Directing Cell Division During Development

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Several evolutionarily conserved proteins constitute a universal mitotic trigger that is precisely controlled during the orderly cell divisions of embryogenesis. As development progresses, the mechanisms controlling this trigger change. Early divisions are executed by maternally synthesized gene products, and in *Xenopus* they are timed by the accumulation and periodic degradation of cyclin, a trigger component. Later, the zygotic genome assumes control, and in *Drosophila*, zygotic transcription is required for production of another trigger protein, the product of *string*. After this transition to zygotic control, pulses of *string* transcription define the timing of highly patterned embryonic cell divisions and cyclin accumulation is not rate limiting.

GROWTH AND CELL PROLIFERATION MUST BE REGULATED to produce an organism having a defined form and coherent structure. The speed of the cell division cycle varies enormously, but does so in an orderly fashion. In a proliferative phase, during which eggs of many species generate the cell mass that initiates morphogenesis, the mitotic cycle can be extraordinarily fast, as short as 8 min in *Drosophila*. In contrast, on reaching a final stage of differentiation, many cell types, such as the nerves of higher organisms, cease dividing altogether. In addition to the speed of the cell cycle, the symmetry and orientation of division contribute to differentiation and the structuring of tissues. Female meiosis provides a striking example of deviation from symmetric division. To produce a gamete, meiosis must segregate the haploid chromosome complements. Males generally use all four haploid products to make sperm, whereas in many species females commit their resources to the production of one large egg. The three unused chromosome complements are discarded in small cells called polar bodies. In frogs the volume of a polar body is about $1/10^7$ that of the egg. Although seldom so dramatic, asymmetric divisions are common and probably contribute to the distinctions that appear when a cell divides to create two different cell types. Finally, during embryonic mitosis the orientation of division planes places cells in their appropriate positions. For example, in grasshopper embryos neuronal stem cells not only divide asymmetrically to give a nerve cell precursor (which is small) and a new stem cell (which is large), but also divide in a precise orientation so that the nerve cell precursor comes to lie internal to the more superficially located stem cell. Continued polarized divisions of the stem cell produce an ordered series of neuronal precursors, with the first born being the most internal. This polarity appears to influence the ordered structure of the nervous system since each neuronal precursor gives rise to different types of neuronal cells (*1*).

In this article we focus on evidence from our laboratory that some of the controls orchestrating the precise timing of embryonic cell division act on the components of an evolutionarily conserved mitotic trigger. There is more than one way to control this trigger, and distinct modes of regulation are used at different developmental stages (*2*).

The Universal Mitotic Trigger

Before committing to division, a cell must duplicate all its essential parts. The responsibility for this commitment is vested in an evolutionarily conserved molecular trigger whose key component is a protein kinase (*2*). This kinase derives its name, $p34^{cdc2}$, from its molecular weight and from the cell division cycle gene, *cdc*2, that encodes it in the fission yeast *Schizosaccharomyces pombe*. Homologs of this gene have been found in all eukaryotes examined. It is so highly conserved that the human and *Drosophila* homologs can function in *S. pombe* and substitute for the yeast gene (*3*). In all species examined, the level of $p34^{cdc2}$ is roughly constant during the cell cycle of actively proliferating cells, but a number of regulatory proteins constrain its kinase activity to an appropriately timed pulse that triggers mitotic events. This active form of the kinase can be assayed biochemically as histone H1 kinase or as a mitosis promoting factor (MPF) in biological assays (*4*).

Several of the key regulators of the $p34^{cdc2}$ kinase have been identified. Two of these, the products of the *S. pombe* genes *cdc*25 and *cdc*13 are particularly relevant to the studies summarized in this article (Fig. 1). Although the biochemical activity encoded by *cdc*25 is unknown, it is required for removal of phosphate from $p34^{cdc2}$, an essential step in kinase activation (*5*). The action of *cdc*25 is opposed by an inhibitory activity encoded by *wee*1 (*6*). The other important activator, the *cdc*13 product, appears to interact directly

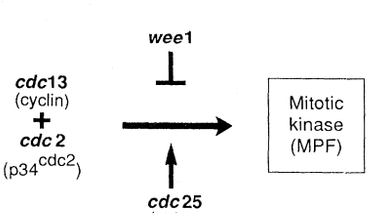

Fig. 1. The components of the mitotic trigger. The genetic designations of the *S. pombe* genes that play a central role in triggering mitosis are shown in boldface, and names used to designate the homologous genes or protein products in other species are in parentheses. Activation of the *cdc*2 product to produce the mitotic kinase or MPF requires *cdc*13 and *cdc*25 and is inhibited by *wee*1.

