Journal of Cell Science 107, 2729-2738 (1994)
Printed in Great Britain © The Company of Biologists Limited 1994

Expression of N-terminally truncated cyclin B in the *Drosophila* larval brain leads to mitotic delay at late anaphase

Georgina Rimmington, Brian Dalby* and David M. Glover†

Cancer Research Campaign Cell Cycle Genetics Group, Department of Anatomy & Physiology, Medical Sciences Institute, University of Dundee, Scotland DD1 4HN, UK

*Present address: Division of Cellular and Molecular Medicine, UCSD School of Medicine, 9500 Gilman Drive, La Jolla, California 92093-0683, USA
†Author for correspondence

SUMMARY

We have introduced an N-terminally truncated form of cyclin B into the *Drosophila* germ-line downstream of the yeast upstream activator that responds to GAL4. When such lines of flies are crossed to lines in which GAL4 is expressed in imaginal discs and larval brain, the majority of the resulting progeny die at the late pupal stage of development. Very rarely (<0.1% of progeny) adults emerge that have a mutant phenotype typical of flies with mutations in genes required for the cell cycle; they have rough eyes, deformed wings, abnormal bristles, and die within hours of emergence. The brains of third instar larval progeny show an abnormally high proportion of mitotic cells containing overcondensed chromatids that have undergone anaphase separation, together with cells that cannot be assigned to a particular mitotic stage. Immunostaining indicates that these anaphase cells contain moderate levels of cyclin B, suggesting that persistent p34^{cdc2} kinase activity can prevent progression from anaphase into telophase.

Key words: anaphase, *Drosophila*, mitosis, truncated cyclin B

INTRODUCTION

The mitotic cyclins were originally identified in the eggs of marine invertebrates as proteins that accumulate during the cell cycle to be degraded around the time of the metaphase-anaphase transition (Evans et al., 1983; Swenson et al., 1986; Standart et al., 1987). Both A- and B-type mitotic cyclins can associate with the p34 kinase encoded by homologues of the fission yeast gene *cdc2* (see Nurse, 1990 for review). B-type cyclins associated with p34^{cdc2} kinase are components of the universal maturation promoting factor (MPF) isolated from *Xenopus* and starfish oocytes (Draetta et al., 1989; Labbé et al., 1989; Meijer et al., 1989; Gautier et al., 1990). Entry into mitosis is dependent upon the phosphorylation state of p34^{cdc2} kinase and its association with a cyclin subunit. Cyclin destruction, which occurs through ubiquitin-dependent proteolysis (Glotzer et al., 1991), is necessary for the inactivation of p34^{cdc2} kinase (Murray et al., 1989; Félix et al., 1990).

In cellularised *Drosophila* embryos and diploid larval cells, the mitotic cyclins behave as in other multicellular eukaryotes with cyclin A being destroyed slightly earlier than cyclin B (Whitfield et al., 1990). However, neither cyclin appears to be completely degraded in the rapid mitotic cycles of the syncytial embryo, suggesting that p34^{cdc2} kinase modification may play a greater role in regulating these divisions, although some localised breakdown and resynthesis of cyclins may occur (Maldonado-Codina and Glover, 1992). Embryos homozygous for mutations in the cyclin A gene can progress through the syncytial cycles using maternally provided wild-type protein, but their development arrests in cell cycles 15-16 (Lehner and O'Farrell, 1989). This appears to be the result of a mitotic defect, although in other organisms an additional S-phase role has been suggested for cyclin A that can also form complexes with p33^{cdk2} (Fang and Newport, 1991). Mutations in the *Drosophila* cyclin B gene at 59B have yet to be identified, although Knoblich and Lehner (1993) have generated a small deficiency that uncovers this chromosomal interval. Embryos homozygous for this deficiency do not undertake mitosis beyond cycle 16, and they show subtle abnormalities of their mitotic spindles. Embryos deficient for this chromosomal interval and also carrying cyclin A mutations arrest before mitotic cycle 15, that is to say at an earlier cycle than mutations in either gene alone (Knoblich and Lehner, 1993; Lehner and O'Farrell, 1989).

The degradation of the mitotic cyclins requires a conserved sequence, 'the destruction box', located in the N-terminal domain of the molecule (Glotzer et al., 1991). When an N-terminally truncated form of sea urchin cyclin B was introduced into frog eggs or extracts derived from them, neither MPF inactivation, chromosome decondensation, or cytokinesis occurred, and this was described as arresting cells in metaphase. The injection of mRNA encoding such N-terminally deleted cyclin B into fertilised eggs led to arrest with a single set of condensed chromosomes (Murray et al., 1989). Similar findings were reported in clam egg extracts and in human tissue culture cells, although in the latter case a wider variety of mitotic defects

were described (Luca et al., 1991; Gallant and Nigg, 1992). As the destruction of first cyclin A and then cyclin B takes place about the time of the metaphase-anaphase transition (Evans et al., 1983), this led to the widely-expressed feeling that cyclin degradation was necessary for this transition that could be equated with the exit from mitosis. However, when the mitotic cycle is arrested with drugs that destabilise microtubules, cyclin A is degraded, whereas cyclin B continues to accumulate (Minshull et al., 1989; Whitfield et al., 1990). This led Whitfield et al. (1990), to suggest that cyclin B degradation was dependent upon the correct functioning of the spindle at the metaphase-anaphase transition.

In this paper we address this question for the first time within an intact metazoan by examining the effects of the expression of an N-terminally deleted form of cyclin B. We show that this results in an accumulation of cells in late anaphase.

MATERIALS AND METHODS

Generation of an N-terminal deletion in the cyclin B gene

The N-terminally truncated form of cyclin B was derived from a 2.7 kb cyclin B cDNA in pKS; 140 amino acids were deleted from the coding region of cyclin B by PCR amplification using primers oriented so that the entire pKS B2.7 plasmid was amplified except for the region to be deleted. Template DNA was prepared by digestion of pKS B2.7 with *Nco*I. This restriction site lies within the region to be deleted. A PCR reaction was set up using primers 5′GCCCACCATTTTGATTGCTTCTTT3′ (complementary to the sequence encoding the N-terminal 3 amino acids of the cyclin B protein and 15 nucleotides of the 5′ untranslated region) and 5′AGTTCTTCCGAGAACGTGAACGAG3′ (complementary to a sequence 420 nucleotides within the coding region of the cyclin B cDNA). DNA was recircularised using T4 DNA ligase, and then digested with *Nco*I in order to render religated template DNA incapable of transformation. The entire ligation was transformed into *E. coli* TG2 cells. Clones were sequenced using the Bluescript KS primer to allow sequence to be read across the ligated deletion breakpoint. The sequence obtained was compared with that of Whitfield et al. (1990) and was found to be identical except for the expected deleted region. The deleted cDNA was subcloned into the vector pUAST (Brand and Perrimon, 1993). The vector DNA was digested with *Eco*RI, end filled and digested with *Not*I. The deleted cDNA was digested with *Hin*dIII, end filled and then digested with *Not*I, in order to release the insert. Following ligation, recombinant clones were identified by hybridisation to a 1.2 kb *Eco*RI-*Eco*RV cyclin B coding region fragment.

