissociation of the lamins does not occur uniformly over the nuclei, but begins on their apical sides and moves down through the nuclei. Double immunofluorescent labeling to detect both lamins and cyclin B in a set of optical sections through nuclei confirms that cyclin B is at its highest concentration on the apical side of the nuclei in the region in which nuclear envelope breakdown begins (data not shown).

Cyclin B Is Particularly Abundant in the Regions of the Astral Microtubules

A series of optical sections showing the distribution of cyclin B relative to spindle microtubules in proximity to mitotic nuclei in cycle 12 is shown in Fig. 2. The position of the nucleus can be seen as a "hole" in the staining pattern. As expected from the optical sections presented in Fig. 1, the cyclin B protein is concentrated on the apical side of the nucleus (Fig. 2, sections *–1*, *–2*, and *–3*). The spindle microtubules are predominantly contained within the top 2–3 μm, within which sections the punctate staining given

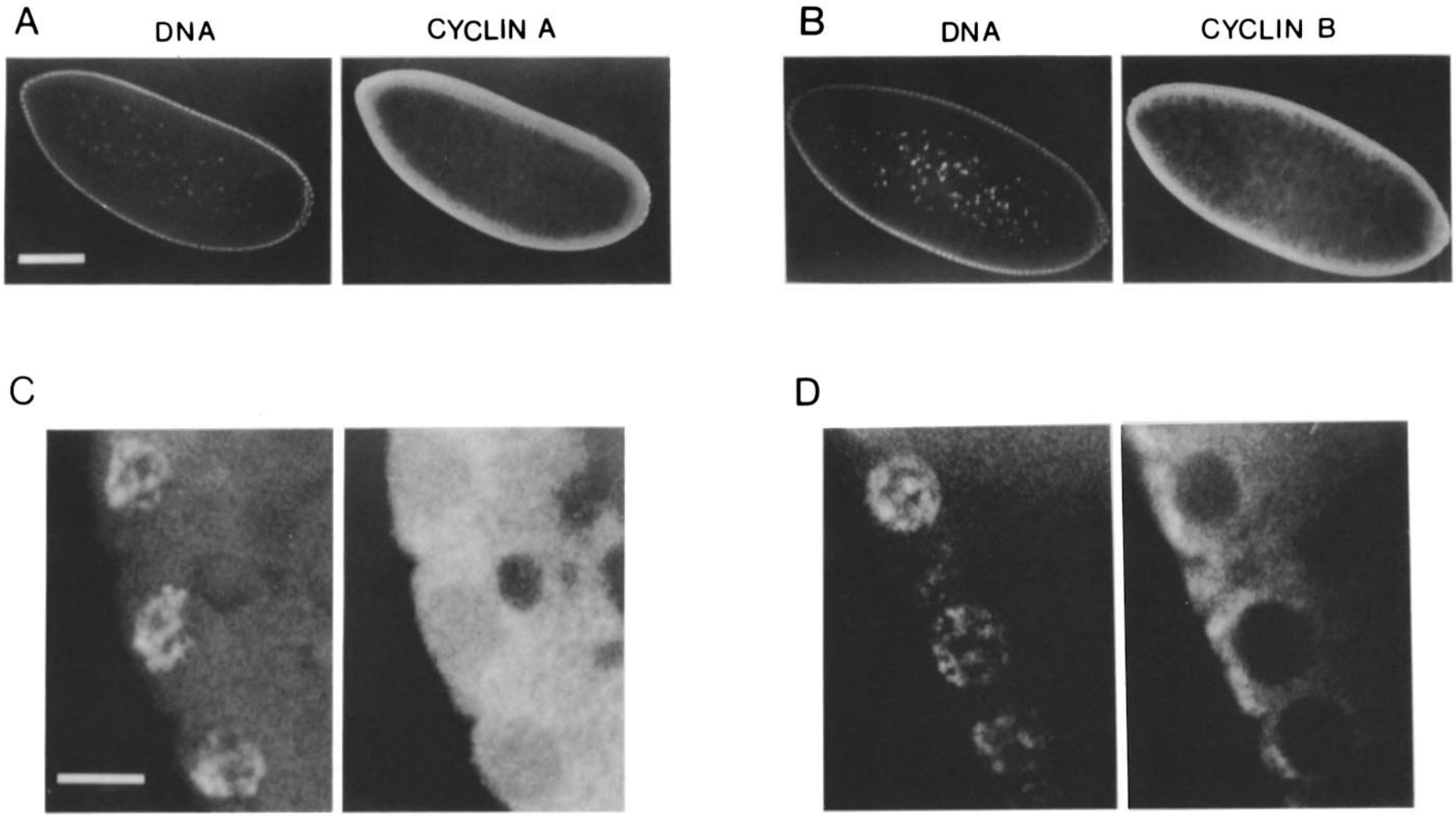

Figure 1. Distribution of cyclin A and B at the cortex of syncytial blastoderm embryos. Each of these paired micrographs shows an optical section through a whole mount preparation of a *Drosophila* embryo in which the left and right hand panels show DNA stained with propidium iodide, and the corresponding cyclin distribution, respectively. The embryos in *A* and *C* have been stained using the rabbit anti-cyclin A antibody, RB270, and those in *B* and *D* with the rabbit anti-cyclin B antibody, RB271, as described by Whitfield et al. (1990). *A* and *B* show whole embryos at cycle 12 in which the mitotically active nuclei are in a monolayer at the surface, and the polyploid yolk nuclei in the interior. The pole cells can be seen at the posterior of the embryo which is to the right of each of the upper panels. *C* and *D* show higher magnification of the cortex of embryos at this stage. Bars: (*A* and *B*) 100 μm; (*C* and *D*) 10 μm.

