Brief Report

Mitotic Regulators Govern Progress through Steps in the Centrosome Duplication Cycle

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Abstract. Centrosome duplication is marked by discrete changes in centriole structure that occur in lockstep with cell cycle transitions. We show that mitotic regulators govern steps in centriole replication in *Drosophila* embryos. Cdc25^{string}, the expression of which initiates mitosis, is required for completion of daughter centriole assembly. Cdc20^{fizzy}, which is required for the metaphase-anaphase transition, is required for timely disengagement of mother and daughter centrioles. Stabilization of mitotic cyclins, which prevents exit from mitosis, blocks assembly of new daughter centrioles. Common regulation of the nuclear and centrosome cycles by mitotic regulators may ensure precise duplication of the centrosome.

Key words: centrosome • duplication • cell cycle • coupling • *Drosophila*

A microtubule nucleator, the centrosome, lies at each pole of the mitotic spindle where it contributes to the organization and orchestration of mitosis (Mazia, 1984). Chromosome missegregation and aneuploidy can result because of aberrant numbers of centrosomes at mitosis (Sluder and Begg, 1985; Sluder and Rieder, 1985b; Sluder et al., 1986). Hence, it is important that cells have two centrosomes at mitosis.

Centrosome number is maintained through cell generations by the centrosome duplication cycle. Interphase *Drosophila* cells have two centrosomes (unlike mammalian cells) as a result of centrosome splitting during mitotic exit (Callaini and Riparbelli, 1990; Callaini et al., 1997) (see Fig. 5). Cylindrical microtubular structures, called centrioles, lie at the heart of the otherwise amorphous centrosome (Sluder and Rieder, 1985a; Sluder et al., 1989; Maniotis and Schliwa, 1991). Distinct transitions in centriole structure underlie centrosome duplication and occur in step with major cell cycle transitions (Robbins et al., 1968; Kuriyama and Borisy, 1981; Vorobjev and Chentsov Yu, 1982; Alvey, 1985; Callaini and Riparbelli, 1990; Callaini et al., 1997) (see Fig. 2 C). In *Drosophila* embryos, at metaphase, the two centrioles within each centrosome are close and perpendicular to each other. During mitotic exit, the centrioles of a pair, invariably separate (henceforth referred to as disengagement), with each centriole organizing a centrosome. Daughter centriole assembly, which initiates during S phase, produces a procentriole that is shorter and lacks the detailed structural features of the mature centriole. Acquisition of the mature structure and size (completion of daughter centriole assembly) occurs upon entry into mitosis. The transitions in the centriole structure might be coupled to cell cycle transitions, which would ensure duplication of the centrosome precisely once in the cell cycle.

Cell cycle transitions are triggered by changes in cyclin-dependent kinase (Cdk)[1] function. Entry into mitosis is triggered by Cdk1 associated with mitotic cyclins A, B, and B3 (Murray and Kirschner, 1989; Lehner and O'Farrell, 1989, 1990; Knoblich et al., 1994; Jacobs et al., 1998), and exit from mitosis requires destruction of mitotic cyclins (Murray et al., 1989; Glotzer et al., 1991; Sigrist et al., 1995). The postblastoderm cycles, which follow cellularization of the embryo, lack a G1 phase but have a regulated G2. Entry into mitosis is regulated by expression of the Cdc25^{string} phosphatase (Edgar and O'Farrell, 1989, 1990) (see Fig. 1), which activates Cdk1 (Kumagai and

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1. *Abbreviations used in this paper:* APC, ANAPHASE promoting complex; Cdk, cyclin-dependent kinase; VE, ventral epidermis.

Dunphy, 1991; Edgar et al., 1994b). Exit from mitosis requires Cdc20^{fizzy}, which activates the anaphase promoting complex (APC) (King et al., 1996; Sigrist et al., 1995). The APC targets cyclins and other substrates for destruction (King et al., 1996) (see Fig. 5) via recognition of a destruction box sequence, whose deletion renders the corresponding substrate stable (Glotzer et al., 1991). Expression of nondegradable mitotic cyclins blocks mitotic exit without blocking destruction of other APC substrates (Sigrist et al., 1995).

We have asked whether steps in the centrosome cycle require activity of mitotic regulators. We report that completion of daughter centriole assembly requires Cdc25^{string} function, timely centriole disengagement requires Cdc20^{fizzy}, and initiation of centriole replication requires downregulation of mitotic cyclins. These requirements for mitotic regulators might result from or might produce the coupling of the centrosome cycle to the cell cycle.

