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Cell cycle roles for two 14-3-3 proteins during *Drosophila* development

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Summary

Drosophila 14-3-3ε and 14-3-3ζ proteins have been shown to function in RAS/MAP kinase pathways that influence the differentiation of the adult eye and the embryo. Because 14-3-3 proteins have a conserved involvement in cell cycle checkpoints in other systems, we asked (1) whether *Drosophila* 14-3-3 proteins also function in cell cycle regulation, and (2) whether cell proliferation during *Drosophila* development has different requirements for the two 14-3-3 proteins. We find that antibody staining for 14-3-3 family members is cytoplasmic in interphase and perichromosomal in mitosis. Using mutants of cyclins, Cdk1 and Cdc25^{string} to manipulate Cdk1 activity, we found that the localization of 14-3-3 proteins is coupled to Cdk1 activity and cell cycle stage. Relocalization of 14-3-3 proteins with cell cycle progression suggested cell-cycle-specific roles. This notion is confirmed by the phenotypes of 14-3-3ε and 14-3-3ζ mutants: 14-3-3ε is required to time mitosis in undisturbed post-blastoderm cell cycles and to delay mitosis following irradiation; 14-3-3ζ is required for normal chromosome separation during syncytial mitoses. We suggest a model in which 14-3-3 proteins act in the undisturbed cell cycle to set a threshold for entry into mitosis by suppressing Cdk1 activity, to block mitosis following radiation damage and to facilitate proper exit from mitosis.

Keywords

Drosophila; Cell Cycle; Checkpoint; Mitosis; 14-3-3

Introduction

14-3-3 proteins are small (~30 kDa), abundant proteins that are present in organisms ranging from yeast to human (Shaw, 2000). 14-3-3 dimers bind phosphorylated epitopes on other proteins, presumably stabilizing this modification; whether this is an activating or an inhibitory act might depend on how phosphorylation affects the target protein (Muslin et al., 1996; Yaffe et al., 1997). Consensus 14-3-3-binding sites appear on a diverse array of proteins, many of which are known to bind 14-3-3 proteins in vivo and in vitro (Yaffe et al., 1997). These include Cdc25 of yeast, *Xenopus* and human, and cyclin B1 of human, proteins that are required for mitosis (Chan et al., 1999; Kumagai and Dunphy, 1999; Lopez-Girona et al., 1999; Peng et al., 1997). Consistent with these data, several reports implicate 14-3-3 proteins in the regulation of mitosis. In *Schizosaccharomyces pombe*, two 14-3-3 proteins (Rad24 and Rad25) act to

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inhibit mitosis in the presence of damaged DNA (Ford et al., 1994; Lopez-Girona et al., 1999). Similarly, 14-3-3σ in human cells acts to inhibit mitosis after DNA damage, by binding cyclin B1-Cdk1 complexes (Chan et al., 1999). The activities attributed to 14-3-3 proteins involve the regulation of mitosis in response to checkpoint activation. Whether 14-3-3 proteins function in normal cell cycle progression remains unknown.

In contrast to other systems, studies of 14-3-3 proteins in *Drosophila* have focused primarily on their roles in RAS/MAPK signaling and in neuronal differentiation (Chang and Rubin 1997; Kockel et al., 1997; Li et al., 1997; Skoulakis and Davis 1998). *Drosophila* 14-3-3ε, for example, is required for Sevenless signaling during photoreceptor differentiation in the adult eye, whereas 14-3-3ζ (Leonardo) functions in Torso signaling during the differentiation of the embryonic termini.

Because of conserved roles for 14-3-3 proteins in cell cycle regulation in yeast and human, we asked whether *Drosophila* 14-3-3ε and 14-3-3ζ also have roles in cell cycle regulation, in addition to their function in signaling. To this end, we analyzed the localization of 14-3-3 proteins during the cell cycle and tested whether mutation of 14-3-3ε and 14-3-3ζ results in cell division defects. Our results suggest that 14-3-3 proteins function both during interphase (to time the entry into mitosis) and in mitosis (to facilitate chromosome separation). Moreover, these functions are required for progression through unperturbed cell cycles as well as in response to irradiation.

Materials and Methods

Fly stocks

All fly stocks used here have been described before. The 14-3-3ε mutant l(3)j2B10 is the result of a P-element insertion in the first intron and results in a strong loss of function allele (Chang and Rubin 1997). 14-3-3ε heterozygotes were crossed to a deficiency stock (Df(3R)Cha7; stock # BL-3011) to generate trans-heterozygotes of l(3)j2B10 and the deficiency (both males and females), which were then the source of '14-3-3ε mutant' embryos in all experiments described here. This cross is necessary because of a lethal mutation on the l(3)j2B10 chromosome (Chang and Rubin 1997). Because the trans-heterozygous females we collect were not necessarily virgins, they were mated with trans-heterozygous males for at least one week before 14-3-3ε mutant embryos were collected, to bias paternity towards trans-heterozygous males over heterozygous siblings. Consequently, most of the embryos examined lack both maternal and zygotic sources of 14-3-3ε. The phenotypes described here for 14-3-3ε mutant embryos were seen for most, if not all, such embryos (we have not seen, but also have not directly tested for, a zygotic contribution to the phenotypes described in this report). Standard FLP protocols were followed to make germ line clones of 14-3-3ζ using a previously characterized mutant, P1188, a P-element insertion allele (Chou and Perrimon 1996; Hou et al., 1995; Li et al., 1997; Skoulakis and Davis 1996). Transgenic stocks carrying stable cyclins and Cdk1AF under the control of heat-inducible (hs) promoters have been described (Sprenger et al., 1997; Su et al., 1998). Stg^{7B} (Edgar and O'Farrell, 1989) and Stg^{7B} with two copies of hs-Cdk1AF transgene (N. Yakubovich and P.H.O., unpublished) were used. Wild type in all experiment is of Sevelen strain.

Irradiation and heat shock

For irradiation, embryos were collected for 2 hours on grape agar plates at 25°C and aged for 2 hours to reach cycle 14. Embryos were irradiated at 2.2 rad sec^{-1} in a TORREX120D X-ray generator (Astrophysics Research, Long Beach, CA) by placing agar plates facing up on shelf 6. The generator was set at 5 mA and 115 kV. Embryos were fixed as described below.

For the induction of stable cyclins A and B, embryos were collected for 30 minutes and aged for 2 hours 45 minutes at 25°C. Embryos were heat shocked by floating the grape-agar plates on water in a 37°C water bath for 30 minutes. Embryos were allowed to recover for 1 hour 15 minutes at 25°C before fixing.

For the analysis of *stg* mutants, embryos were collected for 2 hours and aged for 2 hours at 25° C before fixing. The *stg* homozygous mutants were identified by the lack of mitotic cells. To heat shock Cdk1AF in a *stg* mutant background, embryos were collected for 2 hours and aged for 4 hours at 25°C before being heat shocked as described above. The longer aging time before heat shock allows cells in the *stg* homozygous embryos to remain arrested in cell cycle 14, whereas cells of their siblings have progressed through two additional divisions. This results in a cell size difference that allows us to identify *stg* homozygous embryos. Heat-shocked embryos were allowed to rest for 30 minutes at 25°C before fixing.

Fixing and staining

Embryos were dechorionated with 50% bleach for 2 minutes and fixed for 20 minutes in a bilayer of heptane:PBS + 5% formaldehyde. For stable cyclin experiments, embryos were first incubated for 30 seconds in a bilayer of heptane:PBS + 5 μM Taxol before formaldehyde was added to reach 5% of the PBS layer. Fixed embryos were blocked in PBT + 3% normal goat serum for at least 1 hour before staining with antibodies. PBT is PBS (140 mM NaCl; 2.6 mM KCl; 10 mM Na_2HPO_4; 1.8 mM KH_2PO_4; pH7.4) + 0.2% Tween. Primary antibodies were used at the following dilutions: affinity-purified rabbit anti-14-3-3ε antibody (Tien et al., 1999), 1:200 – 1:300; purified rabbit IgG against 14-3-3 proteins (Catalog # SC-629; Santa Cruz Biotechnologies), 1:300; affinity-purified rabbit polyclonal anti-phospho-histone H3 (Upstate Biotechnologies), 1:1000. SC-629 antibody was generated against the sequence DKSELVQKAKLAEQAERY. The corresponding regions in *Drosophila* proteins are DKEELVQKAKLAEQSERY (ζ) and ERENNVYKAKLAEQAERY (ε). Primary antibodies were detected by staining with FITC- or rhodamine-conjugated secondary antibodies (Jackson) diluted 1:500 and pre-absorbed against a large volume of fixed embryos to reduce non-specific binding. Embryos were also stained with 10 μg ml^{-1} bizbenzimide (Hoechst33258) in PBT for 10 minutes to visualize DNA. In some cases, embryos were stained with 500 ng ml^{-1} FITC- or rhodamine-conjugated wheat-germ agglutinin (WGA; Molecular Probes) in PBT, to visualize the nuclear envelope.