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with $p34^{cdc2}$ (7). It is homologous to the cyclins, proteins whose levels cycle because they are abruptly degraded at mitosis (*8*). In a number of higher organisms, two cyclin types have been identified, cyclin A and cyclin B (*9*). We treat the A and B type cyclins as one regulator (but see below).

Work in several systems, but especially in frogs, has suggested the model that mitosis is triggered by the accumulation of cyclin to a threshold and that mitotic degradation resets the system (*10*). However, our work in *Drosophila* indicates that after gastrulation the time of entry into mitosis is not controlled by cyclin accumulation but by the other positive regulator, the homolog of *cdc*25. After reviewing the evidence for this second mode of control, we suggest how an embryo might switch from one mode of control to another.

Patterned Cell Division

Drosophila embryos begin development with 13 extraordinarily rapid nuclear division cycles that occur without cell division (Fig. 2). The nuclei progress in near synchrony from mitosis to DNA synthesis and back to mitosis with no intervening gap phases, that is, G_1 or G_2. After mitosis 13, the nuclei, together with the cortical layer of cytoplasm, are cellularized to produce the cellular blastoderm, a monolayer of about 5000 cells that surrounds a syncytial yolk mass (*11*). These cycle 14 cells begin DNA synthesis with no intervening G1 phase and progress through S phase in near synchrony, but the cells occupying different positions exhibit G_2 phases that differ in length, from 30 to greater than 150 min (*12–14*).

Cells enter mitosis 14 in an intricate and reproducible sequence (*13, 14*). In an analysis of embryos stained with an antibody for tubulin (for example, Fig. 2), Foe (*13*) identified discrete groups of cells, or domains, that undergo mitosis in near synchrony at characteristic times. What guides this detailed pattern?

The Patterning Genes

Spatially localized regulatory molecules direct pattern formation in *Drosophila* (*15, 16*). The distributions of many such regulators have been defined in the roughly football (American style)-shaped blastoderm embryo. Many of these regulators are expressed in striped patterns, each distinguished by the width, phase, or repeat frequency of the stripes. Some of these stripes cut across the long axis of the football, thereby producing anterioposterior subdivisions. Others appear more like racing stripes running along the long axis to produce dorsoventral subdivisions of the embryo. Just as pairs of coordinates, latitude and longitude, define positions on a globe, combinations of localized regulators can specify positions within the embryo (*16*).

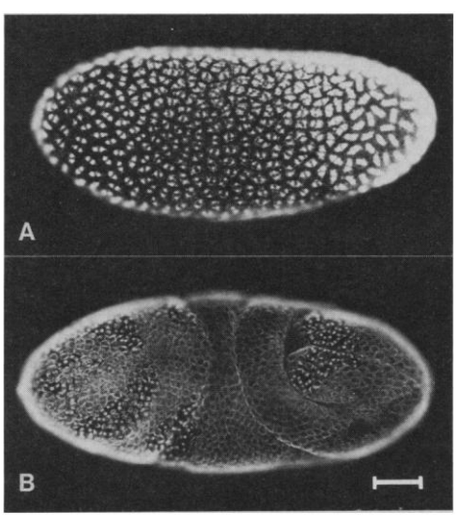

Fig. 2. Mitosis in *Drosophila* embryos before and after the transition from maternal to zygotic control. Whole fixed embryos were immunofluorescently stained with an antibody to tubulin. The localized bright staining of mitotic spindles is easily distinguished from the more diffuse lattice of staining seen in groups of interphase cells. In a syncytial embryo (**A**), all the nuclei divide in near synchrony. In contrast, mitosis 14 (**B**) occurs in a distinctive pattern. Scale bar, 50 μm.

Mutations in the patterning genes alter the map of mitotic domains (*17*). Thus, these genes must directly or indirectly govern the spatially patterned mitotic times. However, there is a slight difficulty in understanding how this control might operate. No individual patterning gene is expressed in a pattern that corresponds to the map of mitotic domains. Rather, the mitotic domains exhibit incomplete parallels with the patterns of expression of a number of these regulators. We consequently expect that the localized patterning regulators will act in combinations to direct mitoses.

