

# Checkpoints: Controls That Ensure the Order of Cell Cycle Events

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The events of the cell cycle of most organisms are ordered into dependent pathways in which the initiation of late events is dependent on the completion of early events. In eukaryotes, for example, mitosis is dependent on the completion of DNA synthesis. Some dependencies can be relieved by mutation (mitosis may then occur before completion of DNA synthesis), suggesting that the dependency is due to a control mechanism and not an intrinsic feature of the events themselves. Control mechanisms enforcing dependency in the cell cycle are here called checkpoints. Elimination of checkpoints may result in cell death, infidelity in the distribution of chromosomes or other organelles, or increased susceptibility to environmental perturbations such as DNA damaging agents. It appears that some checkpoints are eliminated during the early embryonic development of some organisms; this fact may pose special problems for the fidelity of embryonic cell division.

THE CELL CYCLE IS OFTEN CONSIDERED TO BE COMPOSED of four phases, the gap before DNA replication ( $G_1$ ), the DNA synthetic phase (S), the gap after DNA replication ( $G_2$ ), and the mitotic phase, which culminates in cell division (M). Although this formulation is useful and can serve as an organizing principle, the cell cycle is seen on closer examination to be more complex. A large number of macromolecular components are assembled, activated, or moved; a sequence of events involving one organelle (such as the centrosome) may occur throughout all four stages of the cell cycle, and independent sequences are coordinated with one another.

Biochemical, genetic, and cytological research in the last decade has greatly increased our appreciation of the structural and functional complexity involved in numerous cell cycle processes such as DNA replication (1, 2), chromosome organization (1, 3), centrosome duplication and movement (4), the dynamic organization of the mitotic spindle (5), chromosome movement on the spindle (6), nuclear envelope breakdown and reformation (7), organelle duplication and distribution (8), and the establishment of the site of cell division (9). Each of these processes involves cellular organelles that are present in small numbers and whose accurate reproduction and

distribution is important for cell viability. While executing these events, the cell must avoid or correct errors that lead to the production of nonfunctional organelles and those that lead to the production of or distribution of the wrong number of organelles.

Recent results comparing somatic and embryonic cell cycles (10) have revealed basic similarities as well as striking differences in how events are controlled. In the early embryonic cell divisions of *Xenopus*, the initiation and order of events of the cell cycle is determined by cyclic activation of maturation promoting factor (MPF), and the events occur independently of one another. This subject is reviewed by Murray and Kirshner in this issue of *Science* (11). In the cell cycle of yeast and the somatic divisions of many metazoan organisms, an additional principle appears to operate. Although MPF plays an initiating role in the somatic cell cycle, the order of events is ensured by dependent relationships; the initiation of late events is dependent on the completion of early events. We think dependent relationships seen in somatic cells are a key element in understanding the high fidelity of organelle reproduction and distribution during cell division. The purpose of this article is to consider how these dependent relationships are achieved and the consequences incurred by the cell upon their elimination.

## Dependent Relationships in the Cell Cycle

The existence of dependent relationships is not usually apparent simply by observing the normal cell cycle. Dependencies are revealed by perturbations of specific events, that is, by the application of chemicals, by the study of mutants that specifically inhibit one event in the cell, or by surgical and cell fusion techniques (12). For example, specific chemical inhibition of DNA replication prevents nuclear division and cell division in most cell types including bacteria, fungi, and vertebrate somatic cells. Mutants that block specific events in the cell cycle provide additional resolution of dependent relationships. In the yeasts *Saccharomyces cerevisiae* (13) and *Schizosaccharomyces pombe* (14), many mutants have been isolated and characterized that appear to have defects at specific stages of the cell cycle. In *S. cerevisiae*, temperature-sensitive mutations exist that block bud formation, spindle pole body enlargement and division (15), spindle pole body separation and migration (16), tubulin assembly (16), spindle elongation, initiation of DNA replication, DNA elongation (17), DNA ligation (18), chromatin assembly (19), bipolar association of chromosomes (20), sister chromatid decatenation (21), sister chromatid separation (22), nuclear division, and cytokinesis (13). The phenotypes of the mutants suggest that most of these events are ordered into a few dependent pathways. For example, the sequence of events that encompass spindle pole body duplication and segregation as well as those comprising chromo-