Cytological studies on larval brains

Crosses were set up at 18°C. β-Galactosidase activity was detected in third instar larval heads turned inside-out in ice-cold PBS, in order to remove excess fat and salivary glands. Brains and discs, still attached to the cuticle, were transferred to formaldehyde fixative for 15 minutes, rinsed in PBS and developed in staining solution containing 1/40th the volume of 8% X-Gal in DMSO at 37°C (see also Gonzalez and Glover, 1994). Squashed and whole mount preparations of larval brains were prepared for chromosome staining and immunostaining as described by Gonzalez and Glover (1994). For brain squashes, optic lobes were dissected away from the ventral ganglion as this is the region of the brain where the highest level of β-galactosidase activity is detected in line 69B. Microtubules and cyclin B were visualised using the YL1/2 anti-tubulin rat monoclonal antibody (Kilmartin, 1982) and the rabbit polyclonal Rb271 (Whitfield et al., 1990), respectively. FITC-secondary antibodies were obtained from Jackson immunochemicals. Immunofluorescence microscopy was carried out using a Nikon optiphot microscope with the Bio-Rad MRC600 confocal scanning head.

RESULTS

An enhancer trap line that expresses GAL4 in the larval brain

We wished to examine the consequences of the ectopic expression of N-terminally deleted cyclin B during *Drosophila* development, and anticipated this would result in a dominant cell cycle phenotype, thus necessitating the use of a conditional promoter. Previous studies of ectopically expressed genes in *Drosophila* have utilised the temperature-regulated promoter of the *hsp70* gene. As heat shock per se can have profound effects upon cell cycle progression (Maldonado-Codina et al., 1993), we chose to utilise the system developed by Brand and Perrimon (1993) to regulate the expression of N-terminally deleted cyclin B. In order to mediate expression, two lines of flies are crossed: one line carries the yeast gene for the transcriptional activator GAL4 under developmental control, whereas the other contains the truncated cyclin B gene linked to the GAL upstream activating sequence (UAS). We utilised the line 69B (Brand and Perrimon, 1993) previously shown to be expressed in larval imaginal tissues (Fig. 1). In order to determine whether this line gave suitable expression of GAL4 in larval brain, a tissue highly suited for the study of mitosis in *Drosophila*, we crossed the 69B line to a line of flies carrying the *E. coli lacZ* gene downstream of the UAS element. The expression of β-galactosidase activity in the larval brains of the resulting progeny is shown in Fig. 2A. Extensive

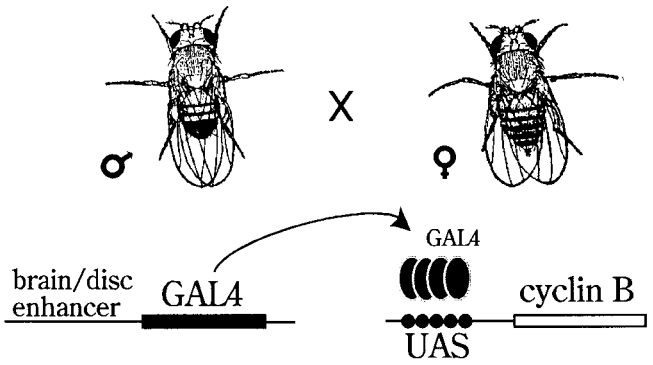


Fig. 1. Ectopic expression of an N-terminally truncated form of cyclin B using a GAL4 enhancer trap line. This two part system allows for conditional expression of a potentially dominant lethal construct (Brand and Perrimon, 1993). One line of flies contains the sequence coding for the yeast GAL4 protein, inserted as a result of P-element-mediated transposition in the vicinity of enhancer sequences that direct the expression of GAL4 in specific tissues at a particular time in development. We have used the line 69B, previously shown to give expression in imaginal discs (Brand and Perrimon, 1993), and which we now show also to be expressed in the larval brain. The other line contains an N-terminally truncated cyclin B gene downstream of UAS sequences to which GAL4 may bind and activate transcription. Both lines of flies are kept as homozygous stocks that are crossed to generate the larvae in which transcription of the N-terminally deleted cyclin B is activated in brains and discs by GAL4.