Controlling the Mitotic Trigger

Because many of the patterning genes encode regulators of transcription (*18*), we speculated that they would control cell division by regulating expression of a gene whose product is limiting for progression to mitosis 14. Most genes could not serve this role as a "trigger gene" because their gene products are presynthesized and prepackaged. They are made maternally and packaged into the egg in amounts sufficient for most of embryonic development. Indeed, the first 13 mitotic cycles occur in the presence of α-amanitin, an inhibitor of transcription (*19*), and no known zygotic mutation blocks these early mitoses. Only one gene, *string*, is known to be needed for the next mitosis (*20, 21*). In *string* mutant embryos, cells arrest in G_2 of cycle 14, but other processes including major steps of morphogenesis continue. Development becomes abnormal as the repercussions of the failure to divide perturb subsequent events. *String* was an obvious candidate for the gene encoding the trigger governing progression to mitotis 14.

String *encodes a conserved mitotic trigger component.* The *string* gene was cloned and the levels of *string* RNA measured. There is an abundant maternal *string* transcript that is stable throughout the first 13 rapid nuclear division cycles, but this abruptly disappears after mitosis 13 (Fig. 3) (*12*). The *string* transcripts reaccumulate during cycle 14. This reaccumulation is the consequence of zygotic expression. In situ hybridization of *string* probes to the RNA in whole fixed embryos shows that the zygotic *string* transcript accumulates in a complex spatial pattern that changes rapidly with time (Fig. 3) (*12*). In each cell the onset of *string* RNA accumulation precedes mitosis by about 20 min. Thus, the pattern of *string* RNA accumulation anticipates the pattern of the entry into mitosis (*12, 13*). The mutant phenotype (cell cycle arrest before mitosis 14) and the correlation of the pattern of *string* expression with the pattern of entry into mitosis suggest that the *string* gene product controls the mitotic patterns. Sequence analysis of *string* offers support for this proposal. The *string* gene is homologous to the *S. pombe cdc*25 gene, and the *string* cDNA clone can substitute for the yeast gene (*12*).

The time of string *transcription is regulated.* We have seen that embryonic division 14 is highly patterned and have proposed that the timing of this division is defined by the time of *string* RNA accumulation, and that *string* RNA accumulation is, in turn, controlled by localized transcriptional regulators. We have explored one of the tenets of this model, that *string* gene transcription is regulated by the patterning genes.

A newly developed method allows us to ask whether the pattern of *string* RNA accumulation is due to regulation of transcriptional

rate or control of RNA stability. Immunocytochemical detection of in situ hybrids allows high-resolution detection of RNA in whole fixed embryos (*22*). In the nuclei that are actively transcribing *string* we detected two intensely staining "dots" that appear to represent nascent transcripts. (These are presumably in structures analogous to the "Christmas trees" of transcription fibers decorating transcription units seen in samples spread for electron microscopy.) The nuclear dots provide a method of assessing ongoing transcription of specific genes in individual cells of the embryo (Fig. 4). The *string* nuclear dots appear in a pattern that parallels, but slightly anticipates, cytoplasmic accumulation of *string* RNA. This finding suggests regulation at the level of transcription. According to our present model we expect that both *string* RNA and protein are unstable so that their levels closely follow the rates of *string* transcription. We have not yet directly tested this prediction.

If transcriptional regulation is the key to the timing of mitoses, what governs the spatiotemporal pattern of *string* transcription? Our preliminary work is consistent with the notion that combinations of patterning genes control *string* expression. For example, the *twist* gene is a patterning gene that is expressed in a broad ventral stripe along the anterior-posterior axis of the blastoderm embryo. *Twist* function is required for cells in this region to follow their normal fate and produce mesoderm. This region of prospective mesoderm is an early domain of *string* RNA accumulation and constitutes mitotic domain 10. Expression of *string* in this region is missing in a *twist* mutant, and the cells fail to divide on schedule. Thus, the *twist* gene controls one aspect of *string* expression, and the failure to divide is consistent with the dependency of mitosis on *string*. We suspect that other patterning genes will control other aspects of *string* expression.