Materials and Methods

Fixation and Antibody Staining

Embryos were fixed (37% formaldehyde) and stained with purified polyclonal anti–γ-tubulin (made against a peptide present in the maternal isoform of γ-tubulin [QIDYPQESPAVEASKAG]), anti–β-tubulin antibody (1:100 dilution; Amersham). Fluorescent secondaries were used at 1:300 (Jackson ImmunoResearch Laboratories, Inc.). DNA was stained with 10 μg/ml of bisbenzamid (Hoechst 33258). Fluorescence was observed on a Leica DMRD or an Olympus 1X70 microscope. Photographs were taken on the Leica using a Photometrics CH250 CCD and 35-mm cameras. Three-dimensional data sets of centrosomes were collected using Deltavision software (Applied Precision, Inc.), based on an Olympus 1X-70 microscope with a Photometrics PXL-cooled CCD camera. Data sets were projected onto a single plane and exported to Adobe Photoshop for further processing.

Heat Induction

Embryos were collected for 2 h from a stock carrying transgenes encoding nondegradable versions of the *Drosophila* mitotic cyclins, cyclin A and cyclin B (homozygous for both transgenes) (Sprenger et al., 1997). After aging the collection for 2 h at 25°C, stable cyclins were induced by floating the agar collection plates on 37°C water for 30 min (heat shock). The embryos were allowed to recover for 1–2 h and fixed.

string and *fizzy* Mutants

string7B (Edgar and O'Farrell, 1989) and *fizzy1* (Dawson et al., 1993; Sigrist et al., 1995) are amorphic alleles.

Preparation of Embryos for EM

Embryos were prepared for EM by high pressure freezing, followed by freeze substitution (McDonald, 1994), or by slight modifications of a glutaraldehyde fixation protocol (Callaini and Riparbelli, 1990; Callaini et al., 1997) (*string* and *fizzy* mutants). Thin serial sections (80–90 nm) cut on a Leica ultramicrotome were stained and examined on a Philips EM 400 at 80 kV, and the images were recorded on film. Though only one section is shown, all centrioles were reconstructed from thin serial sections to assess separation and orientation of centrioles.

Embryos were stained with Hoechst and homozygous *fizzy* mutant embryos were identified based on their abnormally high mitotic index during late stage 11 and stage 12. *string* embryos were identified by a low cell density and the absence of mitotic figures.

Cell cycle stages in wild-type embryos, prepared for EM, were identified based on the morphology of the associated nuclei. Cells with condensing/condensed chromatin not fully aligned at the metaphase plate were identified as prophase; cells with a mitotic spindle and aligned chromosomes as metaphase; cells containing decondensed chromatin in nuclei that are separated away from each other as telophase; and cells with intact nuclear membranes containing decondensed chromatin as interphase cells.

Results

Maturation of Daughter Centrioles Requires Cdc25^{string}

Despite differences in lengths of G2, cells enter mitosis with only two centrosomes, suggesting that centrosome replication is coupled to cell cycle progress, or that centrosome replication is independently, yet appropriately, regulated (e.g., by a developmental timer) (Edgar et al., 1994a). To distinguish between these possibilities, we monitored centrosome duplication in *string* mutant embryos that arrest in G2 but continue to develop. Arrested cells can be driven into mitosis by expression of Cdc25^{string} transcribed from a heat shock–inducible promoter on a transgene (Edgar and O'Farrell, 1990).

After delaying cells in G2 beyond their normal time of mitosis, we induced expression of Cdc25^{string}. β-Tubulin staining of the resultant mitotic cells (see Fig. 2 C) revealed no significant spindle abnormalities (99.4% bipolar, n = 1,350) (data not shown). Immunostaining for γ-tubulin revealed two foci of staining, one at each pole of 42 preanaphase spindles (see Fig. 2 D). In the absence of coupling, we would expect to see four centrosomes in cells that had remained in G2 beyond their normal time of en-

Table I.

Mutant/perturbation	Embryonic phenotype	Centriolar phenotype
Fig. 2	Cells arrest in G2 of cycle 14	Two centriole pairs per cell: >95%
Cdc25^{string} mutants	while development continues well after the time of mitosis 14	of pairs have incompletely assembled daughters (n = 22)
Fig. 3	<5% of cells in VE in metaphase (late stage 11)	Centriole pairs disengage after metaphase
Wild type (late stage 11)		
Cdc20^{fizzy} mutants (late stage 11)	>80% of cells in VE held up in metaphase	62% of centriole pairs at the spindle poles are engaged (n = 21)
Cdc20^{fizzy} mutants (early stage 12)	>95% of cells in VE in mitosis	100% centriole pairs disengaged
Cdc20^{fizzy} mutants (midstage 12)	>95% of cells in VE in mitosis	100% centriole pairs disengaged
Fig. 4	Most epidermal cells arrested in mitosis 14	100% centriole pairs disengaged (n = 17)
Heat shock promoter driven ectopic expression of stable cyclins, A and B	beyond their normal time of mitotic exit	and 100% centrioles not associated with procentrioles (n = 39)
Control wild-type embryos exposed to heat shock	Development and cell cycle progression continues	Procentriole formation not perturbed