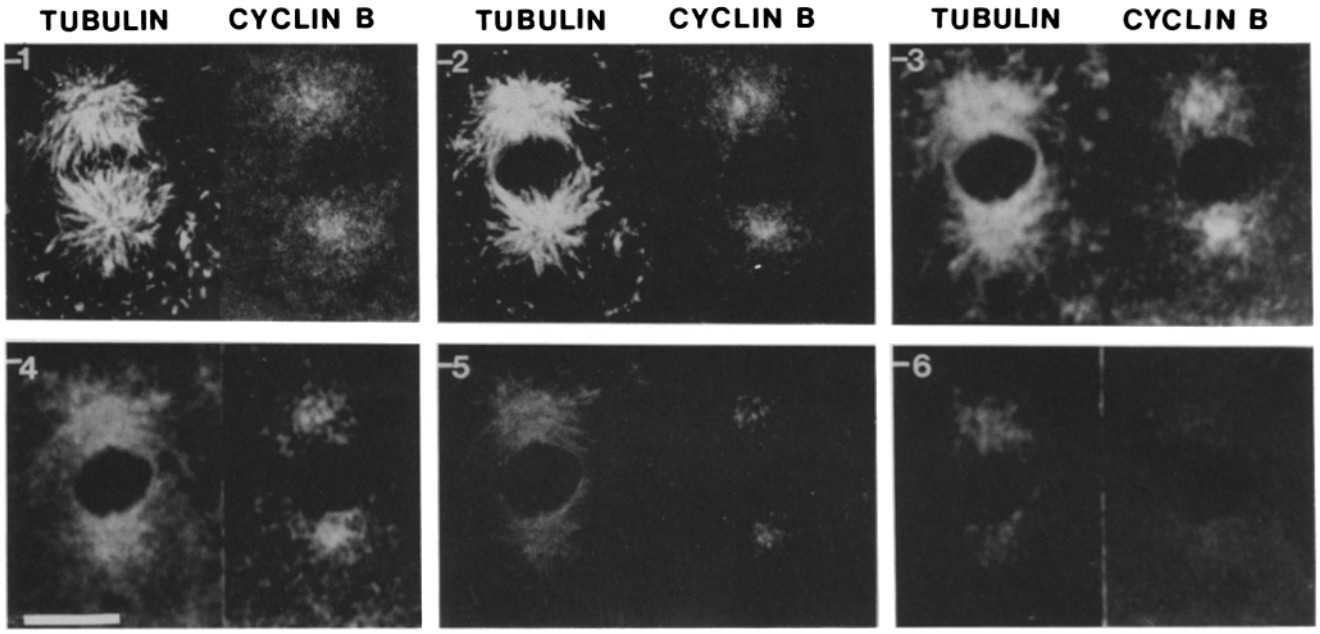

Figure 2. Distribution of cyclin B in relation to spindle microtubules. Panels *-1* to -6 are paired confocal micrographs of serial optical sections at 1-μm intervals of a single mitotic nucleus stained with the rat antitubulin mAb, YL1/2 left-hand micrographs, and the rabbit anti-cyclin B antibody, RB271 (Whitfield et al., 1990) right-hand micrographs. Section -1 is the most apical, with subsequent sections "cutting" progressively deeper into the interior of the embryo. Bar, 5 μm.

by the anti-cyclin B antibodies is largely, but not exclusively, concentrated around the astral microtubules. This association of the cyclin B protein with the polar regions is even more striking in the adjacent deeper sections (sections *-3* and *-4*). Cyclin B staining is, however, weak on the basal side of the nucleus (sections *-5* and *-6*).

Both our own preliminary observations (Whitfield et al., 1990), and those of Lehner and O'Farrell (1989, 1990) using conventional fluorescence microscopy had suggested that if breakdown of the two cyclins occurred in syncytial embryos at each anaphase, then it was incomplete. Thus the behavior of the cyclins appeared to differ in terms of its cyclical degradation from its observed behavior in other species (reviewed in Hunt, 1989), and in cellularized *Drosophila* embryos (Lehner and O'Farrell, 1989, 1990; Whitfield et al., 1990). We were therefore curious whether confocal microscopy would offer sufficient spatial resolution to detect any localized cyclical degradation of the two cyclins at the metaphase-anaphase transition in syncytial embryos. We therefore selected embryos at different stages of the 12th mitotic cycle and carried out optical sectioning to observe the distribution of cyclin B. Cytoplasmic staining of cyclin B was seen at all mitotic stages. The staining was brightest on the apical side of the dividing nuclei, particularly in the vicinity of microtubules around the centrosome. In Fig. 3 we present the staining at the single focal plane through nuclei in different phases of mitosis, showing that anti-cyclin B staining is bright at the level of the mitotic spindle particularly in clouds around the poles.