It is more difficult to determine whether the patterning genes act directly on the *string* gene. An intermediary might be involved. For example, patterning genes might act to govern synthesis of a transcription factor for *string* that in turn drives *string* expression. However, the following evidence argues against this model for indirect control. If there is an intermediary, deletion of this gene should block *string* expression. We have analyzed a number of deletions that together cover 80% of the genome but have not yet found a deletion that eliminates zygotic *string* expression (except deletions removing *string* itself). This analysis supports our suggestion that no single regulator takes responsibility for directing *string* expression; rather, each of the localized transcription factors influences its expression.

The final levels of *string* RNA in most mitotic domains are comparable, and mitosis follows the initiation of *string* transcription by about 20 to 25 min. Thus, in these domains the time required to accumulate an effective level of the *string* product and the lag time required to execute steps leading to mitosis are similar. Mitotic domain 10 is unusual in that *string* RNA levels are lower, and 35 min lapses between initial expression and mitosis. Perhaps it takes longer to accumulate *string* protein to threshold levels in this domain. Thus, the rate of *string* expression might contribute to the timing of mitosis. Nonetheless, in most domains the key parameter controlling the timing of mitosis is the time at which *string* transcription begins.

Two Models for the Mitotic Trigger

Our studies of *string* suggest a simple model for the regulation of mitotic times (Fig. 5A). The *string* gene is regulated to begin transcription at specific times (for example, T1 and T2 in Fig. 5A) in different cells. Mitosis then follows after a lag during which the *string* protein product first accumulates, then activates the $p34^{cdc2}$ kinase, which in turn initiates mitotic events. Cells initiating *string* RNA synthesis at the different times T1 and T2 will enter mitosis at different times, M1 and M2.

A substantially different model is based on periodic cyclin degradation with constant synthesis (Fig. 5B). According to this model the speed of a cell cycle is determined by the rate at which cyclin accumulates; mitosis and the time of induced cyclin degradation would be determined by the time at which cyclin reaches a threshold. Consequently, cells undergoing mitosis at times M1 and M2 would accumulate cyclin at differing rates. This model was tested by cloning the cyclin A and cyclin B genes of *Drosophila*, preparing antibodies to the encoded proteins, and examining the accumulation of these proteins in cells of cycle 14 embryos. We found that cells undergoing mitosis at different times accumulate cyclin A and cyclin B at the same rate (*23*). This indicates that the simple model for cyclin control of mitotic times does not apply to cycle 14 *Drosophila* embryos.