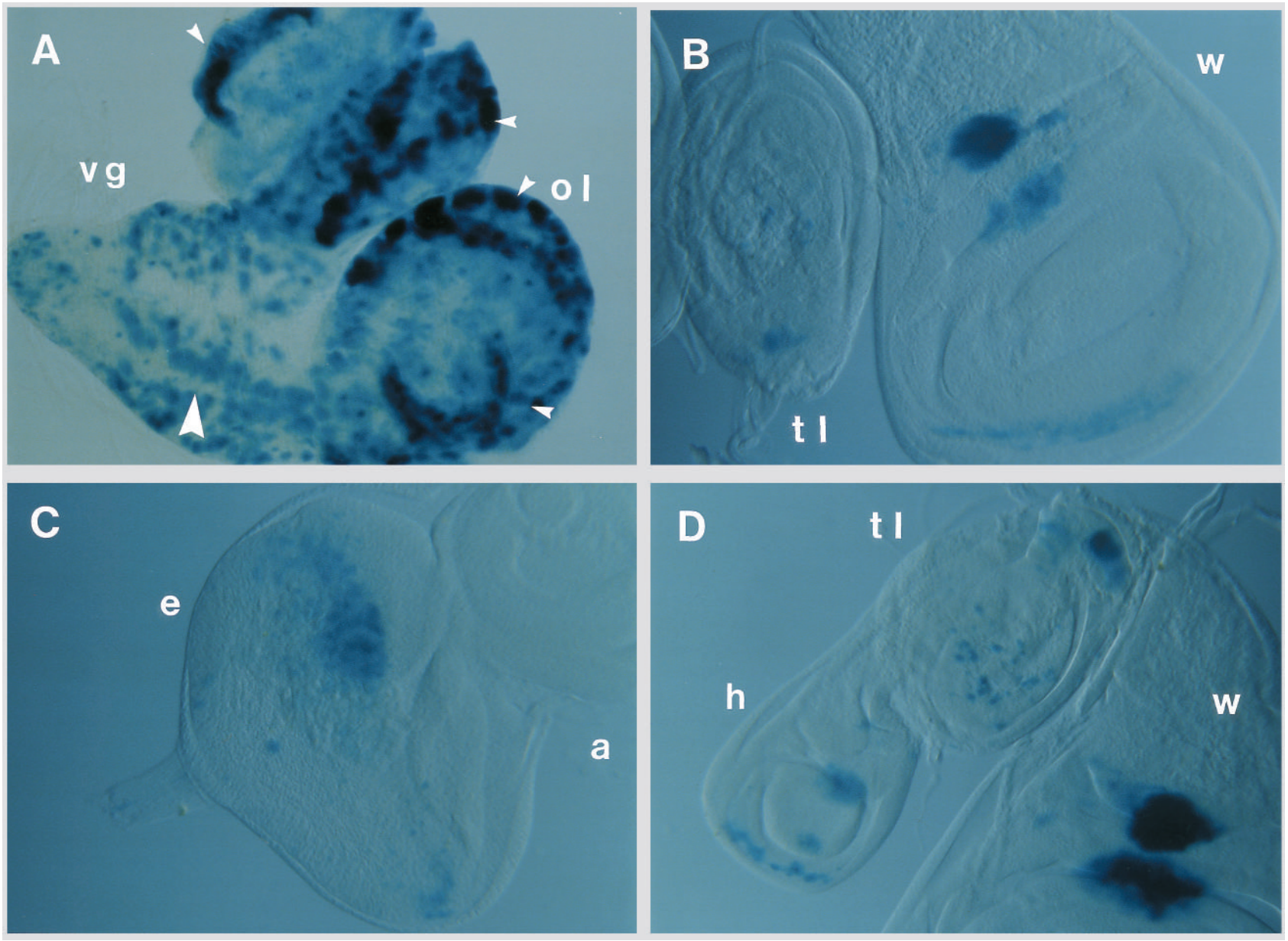


Fig. 2. Expression pattern of the 69B GAL4 enhancer trap line in third instar larval brain and imaginal discs. The panels show the expression of β-galactosidase activity in the brains and discs of larvae derived from the 69B enhancer-GAL4 line crossed with a line carrying the *E. coli lacZ* gene downstream of the UAS element. (A) In the larval brain, β-galactosidase activity is seen in a subset of cells in the proliferative centres (small arrowheads) of the optic lobes (ol), and in subsets of neuroblasts (large arrowhead) of the ventral ganglion (vg). (B) Expression in the wing (w) and third leg (tl) discs. (C) Expression in the eye (e) antennal (a) disc. (D) Expression in the haltere (h) disc.

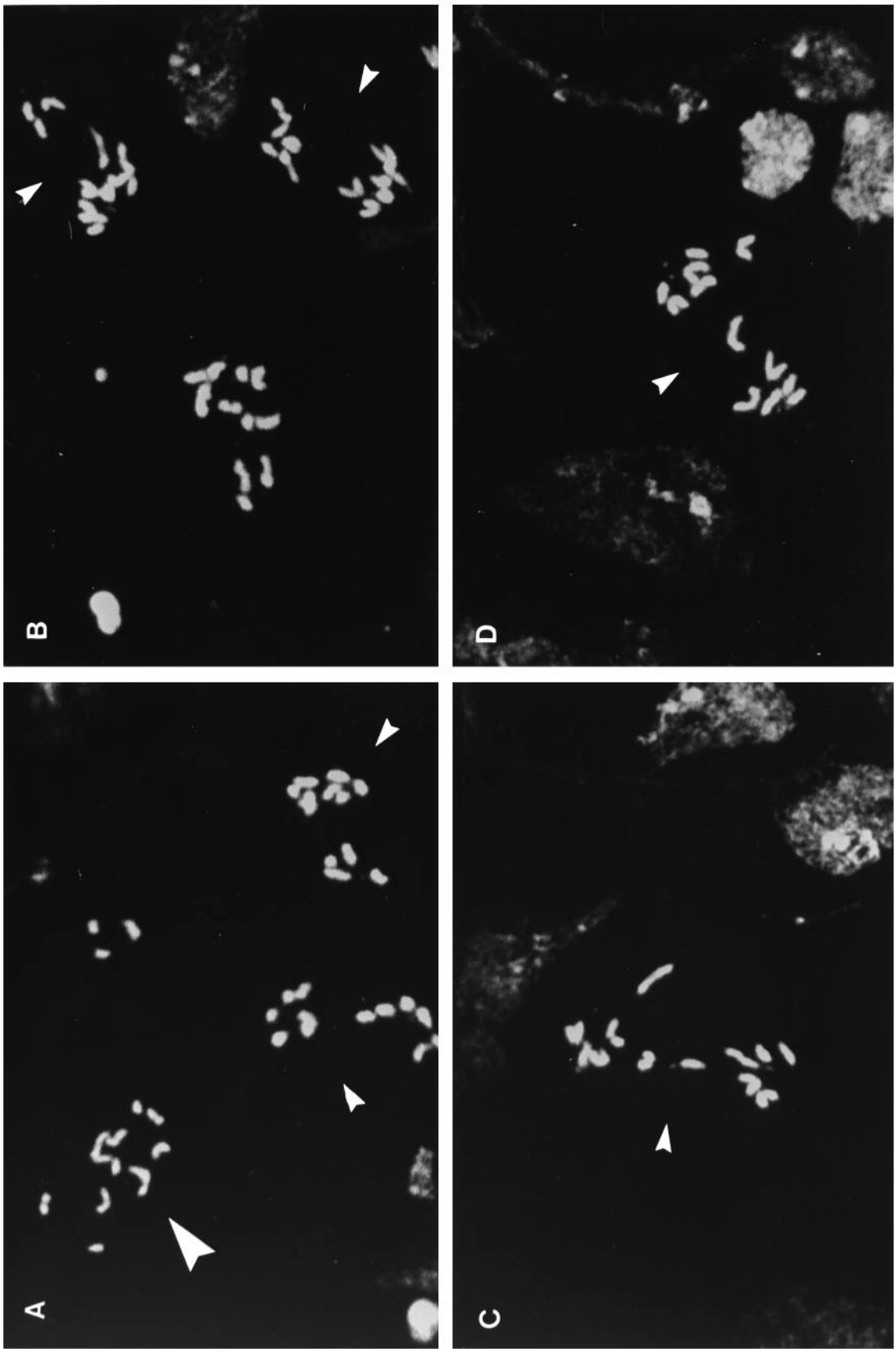


Fig. 3. Mitotic defects in squashed preparations of third instar larval brains showing ectopic expression of an N-terminally truncated form of cyclin B. (A-D) Propidium iodide-stained brain squashes prepared as described by Gonzalez and Glover, 1994. (A,B) Examples of cells at late anaphase in which overcondensed chromatids are present in well separated groups (small arrowheads). Some cells cannot easily be staged with respect to mitotic phases (e.g. large arrowhead in A). (C,D) Anaphase cells containing lagging chromatids (arrowheads).