As the variation in staining pattern at different focal planes given by the rabbit anti-cyclin B antibody is subtle, we were concerned to demonstrate that this pattern truly reflects the distribution of cyclin B protein. Although this antiserum recognizes a very strong band corresponding to the 65-kD cyclin B protein in Western blotting experiments, it does detect a number of other minor bands (see Fig. 7, for example). We therefore carried out immunostaining using various mouse antisera raised against cyclin B fusion proteins. These also recognize a major band of 65 kD by Western blotting, and some very minor bands of different molecular mass to those recognized by the rabbit antiserum. These mouse antisera have not only allowed us to confirm the distribution of cyclin B protein, but have also enabled us to carry out double immunofluorescent labeling together with a rabbit antibody, RB188 (Whitfield et al., 1988), that detects the *Drosophila* centrosome (Fig. 4). This indicates that, in addition to its presence throughout the cytoplasm, cyclin B is most abundant in a cloud that surrounds the centrosome, within the region occupied by astral microtubules. This pericentrosomal concentration of cyclin B is consistent with data obtained in fission yeast (Alfa et al., 1990), and mammalian cells (Pines and Hunter, 1991), and could suggest a role for the cyclin B protein in directing cdc2 kinase to a microtubule or microtubule-associated substrate (see Discussion). The distribution of the protein is indeed similar to the distribution of the maternal transcripts, strengthening the previous suggestions that either nascent cyclin B peptides might "drag" the polysomes upon which it is being translated into the vicinity of the spindle poles, or that transcripts themselves are targeted to the region in which their product is required (Raff et al., 1990).

Cyclin A Associates with Chromatin during the Mitotic Cycle

We have also examined the distribution of cyclin A in optical sections through interphase nuclei during the 12th mitotic

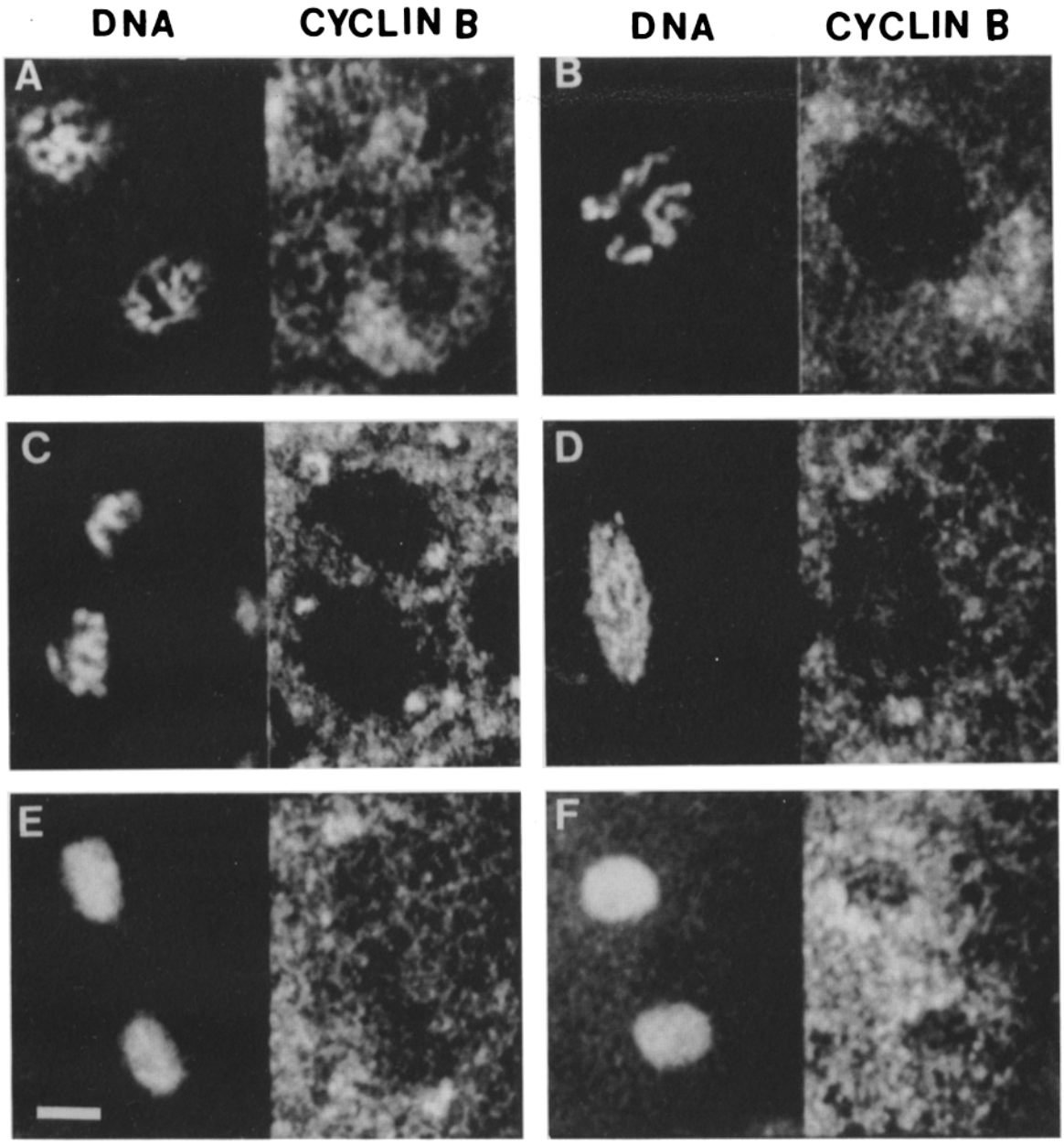

Figure 3. Distribution of cyclin B during the mitotic cycle. The left and right hand sides of each panel show DNA stained with propidium iodide, and cyclin B revealed by indirect immunofluorescence using the RB271 antibody Whitfield et al. (1990), respectively. *A–F* show the following phases of mitosis: (*A*) early prophase; (*B*) late prophase; (*C*) metaphase; (*D*) anaphase; (*E*) late anaphase-early telophase; (*F*) late telophase-early interphase. Each paired micrograph is an optical section taken at a similar depth of 4–5 μm. Bar, 2 μm.

