Cell cycle progression and cell division are sensitive to hypoxia in *Drosophila melanogaster* embryos

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**Douglas, Robert M., Tian Xu, and Gabriel G. Haddad.** Cell cycle progression and cell division are sensitive to hypoxia in *Drosophila melanogaster* embryos. *Am J Physiol Regulatory Integrative Comp Physiol* 280: R1555–R1563, 2001.—We and others recently demonstrated that *Drosophila melanogaster* embryos arrest development and embryonic cells cease dividing when they are deprived of O₂. To further characterize the behavior of these embryos in response to O₂ deprivation and to define the O₂-sensitive checkpoints in the cell cycle, embryos undergoing nuclear cycles 3–13 were subjected to O₂ deprivation and examined by confocal microscopy to analyze in vivo, real-time analysis of embryos carrying green fluorescent protein–kinesin demonstrated that cells arrest at two major points of the cell cycle, either at the interphase (before DNA duplication) or at metaphase, depending on the cell cycle phase at which O₂ deprivation was induced. Immunoblot analysis of embryos whose cell divisions are synchronized by inducible String (cdc25 homolog) demonstrated that cyclin B was degraded during low O₂ conditions in interphase-arrested embryos but not in those arrested in metaphase. Embryos resumed cell cycle activity within ~20 min of reoxygenation, with very little apparent change in cell cycle kinetics. We conclude that there are specific points during the embryonic cell cycle that are sensitive to the O₂ level in *D. melanogaster*. Given the fact that O₂ deprivation also influences the growth and development of other species, we suggest that similar hypoxia-sensitive cell cycle checkpoints may also exist in mammalian cells.

green fluorescent protein–kinesin; cell cycle arrest; O₂ deprivation; invertebrates; metaphase; S phase

**Materials and Methods**

Materials

5′-bromo-2-deoxyuridine (BrdU), propidium iodide, Shields and Sang M3 insect medium, and the α-tubulin antibody were obtained from Sigma (St. Louis, MO). The anti-BrdU antibody was purchased from Becton-Dickinson (San Jose, CA). The antibody to cyclin B (Rb 271) was kindly provided by Douglas, Robert M., Tian Xu, and Gabriel G. Haddad.

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Fly Stocks

Initial studies were performed utilizing wild-type flies of the Canton S strain (Bloomfield Center, IN). Flies containing the GFP-kinesin construct Ma-GFP2P (II). The heat shock String fly strain [y, D[w]/67; HS-STG3] was provided by Dr. Bruce A. Edgar (4).

Egg Collection and Preparation

Fly stocks were maintained in population cages at 25°C under standard conditions. Embryos were collected on apple juice plates supplemented with a small amount of yeast paste. Generally, the first two collections of the day were discarded and subsequent collections were made at 30 min, 1, 2, or 4 h. Embryos were rinsed from the agar plates into a Nytex mesh holder with a solution containing 0.7% NaCl and 0.02% Triton X-100 (TX-100; Sigma). They were then thoroughly rinsed with distilled deionized H2O (DDW). Embryos were then dechorionated in 50% domestic bleach (sodium hypochlorite; Chlorox, Oakland, CA) in DDW for 2 min and again rinsed thoroughly with DDW prior to any experimental manipulation. Twenty-three embryos were analyzed in detail with in vivo confocal imaging.

Observing Living Embryos During Normoxia and Hypoxia

Dechorionated GFP-kinesin fly embryos were mounted onto coverslips, according to Schubiger and Edgar (29), and each coverslip was placed into a specially designed perfusion chamber. The embryos were then enclosed within the perfusion chamber with a circular Thermaxan plastic coverslip (15-mm diameter; Nalge Nunc International, Naperville, IL), immediately perfused with normoxic insect medium, and viewed with a Bio-Rad MRC-1024 confocal microscope system (Bio-Rad Laboratories, Hercules, CA). Images were captured every 30–40 s from GFP-kinesin embryos for 1 or 2 cell cycles under control, normoxic conditions before switching to an hypoxic insect medium. The hypoxic medium had been previously bubbled with 100% N2 for 2 h before the experiment and was continuously bubbled with the same gas throughout the experiment. Measurements of PO2 were made during some experiments, and PO2 was maintained at 10–20 mmHg. Perfusion with the hypoxic solution was maintained for 30 min, 1 h, or 2 h. After the hypoxic period, perfusion with normoxic insect medium was reinitiated, and, on the resumption of cell cycle activity, embryos were observed for an additional one or two cell cycles during reoxygenation.