Live observations

Embryos were collected on grape-juice agar plates, dechorionated with 50% bleach for 2 minutes, rinsed thoroughly with ddH_2O, and placed on a glass coverslip using a soft brush. Excess water was removed and a thin layer of Halocarbon 700 oil (Sigma) was placed on embryos. The coverslip was inverted and placed over a hole in the center of a microscope slide such that oil-covered embryos were otherwise exposed to air. Time-lapsed pictures were taken using a 10× or a 20× objective on a Leica DMR scope and a Sensicam CCD camera.

Image analysis

All images except Fig. 2D were collected on a Leica DMR fluorescence microscope attached to a Sensicam CCD camera, using Slidebook software (Intelligent Imaging Innovations). Images were compiled using Photoshop. Data in Fig. 2D were captured on an Olympus fluorescence microscope using Deltavision software. Optical sections were then deconvolved to exclude out-of-focus information using the same software, and flattened into a two-dimensional projection for presentation.

Results

Subcellular localization of 14-3-3 proteins changes during the cell cycle

Many cell cycle regulators show a change in their properties, such as modification, location or activity, coincident with the cell cycle transitions they regulate. For instance, DNA replication factors change location within the cell as DNA synthesis progresses, and mitotic cyclins localize to the nucleus as cells enter mitosis and are degraded as cells exit mitosis (Sigrist et al., 1995; Su and O'Farrell, 1997). Thus, we might expect the localization of 14-3-3 proteins to change in concert with cell cycle progression if these proteins have a role in the cell cycle.

Embryogenesis in *Drosophila* begins with 13 synchronous nuclear divisions that occur in a common cytoplasm, a syncytium (Foe et al., 1993). Staining of fixed syncytial-stage *Drosophila* embryos with an affinity-purified antibody to 14-3-3ε shows dispersed staining in interphase and predominantly nuclear staining at entry into mitosis (Fig. 1E; Fig. 2A). Because the antibody detects no staining in 14-3-3ε mutants (Tien et al., 1999), this staining reflects the distribution of 14-3-3ε protein. The antigen remains concentrated near chromosomes throughout metaphase, in the absence of an intact nuclear envelope (Fig. 1F). This behavior is different from that of nuclear proteins such as replication factors, which disperse into the cytoplasm when the nuclear envelope is disrupted in metaphase (for example, Su and O'Farrell 1997). Our observations instead suggest a sequestration of 14-3-3ε around chromosomes, rather than localization by nuclear import and retention by the nuclear envelope. The antigen remains in the proximity of chromosomes in early anaphase and disperses in late anaphase/telophase as cells exit mitosis (Fig. 1H). These data reveal a cycle of localization of 14-3-3ε that includes perichromosomal location in mitosis.

After the syncytial cycles, embryos cellularize and undergo three more cell divisions that are no longer synchronous. A similar localization pattern for 14-3-3ε is seen in cellularized embryos, although exclusion from nuclei during interphase is more pronounced in the cellular cycles than in the syncytial cycles (Fig. 1J-N; Fig. 2A).

To determine whether the second *Drosophila* 14-3-3 homolog, 14-3-3ζ, also localizes in a similar manner, we stained embryos with a commercial antibody that recognizes 14-3-3ζ and to a lesser extent 14-3-3ε (Skoulakis and Davis 1998) (T.T.S., unpublished; see also Materials and Methods). This antibody detected a changing pattern of localized staining that paralleled the pattern detected by the 14-3-3ε-specific antibody. For example, Fig. 1F,G shows metaphase staining with 14-3-3ε-specific antibody and commercial 14-3-3 antibody, respectively. We conclude that the two 14-3-3 proteins have similar programs of localization during the cell cycle. The cycle of localization of 14-3-3 proteins, and their perichromosomal location in mitosis suggests that they have roles in mitotic progression.

Cdk1 activity and mitotic progression dictates 14-3-3ε localization

Localization of 14-3-3ε and 14-3-3ζ to the nucleus occurs during prophase, and is thus temporally correlated with the activation of mitotic kinase, Cdk1. To test whether this localization is coupled to Cdk1 activation, we examined 14-3-3ε localization in *string* mutants. The *stg* gene encodes a homolog of Cdc25, a phosphatase that is required for activation of Cdk1 and entry into mitosis. In homozygous *stg* mutant embryos from heterozygous mothers, 13 syncytial cycles are driven by maternally deposited *stg*. Following cellularization, cells arrest in G2 of cycle 14 because embryonic *stg* is required to activate Cdk1 at entry into M14 (Edgar and O'Farrell, 1989). In G2-arrested cells of *stg* mutant embryos, 14-3-3ε remains excluded from the nucleus (Fig. 2A,B). We conclude that *stg* activity is required, either directly or indirectly, for relocalization of 14-3-3ε.

To distinguish whether it is *stg* or consequent Cdk1 activity that promotes 14-3-3 relocalization, we induced a mutant form of Cdk1, Cdk1AF, in *stg* mutants. Cdk1AF has substitutions in amino acid residues that are normally dephosphorylated by Stg and can therefore bypass the requirement for *stg* (Sprenger et al., 1997). Expression of Cdk1AF in *stg* mutants drives G2-arrested cells into mitosis (N. Yakubovich and P.H.O., unpublished). We found that 14-3-3ε localizes to the nucleus and to regions near metaphase chromosomes in Cdk1AF-induced mitoses (Fig. 2C), just like in wild-type mitoses. Although the concentration of antigen near the chromosomes is qualitatively similar to that of the wild type, it is not as robust. This is consistent with previous observations that mitotic induction by Cdk1AF is less robust than in the wild type, as indicated by slower kinetics (N. Yakubovich and P.H.O., unpublished). These data suggest that, although Cdk1 activity drives the localization of 14-3-3ε to chromosomes, *stg* is dispensable for this process.

The failure of nuclear 14-3-3 to disperse upon disruption of the nuclear membrane during mitosis could be due to anchoring of 14-3-3 or could simply reflect slow dispersal and a rapid mitosis. We tested these possibilities by arresting mitotic progression with stable cyclins. *Drosophila* mitotic cyclins A and B, both partners of Cdk1, are degraded during metaphase and anaphase, respectively (Sigrist et al., 1995). Introduction of cyclin mutants that are refractory to degradation arrests cells at specific points within mitosis, at metaphase in the case of stable cyclin A (A^s) and in early anaphase in the case of stable cyclin B (B^s) (Sigrist et al., 1995; Su and O'Farrell, 1997). In these arrests, 14-3-3ε was found to be concentrated around chromosomes, just as in wild-type metaphase and early anaphase (Fig. 2D). Thus, stabilization of Cdk1 activity in this experimental context is sufficient to stabilize 14-3-3ε localization near chromosomes. These data further suggest that the dispersal of 14-3-3ε we see at the end of mitosis in wild-type embryos requires a decline in Cdk1 activity, and argues that 14-3-3ε is specifically localized during mitosis, although the structural identity of the site of localization is mysterious.

In embryos that have been exposed to DNA-damaging agents such as ionizing radiation, cells delay entry into mitosis. Previous data indicate that this delay is achieved by inhibition of Cdk1 activity (Su et al., 2000). If Cdk1 activity is required for the nuclear localization of 14-3-3 proteins, as suggested by *stg* mutants, we would expect this movement to be blocked in irradiated embryos. This was indeed the case: 14-3-3ε remained excluded from nuclei after irradiation (Fig. 2E). Collectively these data suggest that the dynamic mitotic cycle of 14-3-3 localization is coupled to the cycle of Cdk1 activation and inactivation that occurs at entry into and exit from mitosis, respectively.