cycle (Fig. 5). As was also seen in the sections presented in Fig. 1, cyclin A is present in high concentrations throughout the cortical cytoplasm. In interphase, the staining is predominantly cytoplasmic, although very weak punctate staining can be observed in the nuclei. The staining persists throughout this cortical region at similar levels of intensity throughout mitosis, but there is a striking redistribution of the antigen. Fig. 6 shows sections of nuclei at different mitotic stages at a single focal plane corresponding to a depth of 4–5 μm from the surface of the embryo (about mid-way through the nuclei). The strong cytoplasmic staining at interphase (Fig. 5 *A*) persists through early prophase (Fig. 5 *B*) but appears to become more punctate. By late prophase, (Fig. 5 *C*) the staining has become much more uniform throughout the nucleus and cytoplasm, and the antigen has begun to show association with chromatin. The chromosomal association is very clear by metaphase (Fig. 5 *D*). Cyclin A is not completely degraded in anaphase (Fig. 5 *E*) and strong chromatin-associated staining can be seen both at this stage and at late anaphase and telophase (Fig. 5 *F*). The antigen then redistributes between nucleus and cytoplasm and is predominantly cytoplasmic during the next interphase.

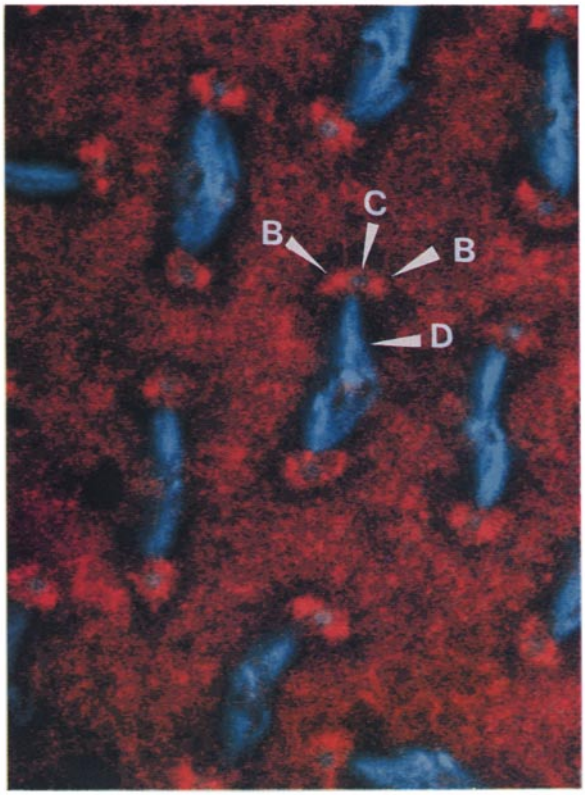

Figure 4. Distribution of cyclin B with respect to the centrosome. A field of anaphase figures from a syncytial blastoderm embryo in which cyclin B (*red*) has been revealed by indirect immunofluorescence using a mouse anti-cyclin B primary antibody, W5, and an FITC-conjugated anti-mouse IgG as secondary antibody. Centrosomes have been stained using the rabbit antiserum RB188 (Whitfield et al., 1988), and Texas red–conjugated anti-rabbit IgG as secondary antibody. Chromosomes have been stained with propidium iodide. The fluorescence of both Texas red and propidium iodide is detected in the same channel of the scanning head (model MRC-500; Bio-rad Laboratories) and has been assigned blue in the computer analysis of the image. If centrosomes are stained with the same primary antibody, but with an FITC-labeled second antibody, their staining shows no overlap with propidium-stained chromosomes at anaphase. *B*, cyclin B staining; *C*, centrosome staining; *D*, DNA staining.

Cyclins Persist after Injection of Cycloheximide

In cells in which the cyclins are completely degraded during mitosis, protein synthesis is required for the regeneration of cyclins in subsequent cycles. We have attempted to determine whether additional cycles occur in the syncytial *Drosophila* embryo after the injection of cycloheximide. We injected the drug during the interphase of cycle 9, following which one would normally see cyclical budding events as nuclei divide at the cortex of the embryo. Similar experiments of Edgar and Schubiger (1986) suggested that this resulted in cell cycle arrest in the S-phase immediately after injection. We see the regression and return of cortical buds for up to two cycles subsequent to injection, which could indicate that persisting cyclins can be used in subsequent cycles. The embryos then arrest with buds at a maximal size corresponding to the interphase state. The nuclei underlying these buds become visibly swollen. The staining of such embryos to reveal DNA shows that the nuclei have some degree of localized chromatin condensation and are about twice the diameter of untreated nuclei. The localization of the two cyclins is dramatically altered within cycloheximide-treated embryos. Cyclin A is seen at high levels inside the nucleus, in contrast to its cytoplasmic localization in nontreated interphase embryos. Cyclin B shows a similar distribution (Fig.