expression is seen in the optic lobes especially where they abut the ventral ganglion. In the ventral ganglion expression appears to be restricted to cells in the thoracic region. This pattern overlaps with, but is not identical to, the distribution of endogenous cyclin B transcripts, which are seen predominantly in the cells of the proliferative centres of the optic lobes as well as in giant neuroblasts of the ventral ganglion. In addition the GAL4 enhancer drives *lacZ* expression in cells in discrete regions of the imaginal discs (Fig. 2B-D).

N-terminally truncated cyclin B leads to an increase in anaphase figures

We therefore generated lines of transformed flies that, in addition to the endogenous cyclin B gene, also carried an N-terminally truncated cyclin B gene downstream of the GAL4 UAS element. We made a 5′ truncation of the cyclin B gene that removes the coding capacity for the N-terminal 140 amino acids, which includes the potential destruction motif RAALGDLQN (amino-acids 37-45). This was cloned into the vector, pUAST, which places the gene downstream of the UAS sequences, and introduced into the *Drosophila* genome by P-element-mediated germ-line transformation. The majority of the progeny of crosses between the transformed flies and the 69B GAL4-expressing line survived only to the late pupal stage of development with less than 0.1% surviving into early adulthood, suggesting that imaginal cells and cells of the CNS were failing to proliferate (Gatti and Baker, 1989). The few adult progeny observed were smaller than wild type and displayed a phenotype indicative of abnormal cell divisions. Defects include rough eyes, deformed wings and abnormal bristles. In order to look at the nature of the defects in progression through the mitotic cycle, we examined chromosomes in squashed preparations of third instar larval brains (Figs 3 and 4). Ectopic expression of the truncated cyclin B has little effect on the mitotic index in the brain overall suggesting either that expression of significant levels of mutant cyclin has not occurred at earlier stages of development of the larval CNS and so abnormal mitoses have not accumulated, or that the protein is resulting in a delay rather than a block to cell cycle progression. However, it is difficult to rule out the possibility that the spatially regulated pattern of GAL4 expression has led to an increased mitotic index in localised brain regions, the morphology of which is destroyed by the squashing procedure. In control brains from the GAL4-enhancer trap line 69B the mitotic index and distribution of figures at various stages of the cycle is comparable with other laboratory wild-type strains, and shows a low proportion of anaphase figures. On the other hand, the most striking feature of brains showing ectopic expression of truncated cyclin B is the very high proportion of anaphase stages. The majority of these are at late stages of anaphase (Fig. 3A and B) and show a degree of chromosome condensation that suggests they have been delayed at this stage of mitosis. Some abnormal looking anaphase figures show lagging chromatids (Fig. 3C and D), whereas others appear similar to wild-type anaphase figures. An additional category of cells have highly condensed chromosomes that cannot be assigned to any particular mitotic stage. Finally, there are a small proportion of cells with various degrees of polyploidy suggesting that other defects in chromosome segregation and spindle behaviour can occur.

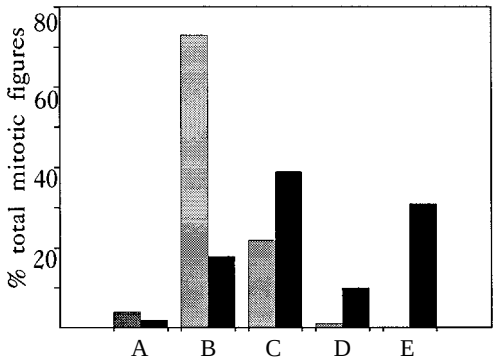
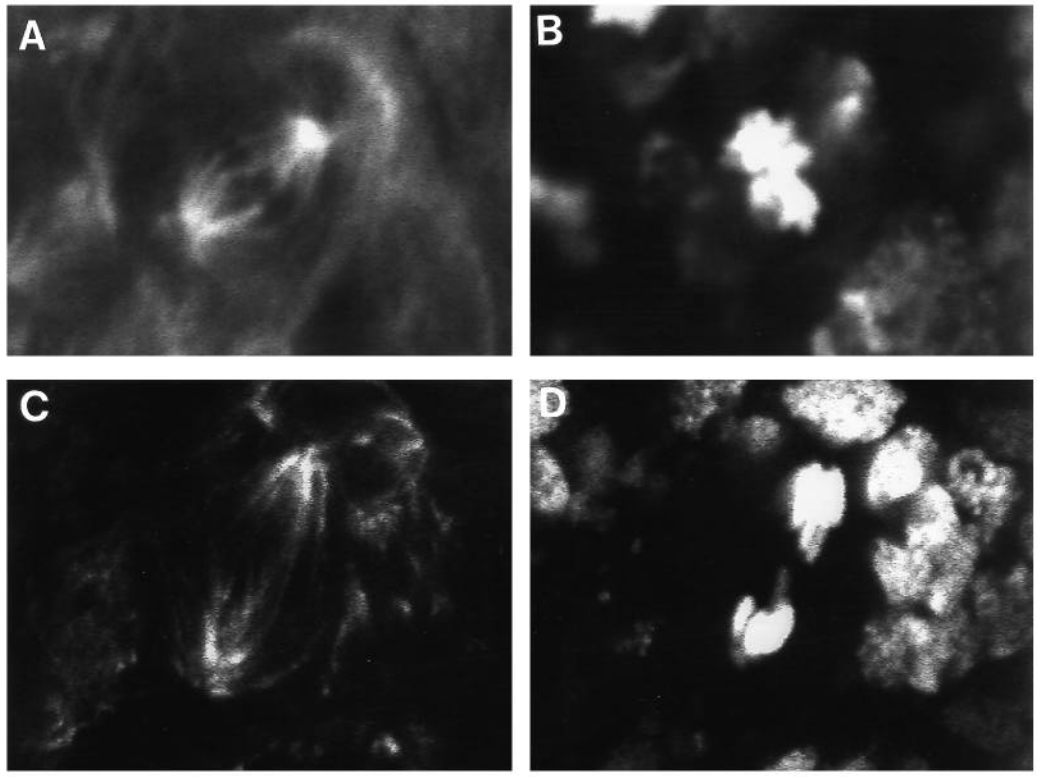