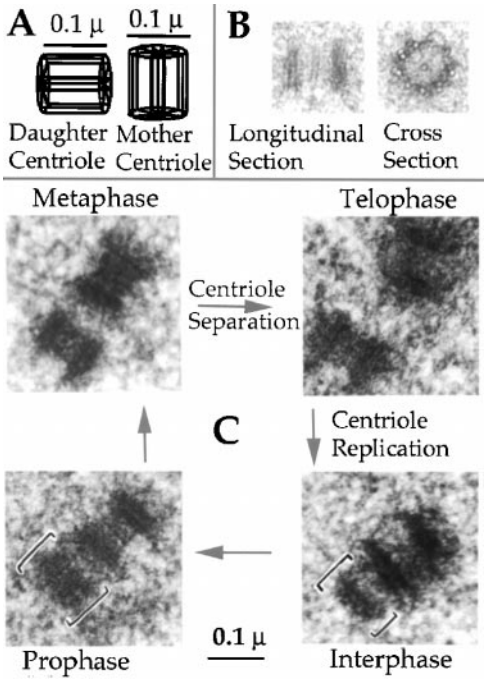

Figure 1. Centriole duplication cycle. (A) A schematic of *Drosophila* centrioles, which are 105 nm in diameter (measured as the distance between the complete inner tubules) and ~110 nm in length (measured as the length of the tubules), and separated by a distance of 46–58 nm. (B) EM longitudinal and cross-sectioned centrioles obtained from high pressure frozen *Drosophila* embryos. Note that the strongly staining aspect of a centriole in longitudinal thin section appears as parallel lines representing the opposite sides of the cylindrical centriole. (C) The centriole pair undergoes discrete transitions during the cell cycle. Mother and daughter centrioles disengage and move apart as cells exit metaphase. Centriole appearance of a procentriole coincides with entry into S phase, and this daughter (brackets) is complete only upon entry into mitosis.

try into mitosis. We conclude that centrosome duplication does not continue during a G2 arrest.

To assess the ultrastructure of the centrioles during the G2 arrest, we examined *string* mutant embryos by EM. In 21 out of 22 centriole pairs (Table I), the daughters were significantly shorter than the mothers and their tubular fine structure was not obvious (e.g., compare Fig. 2 E to wild-type mitotic centrioles in Fig. 1). We conclude that Cdc25^{string} is required, directly or indirectly, for the completion of daughter centriole assembly.

Depleting fizzy Delays Centriole Separation

We investigated for a role of the APC in the centrosome cycle by examining centrioles in embryos mutant for the APC activator, Cdc20^{fizzy}. *fizzy* embryos exhibit mitotically arrested cells in the ventral epidermis (VE) during cell cycle 16 (Dawson et al., 1993; Sigrist et al., 1995). However, morphogenesis continues, providing a timer to estimate the duration of the mitotic arrest.

We scored centriole pairs as engaged, if they were at right angles to each other and closely spaced (48–56 nm). In late stage 11 *fizzy* embryos, >80% of cells in the VE are arrested at metaphase (Fig. 3, A–D, and Table I) and 62% of the metaphase cells (n = 21, from two embryos) contained engaged centrioles (Fig. 3 G). If centriole disengagement was unperturbed in *fizzy* mutants, only cells traversing metaphase should have engaged centrioles. Since fewer than 5% of the VE cells are normally in metaphase at any time (in wild type), <5% of the cells in *fizzy* mutant embryos would be traversing metaphase. Since ~50% of cells in the VE contained orthogonal centrioles (62% of the metaphase cells), we conclude that centriole disengagement is delayed in metaphase cells arrested as a result of Cdc20^{fizzy} depletion.