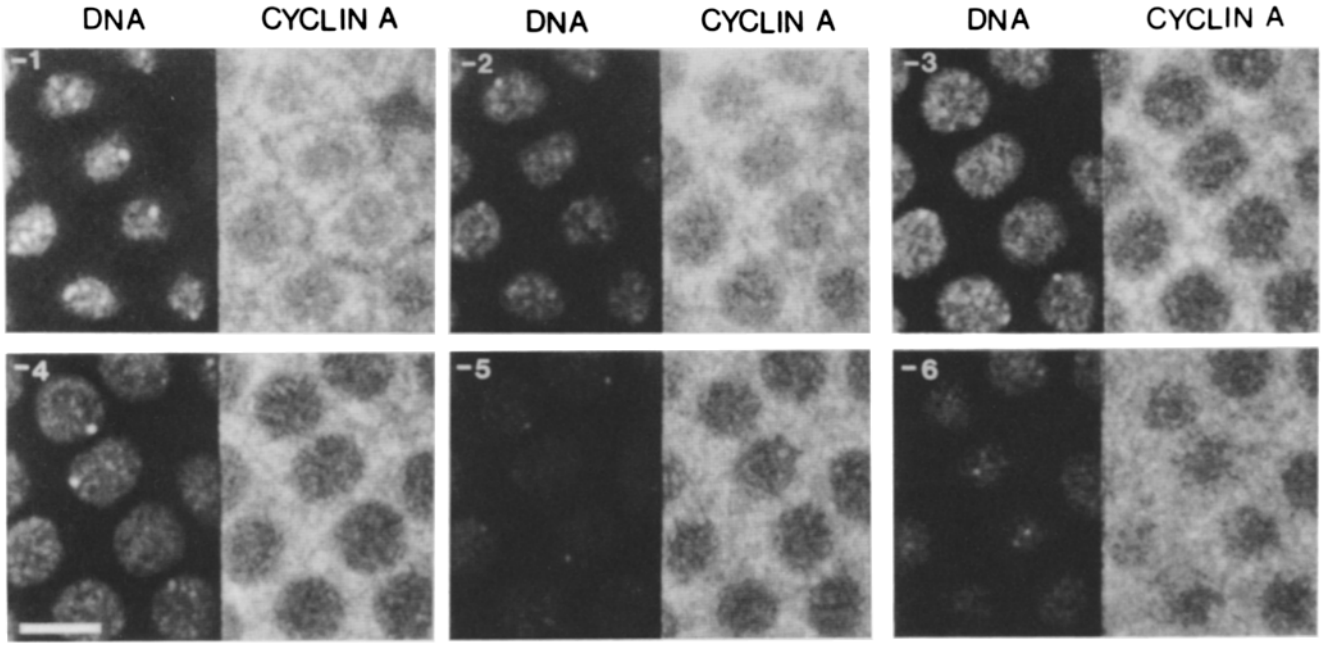

Figure 5. Cyclin A is distributed throughout the cortical cytoplasm at interphase. Panels *−1*–*−6* are paired confocal micrographs of serial optical sections at 1-μm intervals of a field of cortical nuclei in the interphase of cycle 12. The left and right micrographs of each panel show staining with propidium iodide to reveal DNA, and with the rabbit antibody, RB270 Whitfield et al. (1990) to reveal cyclin A. Section -1 is the most apical, with subsequent sections "cutting" progressively deeper into the interior of the embryo. Bar, 10 μm.