Fig. 4. The distribution of mitotic figures in wild-type brains and in brains expressing the N-terminally truncated cyclin B. The wild-type flies are from the line homozygous for the 69B GAL4 insertion. A total of 175 mitotic figures were scored from these larvae (bars with light shading). A total of 251 mitotic figures were scored from mutant brains (bars shaded black). In the brains showing the dominant mutant phenotype, the individual categories could be subdivided as follows. The anaphase group comprised 13% early and 23% late anaphase figures showing normal levels of chromosome condensation, and 64% late anaphase figures with abnormally high degrees of chromosome condensation. Prometaphases and metaphases were classified as abnormal if they showed some degree of overcondensation (17%) or if they were aneuploid (51%) or polyploid (32%). Of those cells at an unidentifiable mitotic stage, the majority (78%) appear to have a 4N complement of chromosomes, the remainder being polyploid. A, prophase; B, (pro)metaphase; C, anaphase; D, abnormal (pro)metaphase; E, unidentified stages.

Overcondensed late anaphase chromatids are associated with bipolar spindles

In order to determine whether we could see other mitotic abnormalities arising from the expression of the N-terminally truncated cyclin B, we carried out immunostaining of whole-mount preparations of larval brain to visualise arrays of microtubules. Examples of representative fields from mutant brains are shown in Figs 5 and 6. Wild-type metaphase and anaphase figures are shown in Fig. 5A,B and C,D, respectively. Anaphase figures from brains expressing N-terminally truncated cyclin B (Fig. 5E-H) can show abnormal separation of condensed chromatin masses. The large arrowhead in Fig. 5F points to an anaphase figure in which chromosomes have attained their usual degree of condensation. The adjacent spindle (small arrowhead) has two separated sets of highly condensed chromatids. The spindle in Fig. 5H has highly condensed anaphase chromatids; compare the dot-like appearance of the chromatids in this cell with the wild-type anaphase chromatids in Fig. 5D where chromosome arms can be discerned. Well-separated extremely highly condensed chromatids suggest a cell has been in mitosis for an unusually long period of time. A series of optical sections of another field is shown in Fig. 6. Three bipolar mitotic figures can be seen in this field lying on different focal planes (see Figure legend). Of these, two cannot be assigned to any particular mitotic stage and show unusual arrangements of chromosomes (small and medium arrows), whereas the third is a late anaphase figure with unsually highly condensed chromatids. This cell appears to be polyploid, as was previously observed with cells in the squashed preparations indicating that



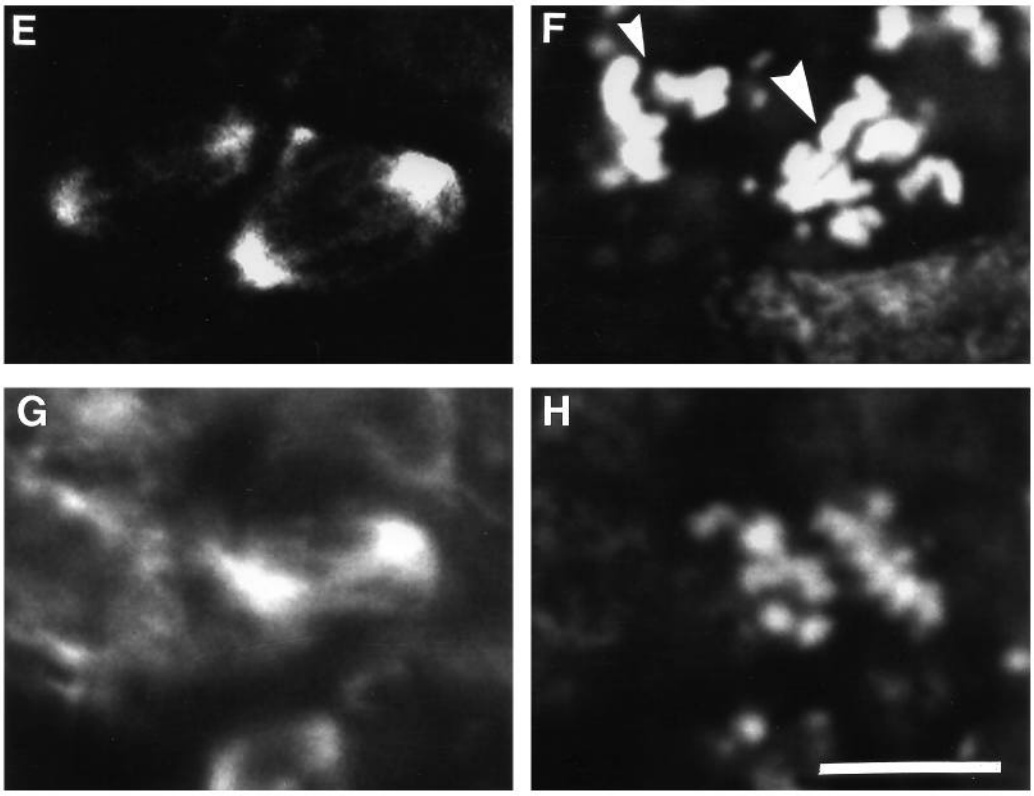


Fig. 5. Mitotic spindles in wild-type brains (A-D), and brains expressing truncated cyclin B (E-H). Whole mount preparations of brains were fixed and stained with propidium iodide to reveal chromosomes (B,D,F,H), and microtubules (A,C,E,G) (see Materials and Methods). Arrowheads point to anaphase figures (see text). Bar, 10 μm.