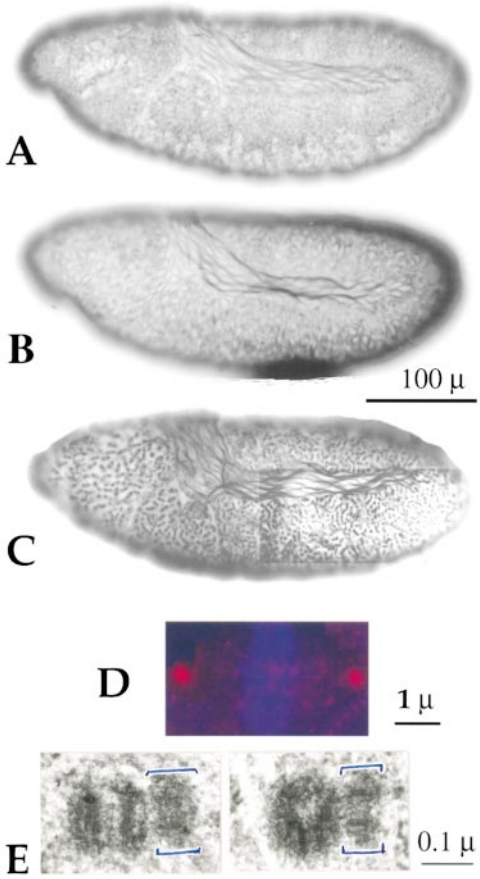

Figure 2. Arrest of centriole maturation in *string* embryos. Embryos are oriented anterior to the left and dorsal up, and are stained for β-tubulin (A–C), or for DNA (Hoechst) and γ-tubulin (D). (A) A wild-type embryo, in which interphase cells show cytoplasmic staining, whereas mitotic cells show a clear cytoplasm and dense spindle staining. Most cells are interphase of cycle 16. (B) A *string* embryo in which cells are arrested in G2 of cycle 14. (C) A *string* embryo after induction of a Cdc25^{string} transgene shows synchronous mitoses after a 3-h arrest in G2. By this time, cells in wild-type embryos have undergone mitosis 14. The spindles in these induced mitoses are bipolar (99.4%, n = 1,350). (D) Preanaphase (metaphase shown) figures in this induced mitosis have one centrosome at each spindle pole. (E) Centriole pairs in arrested *string* embryos have an incomplete daughter centriole (21/22 serially sectioned centrioles from two embryos). The immature daughter is bracketed in the examples shown. Compare the immature daughter centrioles in *string* mutant embryos to the daughter centrioles of wild-type centriole pairs in mitosis (Fig. 1).

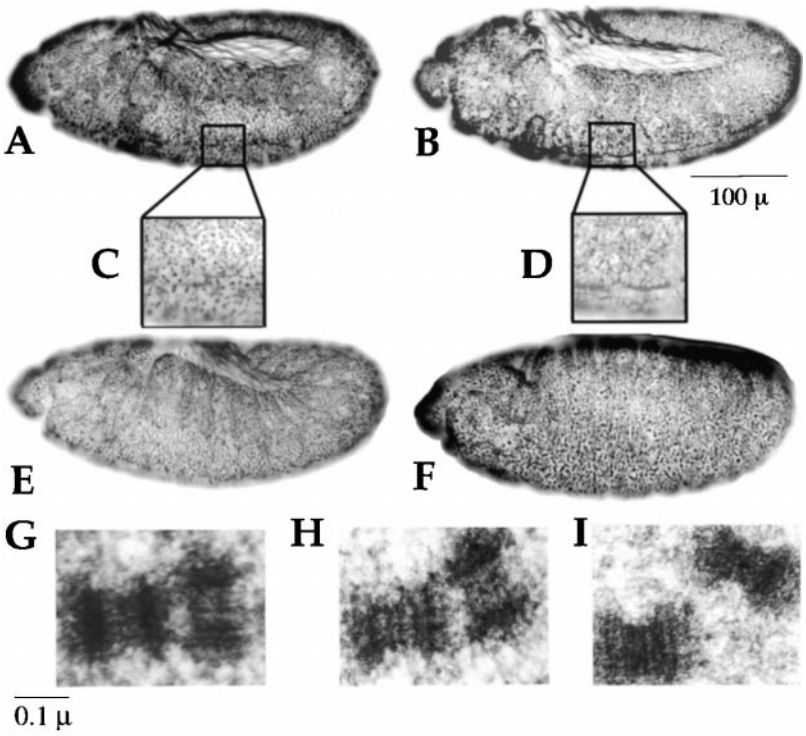

Figure 3. Centriole disengagement is delayed in *fizzy* mutant embryos. *fizzy* and wild-type (sevelen) embryos stained for β-tubulin are tilted (dorsal away) slightly for visualization of arrested (ventral) cells. (A and C) late stage 11 *fizzy* embryos have >80% of ventral epidermis (VE) cells in metaphase versus <5% in wild-type embryos shown in B and D. (E and F) early (E), and midstage 12 (F) *fizzy* embryos have most VE cells in mitosis, whereas there are virtually no mitotic cells in the epidermis of wild-type embryos at stage 12 (not shown). G–I show examples of centrioles in the *fizzy* mutant at late stage 11 (G), early stage 12 (H), and midstage 12 (I). During late stage 11, 62% of serially sectioned and reconstructed centriole pairs were orthogonal (n = 21, from two embryos), while at the later stages, 100% of centriole pairs were disengaged. At midstage 12, they had separated slightly.

Older *fizzy* embryos exhibited disengaged centrioles. In early stage 12 embryos, corresponding to ~30 min of arrest, all observed mother and daughter centrioles appeared disengaged but not widely separated (Fig. 3, E and H). During midstage 12, corresponding to ~1 h of arrest, all observed centriole pairs had disengaged and separated (Fig. 3, F and I). Thus, disengagement and separation of centrioles, events that normally occur within a couple of minutes after metaphase, occur more slowly in the *fizzy* mutant. We conclude that Cdc20^{fizzy} is required, directly or indirectly, for timely centriole disengagement.