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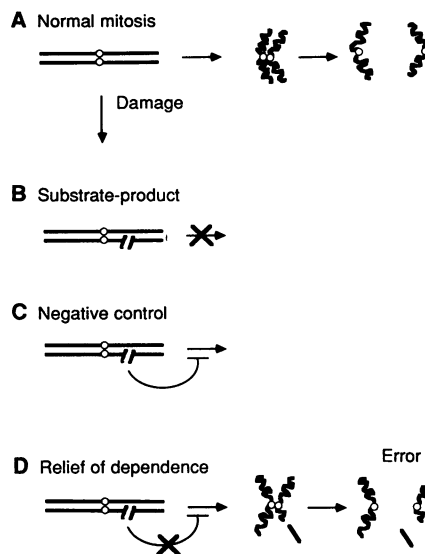
some replication and segregation constitute one dependent pathway.

What principles does the cell use to establish an ordered pathway of events? Does the existence of such order imply the existence of control mechanisms that enforce order? Since many of the events of interest in the cell cycle are those involving the assembly of macromolecular complexes, it may be informative to consider the principles that have been gleaned from the extensive investigations of another case of macromolecular assembly—the formation of a bacteriophage particle (23). Bacteriophage T4 is constructed from three components—the head, tail, and tail fibers—each of which is assembled by an invariant pathway. All structural proteins are synthesized at the same time, and unassembled proteins remain unassociated until the partially assembled structure becomes ready for their addition. At this stage, reactive sites that accommodate the assembly of the next component are created by the addition of the previous protein in the assembly pathway. For example, tail tube subunits do not associate with themselves until the baseplate is assembled; at this stage some tail tube subunits associate with the baseplate, and these provide a seed for the polymerization of additional identical subunits to form a long helical polymer. Similarly, assembly of the bacteriophage T4 baseplate itself occurs by an invariant pathway where each step utilizes the previous step as substrate and in turn provides the substrate for the next step (24). The important lesson from bacteriophage assembly is that a very complicated series of morphogenetic events can be ordered by a principle intrinsic to the components themselves without requirement for extrinsic control mechanisms. We shall refer to this type of ordered pathway as substrate-product order.

The maturation of bacteriophage  $\lambda$  DNA during packaging into the phage head provides an example of dependence due to a control mechanism. Concatameric DNA is not cut by the enzyme terminase, unless phage proheads are present to package the DNA. This dependence of DNA cutting on the presence of proheads could have been enforced by substrate-product order if terminase was activated by binding to proheads; however, this is not the mechanism, rather a trans-acting inhibitor prevents terminase from cutting in the absence of proheads, and this dependence on proheads can be relieved by eliminating the inhibitor (25).

Likewise, the dependency of late events in the cell cycle on the completion of early events may be due to either substrate-product order or to a control mechanism (Fig. 1). If, for example, replicated chromosomes are essential substrates for mitosis, then the dependence is due to substrate-product order; alternatively, if the dependence is due to an inhibitor of mitosis produced in response to unreplicated chromosomes, then we would say the dependence is due to control. Control might also be exerted by an activator; for example, completion of DNA synthesis might produce an activator of mitosis. Since it is difficult to distinguish control by activation from substrate-product order by an empirical test, we will concentrate our discussion of control mechanisms on those that act by inhibition. We use the term control to include regulation at any level: transcription, translation, or posttranslation.