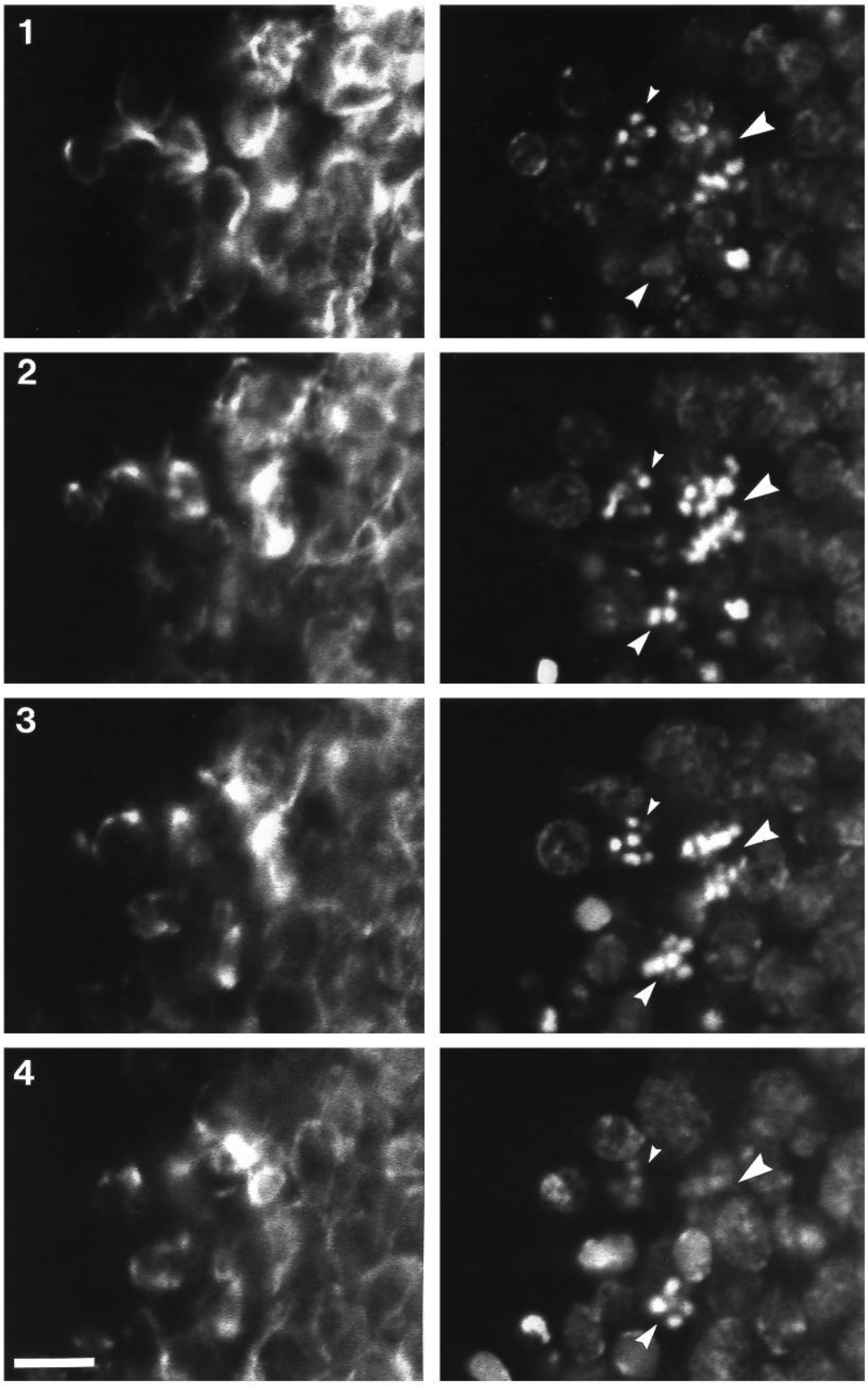


Fig. 6. Optical sections of a brain expressing N-terminally truncated cyclin B. The whole mount brain preparation was fixed and stained with propidium iodide to reveal chromosomes (right hand panels), and microtubules (left hand panels). The sections represent successive 1 μm intervals. Bar, 10 μm. The three arrowheads point to chromosomes associated with bipolar spindles that are most intensely stained in section 2 (small arrowhead), section 3 (medium arrowhead), and equally in sections 2 and 3 (large arrowhead). See Results for further details.