14-3-3ε regulates the entry into mitosis in *Drosophila* embryos

Although the localization data suggest that 14-3-3 proteins have a role in mitosis, it is only correlative evidence. To test for cell cycle roles of 14-3-3 proteins more directly, we examined previously described mutants in 14-3-3ε and 14-3-3ζ, paying particular attention to embryonic cell cycles. 14-3-3ε is dispensable for viability (Chang and Rubin 1997) and so we were able to generate homozygous mutant adults and analyze their embryos (see Materials and Methods). The 14-3-3ε mutant embryos progressed through the first 13 mitotic cycles and cellularized without obvious defect. We compared the timing of the mitosis 14 in these mutant embryos to the timing in wild-type embryos. Unlike the earlier synchronous divisions, the time of entry into mitosis 14 is spatially programmed in wild-type embryos so that local cohorts of cells called ‘mitotic domains’ enter mitosis according to a stereotyped schedule (Foe et al., 1993). Concurrent gastrulation movements change the shape of the embryo. Thus, in wild-type embryos at a particular stage in gastrulation (i.e. exhibiting a specific morphology), specific cells are in mitosis (Foe, 1989). For example, in the embryo in Fig. 3A, germ-band elongation (a major morphogenetic event at this stage) has barely begun and pole cells are still near the

posterior end of the embryo. Very few cells are in mitosis at this time. By contrast, in 14-3-3ε mutants at the same stage in gastrulation (Fig. 3B), cells of mitotic domains 1-5 are seen to be entering mitosis, indicating an advancement of mitosis in 14-3-3ε mutants. This pattern of divisions is seen in wild-type embryos of more advanced gastrulation (Fig. 3C), indicating that the relative programs of mitoses are conserved. Similar observations are made at later stages in gastrulation also. Thus, the division pattern of 14-3-3ε mutant embryo in Fig. 3D is more advanced than in wild-type embryos of similar gastrulation (Fig. 3C) but is similar to wild-type embryos of more advanced gastrulation (Fig. 3E). These data indicate a premature entry of 14-3-3ε mutant cells into mitosis relative to gastrulation; moreover, the mutation advances the entire schedule of mitosis without disrupting the relative order of mitosis in different positions within the embryo.

In the above analyses, we are comparing the timing of mitosis with respect to the extent of gastrulation. The results are consistent with either retardation of morphogenesis or acceleration of entry into mitosis. To address these possibilities, we directly measured the rate of germ-band elongation and found it to be indistinguishable in wild-type and mutant embryos (Fig. 3I). Based on the failure to detect a delay in developmental progression, we infer that loss of 14-3-3ε advances the schedule of entry into mitosis 14.

In wild-type embryos, the time of entry into mitosis 14 is delayed following treatment with DNA-damaging agents (Su et al., 2000); this is detected by the failure of particular mitotic domains to divide at the expected stage of gastrulation (Fig. 3F). To determine whether 14-3-3ε is required for this delay of mitosis, we examined 14-3-3ε mutant embryos after exposure to X-rays. We found that irradiated 14-3-3ε embryos showed mitotic cells in all domains and that each mitotic domain made its appearance at the same stage of embryogenesis as is did in non-irradiated 14-3-3ε mutant embryos (Fig. 3D,G). We conclude that 14-3-3ε mutant embryos are unable to delay mitosis fully and infer that 14-3-3ε normally functions to delay mitosis after DNA damage. However, the number of mitotic cells in irradiated 14-3-3ε mutants might be lower than that in non-irradiated mutant embryos. Thus, the loss of mitotic regulation in irradiated 14-3-3ε mutants might be partial.

14-3-3ζ is required for syncytial divisions

In contrast to 14-3-3ε, 14-3-3ζ is required for viability (Skoulakis and Davis, 1996). Homozygous embryos from heterozygous parents die before hatching. These embryos, however, appear to have intact mitotic regulation: all progeny from heterozygous parents show no apparent defects in the timing of M14, and these embryos also delayed mitosis after irradiation (data not shown). We cannot, however, rule out the possibility that maternal 14-3-3ζ from heterozygous mothers persists through the three post-blastoderm divisions. Therefore, we made attempts to remove the maternal supply by creating genetic mosaics.

We used standard techniques to create mosaics in the germ line of heterozygous 14-3-3ζ mutant females (Chou and Perrimon, 1996). In this technique, recombination events in the mother create 14-3-3ζ homozygous mutant cells in the ovary (and elsewhere in the body). A dominant mutation that disrupts oogenesis is present on the homolog with a functional 14-3-3ζ gene and so all eggs that are produced lack this chromosome and are therefore deficient in maternally supplied 14-3-3ζ gene product (‘14-3-3ζ-deficient embryos’). Analysis of such embryos revealed an essential role for 14-3-3ζ in syncytial divisions, in agreement with a previous study (Li et al., 1997). This previous study, however, focused on a fraction of embryos that progressed beyond syncytial cycles in order to study the role of 14-3-3 proteins in the differentiation of cellularized embryos. We focus here on defects seen during syncytial cycles.

In a 24 hour collection, most 14-3-3ζ-deficient embryos (69±9% from three separate collections) fail to cellularize. To examine cell division defects in these embryos, we fixed and

stained 0-3-hour-old embryos (a population enriched in syncytial stages) with a DNA dye and an antibody to α-tubulin (to visualize microtubules). We noted division defects in almost all (54 of 59) 14-3-3ζ-deficient embryos examined. The defects include DNA bridges between telophase sister nuclei, pronounced asynchrony in division within a single embryo, free microtubule-organizing centers (MTOC) that are not associated with nuclei, loss of nuclei from the cortical monolayer of nuclei and larger than normal yolk DNA masses (Fig. 4; and data not shown).

To understand how loss of 14-3-3ζ can result in such diverse defects, we looked for the earliest visible defect, with the hope of uncovering the primary defect. We found chromosome bridges interconnecting DNA masses in embryos as early as telophase of the fourth embryonic mitosis (Fig. 4B,E). This defect is seen with varying frequency in all syncytial embryos examined, although not all dividing nuclei pairs within each embryo show a chromosome bridge.

Analysis of older syncytial embryos, in which the superficial location of nuclei allows the visualization of microtubules, suggested that mitotic spindles formed. Moreover, these spindles appear to be functional as judged by the segregation of chromosome masses that are still linked by DNA bridges to opposite spindle poles (Fig. 4C,F,G). What appear to be attempts at forming mid-bodies are also visible between segregating nuclei despite the presence of chromosome bridges (Fig. 4C,F,G). These observations suggest that 14-3-3ζ-deficient embryos retain spindle function but chromosome segregation often does not go to completion because DNA masses cannot be fully resolved.

If the inability to separate and segregate chromosomes fully during the earliest mitoses is the primary defect in 14-3-3ζ-deficient embryos, this can explain the pleiotropic defects seen later. Incomplete chromosome separation would invariably lead to aneuploid or damaged nuclei. Such nuclei in the *Drosophila* syncytium are culled by exile into the center of the embryo and become incorporated into yolk, whereas their centrosomes are left behind (Debec et al., 1996); abandoned centrosomes could account for free MTOCs we observed. Culling during cortical syncytial cycles (cycles 10-13, which occur after nuclei have finished migrating to the embryo cortex) would lead to a loss of nuclei from the cortical layer, whereas the addition of culled nuclei to the central pool of DNA would account for larger than normal yolk DNA masses we observed. The remarkable ability of syncytial embryos to adjust to early mitotic defects (Debec et al., 1996) might account for the fraction of embryos that successfully progress to later stages.

As noted above, ~30% of embryos progressed beyond syncytial cycles and cellularized. We see severe gastrulation defects in these, as described before (Li et al., 1997). Therefore, these embryos are unsuitable for studies of mitotic timing such as that in Fig. 3.