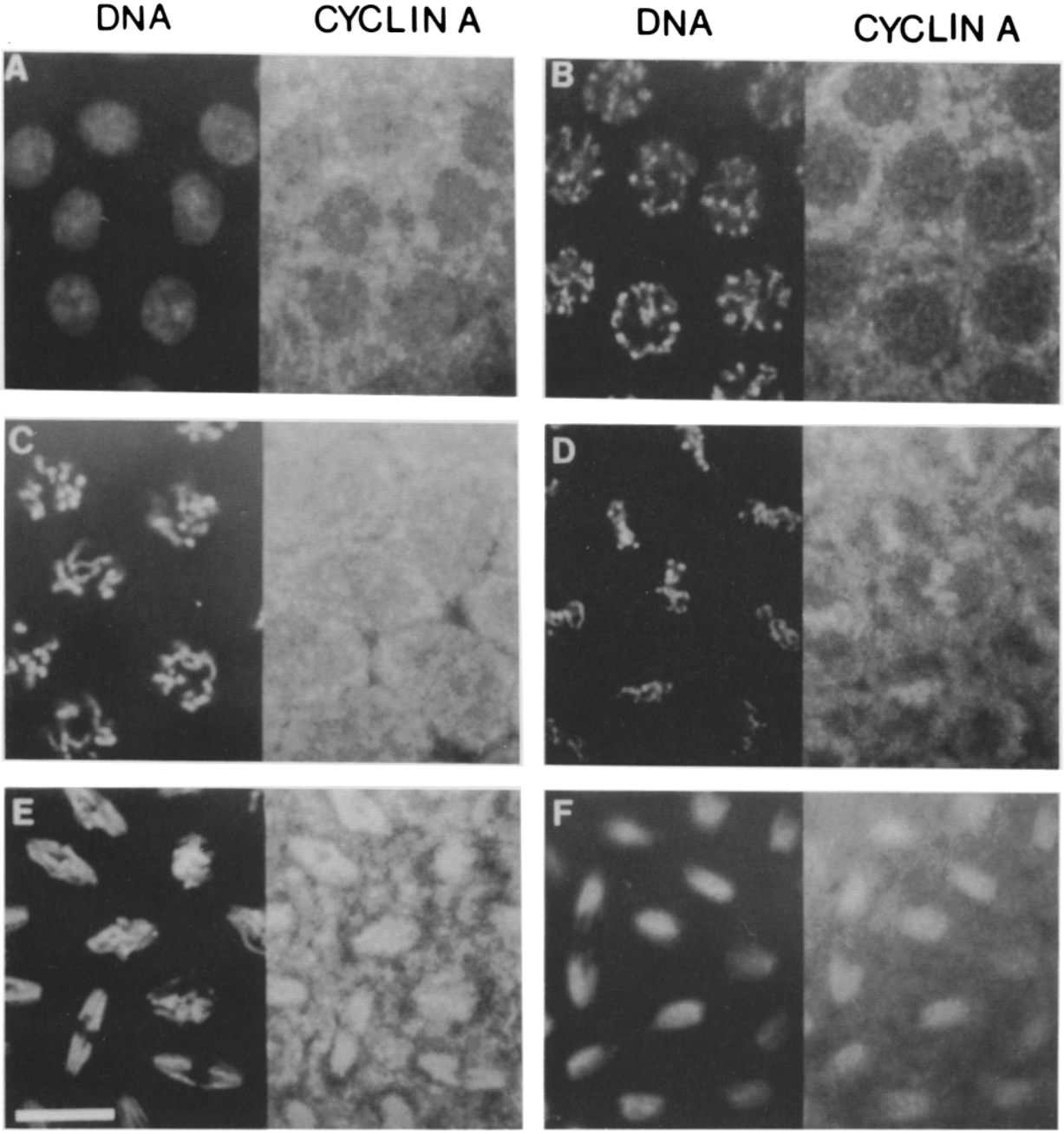

Figure 6. Distribution of cyclin A during the mitotic cycle. The left and right hand sides of each panel show DNA stained with propidium iodide, and cyclin A revealed by indirect immunofluorescence using the RB270 antibody (Whitfield et. al., 1990), respectively. *A–F* show the following phases of mitosis: (*A*) interphase; (*B*) prophase; (*C*) late prophase; (*D*) metaphase; (*E*) anaphase; (*F*) late anaphase-early telophase. Each paired micrograph is an optical section taken at a similar depth of 4–5 μm. Bar, 10 μm.

7), although on occasion we have seen some cytoplasmic staining together with single punctate nuclear distribution (not shown). When such cycloheximide embryos are stained with the antibody RB188 which recognizes the centrosomal Bx63 antigen (Whitfield et al., 1989), we do not see discrete centrosomes, but instead the nuclear staining that is normally given by this antibody at interphase is accentuated. Western blotting of extracts from cycloheximide-treated embryos indicates that the levels of cyclin B (Fig. 7) and cyclin A (not shown) are not significantly diminished for up to 90 min after injection of the drug. The normal cell cycle length at this stage is between ~10 and 14 min. As cycloheximide prevents the de novo protein synthesis, we infer that neither cyclin is completely degraded following these conditions of cell cycle arrest.

Discussion

Do the different distributions of the two cyclins which we describe in the syncytial *Drosophila* embryo suggest they are targeting $p34^{cdc2}$ kinase to specific subcellular locations? We cannot address this question directly because antibodies