Immunocytochemical and Immunoblot Analysis of the Effect of Hypoxia on Drosophila Embryos

Hypoxia chamber. Dechorionated embryos were placed into a specially designed hypoxia chamber that allowed the manipulation of the embryos while totally preserving the hypoxic environment. Embryos were then subjected to hypoxia for varying periods of time (20, 30, 60, and 120 min). At the end of the hypoxic exposure, embryos were either permeabilized and fixed within the chamber for immunocytochemical analysis or quickly removed and frozen in liquid N2 for immunoblot analyses. The appropriate controls were also run concurrently in room air or bubbled with 21% O2.

BrdU incorporation studies. With cell and life cycles as short as those of the D. melanogaster, the collection of embryos of a similar age is rather tedious. This limitation was circumvented by the use of a heat shock String (stg) fly strain. We utilized a fruit fly strain carrying a heat shock protein 70 promoter-String (cde25 homolog) construct that permits embryonic cells resting in G2 to be heat-pulsed into an early but well-synchronized mitosis and subsequent S phase. HS-STG3 embryos were then aged for 130 min to generate embryos that had completed the first 13 cell cycle divisions and had entered the first G2 gap phase of Drosophila embryogenesis in interphase 14. During the aging process, the embryos were dechorionated and permeabilized with 100% n-octane (Sigma) to permit BrdU incorporation. Two groups of embryos, one to be exposed to hypoxia and the other to serve as a heat shock control, were then placed in an incubator at 37°C for 40 min. A heat shock pulse of this duration induces global stg mRNA and STG protein expression within the embryo and synchronously drives all G2 cells into mitosis within ~5–10 min of returning the embryos to room temperature (RT). Mitosis is generally completed at 10–20 min and a singular S phase that lasts for 45 min immediately follows this "premature" mitosis. The cells then enter another G2 and remain there for 70–170 min before resuming the normal developmental program (4). Heat-pulsed HS-STG3 embryos were therefore simultaneously exposed to chamber hypoxia, as described above, and incubated in insect medium containing the thymidine analog BrdU (1 mg/ml) for 20–30 min so we could assess DNA-replication activity during O2 deprivation. HS-STG3 embryos that were neither heat shocked nor exposed to hypoxia and those that were heat shocked but maintained in room air served as parallel controls. Embryos were then either permeabilized and fixed within the hypoxia chamber for BrdU immunocytochemistry or removed quickly and frozen in liquid N2 for immunoblotting.