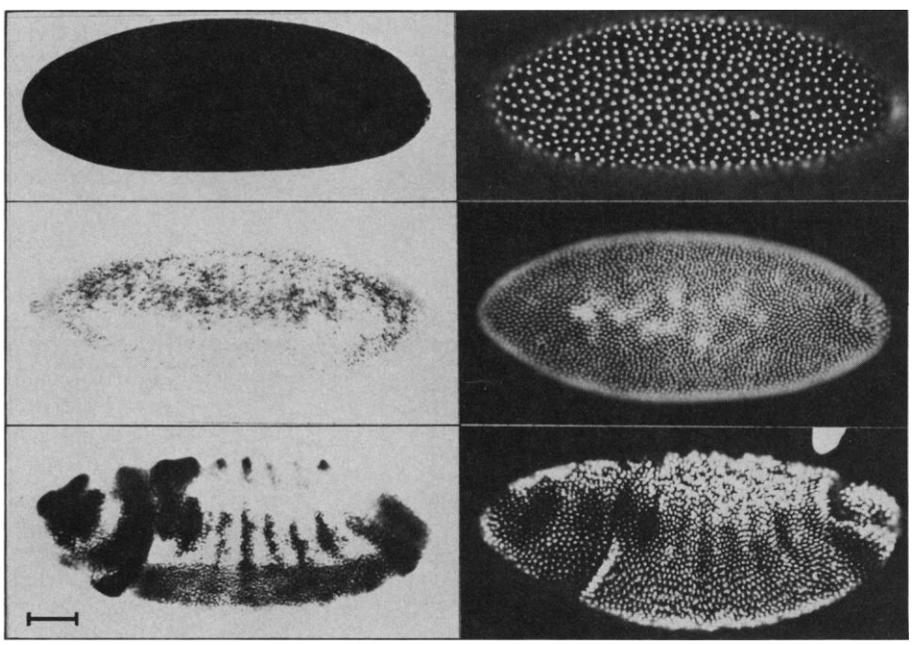

Fig. 3. The transition from maternal to zygotic supply of *string* RNA. Whole, fixed, and permeabilized embryos were hybridized with a *string*-specific DNA probe tagged with digoxigenin. The location of *string* RNA is revealed by immunohistochemical staining with antibodies specific for digoxigenin (left panels). Embryos were also stained with a DNA-specific fluorescent dye to reveal the distribution of nuclei (right panels). Three embryos (all similarly stained, photographed, and printed) are shown. An early embryo (top row) has a very large amount of relatively uniformly distributed *string* RNA. This RNA is gone by the beginning of cell cycle 14 (middle row). Zygotic expression of *string* RNA in later cycle 14 embryos is patterned (bottom row). The pattern faintly evident in the DNA staining (bottom right panel) only represents quenching by the immunohistochemical stain. The patterned mitoses begin about 10 min later than the stage shown here. Scale bar, 50 μm.

An adjustment of the model for the cyclin-driven oscillator makes an attractive alternative. Perhaps cyclin accumulates at similar rates in all cells, but the cells differ in the threshold at which mitosis and cyclin degradation are induced. As a test of this model, mutations resulting in reduced rates of cyclin A accumulation were examined. The timing and the pattern of mitosis were just as in wild-type embryos. This argues either that cyclin A is irrelevant to mitosis 14 or that it is produced in excess. Since cyclin A mutations cause a cell cycle arrest once the embryo runs out of its maternal store of wild-type cyclin A RNA, it appears that cyclin A is essential for cell cycle progression (*23*). We consequently conclude that cyclin A is produced in excess during cycle 14. An equivalent test of cyclin B has not been performed, but we suggest that it will also be produced in excess.

In conclusion, whereas we were unable to demonstrate a role for cyclins in the control of the timing of mitosis 14, we have three arguments that *string* is involved: *string* mutants arrest before mitosis 14, the expression pattern of *string* predicts the pattern of mitoses, and the *string* product is homologous to a yeast regulator of mitosis. We consequently favor a model in which *string* expression is the trigger that times mitosis 14. This model can be tested by determining whether ectopic expression of *string* is sufficient to drive ectopic mitoses.

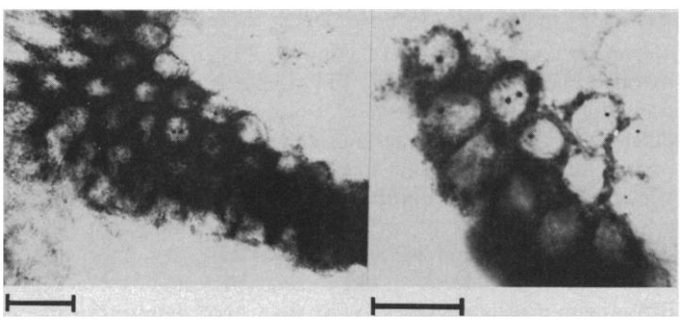

Fig. 4. In situ detection of nascent transcripts. Immunohistochemical detection of hybrids reveals the distribution of *string* RNA within cells of a cycle 14 embryo. Intense staining is seen in the cytoplasms of expressing cells. Except for two intense dots in each nucleus (not all are detected in this focal plane), stain is absent from the nuclei. These dots appear to represent nascent transcripts (*38*). Consequently, this staining allows us to independently assess *string* RNA accumulation and ongoing *string* transcription in each cell. Because dots are absent in the nuclei lying outside domains of *string* expression, we conclude that *string* transcription is regulated. The two panels show two different fields of cells; the right panel is a slightly higher magnification. Scale bars, 10 μm.