Discussion

Localization of 14-3-3 proteins is coupled to cell cycle progression

In *Drosophila*, 14-3-3ε and 14-3-3ζ function in RAS-mediated signaling but are thought to act between RAS and RAF in the cytoplasm (Chang and Rubin 1997; Kockel et al., 1997; Li et al., 1997). In a previous study of 14-3-3ε localization in the embryo, this protein was reported to become nuclear-localized in infolding cells (Tien et al., 1999). However, a close examination of the published data revealed that the localization was in pre-mitotic cells (the publication featured mitotic domain 14 that borders the ventral furrow). In fact, a close correspondence of cells that show nuclear-localized 14-3-3ε in this publication (Tien et al., 1999) and cells that compose the mitotic domains (Foe 1989) is what led us to examine further the role of 14-3-3 proteins in the cell cycle. Using the same antibody and the same conditions, we demonstrate similar staining patterns (Tien et al., 1999). We are, however, offering a different interpretation

of these data. We find no correlation of the localized staining with the movement of cells or folding of the epithelium. Instead, our findings that 14-3-3 proteins localize to the perichromosomal region during mitosis and that this localization is coupled to Cdk1 activity demonstrate that localization is coupled to cell cycle progression and suggest that 14-3-3 proteins have a cell cycle role.

One striking set of data presented here concern the localization of 14-3-3 proteins to the neighborhood of chromosomes in mitosis. Although the perinuclear localization of *Drosophila* 14-3-3 proteins is unprecedented, the interphase location and activity are consistent with reports from other systems. *S. pombe* Rad24 remains exclusively cytoplasmic throughout the cell cycle and this localization appears to be important for blocking mitosis upon checkpoint activation (Lopez-Girona et al., 1999). Similarly, it has been proposed that cytoplasmic human 14-3-3σ inhibits mitosis by retaining Cdk1/cyclin B in the cytoplasm (Chan et al., 1999). Like their homologs in other systems, *Drosophila* 14-3-3 proteins are cytoplasmic in interphase, and our analysis of mutations suggests that *Drosophila* 14-3-3ε also inhibits entry into mitosis in response to activation of DNA damage checkpoint in embryos. This is in agreement with its proposed role in other species and consistent with a recent report of a role for 14-3-3ε in preventing mitosis after DNA damage in *Drosophila* larvae (Brodsky et al., 2000).

Role for 14-3-3ε in normal cell cycle progression

In addition, our observations indicate a role for 14-3-3ε in the normal timing of embryonic mitoses. The precise schedule of mitotic times of cells in various positions in the *Drosophila* embryo allows us to detect deviations from normal timing that are as small as a few minutes. Defects can occur in the normally rigid stereotypical order with which different regions of the embryo progress into mitosis. For example, recent reports described the premature mitosis of mesodermal cells, normally domain 10, in a mutant *tribbles* (Grosshans and Wieschaus, 2000; Mata et al., 2000; Seher and Leptin, 2000). When we examined embryos deficient in 14-3-3ε, we found a different type of timing defect. The normal order of the mitotic domains was retained, but the entire schedule of mitosis was advanced relative to germ-band extension, a major morphological marker of developmental progression. Because there was no detectable slowing of germ-band extension in 14-3-3ε mutant embryos, we infer that mitosis is advanced in embryos that lack 14-3-3ε. Thus, 14-3-3ε might set physiologically relevant thresholds for entry into mitosis in *Drosophila*, and this activity might be amplified in response to irradiation. *S. pombe* mutants in a 14-3-3 homolog show smaller cell size at division; because cellular growth in this organism occurs mainly in G2, it has been proposed that G2 is shorter in these 14-3-3 mutants (Ford et al., 1994), although precise measurements of this period have not been reported. Thus, it remains to be seen whether 14-3-3 proteins have a similar ability to set the threshold for normal mitosis in other species where only its checkpoint function has been detected.

Mutations in 14-3-3ζ uncover an early role for 14-3-3 proteins

We find that 14-3-3ζ mutants show defective mitoses in the syncytium, indicating a requirement for this protein in syncytial divisions. Embryos that lack checkpoint functions such as Grapes (Chk1 homolog) and Mei-41 (an ATR homolog) also show mitotic defects, and it has been proposed that these defects are secondary to entry into mitosis with unreplicated DNA (Sibon et al., 2000; Yu et al., 2000). However, loss of 14-3-3ζ functions affects early cycles. By contrast, the dramatic phenotypes of checkpoint defects occur at later syncytial stages (around cycle 12) when checkpoints are thought to become essential to postpone mitosis as S phase takes longer to complete. Thus, the early phenotype of 14-3-3ζ mutant embryos suggests that 14-3-3ζ has roles beyond its likely function in the checkpoint. Perhaps, like 14-3-3ε, 14-3-3ζ might contribute to the normal timing of mitosis even when checkpoints are not operating. Alternatively, incomplete separation of chromosomes in 14-3-3ζ mutants could

indicate a more direct involvement of 14-3-3ζ in mitotic progression, an idea that is supported by the localization of the proteins around the mitotic chromosomes and their dispersal after chromosome separation. A direct test of these models will require specific inactivation of 14-3-3ζ in mitosis (as opposed to interphase), for which we lack the means at present.

Drosophila 14-3-3ε and 14-3-3ζ have documented roles in RAS signaling. Recent data implicate a MAP kinase pathway in cell cycle control in *Xenopus* (Chau and Shibuya, 1999; Guadagno and Ferrell, 1998; Takenaka et al., 1998), raising the possibility that *Drosophila* 14-3-3 proteins function through a MAPK pathway to affect their cell cycle roles. We think that this is unlikely because treatment of *Drosophila* embryos with pharmacological inhibitors of MAPK pathway did not phenocopy either 14-3-3ε or 14-3-3ζ mutations (N. Ahn and T.T.S., unpublished).

Regardless of the mechanism of action of 14-3-3ζ, it is notable that it has essential cell cycle roles in the absence of perturbations that normally provoke checkpoint responses. This reinforces other findings in *Drosophila* and in mammals that suggest that functions normally considered to be checkpoint functions have essential roles in regulating the cell cycle early in development (Brown and Baltimore, 2000; de Klein et al., 2000; Fogarty et al., 1994; Liu et al., 2000; Sibon et al., 1999).

Model for the interplay of cyclin/Cdk1 activity and 14-3-3 protein function

Based on the cytoplasmic localization of 14-3-3ε and cyclin/Cdk1 during interphase (Fig. 4) (Sigrist et al., 1995), we propose that 14-3-3ε acts to keep Cdk1 in check during interphase. As Cdk1 becomes active (owing to the accumulation of its activator Stg or after recovery from DNA damage) and cells enter mitosis, accumulating cyclin/Cdk1 activity promotes and maintains, probably indirectly, 14-3-3 protein localization near chromosomes. Upon the transition to anaphase, the localized 14-3-3 proteins can contribute to chromosome separation. The decline in Cdk1 activity allows 14-3-3 proteins to return to their interphase distribution. Thus, during interphase, 14-3-3ε can act to keep Cdk1 inactive in the cytoplasm but, once Cdk1 is active, it can act in turn to localize 14-3-3 proteins in preparation for their action during the exit from mitosis (Fig. 5). We failed to detect a physical interaction between 14-3-3 proteins and *Drosophila* homologs of cell cycle regulators known to interact with 14-3-3 proteins in other systems ($Cdc25^{string}$ and cyclin B) under several experimental conditions (A.P. and T.T.S., unpublished). Thus, understanding the mechanism of 14-3-3 action might require the identification of novel target molecules.

Embryonic stage-specific requirement for 14-3-3ε and 14-3-3ζ

Our results do not rule out the possibility that 14-3-3ζ also functions to regulate the entry into mitosis in cellular embryos. We cannot address this possibility because 14-3-3ζ mutants arrest before G2/M control is first seen in embryogenesis, and the fraction of embryos that do progress to cellular stages are too defective with respect to cell cycle progression and gastrulation. In addition, the fact that these embryos progressed to cellular stages might reflect an incomplete loss of maternal 14-3-3ζ as previously proposed (Li et al., 1997), thus precluding meaningful experiments. What is certain, however, is that 14-3-3ε cannot substitute for 14-3-3ζ during the nuclear divisions of syncytial stages, and that 14-3-3ζ cannot substitute 14-3-3ε for regulating the entry into mitosis during cellular stages.