Whole mount immunocytochemistry. For immunocytochemical and immunoblot studies, we used cellularized rather than syncytial embryos because we needed to synchronize the cell cycle stage among embryos. This necessitated the use of HS-STG3 embryos, which relies on aging embryos until interphase 14. Whole mount immunocytochemistry of embryos was performed essentially as described by Fehon et al. (9). Embryos were permeabilized by shaking in 100% heptane (Sigma) for 30 s before being transferred to a solution of 50% heptane and 2% paraformaldehyde (for BrdU) or 37% formaldehyde (for α-tubulin) in PBS for 30 min. The vitelline membrane was removed by incubation in methanol, and, if the embryos were not immediately processed for immunocytochemistry, they were stored at −20°C. Rehydrated embryos were rinsed three times for 5 min each in PBS, 0.2% BSA, and 0.1% TX-100 (PBT). They were then incubated in 10% normal donkey serum (NDS) and 0.3% TX-100 in PBT for at least 1 h at RT. Embryos were incubated in primary antibody in 10% NDS and 0.3% TX-100 in PBT overnight at 4°C. After being rinsed in PBT, embryos were incubated in the secondary antibody, also in 10% NDS, 0.3% TX-100, and PBT for 2 h at RT. Embryos were finally rinsed twice 5 min each with PBT and once for 5 min in PBS before being mounted onto slides in Vectashield (Vector, Burlingame, CA) and placed on a coverslip. DNA staining performed in conjunction with antibody staining involved incubation with 200 μg/ml RNase A (Sigma) during the primary antibody step as well as in the three rinses in PBT after the secondary antibody step. Embryos were then incubated in 10 μg/ml propidium iodide in PBT for 3–5 min and thereafter rinsed twice, 5 min each, in PBT and once for 5 min in PBS prior to mounting.
Immunoblotting. For Western blotting, dechorionated embryos were homogenized in Laemmli sample buffer (2% SDS, 10% glycerol, and 67 mM Tris, pH 6.7) containing protease inhibitors. Embryo samples were assayed for protein content (BCA Assay, Pierce Chemical, Rockford, IL), and 10 μg of total protein was loaded per lane for SDS-PAGE and subsequent transfer to polyvinylidene fluoride membranes (Immobilon-P, Millipore, Bedford, MA). Proteins were detected with secondary antibodies conjugated with horseradish peroxidase and the enhanced chemiluminescence system (Amerham, Little Chalfont, UK) as described previously (14). Scanning densitometry of immunoblot films was performed on a Personal Densitometer SI scanner (Molecular Dynamics, Sunnyvale, CA) and analyzed with ImageQuaNT image-analysis software (Molecular Dynamics).

Data Analysis

Data are reported as means ± SE.

RESULTS

Live Confocal Imaging of Early Embryogenesis With and Without O2

In vivo confocal imaging of cell cycle activity in Drosophila embryos under control, normoxic conditions. Embryos undergoing nuclear division cycles 3–13 were imaged on the confocal microscope while being perfused with Shields & Sang M3 insect medium. After an equilibration period of 10–15 min, GFP fluorescence is seen along the extent of the spindle fiber apparatus, inclusive of astral fibers at particular stages during the cell cycle, and is localized to centrosomes throughout the cell cycle (Fig. 1). The first evidence of mitotic activity is the appearance of relatively amorphous spindle fiber apparatuses extending between the two polar centrosomes (prometaphase stage of mitosis, as seen in Fig. 2G). Within the subsequent few minutes, GFP fluorescence increases markedly and distinct spindle fiber bundles separated by a dark central portion (paired sister chromatids) become evident, as cells enter metaphase proper (Fig. 1A). Embryonic cells remain in metaphase for ~2.5 min but, shortly after, the spindle fibers begin to separate and move to opposite poles of the energid (nuclei and associated cytoplasm) (1) at a time when the astral fibers surrounding the centrosomes become apparent (metaphase to anaphase, Fig. 1B). As the spindle fibers continue to migrate outward during the anaphase to telophase transition (Fig. 1C), spindle fibers become less ovoidal and more rectangular. During telophase and cytokinesis (Fig. 1, D and E), the two new daughter nuclei can also be detected as darkened circles outlined by a halo of fluorescence. The centrosomes of the daughter energids, which are initially located on one side of the cell, then replicate and can be seen to migrate along the perimeter of the two newly forming daughter cells to assume polar positions around the nucleus (Fig. 1F). About 12 min from the start of metaphase, most or all of the energids of the embryo have entered S phase (Fig. 1F), where the dark circles represent the new...
daughter nuclei. The manipulation of the embryos under these experimental conditions did not have any deleterious effects on subsequent development.

Effect of hypoxia on cell cycle activity of GFP-kinesin embryos. In these experiments, hypoxia exposure of 30, 60, or 120 min was initiated at a variety of points during the cell cycle. Arrest was achieved both with 100% N₂ only and also with solutions bubbled with 5%O₂ in 95% N₂. Two major arrest stages resulted from these in vivo perfusion hypoxia experiments.