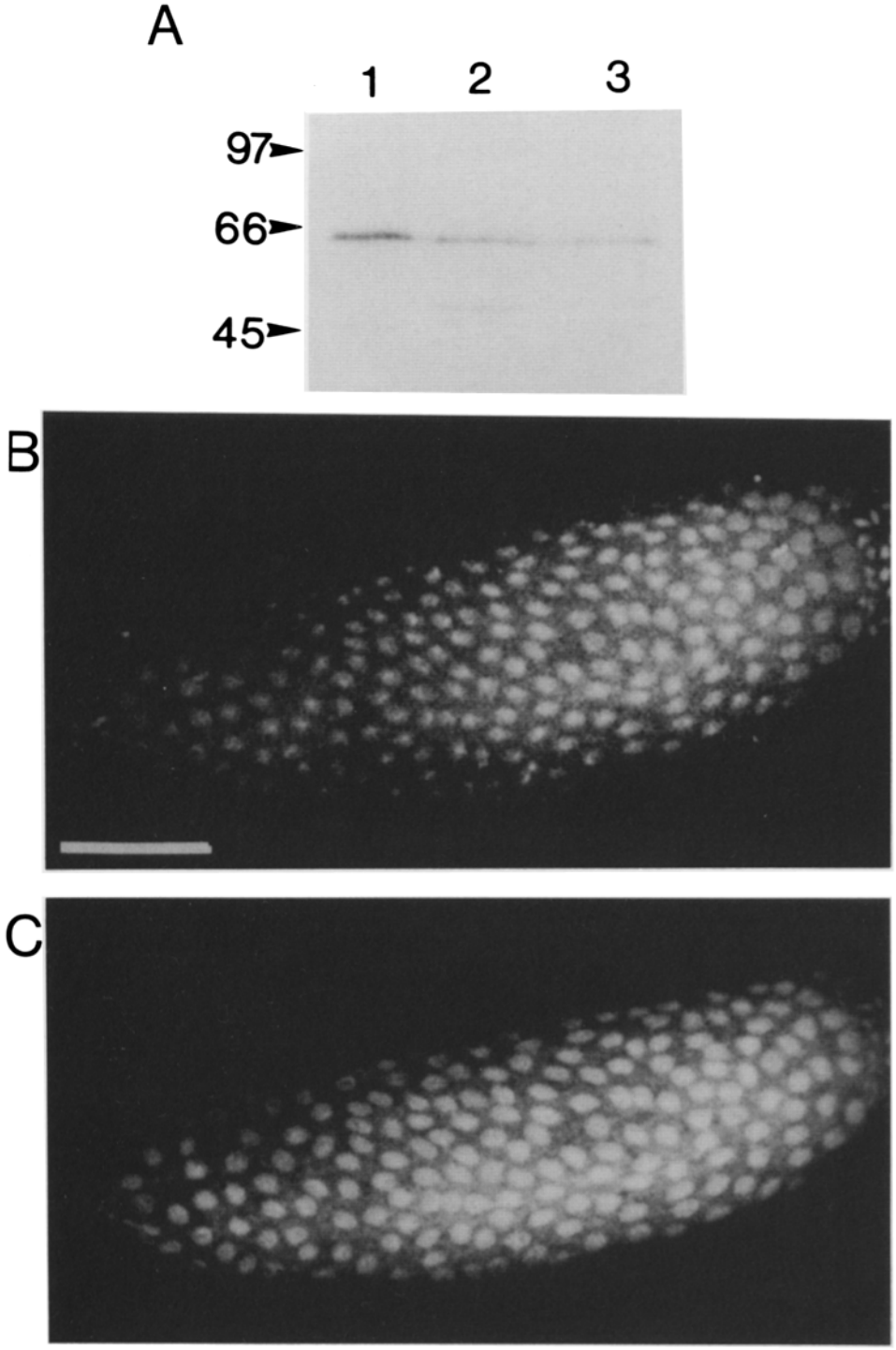

Figure 7. Cyclin B remains high in cycloheximide-arrested embryos. *A* shows a Western blot probed with rabbit anticyclin B antiserum (Whitfield et al., 1990). Lane 1 contains an extract of 0–4 h embryos, and shows that the antibody recognizes a major band of 65 kD. Lane 2 contains an extract of 20 hand-picked control embryos at division cycle 9 or 10 otherwise processed in the same way as cycloheximide-injected embryos. An extract from hand-picked 20 embryos injected with cycloheximide at nuclear cycle 9 or 10 is shown in lane 3. A volume of drug corresponding to ~1/50 egg volume was injected at a concentration of 100 μg/ml. Extracts were made 90 min after drug injection. A cycloheximide-injected embryo stained with propidium iodide to reveal DNA is shown in *B*. The staining of this same embryo with rabbit anti-cyclin B (primary) and fluorescein-labeled goat anti-rabbit (secondary) antibodies is shown in *C*. Bar, 100 μm.

such as those directed against the conserved "PSTAIRE" domain of $p34^{cdc2}$ recognize many proteins in *Drosophila* embryos. We are currently raising antibodies to *Drosophila* $p34^{cdc2}$, but as there is a second gene encoding a similar protein (Lehner and O'Farrell, 1990), the specificity of such antibodies will need careful characterization. The ability of antibodies raised against conserved regions of $p34^{cdc2}$ to recognize other molecules associated with cyclins has been demonstrated in human cells by Pines and Hunter (1990*b*). Moreover, the distribution of $p34^{cdc2}$ is likely to be complex since it can exist alone and also in association with other molecules, such as the G1 cyclins, so far best characterized in yeast (Hadwiger et al., 1989; Richardson et al., 1989; Wittenberg et al., 1990). Nevertheless, in spite of the complexity of $p34^{cdc2}$ distribution that we might now expect, earlier experiments have shown similar patterns of its distribution to those we now present for the two mitotic cyclins in *Drosophila*. In particular, localization of cdc2 at centrosomes in mammalian cells was previously noted by two groups (Riabowol et al., 1989; Bailly et al., 1989). Indeed,

universal targeting of cyclin B to centrosomes and spindle microtubules is suggested not only from our present observations, but also from recent immunostaining experiments in fission yeast reported by Alfa et al. (1990), and observations on the association of cyclin B with the mitotic apparatus in mammalian cells (Pines and Hunter, 1991). An interaction of cyclin B with microtubules was first suggested by the work of Booher and Beach (1988) who observed that a mutation in the gene for the cyclin B homologue of *S. pombe* (*cdc13-117*) caused the mutant strain to become hypersensitive to thiabendazole, an inhibitor of tubulin polymerization. The behavior of microtubules is tightly coupled to the G2-M transition, and several inhibitors of tubulin polymerization are well known to block the cell cycle in a metaphase-like state with no mitotic spindle. In systems in which the cyclins normally undergo cyclical degradation, blocking the cycle with drugs that destabilize microtubules causes cyclin B to accumulate to high levels (Westendorf et al., 1989; 1990; Minshull et al., 1989). The degradation of cyclin A on the other hand is not affected by such a block. The precise role of cdc2 kinase in modifying microtubules is not clear. However, Verde et al. (1990) have shown that the long, rapidly growing microtubules nucleated by centrosomes in interphase extracts of *Xenopus* eggs become shorter and have slower elongation rates characteristic of mitotic extracts in proportion to the amount of cdc2 kinase added. Taken together, the available data thus suggests that cyclin B may act in vivo to target $p34^{cdc2}$ kinase to the astral microtubules where it would play an essential role in modifying microtubule dynamics upon formation of the mitotic spindle.