How can one distinguish extrinsic control by inhibition from substrate-product order? The existence of a control mechanism is suggested when one finds chemicals, mutants, or other conditions that relieve a dependent relationship; that is, conditions that permit a late event to occur even when an early, normally prerequisite event, is prevented. We term such an observation “relief of dependence.” This argument rests on the assumption that if dependence is intrinsic to the mechanism of assembly as it is for the bacteriophage T4 tail, then one would not be likely to relieve this dependence by mutational inactivation of a gene product. For example, one would not likely find a mutation that would relieve the need for an early protein in the assembly sequence [however, see (26)]. Similarly, if



**Fig. 1.** Models illustrating the dependence of mitosis on replicated and undamaged DNA. (A) A replicated, undamaged chromosome passes through chromosome condensation and segregation. (B, C, and D) A chromosome sustaining a double-strand break is illustrated; however, a gap or a single-strand lesion remaining after DNA replication would be expected to have the same consequences. (B) The broken chromosome is an inadequate substrate for chromosome condensation (or for some later step) and mitosis is blocked. (C) The DNA break creates a signal that inhibits chromosome condensation. (D) A mutation (like *rad9*) eliminates the negative inhibition so the damaged chromosome passes through mitosis and an error occurs.

the dependence of nuclear division on DNA replication in the cell cycle is due to an intrinsic requirement of replicated chromosomes in the nuclear division machinery, we would not expect to relieve this dependence by mutation. We are aware of the fact that this empirical criterion cannot be taken as rigorous evidence for the existence of a control mechanism. One can imagine rare mutations that will alter a protein in such a way as to permit it to assemble without its normal substrate, but most mutations will eliminate a function. We suggest that a finding of “relief of dependence” provides *prima facie* evidence for control, especially when the mutation is shown to eliminate the function of a protein. By the relief of dependence criterion, a number of control mechanisms have recently been identified in the cell cycle. We call these control mechanisms checkpoints (27), because they appear to have the role of checking to see that prerequisites (such as DNA replication in the case of mitosis) have been properly satisfied.

## Checkpoints in the Cell Cycle

We discuss in this section a few cases where sufficient evidence exists to suggest that the dependence of a late event in the cell cycle on an early event is due to a checkpoint. We will describe first in some detail a control mechanism that we have worked on, the *RAD9* system in yeast, which is responsible for making mitosis dependent on the completion of DNA replication, and then discuss more briefly a few other control systems.

**Dependence of mitosis on DNA synthesis.** In yeast, mammalian tissue culture cells, *Aspergillus*, and many other eukaryotic organisms, arrest of DNA synthesis by specific inhibitors or by mutational inactivation of replication enzymes prevents mitosis. In yeast, the *RAD9* gene specifies a component of this control system (27).

Temperature-sensitive mutants defective in some DNA replication functions (DNA polymerase I, *cdc17*; DNA polymerase III, *cdc2*; and DNA ligase, *cdc9*) do not normally undergo mitosis at the restrictive temperature. Dependency of mitosis on the completion of DNA synthesis is relieved, however, by a complete deficiency of *RAD9*; if these same *cdc* mutants have a *rad9* gene defect, then the cells continue through mitosis into the next cell cycle at the restrictive temperature (28). A mutant temperature-sensitive for

DNA ligase illustrates the effect of the *RAD9* checkpoint (Fig. 2). The analysis exploits the fact that in *S. cerevisiae* different phases of the cell cycle are accompanied by distinctive changes in daughter bud morphology. During incubation at the restrictive temperature for 3 hours,  $G_1$  (unbudded) cells containing the *cdc9* mutation became blocked before mitosis (large budded cells) (Fig. 2, A and B). Arrested cells had completed the bulk of DNA synthesis [confirmed by analysis of DNA content by flow cytometry (27)], but become arrested presumably because many unligated single-strand lesions remain in the DNA (28). In contrast, *cdc9*  $G_1$  cells that also have the *rad9* defect typically proceed past mitosis and enter the next cell division at the restrictive temperature (Fig. 2, C and D). Analysis of the nuclear morphology of growing cells shifted to the restrictive temperature confirms that *cdc9* cells are blocked before chromosome separation in mitosis (Fig. 2E), whereas *cdc9-rad9* cells are not arrested, but display a distribution of cells in different phases of the cell cycle.