**Interphase arrest.** Embryos exposed to hypoxia from the period just after the metaphase stage of mitosis, e.g., during the metaphase-to-anaphase transition (Fig. 2A) to very early in S phase, continue to progress (Fig. 2, B–D), and do not arrest until S phase (Fig. 2E). At this phase, embryos appeared to have arrested and do not progress for the duration of the hypoxic exposure. If the hypoxia is maintained for 1 h or longer, at ~45–55 min of hypoxic exposure, fluorescent signals generated by the centrosomes generally dissipate to background levels. As can be seen in Fig. 2, the exposure of these embryos to hypoxia does not seem to have any deleterious effects on spindle fiber morphology or movement. Indeed, reoxygenation of embryos resulted in the resumption of cell cycle activity, within ~20 min of the return to normoxia (Fig. 2F). The first evidence of recovery during reoxygenation is usually the marked increase in GFP-fluorescence of the centrosomes within ~5 min (Fig. 2F). Interphase-arrested embryos resume mitotic activity, as evidenced by the appearance of centrosomes followed by amorphous spindle fibers overlying the nuclei during prometaphase (Fig. 2G). Subsequently, embryonic cells divide, as can be seen in Fig. 2H.

**Metaphase arrest.** Embryos exposed to hypoxia very late in S phase, i.e., just after DNA replication but before metaphase proper, arrest in metaphase (Fig. 3). Figure 3A shows an embryo whose energids appear to be in either late prophase or prometaphase. Hypoxia was initiated just as the energids were entering metaphase (Fig. 3B). Unlike the embryo depicted in Fig. 2 (S phase arrest), this embryo arrested in metaphase (Fig. 3, C and D) without migrating through any other stage of mitosis. Two obvious phenomena occurred during a metaphase arrest. 1) The centrosomes lose their GFP-fluorescence intensity over time, as in the previous embryo. Centrosomes and astral fibers, visible in Fig. 3, B and C, are no longer apparent in Fig. 3D. 2) The spindle fiber apparatus undergoes specific and consistent morphological changes during hypoxia. The spindle fiber apparatus assumes a more ellipsoid (thinner) and condensed configuration and demonstrates some loss of fluorescent intensity during hypoxia (Fig. 3, B–D). The energids remain arrested in metaphase for the duration (up to 2 h tested) of hypoxia. On reoxygenation, the first recordable event is the reappearance of the centrosomes, as occurs after an S phase arrest (Fig. 3E). Thereafter, the spindle fiber apparatus demonstrates a marked increase in fluorescent intensity and a widening of its structure. The spindle fiber apparatus then resumes its normal metaphase conformation, and the cells resume cycling, again within ~20 min of reoxygenation (Fig. 3F). It is noteworthy that embryos exposed to hypoxia before metaphase (e.g., during prometaphase) that arrest at metaphase do so in a very short period of time, i.e., 1–2 min.

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**Fig. 2.** In vivo confocal imaging of a GFP-kinesin embryo arrested at interphase during hypoxia. A: control, normoxic embryo at the metaphase-to-anaphase transition of the cell cycle, captured 26 s before hypoxic perfusion. The embryo continues to cycle during hypoxia (B–E) passing through metaphase-anaphase (B), anaphase (C), and telophase-cytokinesis (D) before arresting in S phase (E). The embryo remains arrested for the duration of hypoxia (30 min hypoxic exposure) but demonstrates recovery (increased centrosomal fluorescence at 5 min of normoxia; G) and cell cycle activity (H) within 20 min of normoxia. Magnification, ×63.
AGE DEPENDENCE OF HYPOXIA-INDUCED ARREST. The age of the embryo does not appear to affect the stage at which the embryo arrests. Embryos at all stages of development as well as larvae (data not shown) undergo global arrest of the cell cycle during hypoxia. However, as demonstrated above, the stage of the cell cycle at the time of hypoxia initiation does appear to affect the stage at which the embryo will arrest.