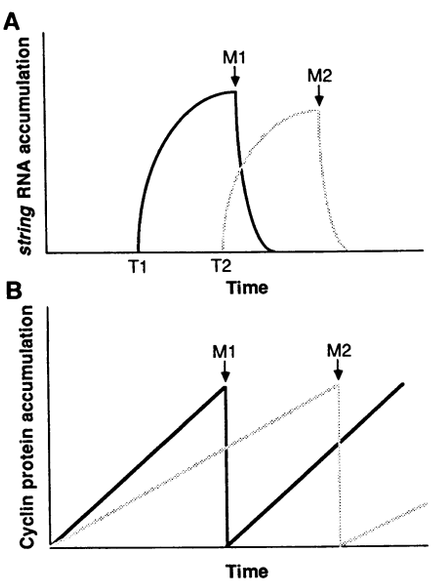

Fig. 5. Models for control of mitotic time by *string* and by cyclin. (**A**) The time of mitosis can be regulated by the timing of *string* gene transcription in different cells. The time of mitosis (M1 and M2) will be determined by the time of initiation of *string* expression (T1 and T2) and the lag time required for *string* transcription to induce mitosis. We presume that the *string* gene product is unstable and consequently depict its accumulation as nonlinear. Observations suggest that *string* transcription is usually discontinued at mitosis and is not reinitiated until later in the subsequent cell cycle. (**B**) In an alternative model, the time of mitosis is controlled by the accumulation and periodic degradation of cyclin. In this case, cells that divide at different times (M1 and M2) would accumulate cyclin at different rates and reach the threshold required for division at different times.

Mitosis in Large Eggs

The production of sufficient *string* transcript to reach the threshold required for mitosis is likely to be a problem for the eggs of many species. The rules of Mendel limit organisms to a single diploid nucleus per egg, whereas the requirements of embryogenesis demand an enormous reserve of the raw materials used during development. The result is a diploid egg cell that, in many species, has an unusually large cytoplasmic volume—in frog about a million-fold that of a normal cell. The inadequacy of the nucleus to direct the rapid development of this huge cytoplasm is illustrated by a calculation described by Woodland (*24*). It would take a single gene about 16 years to make the amount of histone mRNA found in a frog egg. The impotence of the egg nucleus is further illustrated by the observation that early cell division in a number of species does not require the zygotic nucleus. Indeed, enucleated frog or sea urchin eggs can go through a number of abortive cleavages and, as mentioned earlier, *Drosophila* embryos go through the first 13 cycles in the presence of the transcriptional inhibitor α-amanitin (*19, 25*).

Clearly, *string* transcription is not required during these early cleavage divisions. However, there is a large supply of maternal *string* RNA in *Drosophila* eggs. Because this maternal supply of *string* RNA survives for the first 13 mitotic cycles, oscillating levels of *string* RNA cannot be used to coordinate these mitoses. What then serves as the trigger for mitosis? The simplest hypothesis is that another component of the mitotic trigger is limiting and controls activation of the mitotic kinase. The most appealing candidate for this alternative regulator is cyclin. Cyclin RNA is also provided as a stable maternal store. However, the protein level can oscillate as a consequence of periodic degradation. Thus, cyclin is well suited for the control of the early, maternally driven cleavages. Indeed, it appears that cyclin plays exactly this role in the early cleavages of frog eggs (*26*). Unfortunately, no comparable data exist for the early divisions of *Drosophila*. Nonetheless, at present we adopt this view as our simplest hypothesis.

In conclusion, transcriptional regulation of *string* does not control the early cleavage cycles. In frogs and perhaps also in *Drosophila* the cycles appear to be driven by the periodic accumulation and degradation of cyclin.

Controlling the Transition

The transition from maternal to zygotic control of the cell cycle is not simply a consequence of gradual depletion of maternal products and increasing zygotic contributions due to the exponential increase in DNA. Rather, it is a well-coordinated switch that, in *Drosophila*, involves the abrupt degradation of maternal *string* RNA and the consequent transition of cell cycle progression to dependency on zygotic *string* expression.

In several species two phases can be distinguished in the transition to zygotic control. The early, maternally driven cleavage cycles are all the same length. The first phase of the transition begins as these

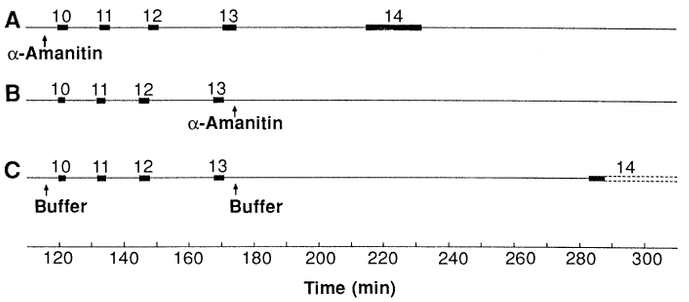

Fig. 6. Time lines of mitotic times illustrating the affect of α-amanitin injection. Heavy lines with numbers indicate the times of mitosis. (**A**) When α-amanitin is injected early enough to block all zygotic transcription, the maternal division program continues, resulting in a premature and defective mitosis 14. (**B**) α-Amanitin injection at a time when *string* RNA levels are very low blocks subsequent cell cycles. (**C**) The normal time course of mitoses is shown. Control injections of buffer at the early or late times did not disturb this time course. (The dotted line segments indicate the asynchrony of the normal mitosis 14.) The scale indicates time after egg disposition at 22°C.

cycles become progressively longer (*27*). The second phase is abrupt and is associated with a large increase in cell cycle length, a loss of synchrony, a dramatic increase in transcription, and initiation of cell movements. This transition has been called the midblastula transition (*28*).