Persistent Mitotic Cyclin Blocks Centriole Replication

We monitored the centrosome cycle upon expression of stable cyclins, which blocks some aspects of mitosis. Destruction box and NH_2 terminally deleted forms of both cyclins A and B (Sprenger et al., 1997) were ectopically expressed when transgenic embryos were exposed to heat shock. Entry into mitosis occurred at the normal time in these embryos, but cells failed to exit mitosis (Sigrist et al., 1995) (Fig. 4, A and B). Immunostaining for γ-tubulin revealed no more than two centrosomes at each pole (50% had two centrosomes, 23% had one, and 27% had one elongated centrosome, n = 51 from two embryos) (Fig. 4 C).

To explore the status of centrioles, we examined stable cyclin-expressing embryos by EM. Centrioles at 17 spindle poles had disengaged with varying degrees of separation (between 80 nm to 1 μm), consistent with the distribution of γ-tubulin foci. Importantly, all observed centrioles (n = 39; Table I), were present as singlets, i.e., were not associated with daughter centrioles (Fig. 4 D). Possible heat shock effects independent of transgene induction (Debec et al., 1990) were investigated by monitoring events in the centrosome cycle upon heat shock of nontransgenic embryos. Both cell cycle progression and procentriole formation continued after heat shock (data not shown). We conclude that stabilization of mitotic cyclins can, directly or indirectly, inhibit production of procentrioles.

Discussion

Cell doubling requires the coordination of many cellular events including DNA replication and centrosome duplication. We show that the activities of mitotic regulators influence progress of the centrosome cycle in addition to the nuclear cycle (Fig. 5). Cdc25^{string}, which induces entry into mitosis, is required for the completion of daughter centriole assembly. Cdc20^{fizzy} triggers chromosome separation by APC-dependent degradation of inhibitors and is required for timely centriole disengagement. Destruction of cyclins at mitosis is required for synthesis of new daughter DNA strands and elaboration of a new daughter centriole. By genetically arresting cell cycle progress at specific points and identifying a correspondingly specific arrest of the centrosome cycle, we have resolved multiple points of coupling between the cell cycle and the centrosome cycle. Each point of coupling might represent direct action of the cell cycle regulator on the centrosome cycle, or a checkpoint induced as a consequence of the cell cycle arrest. Ultimately, available genetic and molecular tools ought to allow identification of the specific events responsible for the observed coupling.

Three Points of Coupling to Mitosis

In *Drosophila* embryos, a procentriole appears during S phase, but its assembly is completed during mitosis (Cal-

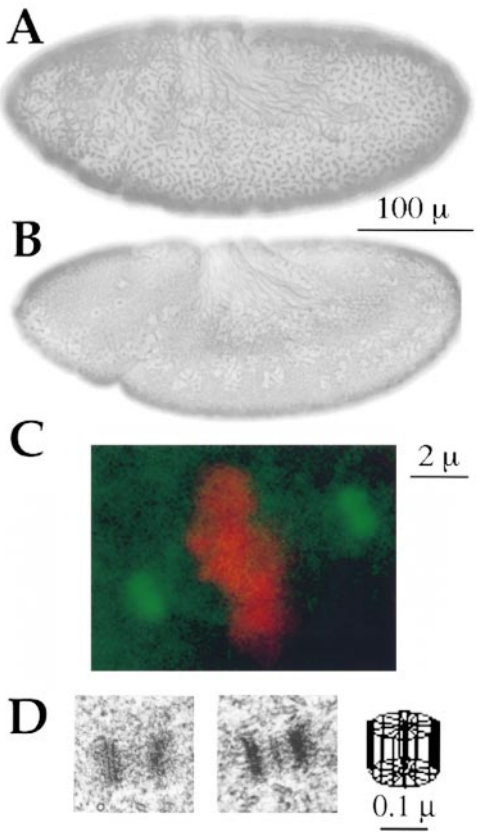

Figure 4. Procentriole formation is inhibited during mitotic arrest induced by expression of stable cyclins A and B. (A and B) β-tubulin–stained embryos. (A) An embryo fixed 2 h after induced expression of stable versions of cyclins A and B. Most epidermal cells are arrested in mitosis. (B) A wild-type embryo at a similar stage of development. (C) High magnification view of cell arrested by expression of stable cyclins. The centrosomes are highlighted by staining for γ-tubulin (green) and DNA is in red. These mitotic cells revealed no more than two centrosomes at each pole (n = 51) (see text). Serial sections reconstruction of these centrosomes (n = 17) by EM revealed that centrioles had disengaged and were not associated with a procentriole (n = 39). As an example, C shows two singlet centrioles from one pole of a stable cyclin arrested mitotic spindle. Note that the pair of strongly staining lines represent the tubules at the opposite wall of one centriole, as is depicted in the cartoon on the right.