CELL CYCLE KINETICS DURING HYPOXIA. We estimated that mitosis, as defined from prometaphase to the completion of cytokinesis, occupies ~3–5 min of the cell cycle. The time course of the full cell cycle, as estimated from one metaphase to the subsequent one, is ~12–15 min under control conditions. In control embryos (n = 5), the duration of mitosis was 321.8 ± 46 s, and the total length of the cycle was 945.2 ± 178 s. During hypoxia, mitotic duration and cell cycle length could not be easily estimated because of the cell cycle arrest. There appears to be no significant alteration in cell cycle times (958.0 ± 85 s) or in mitotic durations (421.5 ± 181.5 s) after reoxygenation (n = 3).

Determination of Hypoxia-Induced Cell Cycle Arrest Points

Effect of hypoxia on the distribution and expression of α-tubulin and propidium iodide. INTERPHASE ARREST. Embryos examined after 2 h of hypoxia demonstrated an arrest at either metaphase or S phase. A gastrula-stage embryo that appears to be arrested in S phase is depicted in Fig. 4A. The chromatin material is stained red by propidium iodide, and the α-tubulin antibody stains membranous material green. All of the cells in the gastrula-stage embryo seem to have arrested at the same stage (S phase) of the cell cycle. The chromatin material has a “prophase” configuration, in that the...
chromatin is condensed and somewhat circular. The α-tubulin stains the plasma membrane and intracellular cytoskeletal elements.

**METAPHASE ARREST.** An embryo exposed to 2 h of hypoxia can be seen in Fig. 4B. All of the cells in this early, precycle-13 embryo are arrested at metaphase. The chromatin, stained red by propidium iodide, is aligned along the equatorial plate of the cells and appears relatively condensed. The spindle fiber apparatus, stained green by the α-tubulin antibody, is remarkably similar in conformation to that observed during metaphase arrest in the in vivo GFP experiments. The spindle apparatus appears thinner and elliptical.

Therefore, both experiments in live and fixed tissues demonstrate that arrests in the cell cycle mediated by hypoxia occur at metaphase or during S phase. The early embryo has no G1 and G2 gap phases; however, the above experiments did not indicate whether hypoxia could interfere with DNA synthesis.

**Effect of hypoxia on the incorporation of BrdU.** In assessing both the live and fixed tissues, we determined that there were essentially two hypoxia-induced arrest points, at the metaphase-to-anaphase transition and at S phase. However, it was not clear from these experiments where embryos arrested, vis-à-vis DNA replication, when they were exposed to O2 lack past metaphase or anaphase. To address this issue, we exposed embryos to hypoxia in a specially designed chamber that permitted the permeabilization and fixation of embryos within the hypoxic environment. To determine the exact time that embryos arrested during S phase, we found it useful to utilize a fly strain that, on entering into the first gap phase in embryogenesis (G2 of S phase 14), could be induced to enter mitosis synchronously. This protocol, which is based on the use of heat shock String overexpression (see MATERIALS AND METHODS), provides gastrula-stage embryos whose cells enter mitosis and the subsequent S and G2 phases simultaneously.

Embryos were collected and allowed to age for 130 min, which would bring them to the G2 phase of interphase 14. They were then heat shocked for 40 min at 37°C. Control embryos that were not subjected to either heat shock or hypoxia demonstrated the incorporation of BrdU into nuclei within discrete mitotic regions (Fig. 5A). BrdU, in green, was detected along the ventral surface, where actively dividing neural precursors reside, and within nuclei of the mitotic domains of the gut region of this gastrula embryo, whereas chromatin material is stained red by propidium iodide. Embryos that were subjected to 40 min of heat shock but no hypoxia (heat shock control) gave evidence of more global BrdU incorporation, indicating that heat shock-induced String expression had driven most of the cells of this gastrula embryo into a premature mitosis and associated S phase (Fig. 5B). Embryos that were exposed to hypoxia within 10–15 min after heat shock showed absolutely no BrdU incorporation (Fig. 5C), indicating that hypoxia prevented these cells from initiating DNA replication. This study verified that hypoxia arrests embryos in interphase before DNA replication.