Conclusions

In summary, three lines of data indicate that *Drosophila* 14-3-3 proteins function in normal cell cycle progression, in addition to checkpoint regulation. These are: (1) cell cycle stage specific localization, which is dictated by Cdk1; (2) advancement of mitotic entry in 14-3-3ε mutants; and (3) defective mitoses in 14-3-3ζ mutants. To our knowledge, this is the first clear

evidence for the requirement for 14-3-3 proteins in normal mitosis in a eukaryote. Furthermore, the fact that mutations in two 14-3-3 proteins lead to different outcomes and at different stages in embryogenesis indicates that these proteins are not functionally redundant. Instead, our results provide strong evidence that, during metazoan development, cell division and its regulation might have different requirements for two members of the 14-3-3 family.

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References

Brodsky MH, Sekelsky JJ, Tsang G, Hawley RS, Rubin GM. *mus304* encodes a novel DNA damage checkpoint protein required during *Drosophila* development. Genes Dev 2000;14:666–678. [PubMed: 10733527]

Brown EJ, Baltimore D. ATR disruption leads to chromosomal fragmentation and early embryonic lethality. Genes Dev 2000;14:397–402. [PubMed: 10691732]

Chan TA, Hermeking H, Lengauer C, Kinzler KW, Vogelstein B. 14-3-3Sigma is required to prevent mitotic catastrophe after DNA damage. Nature 1999;401:616–620. [PubMed: 10524633]

Chang HC, Rubin GM. 14-3-3 epsilon positively regulates Ras-mediated signaling in *Drosophila*. Genes Dev 1997;11:1132–1139. [PubMed: 9159394]

Chau AS, Shibuya EK. Inactivation of p42 mitogen-activated protein kinase is required for exit from M-phase after cyclin destruction. J Biol Chem 1999;274:32085–32090. [PubMed: 10542242]

Chou TB, Perrimon N. The autosomal FLP-DFS technique for generating germline mosaics in *Drosophila melanogaster*. Genetics 1996;144:1673–1679. [PubMed: 8978054]

de Klein A, Muijtjens M, van Os R, Verhoeven Y, Smit B, Carr AM, Lehmann AR, Hoeijmakers JH. Targeted disruption of the cell-cycle checkpoint gene *ATR* leads to early embryonic lethality in mice. Curr Biol 2000;10:479–482. [PubMed: 10801416]

Debec A, Kalpin RF, Daily DR, McCallum PD, Rothwell WF, Sullivan W. Live analysis of free centrosomes in normal and aphidicolin-treated *Drosophila* embryos. J Cell Biol 1996;134:103–115. [PubMed: 8698807]

Edgar BA, O'Farrell PH. Genetic control of cell division patterns in the *Drosophila* embryo. Cell 1989;57:177–187. [PubMed: 2702688]

Foe VE. Mitotic domains reveal early commitment of cells in *Drosophila* embryos. Development 1989;107:1–22. [PubMed: 2516798]

Foe, VE.; Odell, GM.; Edgar, BA. Mitosis and morphogenesis in the Drosophila embryo. In: Bate, M.; Martinez Arias, A., editors. The Development of Drosophila melanogaster. Cold Spring Harbor: CSHL Press; 1993. p. 149-300.

Fogarty P, Kalpin RF, Sullivan W. The *Drosophila* maternal-effect mutation *grapes* causes a metaphase arrest at nuclear cycle 13. Development 1994;120:2131–2142. [PubMed: 7925016]

Ford JC, al-Khodairy F, Fotou E, Sheldrick KS, Griffiths DJ, Carr AM. 14-3-3 protein homologs required for the DNA damage checkpoint in fission yeast. Science 1994;265:533–535. [PubMed: 8036497]

Grosshans J, Wieschaus E. A genetic link between morphogenesis and cell division during formation of the ventral furrow in *Drosophila*. Cell 2000;101:523–531. [PubMed: 10850494]

Guadagno TM, Ferrell JE Jr. Requirement for MAPK activation for normal mitotic progression in *Xenopus* egg extracts. Science 1998;282:1312–1315. [PubMed: 9812894]

Hou XS, Chou TB, Melnick MB, Perrimon N. The torso receptor tyrosine kinase can activate Raf in a Ras-independent pathway. Cell 1995;81:63–71. [PubMed: 7720074]

Kockel L, Vorbruggen G, Jackle H, Mlodzik M, Bohmann D. Requirement for *Drosophila* 14-3-3 zeta in Raf-dependent photoreceptor development. Genes Dev 1997;11:1140–1147. [PubMed: 9159395]

Kumagai A, Dunphy WG. Binding of 14-3-3 proteins and nuclear export control the intracellular localization of the mitotic inducer Cdc25. Genes Dev 1999;13:1067–1072. [PubMed: 10323858]

Li W, Skoulakis EM, Davis RL, Perrimon N. The *Drosophila* 14-3-3 protein Leonardo enhances Torso signaling through D-Raf in a Ras 1-dependent manner. Development 1997;124:4163–4171. [PubMed: 9374412]

Liu Q, Guntuku S, Cui XS, Matsuoka S, Cortez D, Tamai K, Luo G, Carattini-Rivera S, DeMayo F, Bradley A, et al. Chk1 is an essential kinase that is regulated by Atr and required for the G(2)/M DNA damage checkpoint. Genes Dev 2000;14:1448–1459. [PubMed: 10859164]

Lopez-Girona A, Furnari B, Mondesert O, Russell P. Nuclear localization of Cdc25 is regulated by DNA damage and a 14-3-3 protein. Nature 1999;397:172–175. [PubMed: 9923681]

Mata J, Curado S, Ephrussi A, Rorth P. Tribbles coordinates mitosis and morphogenesis in *Drosophila* by regulating string/CDC25 proteolysis. Cell 2000;101:511–522. [PubMed: 10850493]

Muslin AJ, Tanner JW, Allen PM, Shaw AS. Interaction of 14-3-3 with signaling proteins is mediated by the recognition of phosphoserine. Cell 1996;84:889–897. [PubMed: 8601312]

Peng CY, Graves PR, Thoma RS, Wu Z, Shaw AS, Piwnica-Worms H. Mitotic and G2 checkpoint control: regulation of 14-3-3 protein binding by phosphorylation of Cdc25C on serine-216. Science 1997;277:1501–1505. [PubMed: 9278512]

Seher TC, Leptin M. Tribbles, a cell-cycle brake that coordinates proliferation and morphogenesis during *Drosophila* gastrulation. Curr Biol 2000;10:623–629. [PubMed: 10837248]

Shaw A. The 14-3-3 proteins. Curr Biol 2000;10:R400. [PubMed: 10837257]

Sibon OC, Laurencon A, Hawley R, Theurkauf WE. The *Drosophila* ATM homologue Mei-41 has an essential checkpoint function at the midblastula transition. Curr Biol 1999;9:302–312. [PubMed: 10209095]

Sibon OC, Kelkar A, Lemstra W, Theurkauf WE. DNA-replication/DNA-damage-dependent centrosome inactivation in *Drosophila* embryos. Nat Cell Biol 2000;2:90–95. [PubMed: 10655588]

Sigrist S, Jacobs H, Stratmann R, Lehner CF. Exit from mitosis is regulated by *Drosophila* fizzy and the sequential destruction of cyclins A, B and B3. EMBO J 1995;14:4827–4838. [PubMed: 7588612]

Skoulakis EM, Davis RL. Olfactory learning deficits in mutants for *leonardo*, a *Drosophila* gene encoding a 14-3-3 protein. Neuron 1996;17:931–944. [PubMed: 8938125]

Skoulakis EM, Davis RL. 14-3-3 proteins in neuronal development and function. Mol Neurobiol 1998;16:269–284. [PubMed: 9626666]

Sprenger F, Yakubovich N, O'Farrell PH. S-phase function of *Drosophila* cyclin A and its downregulation in G1 phase. Curr Biol 1997;7:488–499. [PubMed: 9210381]

Su TT, O'Farrell PH. Chromosome association of minichromosome maintenance proteins in *Drosophila* mitotic cycles. J Cell Biol 1997;139:13–21. [PubMed: 9314525]

Su TT, Sprenger F, DiGregorio PJ, Campbell SD, O'Farrell PH. Exit from mitosis in *Drosophila* syncytial embryos requires proteolysis and cyclin degradation, and is associated with localized dephosphorylation. Genes Dev 1998;12:1495–1503. [PubMed: 9585509]