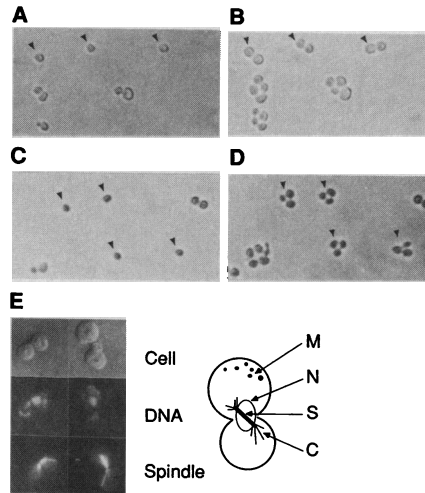
The *cdc9-rad9* double mutant illustrates a principle that we think may apply to many checkpoints, that elimination of the checkpoint may have catastrophic or subtle consequences depending on prevailing conditions. Temporary inactivation of DNA ligase activity is not lethal for most cells, as shown by the ability of *cdc9* mutant cells to retain viability after a brief incubation at the restrictive temperature. In the absence of *RAD9*, however, DNA ligase-deficient cells die much more rapidly at the restrictive temperature (Fig. 3) (29). Therefore, relief of the dependence of mitosis on DNA synthesis is lethal when completion of DNA replication is blocked. Alternatively, if cells are not perturbed by an interruption of DNA replication (or by extrinsic DNA damage, see below), then *RAD9* is not an essential function; cells have indistinguishable growth properties whether the *RAD9* gene is intact or defective (27). The only effect of complete deficiency of the *RAD9* checkpoint is that *rad9* cells lose chromosomes spontaneously at a rate 21 times higher than that of wild-type cells [rate of loss of one chromosome from a disome strain;  $6.0 \times 10^{-4}$  in a *rad9* deletion and  $2.9 \times 10^{-5}$  in *Rad*<sup>+</sup> (27)]. We imagine that these two phenotypes, cell death or chromosome loss, are extremes of the same primary role of this checkpoint, to ensure that chromosomes have been fully replicated and are intact before mitosis is initiated. Thus the order of DNA synthesis and mitosis is apparently established independently of the *RAD9* checkpoint, but the *RAD9* product ensures the order if DNA synthesis is interrupted. An attractive hypothesis to explain how *RAD9* inhibits mitosis is to suggest that it negatively regulates a function essential for mitosis. It will be of interest to determine whether the *RAD9* gene product interacts with MPF or other components known to play essential roles in the initiation of mitosis.

The *RAD9* control system was initially identified in a search for mutations that permit cell division of cells with defective genomes due to damage induced by x-irradiation (27). Mutations in the *RAD9* gene allow cells with DNA damage to proceed through cell division, whereas irradiated wild-type cells arrest in  $G_2$  until the damage is repaired. Mitosis in most other eukaryotic cells (30) is also dependent on undamaged DNA, since x-irradiation and other DNA damaging agents arrest cells before mitosis. The dependence of mitosis on completion of DNA replication is relieved in mammalian somatic cells by caffeine (31). Like *rad9* defects in *S. cerevisiae*, caffeine treatment of irradiated mammalian cells permits their entry into mitosis (and decreases cell viability) and yet has no observable effect on this transition in unirradiated cells (32).