We will review evidence that three factors contribute to the gradual and abrupt phases of this transition. First, there is a titration of the capacity of maternal components to drive rapid cell cycles. This results in gradual slowing of the cleavage cycles. Second, mitosis and the rapid embryonic S phases inhibit transcription. As a consequence, zygotic transcription can start only when the mitotic cycle slows. Third, in *Drosophila*, and perhaps other species, during the gradual increase in cell cycle length some maternal RNAs including *string* are selectively destabilized. Further cell cycles then depend on zygotic expression of *string*.

Experiments in fly, starfish, newt, and frog (*27, 29*) suggest that the midblastula transition is induced by a titration mechanism that measures the increasing number of nuclei. Manipulations that double the nuclear density cause the midblastula transition to occur one cycle early, whereas reductions in nuclear density delay the transition. Because haploid embryos undergo the midblastula transition one cycle later than diploid embryos, it appears that nuclei are counted according to their DNA content or another co-varying parameter (for example, size). Although DNA injection can advance the midblastula transition in frog (*30*), it is not clear how closely the DNA is mimicking the normal titrating component.

In *Drosophila* and in *Xenopus* no zygotic transcription occurs during the early, rapid nuclear cleavage divisions. Because a drug-induced block of cell cycle progression induces precocious activation of zygotic transcription, it appears that the rapid mitoses and S phases of early embryogenesis inhibit transcription (*19, 31, 32*). In *Drosophila* the speed of the early nuclear division cycles slows during cycles 10 (8.8 min), 11 (9.5 min), 12 (12.5 min), and 13 (21 min) (*11*). Zygotic transcription is first detected in cycle 10, and it accelerates as the division cycles slow. Because transcription during these cycles is confined to the later parts of interphase when DNA synthesis is reduced, it seems reasonable to assume that the increase in transcription is a consequence of the lengthened division cycle (*19, 31*). Accordingly, lengthening of the cell cycle leads to activation of zygotic gene expression (*32*).

Coincident with the abrupt transition to zygotic control of cell cycle progression at mitosis 13 in *Drosophila* embryos, a number of maternal RNAs including *string* are destabilized (Fig. 3) (*12, 33*). We tested whether zygotic transcription contributes to termination of the maternal mitotic program by injecting embryos with α-amanitin during mitotic cycle 9, just before the earliest detectable zygotic transcription (Fig. 6). These injected embryos underwent a rapid and almost synchronous cycle 14 as if they were continuing the maternal division program. In contrast, injection of α-amanitin after the loss of maternal *string* RNA completely blocked mitosis 14, as expected, because mitosis requires zygotic transcription for reaccumulation of *string* function. These observations lead us to propose that early zygotic transcription promotes the degradation of some maternal RNAs. Additionally, it appears that the acquisition of a G_2 in cycle 14 is due to the erasure of the maternal RNA, and the period of G_2 represents the time needed for zygotic provision of *string* function.

In summary, the transition to zygotic control appears to be initiated by a titration mechanism that slows the early cleavage stages to allow zygotic gene expression. In parallel, a transition to zygotic control is provoked by selective destabilization of some maternal RNAs, in particular *string*.

After Cycle 14

During the first 3 hours of *Drosophila* embryogenesis we encountered two different modes of regulating the rate of cell cycle progression. Will yet other modes come into play at later stages or in particular cell lineages? The answer is yes. After 16 mitoses, division stops in most cells of the embryo and subsequent larval growth proceeds mainly by expansion of cell volume with continued rounds of DNA replication to produce polyteny. The abbreviated cell cycle that leads to polytenization skips mitosis, and thus is presumably regulated by components other than those of the mitotic oscillator. We detect no *string* or cyclin RNA in these cells. In another example, the cells of imaginal discs behave quite differently. They proliferate throughout larval stages, arresting only as terminal differentiation occurs. At least in the eye disc, many of these cells arrest in G_1 (*34*). Again this regulation must involve controls other than those acting on the mitotic oscillator.