Su TT, Walker J, Stumpff J. Activating the DNA damage checkpoint in a developmental context. Curr Biol 2000;10:119–126. [PubMed: 10679321]

Takenaka K, Moriguchi T, Nishida E. Activation of the protein kinase p38 in the spindle assembly checkpoint and mitotic arrest. Science 1998;280:599–602. [PubMed: 9554853]

Tien AC, Hsei HY, Chien CT. Dynamic expression and cellular localization of the *Drosophila* 14-3-3epsilon during embryonic development. Mech Dev 1999;81:209–212. [PubMed: 10330502]

Yaffe MB, Rittinger K, Volinia S, Caron PR, Aitken A, Leffers H, Gamblin SJ, Smerdon SJ, Cantley LC. The structural basis for 14-3-3:phosphopeptide binding specificity. Cell 1997;91:961–971. [PubMed: 9428519]

Yu KR, Saint RB, Sullivan W. The Grapes checkpoint coordinates nuclear envelope breakdown and chromosome condensation. Nat Cell Biol 2000;2:609–615. [PubMed: 10980701]

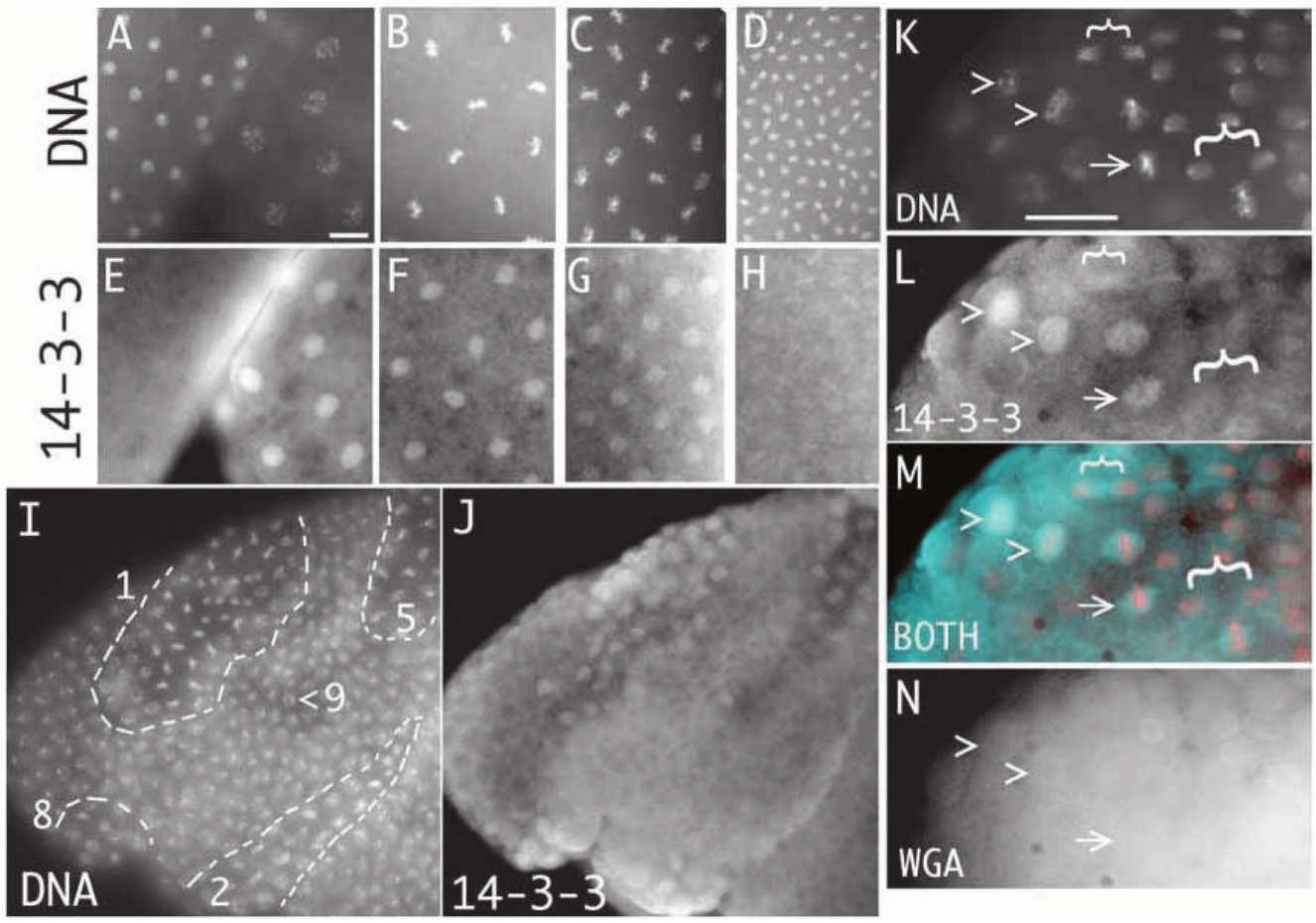

Fig. 1.

Localization of 14-3-3 proteins during cell cycle progression. Wild-type embryos were fixed and stained for DNA and with antibodies to 14-3-3 proteins. Staining with an affinity-purified antibody that is specific to 14-3-3ε shows that 14-3-3ε is dispersed throughout the embryo in interphase of syncytial cycles (left embryo in A and E). The antigen concentration increased in the nucleus in prophase (embryo on the right in A and E) and persists near chromosomes in metaphase (B,F). The staining intensity decreases in anaphase and telophase, with the remaining signal stretching across the dividing nuclei (D,H). A similar cell cycle profile of staining was seen with a commercial pan-specific antibody to 14-3-3 proteins; only the perichromosomal localization in metaphase is shown here (C,G). During cellular cycles (I-N), 14-3-3ε-specific antibody detected a similar pattern of localization as in syncytial cycles, but exclusion from the nucleus is more apparent. (I,J) The antigen is cytoplasmic in all cells except those in the midst of M14. Mitotic domains 1,2,5 and 8 are indicated. A single cell in mitotic domain 9 is initiating mitosis and is also indicated (other domains reside in the rest of the embryo). (K-N) The change in localization during M14. The antigen concentrates in the nucleus in prophase (arrowheads in K-M), at which time the nuclear envelope remains (arrowheads show remnant wheat-germ agglutinin (WGA) staining in N). The antigen persists in metaphase (arrow in K-M) when the nuclear envelope is absent (arrow in N). In anaphase and telophase, the antigen disperses (brackets in K-M), with the remaining signal concentrating in the region between the nuclei pair in anaphase (smaller bracket in K-M). A similar profile of localization was also revealed by the commercial pan-specific 14-3-3 antibody (not shown). Bar, 5.5 μm in A-H, 16.5 μm in I,J and 13 μm in K-N.