We do not suggest that cyclin B-cdc2 complex would be targeted solely to microtubules, and indeed the anti-cyclin B antibody is seen to give staining throughout the apical cytoplasm, although it is concentrated around the polar regions of the spindle. It seems significant that the breakdown of the envelopes of the syncytial blastoderm nuclei first begins on their apical side as indicated by the disruption of lamin staining. Phosphorylation of the lamins is thought to be a critical step in the mitotic depolymerization of the network formed by these proteins underlying the inner nuclear membrane. $p34^{cdc2}$ kinase has been shown to phosphorylate lamins in vitro on sites that become phosphorylated at M-phase in vivo (Peter et al., 1990; Ward and Kirschner, 1990), leading to the suggestion that the lamins are a direct substrate of cdc2 kinase used in mitosis. Thus this predicted substrate of $p34^{cdc2}$ kinase appears to be preferentially degraded in those regions of the syncytial embryos in which we observe the highest concentration of cyclin B. This role for the cyclin B–cdc2 kinase complex has also been suggested by Pines and Hunter (1991) by their observations that whereas cyclin A is associated with the nuclei of human cells as early as S-phase, cyclin B enters the nucleus immediately preceding nuclear lamin breakdown.

The observed concentration of cyclin B in the vicinity of microtubules is very dependent upon their preservation by the fixation conditions used before immunostaining. Microtubules in *Drosophila* embryos are particularly unstable, but can be preserved by the brief inclusion of taxol at the time of fixation without leading to any significant artefactual taxol-mediated microtubule nucleation (Karr and Alberts, 1986; Kellogg et al., 1988). If care is not taken to preserve microtubules, then the centrosomal and spindle association of cyclin B may not be seen. This was the case with our previous observations of the cyclin B distribution in cellularized embryos and neuroblasts in which we took no steps to preserve the mitotic apparatus (Whitfield et al., 1990). We now know that with careful preparation of tissue it is also possible to see cyclin B in the vicinity of the spindle poles.

The subcellular distribution of cyclin A suggests it has a different targeting role to cyclin B. Like cyclin B it does not undergo cyclical rounds of complete degradation in the syncytial embryo, but instead, cyclin A appears to shuttle between nucleus and cytoplasm. In human cells, cyclin A has also been observed to accumulate in the nucleus but this begins much earlier in the cycle during S-phase (Pines and Hunter, 1991), whereas we only see the nuclear association during prophase. This difference in timing may reflect the differences in cycle length in these two systems and the absence of a G2 phase in the syncytial embryo. We note that after cellularization cyclin A does accumulate in early prophase nuclei in a manner more analogous to that seen in mammalian cells (see Fig. 3 of Whitfield et al., 1990).

Suggestions that cyclin A might have a role in S-phase gain support from the observations of the protein in S-phase nuclei by Pines and Hunter (1991). Our observations of nuclear association of cyclin A during S-phase in the syncytial *Drosophila* embryo are very much less striking, but nevertheless, we do see some weak punctate nuclear staining. A role for $p34^{cdc2}$ kinase-cyclin complexes in DNA replication is suggested by the work of d'Urso and Roberts (1990) who have purified an extract from proliferating mammalian cells that when added to an extract of G1 phase cells confers replication competence. This extract contains cdc2 kinase and A and B-type cyclins. The physiological significance of these findings is not clear. The presence of cyclin A in S-phase nuclei need not necessarily reflect its codistribution with $p34^{cdc2}$ kinase. Indeed, there is evidence from Pines and Hunter (1990*b*) that cyclin A can associate with another protein apparently related to cdc2, in which case it may be acting as a targeting subunit for several proteins. Furthermore, cyclin A has also been found to be associated with the Ela gene product of adenovirus in virally transformed mammalian cells (Pines and Hunter, 1990). If it can act as a targeting subunit for several proteins in *Drosophila*, then this would further complicate the interpretation of the staining patterns we observe.

The stability of the two cyclins in the metaphase-anaphase transition in the syncytial *Drosophila* embryo contrasts to their cyclical degradation in later development. The early embryonic mitoses of the *Drosophila* embryo are unusual in their rapidity, their lack of G1 and G2 phases, and in their lack of feedback controls to ensure that M-phase does not occur until S-phase is completed (Raff and Glover, 1988). It is as though the syncytial cycles are permanently cycling M phases to which DNA replication is only loosely coupled. Feedback controls are introduced together with the first G2 phase during cycle 14 and only now does the significant degradation of the two cyclins occur. M-phase is followed immediately by S-phase for cycles 14–16 (Edgar and O'Farrell, 1990). This suggests that in the newly cellularized embryo, cyclin A is not needed, at least for the entry into S-phase. Perhaps the degradation of the two cyclins following the 14th mitosis ensures that the mitotic form of cdc2 kinase is completely inactivated thus preventing immediate

reentry into M-phase after the completion of S-phase as would occur in the syncytium. The ability to undergo rapid successive mitotic cycles in the syncytial embryo might be imagined to be a consequence of the "constitutive" activity of both mitotic cyclins and the mitotic activator *string*, in which case the persistence of the cyclins throughout the syncytial cycles may indicate that the cdc2 complex is permanently "primed" for activity. What then controls cdc2 kinase activity in these rapid cycles? We can only speculate that this is some modification to the kinase itself, most probably its phosphorylation state which is known to regulate activity (see Nurse, 1990). As it is not possible to synchronize the development of a population of *Drosophila* embryos, we are at present unable to undertake a biochemical analysis of the phosphorylation state of cdc2 kinase in order to address this issue.

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