Given that there are a number of modes of regulating the cell cycle, what is the relative importance of the mechanism based on transcriptional control of *string* levels? At one extreme, perhaps the *string* product is needed only for mitosis 14, when the *string* mutants arrest. To test for a later requirement, the wild-type copy of *string* can be removed from heterozygotes by mitotic recombination. The failure to detect clones homozygous for *string* suggests that the gene is essential at late stages (*35*).

Even though *string* is required for later divisions, it might no longer control entry into mitosis: the gene product might be provided in excess as we have suggested for the first 13 nuclear cycles. However, several observations suggest that *string* controls mitosis 15 just as it controls mitosis 14. First, the time of mitosis 15 differs in different cells. Second, DNA labeling experiments fail to detect a G_1 phase in cycle 15 and thus suggest that, as in the case of cycle 14, the length of G_2 is regulated. Finally, in most cells, *string* RNA disappears shortly after mitosis 14 and reaccumulates in a complex pattern that anticipates the pattern of mitosis 15. Similarly, in cycle 16, *string* RNA disappears and reaccumulates. After mitosis 16, *string* expression is limited to a complex pattern of pulses in cells of the nervous system and in cells along the dorsal edge of the ectoderm, the only cells that continue to divide during the stages examined. These observations suggest that *string* expression either

controls entry into mitosis or is tightly coupled to mechanisms controlling mitosis. In summary, while other modes of cell cycle regulation come into play as development progresses, it appears that *string* continues to act as a determinant mediating the developmental programming of mitotic times.

G_2 Versus G_1 Regulation

The importance of regulation of the length of G_2 in *Drosophila* embryos was somewhat of a surprise. Most of the analysis of cell proliferation in eukaryotic systems suggested that the major point of control is in G_1 (*36*). However, these analyses focused on growth control in tissue culture and are not likely to represent the full diversity of control mechanisms. There are only a few examples of embryonic cell cycles in which the level of the control is known. A study in leech provides a rare exception (*37*). As in other organisms, the early cleavage divisions of leech are extremely rapid, lack a G_1 phase, and are presumably executed by maternal products. G_2 phases are present from the outset, and regulation of the length of G_2 occurs very early. Stereotyped changes in G_1, S, and G_2 in the various leech embryonic lineages demonstrate that regulation of cell cycle length occurs at multiple stages. These direct observations of embryonic cell cycles provide another argument that diverse modes of cell cycle regulation are used during development.

Can we find any rationale to explain the observation that G_2 regulation plays an important, if not predominant, role in early development? Division of cultured cells is intimately associated with doubling of cell mass. In these cells limitations of growth lead to a cell cycle arrest at a control point in G_1 (*36*). Because many embryonic divisions subdivide the large egg cytoplasm with little or no growth, the mode of cell cycle regulation in tissue culture may not be well suited to embryogenesis. Indeed, this is suggested by the details of embryonic control of cell division. The orchestrated events of development often demand timely production of new cells. The birth of cells would be most accurately timed by controls acting directly at mitosis: timing control in G_1 would lose precision because of variability in the duration of subsequent stages of the cell cycle. Furthermore, production of distinctly differentiated sister cells and organization of structured tissues also relies on controls that alter the symmetry and orientation of mitosis. These factors suggest that control of mitosis will have a pivotal role in cell divisions associated with developmental patterning. In this article we have summarized evidence for a mechanism in which expression of the *string* gene coordinates the timing of mitosis with the expression of developmental patterning genes.

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32. Inhibition of transcription is an unusual feature of the early embryonic S phases. Transcription continues through the prolonged S phases of most other cells. However, this distinction may only be superficial. In the slower cell cycles of nonembryonic cells, different parts of the genome are replicated at different times during S phase. Thus, in these S phases, continued transcription of the nonreplicating regions of the genome could mask an inhibitory effect of ongoing replication. In contrast to these prolonged S phases, in order to achieve an extraordinarily rapid S phase, early embryonic nuclei initiate replication at numerous sites throughout the genome. Thus, the effectiveness with which DNA synthesis inhibits transcription in early embryonic cells might be due to a quantitative rather than a qualitative peculiarity of these early S phases.
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