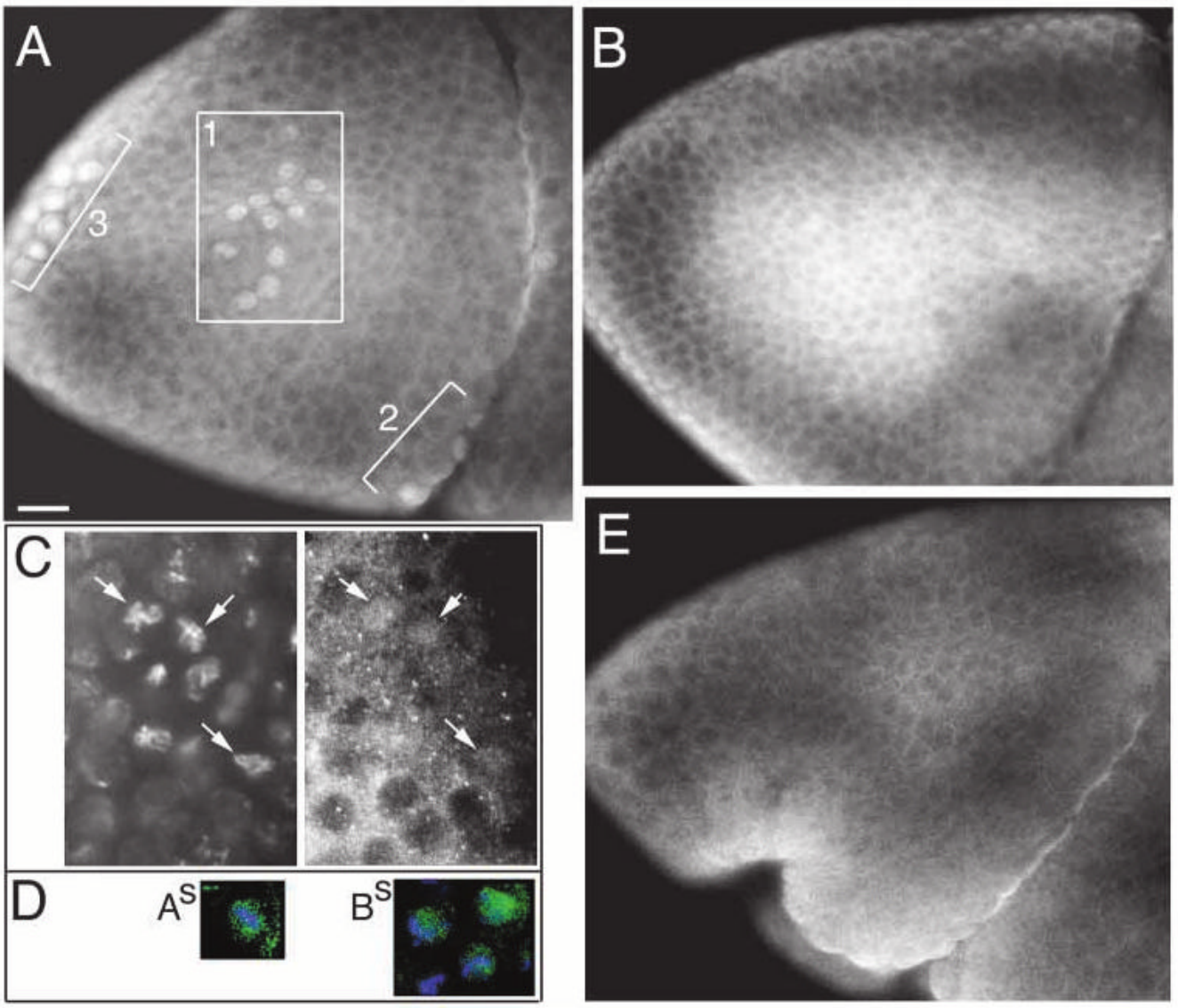

Fig. 2.

Localization of 14-3-3ε is dependent on Cdk1 activity. Embryos were fixed and stained for DNA and 14-3-3ε. (A) Mitotic domains 1, 2 and 3 in the head region of a wild-type embryo is seen initiating M14. Nuclear localization of 14-3-3ε is apparent in these cells. (B) In a *stg* homozygous mutant at a similar embryonic stage, nuclear staining is absent. (C) Expression of Cdk1AF in a *stg* mutant promotes mitosis (DNA on the left) and concentration of 14-3-3ε near chromosomes in metaphase (on the right). (D) In a metaphase arrest induced by non-degradable cyclin A (A^s) and non-degradable cyclin B (B^s), 14-3-3ε (green) remains concentrated near chromosomes (purple). (E) In an irradiated embryo in which cells are delayed from entering M14, 14-3-3ε remains cytoplasmic. Bar, 16.5 μm.

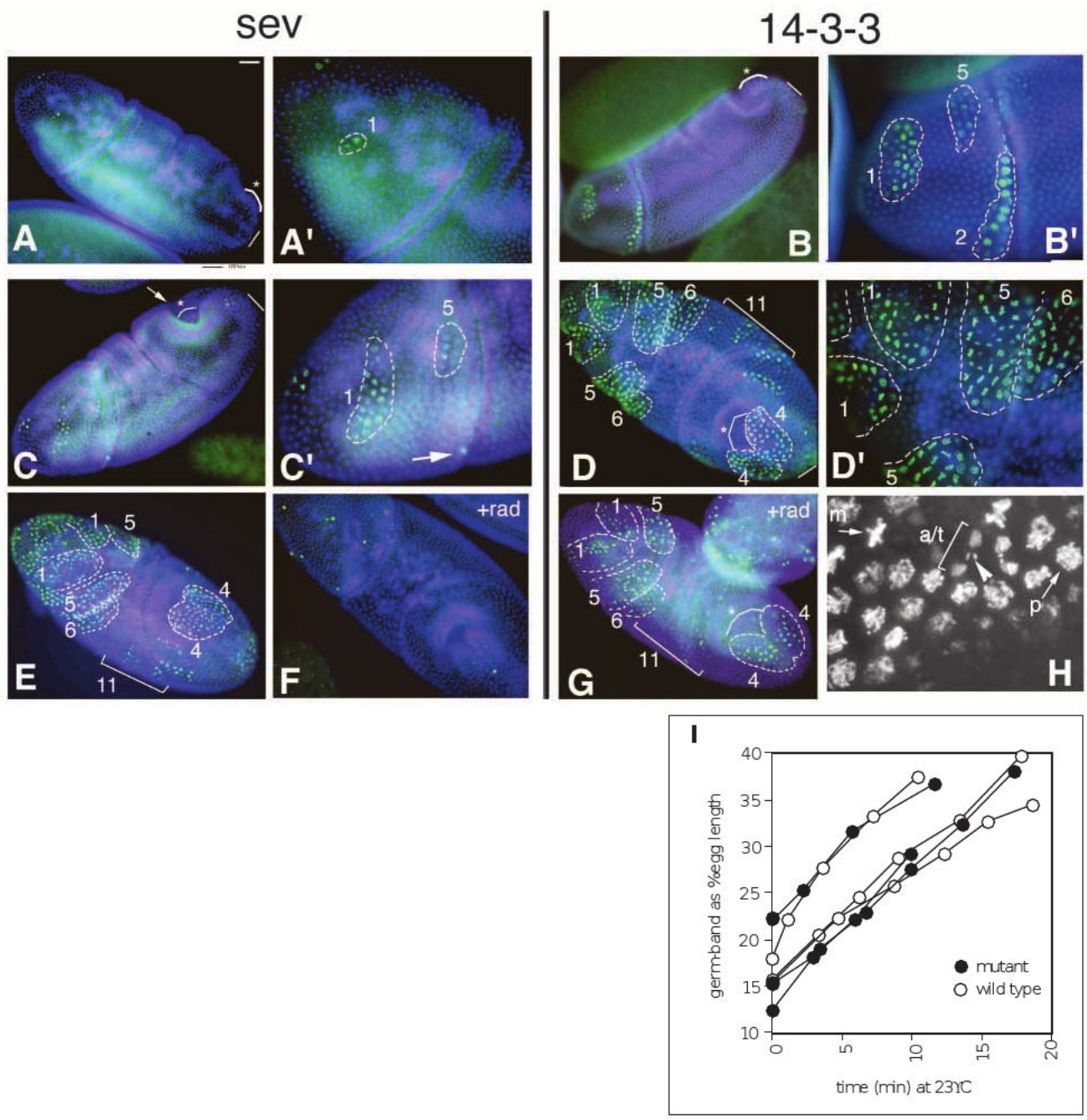

Fig. 3.