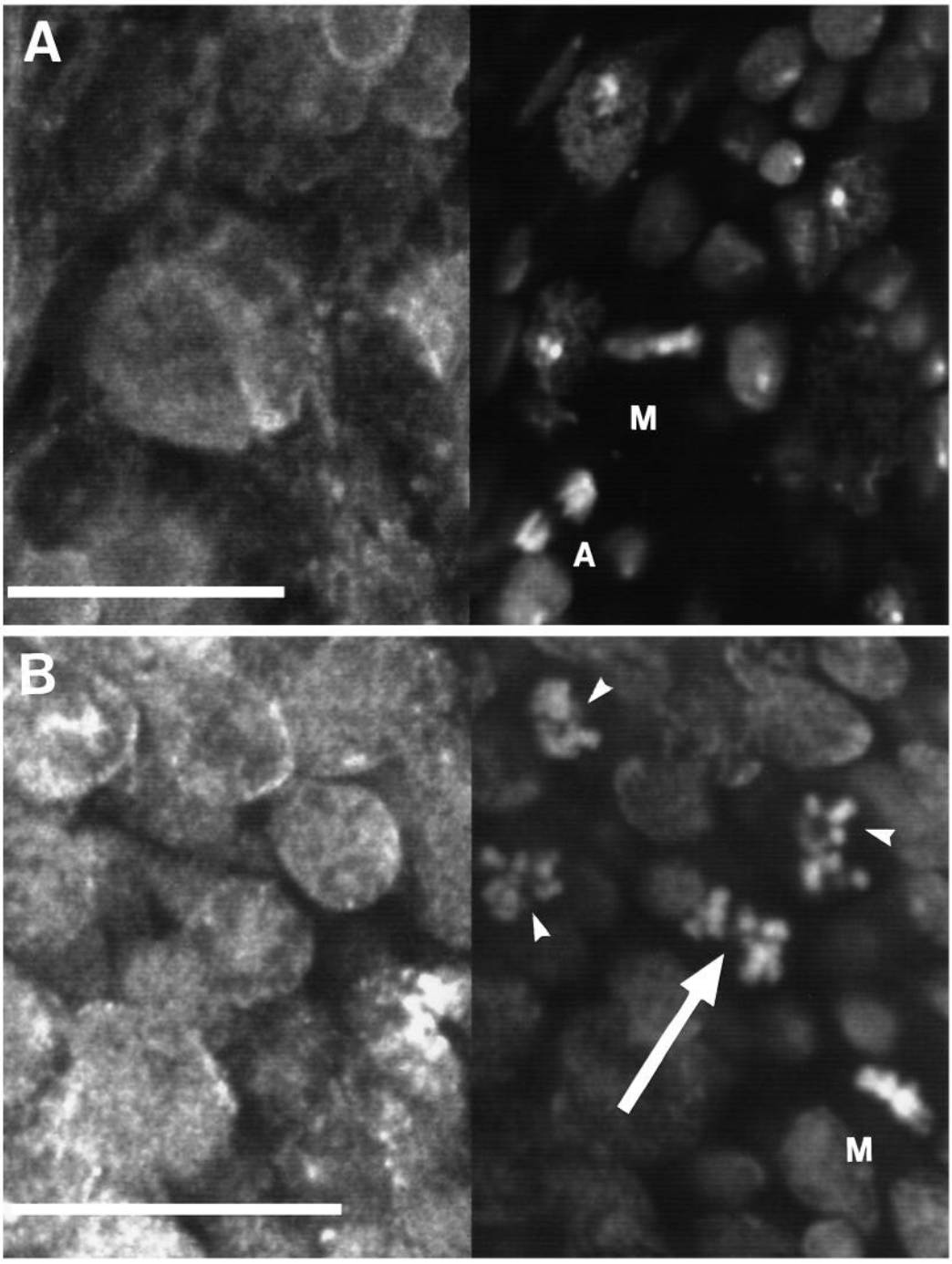


Fig. 7. Cyclin B immunofluorescence in a wild-type brain (A) and a brain expressing truncated cyclin B (B). Whole mount preparations of brains were fixed and stained with propidium iodide to reveal cyclin B (left hand panels) and chromosomes (right hand panels) (see Materials and Methods). Cells in metaphase and anaphase are labelled M and A, respectively. The arrowheads indicate mitotic cells at stages that cannot be classified. The arrow points to an anaphase figure in which chromosomes are highly condensed. Bars: 10 μm (A); 25 μm (B).

at least a sub-population of cells can undertake repeated cycles in the absence of chromosome segregation. None of these cells show the anti-tubulin staining characteristic of the mid-body that appears at telophase suggesting a failure to progress beyond the late stages of anaphase.

Normally the cyclin B immunostaining of larval neuroblasts disappears abruptly at the metaphase-anaphase transition (Whitfield et al., 1990). We carried out immunostaining of larval neuroblasts expressing the truncated cyclin B under GAL4 control to visualise the extent of cyclin B stability in localised regions of the larval brain (Fig. 7). Fig. 7A shows a field of cells from a wild-type brain in which both a giant neuroblast and a ganglion mother cell have been caught in mitosis. The former is in metaphase (labelled M) and shows high levels of cyclin B staining, whereas only low levels of cyclin B are seen in the latter cell, which is in anaphase (labelled A). In brains expressing truncated cyclin B, we see local areas with a high mitotic index in which all cells show cyclin B staining. Several mitotic cells can be seen in the field shown in Fig. 7B, most of which have highly condensed chromosomes. In this field, one cell is in metaphase (labelled M), three are at a stage of mitosis that is impossible to define (arrowheads), and one is an anaphase figure with dot-like condensed chromatids (large arrow).

DISCUSSION

The moderate levels of anti-cyclin B immunofluorescence suggests that the increased frequency of late anaphase figures results from the expression of a form of cyclin B that, by analogy with N-terminally deleted cyclins in other species, is stable and so has accumulated above a critical threshold. The extent of chromatin condensation suggests that these cells have been maintained in a mitotic state for some period of time, since chromosome overcondensation in this tissue is characteristic of mutations and drug treatments that delay progression through the mitotic cycle (Whitfield et al., 1990; Axton et al., 1990). We suggest that this is a consequence of the activity of $p34^{cdc2}$ kinase being maintained at a high level due to its association with the stable B-type cyclin. The work of Verde and co-workers (1990) indicates that $p34^{cdc2}$/cyclin B kinase has a direct effect on microtubule dynamics that is important for the formation and maintenance of the bipolar mitotic spindle. This provides an explanation for the persistence of a bipolar mitotic spindle in cells expressing truncated cyclin B, and of why many cells become delayed at this very late anaphase stage and cannot enter telophase. Some cells do appear to progress further into the mitotic cycle and become polyploid. Although we cannot say whether eventually all cells might be capable of such progression, we note that the expression of an N-terminally truncated B-type cyclin in budding yeast seems to retard rather than arrest progression through mitosis since cell number continues to increase (Ghiara et al., 1991).

The mitotic phenotype of budding yeast over-expressing N-terminally deleted *CLB1* was described by Ghiara et al. (1991). These workers observed arrested cells with two well separated masses of chromatin on an elongated spindle, but point out that it is difficult to assess mitotic stages in the *Saccharomyces* cell cycle. They were therefore unwilling to conclude that this could differ from the metaphase arrest reported by Murray et al. (1989). This phenotype was re-examined by Surana et al. (1993) who also examined the effects of the over-expression of the *CLB2* B-type cyclin or its stable derivative. These authors are more categorical about the mitotic stages in budding yeast, and state that the expression of these constructs results in telophase arrest. Gallant and Nigg (1992) observed that the expression of a stable chicken cyclin B construct in human cells cultured in vitro does not prevent cells from attempting some form of chromosome separation although a clean arrest was not observed. Moreover, Tsuji and colleagues (1992) have described a temperature-sensitive mutant Chinese hamster ovary cell line in which histone H1 kinase activity is maintained at high levels at the later stages of mitosis. During mitosis, the highly condensed sister chromatids separate and do not undergo telophase decondensation but remain associated with a spindle. These reports would seem to differ from the early findings of Murray and colleagues (1989), which have led to the widespread assumption over recent years that the expression of stable cyclin B and consequent maintenance of high levels of $p34^{cdc2}$ kinase activity leads to metaphase arrest. However, a major shortcoming of this work is that it was performed in an in vitro system incapable of spindle assembly. Indeed, the recent finding by Holloway and colleagues (1993) of late anaphase arrest following addition of N-terminally deleted cyclin B to an in vitro system with such capabilities suggests that $p34^{cdc2}$ inactivation is required primarily for telophase to take place. Our experiments are the first to address these issues in living cells in a metazoan, and also show both cells arrested with chromosomes in unusual mitotic configurations together with cells that are not progressing from anaphase into telophase.

If cyclin B degradation is not required for the metaphase-anaphase transition, why is it usually degraded at this point of the mitotic cycle? Cyclin B degradation seems to occur through a ubiquitin-dependent system (Glotzer et al., 1991). Holloway and colleagues (1993) have suggested that chromatid separation could also occur via ubiquitin-mediated proteolysis since an N-terminal fragment of cyclin B seems to compete for this process in a cell-free system. In this case, both processes might be effectively triggered by another signal. We suspect that there are exquisite mechanisms that regulate the timing or local activation of several of these proteolytic processes, since the degradation of cyclin A normally occurs minutes before that of cyclin B at the metaphase-anaphase transition. We would not like to suggest that all functions at the metaphase-anaphase transition are independent of cyclin B degradation and $p34^{cdc2}$ kinase inactivation. However, it seems that chromatid separation can take place independently of this process. Although we cannot assign a specific mitotic stage to many of the arrested cells, a major defect resulting from this ectopic expression of N-terminally truncated cyclin appears to be a failure to undertake telophase following the completion of anaphase.