laini et al., 1997). We found that Cdc25^{string} function, the expression of which drives cells into mitosis, is required for the completion of daughter centriole assembly. To our knowledge, this is the first indication that a G2 arrest does not support daughter centriole maturation. However, it is possible that centriole maturation continues at a slow rate in the absence of Cdc25^{string} function and is accelerated upon its expression. The centriole maturation induced by Cdc25^{string} might be secondary to the activation of mitotic cyclin/Cdk1 and subsequent mitotic events. For example, since several centrosomal proteins are recruited to the nucleus during interphase, nuclear membrane breakdown at mitosis might stimulate centriolar growth by releasing previously sequestered components.

The EM analysis of *fizzy* embryos reveals that disengagement and separation of mother and daughter centrioles are delayed: disengagement for ~10 times the length of metaphase. Requirement for Cdc20^{fizzy} in timely centriole disengagement could reflect a corresponding requirement for degradation of an APC substrate that inhibits centriole disengagement. The block to centriole disengagement might be incomplete because residual maternal Cdc20^{fizzy} could slowly promote centriole disengagement in mitotically arrested cells. Alternatively, centriole disengagement might be timed by activation of Cdc20^{fizzy}, while not absolutely requiring its function. For example, slow Cdc20^{fizzy}-independent separation might reflect APC-independent turnover of an inhibitor of centriole disengagement.

The mitotic cyclins are degraded in a *fizzy*-dependent fashion at the mitotic exit (Sigrist et al., 1995), and could be candidates for the inhibitors of centriole disengagement. However, cyclins A and B persist in *fizzy* mutant embryos at times when we observe disengagement (Sigrist et al., 1995). Additionally, ectopic expression of stabilized cyclins A and B does not block centriole disengagement but inhibits assembly of new procentrioles. Similar results have been obtained upon injection of stable cyclin B into sea urchin embryos (Hinchcliffe et al., 1998). Inhibition of procentriole formation during mitosis may reflect a requirement for nuclear envelope formation (say for nuclear sequestration of inhibitors) or dephosphorylation of certain key proteins upon downregulation of mitotic cyclin/

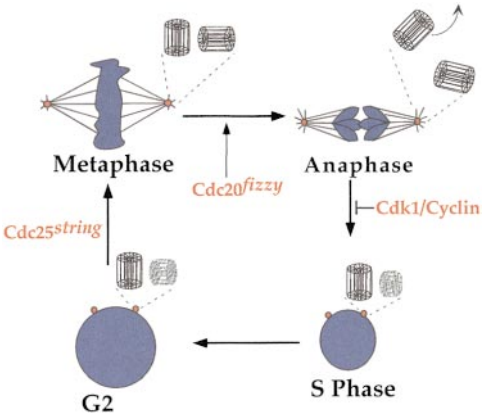

Figure 5. Centrosome, centriole, and nuclear cycles in *Drosophila* embryos. Schematic showing chromatin in blue, centrosomes in red, and centrioles as segmented cylinders. Activation of Cdc20^{fizzy}-dependent degradation triggers the metaphase-anaphase transition and allows exit from mitosis. As cells progress through mitosis, mother and daughter centrioles disengage and separate and centrosomes split into two. Unlike mammalian cells, each interphase cell contains two centrosomes. The postblastoderm mitoses (of cycles 14 and 15) are followed immediately by the S phase, during which procentrioles form. G2 cells enter mitosis upon developmentally controlled expression of Cdc25^{string}. Completion of daughter centriole assembly occurs as cells progress to metaphase. Our results show that Cdc25^{string} is required for completion of daughter centriole assembly, Cdc20^{fizzy} is required for timely centriole disengagement and mitotic cyclin/Cdk1 needs to be downregulated for procentriole formation.

Cdk1. We infer that in an unperturbed cell, downregulation of mitotic cyclin/Cdk1 is essential for procentriole formation.

How might these observed points of coupling be enforced? Cell cycle regulators could contribute rather indirectly to progression through the centrosome cycle. In the yeast *Saccharomyces cerevisiae*, spindle pole body duplication is coordinated with the nuclear cycle by cell cycle–specific transcription of spindle pole body components (Kilmartin et al., 1993). Two of the three points of coupling that we have uncovered in *Drosophila* operate within mitosis, during which there is no transcription (Shermoen and O'Farrell, 1991). Hence, mitotic maturation of daughter centrioles and disengagement of centriole pairs are apparently regulated posttranscriptionally. In contrast, procentriole formation might require new gene expression and would be deferred until transcription resumes upon mitotic exit. While such a mechanism is possible, it would be short circuited by the persistence of maternal gene products, which appears to play a significant role in the progression of the postblastoderm cell cycles.