**Effect of hypoxia on cyclin B expression.** Again taking advantage of the heat shock String protocol, we examined the expression of cyclin B during hypoxia (n = 2). Cyclin B protein expression levels were normalized to α-tubulin protein expression levels and are presented in arbitrary units (AU) (Fig. 6). Control embryos that were heat shocked (Fig. 6, lane 2) had similar levels of cyclin B expression (0.64 ± 0.18 AU) compared with control embryos that were not heat shocked (0.84 ± 0.37 AU; Fig. 6, lane 1), indicating that the heat shock protocol did not have a significant effect on cyclin B expression in these S phase embryos. Levels of cyclin B expression in embryos that were heat shocked and exposed to hypoxia 4 min after the heat pulse (0.75 ± 0.38 AU; Fig. 6, lane 3) were similar to both non-heat-shocked and heat-shocked controls, indicating that these embryos were arrested in metaphase with unde-
graded cyclin B. On the other hand, heat-shocked embryos that were exposed to hypoxia 12 min after the heat pulse (Fig. 6, lane 4) showed lower levels of cyclin B expression (0.38 ± 0.11 AU) than the controls and the 4-min hypoxia group, indicating that they had passed through metaphase and had arrested in S phase with degraded cyclin B.

DISCUSSION

Within the cell cycle of both prokaryotes and eukaryotes are a number of transition states that can be interrupted if certain requirements have not been met or if the regulation of a number of cell cycle regulatory proteins has been altered (5, 15, 26, 28). In this study, we have identified cell cycle checkpoints that are responsive to O2 levels. Indeed, our in vivo analysis of fruit fly embryos containing a GFP-kinesin construct revealed that hypoxia induces cell cycle arrest at either S phase (before DNA duplication) or metaphase. These arrest points are dependent on the stage of the cell cycle when the embryo is exposed to O2 deprivation.

Although there have been two detailed studies on the effect of hypoxia on fly embryos, our study has differed in a number of respects (10, 35). First, this is the first study showing, in live embryos, online cell division of syncytial and cellularized embryos that arrest in very low O2 conditions. Second, although one of the other two studies (10) shows an arrest in the developmental program of the embryos during mitosis, the study did not show any detail regarding the relationship between the start of hypoxia and the stage at which the embryo arrested. Third, unlike the previous two studies, we describe the alterations in the shape of the spindle fibers when embryos are arrested in metaphase and the reversibility of this process. Fourth, unlike our study, in neither of the other studies did the authors investigate cell cycle checkpoint proteins.

Specificity of Hypoxia-Induced Cell Cycle Response

Depriving D. melanogaster embryos of O2 resulted in a consistent and reproducible cell cycle arrest at either S phase or metaphase. Reoxygenation led to the resumption of cell cycle activity within ~20 min, again in a relatively stereotypical fashion. In this study, we
have been able to demonstrate that fruit fly embryos can maintain the arrested state for up to 2 h and resume cell cycle activity on reoxygenation. This can be repeated several times even within the same embryo. It is reasonable then to assume that the presence or absence of O₂ has specific, reproducible effects on cell cycle activity. These data support the idea that O₂ deprivation has a unique mechanism of action on the cell cycle in fruit fly embryos. Furthermore, the rapidity of the induced arrest suggests that there is a direct effect of decreased O₂ levels on the cell cycle machinery rather than an effect on energy stores that would generally take a longer time.