Temperature-sensitive recessive mutations in mouse BHK cells (*tsBN2*) and *Aspergillus* (*bimE7*) qualify as checkpoints since both relieve dependency of mitosis on DNA synthesis (33). In both mutants, cells blocked in DNA synthesis enter mitosis without completing DNA synthesis when mutant cells are shifted to the

restrictive temperature. Death of cells carrying these mutations is probably not due solely to relief of dependence, as *bimE7* mutants shifted to the restrictive temperature are arrested in mitosis. The cellular functions are unknown, but deficiency for either must render inactive a checkpoint that prevents mitosis should DNA synthesis be blocked. Both are suggested to be negative regulators of additional functions essential for mitosis.

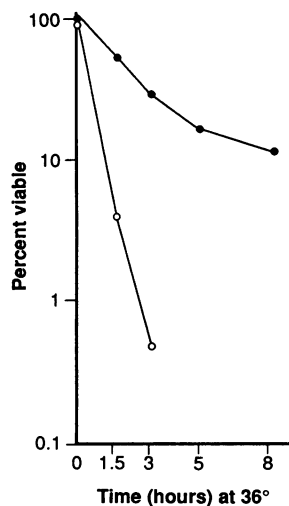
*Dependence of anaphase on metaphase and delay as evidence of a checkpoint.* In the examples of dependence cited above, inhibition of



**Fig. 2.** DNA ligase-defective cells are arrested before mitosis only if the *RAD9* gene is present. Progression in the cell cycle of individual cells was determined by photomicroscopy. (A and B) *cdc9* and (C and D) *cdc9-rad9* cells were grown at the permissive temperature then plated on thin agar slabs. Photographs of the same fields were made at the time cells were initially shifted to the restrictive temperature for DNA ligase (A and C) and 3 hours later (B and D). Unbudded cells have not replicated

DNA and require ligase activity in the first cell cycle. The fate of  $G_1$  (unbudded) cells was quantitated by analysis of several fields; 78% (49 out of 63) of  $G_1$  *cdc9* cells are arrested with one large bud, whereas only 21% (12 out of 57) of  $G_1$  *cdc9-rad9* cells are arrested with one large bud. Most *cdc9-rad9*  $G_1$  cells (79%, 45 out of 57) generated at least a third bud indicating the original  $G_1$  cell had proceeded through mitosis and one progeny cell had initiated bud formation (and DNA synthesis) in the next cell cycle. The fates of cells that were budded at the time of ligase inactivation were also analyzed [note three in (A) and two in (C)]. Most of the budded cells have completed replication and do not require ligase activity in the first cell cycle but do require ligase for the second cell cycle. Eighty percent of budded *cdc9* cells generate two cells each arresting with one large bud, whereas 45% (45 out of 99) of budded *cdc9-rad9* cells generate two cells each with one large bud and 55% (54 out of 99) proceed to a subsequent cell cycle. Combining data from unbudded and budded cells 79% of *cdc9* cells and only 36% of *cdc9-rad9* cells are blocked as large budded cells after 3 hours. Cytological examination (described below) shows that even the few *cdc9-rad9* cells that are arrested with one large bud have proceeded past mitosis, whereas most of the *cdc9* cells have arrested at mitosis. About 40% of the *cdc9-rad9* cells at the time of plating were inviable and did not change their shape after 3 hours [note unchanged large budded cell in the corner of (C) and (D)]; these cells were excluded from quantitative analysis. (E) DNA ligase inactivation results in the arrest of cells before mitosis. *cdc9* cells grown at the permissive temperature (23°C) were shifted to restrictive temperature (36°C) for 3 hours, fixed, and analyzed for cell (top), nuclear (middle), and microtubule (bottom) morphology. Two cells are shown. M, mitochondria; N, nucleus; S, spindle; C, cytoplasmic microtubules. Cell morphology was determined by differential-interference-contrast microscopy and nuclear and microtubule morphologies, by epifluorescence from the DNA-binding dye DAPI (4',6-diamidino-2-phenylindole dihydrochloride) and from FITC-conjugated antibodies to antibodies to tubulin, as described (38). The position of the nuclei and the presence of a short spindle at the neck of each large budded cell has been described (36). When cells were shifted from growth at 23°C to the restrictive temperature for *cdc9*, the percentage of cells with DNA at the neck of a large budded cell increased from 11 to 84% for *cdc9* and only from 11 to 32% for *cdc9-rad9*. As expected, the distribution of cells in other parts of the cell cycle showed that most *cdc9* cells were arrested before mitosis, whereas most *cdc9-rad9* cells were not arrested before mitosis (27). Strains: 598-3 *MAT $\alpha$* , *cdc9-8 ura3 ade2 ade3 leu1 can1 cyh2 sap3 trp1 SCE::URA3* (provided by L. Kadyk), and 7851-3-2 *MAT $\alpha$  cdc9-8 rad9::TRP1 trp1 ura3 leu2 ade3 ade2 his3 leu1*. Both are congenic with A364a. The *RAD9* deletion we constructed by deleting 93% of the internal coding sequence and inserting a DNA fragment containing the *TRP1* gene, and will be described in detail elsewhere.