14-3-3ε mutant embryos enter mitosis prematurely. Wild-type ('sev'; A,C,E,F) and 14-3-3ε mutant ('14-3-3'; B,D,G,H) embryos in embryonic cycle 14 were fixed and stained to visualize DNA (purple) and with an antibody to phosphorylated histone H3, to visualize mitotic cells (H and green in all others). Embryos in F and G were irradiated for 20 minutes before fixing (+rad). Head regions of embryos in A-D are magnified and shown in A′-D′ respectively. The * marks pole cells (enclosed by curved lines) in A-D,G. The numbers refer to mitotic domains (Foe, 1987). Embryos are shown with anterior end to the left. Embryos in A-C have dorsal sides up. Embryos in D-G have dorsal sides rotated towards the viewer. (A-B′) Embryos of a developmental stage in which pole cells are still exposed and still close to the posterior end

(solid line). At this stage, few cells are in mitosis in wild-type embryos (A,A′), whereas cells of domains 1-5 are in mitosis in 14-3-3ε mutants (B; domains 1, 2 and 5 are visible in the view shown in B′). Green stain in the cytoplasm in A′ is due to background signal in this sample. (C,D) More advanced embryos in which pole cells, still exposed, have moved away from the posterior end (solid line) owing to germ-band extension. At this stage, cells of domain 1-5 are entering mitosis in wild-type embryos (C,C′). Domains 1 and 5 are visible in C′; only a single mitotic cell in domain 2 (arrow) is seen in this view, which is rotated towards the viewer with respect to the one in B′. In 14-3-3ε mutant embryos at similar stages, domains 1-11 are in mitosis (D,D′); many cells of earlier domains have now finished mitosis (e.g. domain 1 in D ′). For visual clarity, not all domains visible in these views are numbered. The division pattern in wild-type embryos at this stage (C) is similar to that of 14-3-3ε mutant embryos at an earlier stage (B), indicating the advancement of mitotic program in the latter. (E) In yet more advanced embryos, pole cells are internalized and no longer visible. Domains 1-11 are now in mitosis in the wild-type embryo shown here. The division pattern of this embryo is similar to that of 14-3-3ε embryos at an earlier stage (D), indicating the advancement of mitotic program in the latter. In wild-type embryos of similar stage that had been irradiated, mitotic cells are absent (F), indicating that DNA damage delayed the entry into mitosis. (G) An irradiated 14-3-3ε embryo at similar developmental stage (notice exposed the pole cells) as in D, but with similar division pattern to D and E. Mitotic domains seen in the absence of irradiation (D,E) are also present after irradiation in the 14-3-3ε mutant (G), indicating the failure to delay the entry into mitosis in response to DNA damage. (H) In irradiated mutant embryos, cells that enter mitosis execute all stages of mitosis and often show broken or lagging chromosomes (arrowhead), presumably a result of entry into mitosis with damaged DNA. Abbreviations: a/t, anaphase/telophase; m, metaphase; p, prophase. Bar, 30 μm in A-G, 15 μm in A′, B′ and C′, and 4 μm in H. (I) Germ-band extension in live embryos. The anterior lip of the aminoprotodeal fold (e.g. arrow in C) is defined as the extent of the germ band. Its position is measured from the posterior end of the embryo on photographic images of embryos and expressed as a percentage of the total embryo length. Data from three wild-type and 14-3-3ε mutants (mutant) embryos are shown. The rate of germ band elongation was 1.38±0.4% embryo length per minute in wild-type and 1.38±0.04% in mutant embryos.

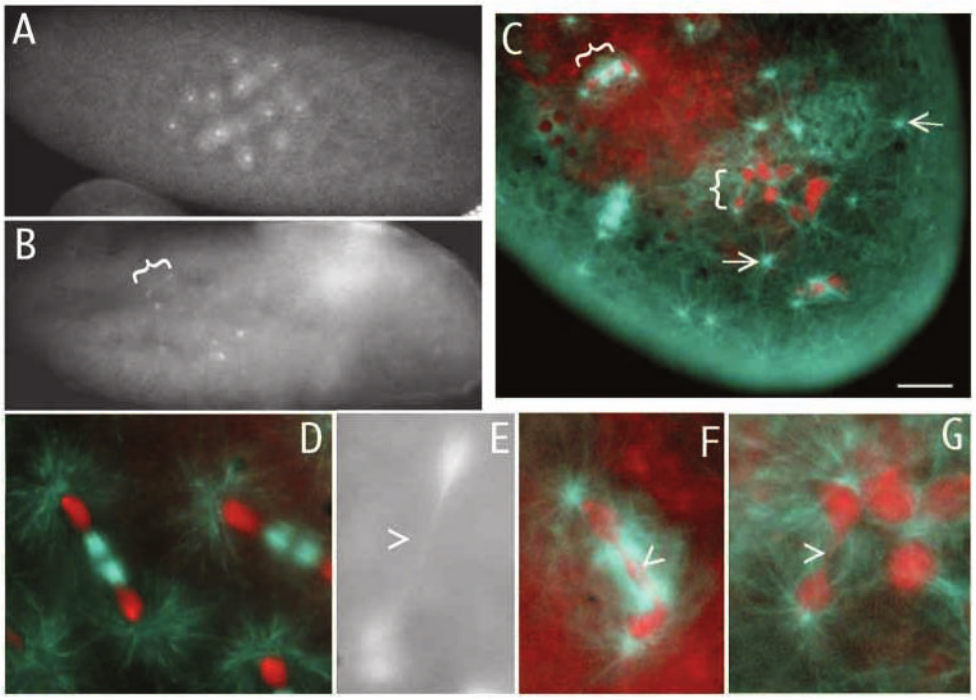

Fig. 4.

14-3-3ζ-deficient embryos fail to execute syncytial divisions properly. Wild-type (A,D) and 14-3-3ζ-deficient (B,C,E-G) embryos were fixed and stained for DNA (red) and an anti-α-tubulin antibody (C-F) to visualize microtubules (blue). (A) A wild-type embryo at the end of fourth embryonic mitosis. Nuclei are clearly separated. Embryonic cycle number is determined by counting nuclei ($n = 2$ at the end of M1, $n = 4$ at the end of M2, etc.). (B) A 14-3-3ζ-deficient embryo at the end of M4 shows nuclei pairs connected by chromosome bridges. One such pair is indicated with a bracket and is shown rotated and magnified in E (arrowhead indicates bridge). (C) A 14-3-3ζ-deficient embryo in a later syncytial cycle. Arrows point to MTOCs that have no obvious nuclei associated with them. Brackets indicate nuclei pairs connected by bridges. These are magnified and shown in F and G. Arrowhead indicates bridges. Notice the presence of robust microtubules around the chromosome bridge in F, the region in which the spindle mid-body normally resides at a similar stage of mitosis in wild-type embryos (D). The red stain in the middle of the embryo in C indicates a large yolk DNA mass. Bar, 15.7 μm in A and B, 8.3 μm in C and 5.5 μm in D-G.

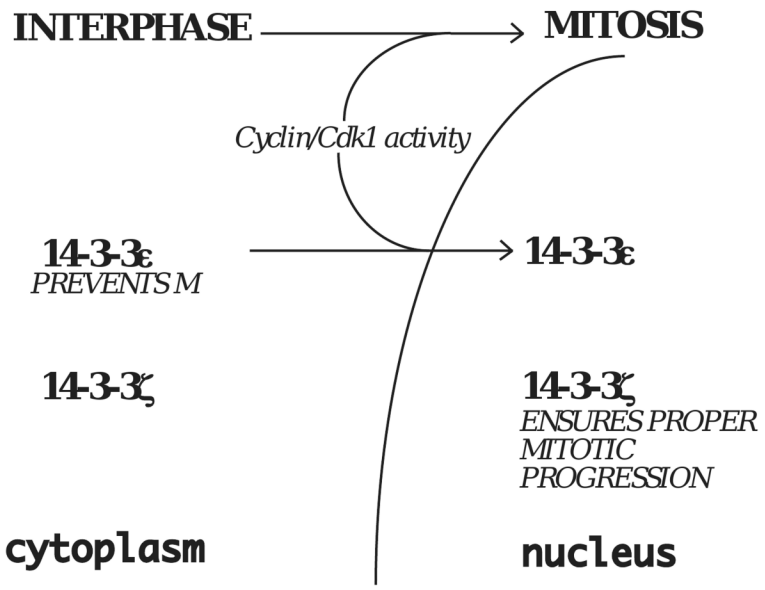

Fig. 5.

A model for roles of 14-3-3 proteins in *Drosophila* cell division. In interphase, 14-3-3 proteins and cyclin A/Cdk1 and cyclin B/Cdk1 complexes are predominantly cytoplasmic. Here, 14-3-3ε acts to keep Cdk1 in check, preventing mitosis in normal or irradiated embryos. As Cdk1 becomes active (owing to the accumulation of its activator Stg or after recovery from DNA damage) and cells enter mitosis, accumulating cyclin/Cdk1 activity promotes and maintains 14-3-3 protein localization near chromosomes. Upon the transition to anaphase, the localized 14-3-3 proteins, and 14-3-3ζ in particular, can contribute to the rapid, effective inactivation of the cyclin/Cdk1. Thus, in interphase, 14-3-3ε can act to keep Cdk1 inactive in the cytoplasm but, once Cdk1 is active, it can act in turn to localize 14-3-3 proteins in preparation for their action during the exit from mitosis.