Possible Mechanisms of Action of Hypoxia-Induced Arrest

Hypoxia-induced S phase arrest, based on in vivo confocal and BrdU incorporation studies, seems to occur before DNA replication. This indicates that DNA replication is sensitive to the availability of O₂ and that at least one or more components of the DNA-replication checkpoint machinery may be hypoxia responsive. Two categories of DNA checkpoints have been described: 1) DNA replication checkpoint and 2) DNA damage checkpoint (8, 33). Because these embryos recover cell cycle activity rather quickly during reoxygenation, it is more likely that the DNA-replication checkpoint is invoked during hypoxia to produce an S phase arrest. A number of DNA-replication checkpoint proteins have been implicated, and some could be important in transducing the hypoxia signal. These would include cyclin-dependent kinases 1 (cdk2), 2, 4, and 6; cyclins A, D, and E; the retinoblastoma protein; and cyclin-dependent kinase inhibitors (11, 21, 22).

A block of the cell cycle at metaphase can occur if the spindle apparatus is improperly assembled or if sister chromatid separation is prevented (5, 28). In our study, hypoxia-exposed embryos specifically arrested at this mitotic spindle checkpoint, as demonstrated in the in vivo confocal and α-tubulin immunocytochemical studies. Therefore, a release from metaphase was inhibited by the hypoxic exposure. The metaphase-to-anaphase transition requires the activation and inactivation of several cell cycle regulatory proteins (3), and the release from metaphase requires the degradation of cyclin B and the inactivation of cdc2 by ubiquitin-dependent proteolytic activity of the anaphase promoting complex (APC) (19, 25, 34). Additionally, proteins such as the securins and separins that control sister chromatid cohesion and separation, as well as a block on the APC, could therefore be involved in the hypoxia-induced metaphase arrest (18, 20). Because we have demonstrated that cyclin B is not degraded during a hypoxia-induced metaphase arrest, it can therefore be hypothesized that O₂ deprivation imposes a block on cyclin B degradation either by maintaining the inactivated state of the APC or preventing activation of the APC. However, as noted in Fig. 6, cyclin B does not appear to be completely degraded after metaphase. It has been reported that cyclins A and B are not completely degraded after metaphase in syncytial (early) embryos (23) and that a 57°C heat shock may also delay the degradation of cyclins A and B in blastoderm (later stage) embryos (24).

Significance

The hypoxia-induced arrest in embryos may have a beneficial value, possibly related to survival of the organism. Besides saving on energy requirements, an arrest during S phase before DNA replication may function to safeguard against DNA-replication errors. Similarly, an arrest during metaphase may serve to protect the cell from errors associated with abnormal spindle morphology and migration, improper chromatid alignment on the spindle apparatus, and abnormal or dysfunctional sister chromatid separation during hypoxia (15, 26). What is still a mystery, however, is the molecular basis for the lack of apparent injury to the organism during resumption of growth after hours, or even days and weeks, of hypoxia/anoxia and developmental arrest as has been described for Drosophila embryos exposed to chronic hypoxia for up to 8 days (35) and brine shrimp embryos maintained in complete N₂ for up to 4 yr (2). Furthermore, because in utero and early postnatal growth is also profoundly affected by O₂ deprivation such as at high altitude, it is very likely that mechanisms similar to those described in this study are also present in other organisms, including mammals. Indeed, it has become clear in the past decade that organisms such as Drosophila and Caenorhabditis elegans share with mammals and even primates the same complements of genes that shape development, growth, and response to stress. This concept has rendered these genetic models very attractive since findings in these models may be generalizable.

Perspectives

That cell cycle and cell division are affected during low O₂ states does not need to be limited to this species. As mentioned above, the shrimp embryo is also affected in a major way. There is indeed evidence that growth in mammals, including humans, is sensitive to low O₂ states. Experiments from our laboratory in the past few years have always demonstrated that rodents exposed to chronic or intermittent hypoxia in early life are smaller. In utero growth and postnatal development can be retarded, and lessons from young patients with cyanotic congenital heart disease have taught us that growth rate can be impaired in these patients. Whether healing from injury and repair mechanisms is also affected by hypoxia remains to be seen and needs additional experimentation.

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