**Fig. 3.** Rapid loss of viability in cells defective both for DNA ligase and for the *RAD9* gene. *cdc9* (●) and *cdc9-rad9* (○) cells growing at 23°C were shifted to the restrictive temperature and viability was determined by plating for viable colonies at the permissive temperature (23°C). The cell viability reported is relative to viability at the time of temperature shift. Results were reproducible in separate experiments and with other congenic strains.

an early event resulted in complete arrest of a late event. It is clear in other cases that inhibition of an early event merely delays a late event. Unless careful studies are carried out the delay might be overlooked. In cases where delay occurs, a checkpoint may exist. The delay in the late event indicates its dependence on the early event; moreover, the eventual occurrence of the late event in the absence of the early event indicates that relief of dependence eventually occurs spontaneously making it unlikely that dependence is due to a substrate-product relationship.

A control mechanism is suggested by the observation that when a chromosome lags in finding its way to the metaphase plate, anaphase is delayed, often until the lagging chromosome arrives at the metaphase plate (34). Perhaps a similar checkpoint is present in yeast cells, since cell division is greatly delayed in those divisions at which loss of centromere-containing plasmids occurs (35); the delay of division occurs at a specific stage in the cell cycle probably corresponding to mitosis.

**Dependence of centrosome duplication on DNA synthesis.** In yeast (36) and mammalian cells (37), inhibition of DNA synthesis by temperature-sensitive mutations in replication enzymes or by aphidicolin, respectively, arrest spindle pole body or centrosome duplication at a stage characteristic of metaphase. A mutation of yeast, *esp1*, blocks DNA replication and nuclear division but not spindle pole body duplication, so that nuclei accumulate with as many as eight spindle pole bodies (38); thus, this essential gene plays a role in preventing spindle pole body duplication in the absence of DNA synthesis.

**Dependence of replicon initiation on other replicons.** In mammalian cells large contiguous regions of DNA, corresponding roughly to the cytogenetic bands of chromosomes, replicate coordinately as a result of the fact that large arrays containing as many as a hundred replicons initiate replication synchronously (39). The initiation of DNA synthesis has been found to be exquisitely sensitive to single-strand lesions (40); one single-strand break may inactivate initiation in as many as a hundred replicons, presumably those adjacent to one another. This result is surprising, since the chromosome is thought to be organized into topological domains corresponding roughly to single replicons. The coordinate inhibition of initiation in clusters of replicons is likely due to a control mechanism, since cells from individuals with the genetic disease, ataxia telangiectasia, a syndrome predisposing individuals to cancer, are resistant to this inhibition (41). In these cells dependency of DNA synthesis on intact template is relieved and might be the cause of radiation sensitivity. In unirradiated wild-type cells, this control may prevent reinitiation of any unligated strands remaining from the previous S phase; replication of a chromatid containing a single-strand lesion

would generate a double-strand break leading to the production of an acentric chromosome fragment.