Centrosome Duplication in Other Systems

The present work demonstrates coupling of three steps in the centrosome duplication cycle to transitions in the cell cycle. However, uncoupled centrosome duplication has been observed in early embryonic systems and tissue culture cell lines in the absence of visible cell cycle progression (Sluder and Lewis, 1987; Gard et al., 1990; Balczon et al., 1995; Hinchcliffe et al., 1998; Lacey et al., 1999). These disparate observations could be explained if coupling is conserved, but unseen local oscillations of activities of cell cycle regulators (maybe even at the centrosome) drive the centrosome cycle. Alternatively, perhaps the coupling itself is differentially regulated in various species and at different stages of development. Thus, the syncytial cell cycles (that precede the postblastoderm cell cycles) may exhibit regulation of the centrosome cycle similar to sea urchin and *Xenopus*. One mechanism that could account for regulated coupling is regulated expression of inhibitors that block specific steps in the centrosome cycle which, in turn, are then inhibited by cell cycle regulators.

Cyclin E has been shown recently to be required for centrosome duplication (Hinchcliffe et al., 1999; Lacey et al., 1999; Matsumoto et al., 1999). During the postblastoderm cell cycles, cyclin E/Cdk2 activity is continually high (Sauer et al., 1995). Our data do not address roles for cyclin E in *Drosophila*, but indicate that its continuous activity is not sufficient for continued centrosome duplication in the absence of the requirements that we have uncovered.

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References

Alvey, P.L. 1985. An investigation of the centriole cycle using 3T3 and CHO cells. *J. Cell Sci.* 78:147–162.

Balczon, R., L. Bao, W.E. Zimmer, K. Brown, R.P. Zinkowski, and B.R. Brinkley. 1995. Dissociation of centrosome replication events from cycles of DNA synthesis and mitotic division in hydroxyurea-arrested Chinese hamster ovary cells. *J. Cell Biol.* 130:105–115.

Callaini, G., and M.G. Riparbelli. 1990. Centriole and centrosome cycle in the early *Drosophila* embryo. *J. Cell Sci.* 97:539–543.

Callaini, G., W.G. Whitfield, and M.G. Riparbelli. 1997. Centriole and centrosome dynamics during the embryonic cell cycles that follow the formation of the cellular blastoderm in *Drosophila*. *Exp. Cell Res.* 234:183–190.

Dawson, I.A., S. Roth, M. Akam, and S. Artavanis-Tsakonas. 1993. Mutations of the fizzy locus cause metaphase arrest in *Drosophila* melanogaster embryos. *Development.* 117:359–376.

Debec, A., A.M. Courgeon, M. Maingourd, and C. Maisonhaute. 1990. The response of the centrosome to heat shock and related stresses in a *Drosophila* cell line. *J. Cell Sci.* 96:403–412.

Edgar, B.A., and P.H. O'Farrell. 1989. Genetic control of cell division patterns in the *Drosophila* embryo. *Cell.* 57:177–187.

Edgar, B.A., and P.H. O'Farrell. 1990. The three postblastoderm cell cycles of *Drosophila* embryogenesis are regulated in G2 by *string*. *Cell.* 62:469–480.

Edgar, B.A., D.A. Lehman, and P.H. O'Farrell. 1994a. Transcriptional regulation of string (cdc25): a link between developmental programming and the cell cycle. *Development.* 120:3131–3143.

Edgar, B.A., F. Sprenger, R.J. Duronio, P. Leopold, and P.H. O'Farrell. 1994b. Distinct molecular mechanism regulate cell cycle timing at successive stages of *Drosophila* embryogenesis. *Genes Dev.* 8:440–452.

Gard, D.L., S. Hafezi, T. Zhang, and S.J. Doxsey. 1990. Centrosome duplication continues in cycloheximide-treated *Xenopus* blastulae in the absence of a detectable cell cycle. *J. Cell Biol.* 110:2033–2042.

Glotzer, M., A.W. Murray, and M.W. Kirschner. 1991. Cyclin is degraded by the ubiquitin pathway. *Nature.* 349:132–138.

Hinchcliffe, E.H., G.O. Cassels, C.L. Rieder, and G. Sluder. 1998. The coordination of centrosome reproduction with nuclear events of the cell cycle in the sea urchin zygote. *J. Cell Biol.* 140:1417–1426.

Hinchcliffe, E.H., C. Li, E.A. Thompson, J.L. Maller, and G. Sluder. 1999. Requirement of Cdk2-cyclin E activity for repeated centrosome reproduction in *Xenopus* egg extracts. *Science.* 283:851–854.

Jacobs, H.W., J.A. Knoblich, and C.F. Lehner. 1998. *Drosophila* cyclin B3 is required for female fertility and is dispensable for mitosis like cyclin B. *Genes Dev.* 12:3741–3751.