**Dependence of DNA reinitiation on mitosis.** Mammalian cells inhibited in mitosis by colchicine (42) or mutations (43) delay for many hours but eventually reconstitute an interphase nucleus and replicate their DNA without completing chromosome segregation. These observations may identify a checkpoint that makes reinitiation of DNA replication dependent on mitosis. This dependence has been reproduced in vitro in extracts of *Xenopus* eggs where added DNA is assembled into a nucleus and replicates once (44); nuclei can rereplicate without completing mitosis if they are treated with agents that make them permeable, an observation that has suggested a specific model for this dependence (45).

**Dependence of mitosis on growth.** The product of the *WEE1* gene of *S. pombe* is not essential, but its presence delays mitosis; deletion of *WEE1* leads to cells that are smaller than normal at mitosis, and increased dosage leads to proportionately larger cells at mitosis (46). This observation may identify a checkpoint that integrates growth and division (11).

**Checkpoints in bacteria.** In *Escherichia coli*, cell division is dependent on completion of DNA replication (47) and on the presence of undamaged DNA (48) as well. The SOS regulatory system of *E. coli*, like the *RAD9* system of yeast, is responsible for the arrest (dependency) of cell division in response to an inhibition of DNA replication and in response to DNA damage (49). DNA damage is recognized (directly or indirectly) by the RecA protein, which in its activated form stimulates proteolytic cleavage of the LexA protein, an inhibitor of *SulA* gene transcription. *SulA* protein inhibits cell division possibly by inhibiting the FtsZ protein. Null mutations of *SulA* are insensitive to division arrest by DNA damage, thus meeting the criterion of a checkpoint. Deficiency for *SulA* generates a higher frequency of anucleate cells when DNA synthesis is inhibited than is the case for wild-type cells (50).

A checkpoint that couples F plasmid replication and segregation to *E. coli* cell division has been identified (51). A gene, *CadB*, carried by stable mini-F plasmids inhibits *E. coli* cell division when replication of the plasmid is inhibited. Cells containing plasmids lacking this function produce plasmid-free cells at much higher frequencies than those containing this function.

## Some Embryonic Cell Cycles Lack Some Checkpoints

Many experimental results suggest that some early embryonic cell cycles are controlled differently than somatic cell cycles (11); the differences may be attributable to the existence of fewer checkpoints in some embryonic cell cycles.

Perhaps the most definitive evidence for a difference in the control of the cell cycle between somatic cells and some embryonic systems arises from the consequences of inhibiting DNA synthesis. As discussed above, chromosome condensation, elaboration of the mitotic spindle, and cytokinesis are all prevented in yeast and other somatic cells when DNA synthesis is inhibited. However, in *Drosophila* one or more aberrant nuclear divisions may occur after inhibition of DNA synthesis (52); centrosomes continue dividing for many divisions up to the time of cellular blastoderm (53), and, although cytokinesis is not completed, the egg surface undergoes periodic budding. Thus, some of the events of cell division are being activated. In early *Xenopus* embryos (54), cell division continues at a normal rate after inhibition of DNA synthesis until the mid-blastula transition; anucleate cells are the products of these divisions.

Some embryonic cells differ from somatic cells in their response to broken DNA. Although somatic cells arrest in G<sub>2</sub> in response to x-

irradiation, some embryos seem to be insensitive to broken DNA since *Rana pipiens* oocytes fertilized with heavily irradiated sperm divide to produce haploid organisms (55) and early cleavage stage *Drosophila* embryos continue nuclear division, DNA replication, and centrosome duplication for several cycles with little or no mitotic delay after x-irradiation (56). We speculate that the *Rana pipiens* and *Drosophila* analogs of the *RAD9* checkpoint are inactive in these embryos.

The *gnu* mutation of *Drosophila* has a phenotype that supports the idea that many cell cycle events are independent of one another in the embryo (57); *gnu* embryos replicate DNA and centrosomes without undergoing nuclear division. In contrast, yeast mutations that block nuclear division also prevent the next round of DNA replication (58) and spindle pole body duplication (36).

Quite dramatic results have been obtained in the study of enucleated embryos where centrosome duplication and cortical contractions characteristic of cytokinesis continue for many cycles, often with normal kinetics (55, 59, 60). However, these results are not necessarily informative with respect to our search for the presence of checkpoints, since these control mechanisms probably require signals generated by the failure of one event that are received by and serve to inhibit some other event; with the nucleus or another organelle removed it is unavailable to send signals. For example, sea urchin embryos have been reported to continue to prematurely condense the chromosomes of fertilizing sperm after enucleation but not after inhibition of DNA synthesis with aphidicolin (62).

## Fidelity in the Embryonic Cell Cycle

The function of checkpoints in the cell cycle is to ensure the completion of early events before late events begin. When checkpoints are eliminated by mutation or other means, cell death, infidelity of chromosome transmission, or increased susceptibility to environmental perturbations (like DNA damaging agents) result.

Since some early embryos (*Drosophila*, *Xenopus*) lack the checkpoint that makes mitosis dependent on DNA replication, the considerations discussed above would predict that the embryonic cell divisions in these organisms might occur with less fidelity than the somatic cell divisions of the same organism. To our knowledge, there is no data available at present on the fidelity of chromosome or other organelle distribution during embryonic cell division in comparison to somatic cell divisions of the same organism; we will, nevertheless, pursue the implications of this thought.

If this prediction of a lower fidelity in some embryonic divisions is verified, we will need to consider why embryonic development has sacrificed fidelity. We suggest that checkpoints would delay cell division in those cells where stochastic problems require correction and would lead to asynchrony in a cell population. It appears that those embryonic systems that have eliminated the checkpoint, making mitosis dependent on DNA replication, are the systems where rapid and synchronous division is evident. Perhaps checkpoints have been eliminated because synchrony and speed were important for embryonic development.

What is the value of fidelity to metazoans and what price would the embryo pay in sacrificing fidelity? Developmental abnormalities (63) and cancer (64) seem to be much greater risks in organisms with chromosomal aneuploidy. It seems unlikely that embryonic systems would incur these risks. We suspect therefore that these embryonic systems have evolved some compensating system to allay increased risk of cancer and developmental aberrations. We suggest that embryonic systems utilizing rapid synchronous divisions will detect and eliminate aneuploid cells and possibly cells that have incurred

errors in the segregation of other organelles. A likely period to look for the deliberate elimination of defective cells is at the time when cell divisions slow down, transcription begins, groups of cells become asynchronous, and gastrulation commences (the mid-blastula transition in *Xenopus* and division fourteen of *Drosophila*). It may be pertinent that nuclei of *Drosophila* embryos that fail to divide or that merge with other nuclei fall into the interior of the egg at this time in embryogenesis and do not contribute to the larval cells (65). The *Drosophila* embryo has the capacity to replace lost nuclei even when the number lost is quite large; when nuclei are inactivated by ultraviolet irradiation before the fourteenth division, compensatory divisions occur that approximately restore the appropriate number of nuclei before cellularization (66). The existence of a monitoring system for aneuploidy does not seem too difficult to imagine in view of the phenomena of dosage compensation (67) and X chromosome inactivation (68), situations in which differences in chromosome ratios are detected.

A variety of abnormal cells arising from infidelity of the mitotic process have been detected in humans including aneuploidy, gene amplification, and multipolar mitoses. Sporadic cases of mitotic infidelity may not merit special attention since many causes are possible. However, when mitotic infidelity is rampant and reproducible as it is in many types of human tumors it may be fruitful to consider perturbations of the checkpoints that normally ensure mitotic fidelity as potential causes.

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## Checkpoints: controls that ensure the order of cell cycle events

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