Kilmartin, J.V., S.L. Dyos, D. Kershaw, and J.T. Finch. 1993. A spacer protein in the *Saccharomyces cerevisiae* spindle poly body whose transcript is cell cycle–regulated. *J. Cell Biol.* 123:1175–1184.

King, R.W., R.J. Deshaies, J.M. Peters, and M.W. Kirschner. 1996. How proteolysis drives the cell cycle. *Science.* 274:1652–1659.

Knoblich, J.A., K. Sauer, L. Jones, H. Richardson, R. Saint, and C.F. Lehner. 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.

Kumagai, A., and W.G. Dunphy. 1991. The cdc25 protein controls tyrosine dephosphorylation of the cdc2 protein in a cell-free system. *Cell.* 64:903–914.

Kuriyama, R., and G.G. Borisy. 1981. Centriole cycle in Chinese hamster ovary cells as determined by whole-mount electron microscopy. *J. Cell Biol.* 91: 814–821.

Lacey, K.R., P.K. Jackson, and T. Stearns. 1999. Cyclin-dependent kinase control of centrosome duplication. *Proc. Natl. Acad. Sci. USA.* 96:2817–2822.

Lehner, C.F., and P.H. O'Farrell. 1989. Expression and function of *Drosophila* cyclin A during embryonic cell cycle progression. *Cell.* 56:957–968.

Lehner, C.F., and P.H. O'Farrell. 1990. The roles of *Drosophila* cyclins A and B in mitotic control. *Cell.* 61:535–547.

Maniotis, A., and M. Schliwa. 1991. Microsurgical removal of centrosomes blocks cell reproduction and centriole generation in BSC-1 cells. *Cell.* 67: 495–504.

Matsumoto, Y., K. Hayashi, and E. Nishida. 1999. Cyclin-dependent kinase 2 (Cdk2) is required for centrosome duplication in mammalian cells. *Curr. Biol.* 9:429–432.

Mazia, D. 1984. Centrosomes and mitotic poles. *Exp. Cell Res.* 153:1–115.

McDonald, K.L. 1994. Electron microscopy and EM immunocytochemistry. *Methods Cell Biol.* 44:411–444.

Murray, A.W., and M.W. Kirschner. 1989. Cyclin synthesis drives the early embryonic cell cycle. *Nature.* 339:275–280.

Murray, A.W., M.J. Solomon, and M.W. Kirschner. 1989. The role of cyclin synthesis and degradation in the control of maturation promoting factor activity. *Nature.* 339:280–286.

Robbins, E., G. Jentzsch, and A. Micali. 1968. The centriole cycle in synchronized HeLa cells. *J. Cell Biol.* 36:329–339.

Sauer, K., J.A. Knoblich, H. Richardson, and C.F. Lehner. 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 P.H. O'Farrell. 1991. Progression of the cell cycle through mitosis leads to abortion of nascent transcripts. *Cell.* 67:303–310.

Sigrist, S., H. Jacobs, R. Stratmann, and C.F. Lehner. 1995. Exit from mitosis is regulated by *Drosophila fizzy* and the sequential destruction of cyclins A, B and B3. *EMBO (Eur. Mol. Biol. Organ.) J.* 14:4827–4838.

Sluder, G., and D.A. Begg. 1985. Experimental analysis of the reproduction of spindle poles. *J. Cell Sci.* 76:35–51.

Sluder, G., and C.L. Rieder. 1985a. Centriole number and the reproductive capacity of spindle poles. *J. Cell Biol.* 100:887–896.

Sluder, G., and C.L. Rieder. 1985b. Experimental separation of pronuclei in fertilized sea urchin eggs: chromosomes do not organize a spindle in the absence of centrosomes. *J. Cell Biol.* 100:897–903.

Sluder, G., and K. Lewis. 1987. Relationship between nuclear DNA synthesis and centrosome reproduction in sea urchin eggs. *J. Exp. Zool*. 244:89–100.

Sluder, G., F.J. Miller, and C.L. Rieder. 1986. Centrosomes are required for the assembly of a bipolar spindle in animal cells. *Annu. NY Acad. Sci.* 466:669–673.

Sluder, G., F.J. Miller, and C.L. Rieder. 1989. Reproductive capacity of sea urchin centrosomes without centrioles. *Cell Motil. Cytoskeleton*. 13:264–273.

Sprenger, F., N. Yakubovich, and P.H. O'Farrell. 1997. S-phase function of *Drosophila* cyclin A and its downregulation in G1 phase. *Curr. Biol.* 7:488–499.

Vorobjev, I.A., and S. Chentsov Yu. 1982. Centrioles in the cell cycle. I. Epithelial cells. *J. Cell Biol.* 93:938–949.