We are grateful to Andrea Brand and Norbert Perrimon for providing transformation vectors, and GAL4 enhancer-trap-lines ahead of publication. We thank Cayetano Gonzalez for his continued interest in this work, and for his help in carrying out brain squashes and in their interpretation, and together with Robert Saunders for comments on the manuscript. The work was supported by the Cancer Research Campaign.

REFERENCES

Axton, J. M., Dombrádi, V., Cohen, P. T. W. and Glover, D. M. (1990). One of the protein phosphatase 1 isoenzymes in *Drosophila* is essential for mitosis. *Cell* **63**, 33-46.

Brand, A. H. and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401-415.

Draetta, G., Luca, F., Westendorf, J., Brizuela, L., Ruderman, J. and Beach, D. (1989). cdc2 protein kinase is complexed with both cyclin A and B: evidence for proteolytic inactivation of MPF. *Cell* **56**, 829-838.

Evans, T., Rosenthal, E., Youngbloom, J., Distel, D. and Hunt, T. (1983). Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. *Cell* **33**, 389-396.

Fang, F. and Newport, J. W. (1991). Evidence that the G1-S and G2-M transitions are controlled by different cdc2 proteins in higher eukaryotes. *Cell* **66**, 731-742.

Félix, M.-A., Labbé, J.-C., Dorée, M., Hunt, T. and Karsenti, E. (1990). Triggering of cyclin degradation in interphase extracts of amphibian eggs by cdc2 kinase. *Nature* **346**, 379-382.

Gallant, P. and Nigg, E. A. (1992). Cyclin B2 undergoes cell-cycle dependent nuclear translocation and, when expressed as a non-destructible mutant, causes mitotic arrest in HeLa cells. *J. Cell Biol.* **117**, 213-224.

Gatti, M. and Baker, B. (1989). Genes controlling essential cell cycle functions in *Drosophila melanogaster*. *Genes Dev.* **3**, 438-453.

Gautier, J., Minshull, J., Lohka, M., Hunt, T. and Maller, J. L. (1990). Cyclin is a component of maturation-promoting factor from *Xenopus*. *Cell* **60**, 487-494.

Ghiara, J. B., Richardson, H. E., Sugimoto, K., Henze, M., Lew, D. J., Wittenberg, C. and Reed, S. I. (1991). A cyclin B homologue in *S.*

cerevisiae: chronic activation of the cdc28 protein kinase by cyclin prevents exit from mitosis. *Cell* **65**, 163-174.

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

Gonzalez, C. and Glover, D. M. (1994). Techniques for studying mitosis in *Drosophila*. In *The Cell Cycle: A Practical Approach*. IRL Press at OUP, Oxford (in press).

Holloway, S. L., Glotzer, M., King, R. W. and Murray, A. W. (1993). Anaphase is initiated by proteolysis rather than by the inactivation of maturation-promoting factor. *Cell* **73**, 1393-1402.

Kilmartin, J. V., Wright, B. and Milstein, C. (1982). Rat monoclonal anti-tubulin antibodies derived by using a new non-secreting rat cell line. *J. Cell Biol.* **93**, 576-582.

Knoblich, J. A. and Lehner, C. F. (1993). Synergistic action of *Drosophila* cyclins A and B during the G2-M transition. *EMBO J.* **12**, 65-74.

Labbé, J. C., Picard, A., Peaucellier, G., Cavadore, J. C., Nurse, P. and Dorée, M. (1989). Purification of MPF from starfish: identification as the H1 histone kinase p34cdc2 and a possible mechanism for its periodic activation. *Cell* **57**, 253-263.

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

Luca, F. C., Shibuya, E. K., Dohrmann, C. E. and Ruderman, J. V. (1991). Both cyclin A60 and B97 are stable and arrest cells in M-phase, but only cyclin B97 turns on cyclin destruction. *EMBO J.* **10**, 4311-4320.

Maldonado-Codina, G. and Glover, D. M. (1992). Cyclins A and B associate with chromatin and the polar regions of spindles, respectively, and do not undergo complete degradation at anaphase in syncytial *Drosophila* embryos. *J. Cell Biol.* **116**, 967-976.

Maldonado-Codina, G., Llamazares, S. and Glover, D. M. (1993). Heat shock results in cell cycle delay and synchronisation of mitotic domains in cellularised *Drosophila melanogaster* embryos. *J. Cell Sci.* **105**, 711-720.

Meijer, L., Arion, D., Golsteyn, R., Pines, J., Brizuela, L., Hunt, T. and Beach, D. (1989). Cyclin is a subunit of the sea urchin M-phase specific histone H1 kinase. *EMBO J.* **8**, 2275-2282.

Minshull, J., Pines, J., Golsteyn, R., Standart, N., Mackie, S., Colman, A., Blow, J., Ruderman, J., Wu, M. and Hunt, T. (1989). The role of cyclin synthesis, modification and destruction in the control of cell division. *J. Cell Sci. Suppl. 12*, 77-97.

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

Nurse, P. (1990). Universal control mechanism regulating onset of M-phase. *Nature* **344**, 503-508.

Standart, N., Minshull, J., Pines, J. and Hunt, T. (1987). Cyclin synthesis, modification and destruction during meiotic maturation of the starfish oocyte. *Dev. Biol.* **124**, 248-258.

Surana, U., Amon, A., Dowzer, C., McGrew, J., Byers, B. and Nasmyth, K. (1993). Destruction of the CDC28/CLB mitotic kinase is not required for the metaphase to anaphase transition in budding yeast. *EMBO J.* **12**, 1969-1978.

Swenson, K. I., Farrell, K. M. and Ruderman, J. V. (1986). The clam embryo protein cyclin A induces entry into M-phase and the resumption of meiosis in *Xenopus* oocytes. *Cell* **47**, 861-870.

Tsuji, H., Matsudo, Y., Ajiro, K., Yasuda, H., Hanaoka, F., Hayashi, A., Utsumi, S., Ohba, Y. and Hori, T. (1992). A temperature sensitive CHO-K1 cell mutant (tsTM13) defective in chromosome decondensation and spindle deconstruction in M phase. *Exp. Cell Res.* **198**, 201-213

Verde, F., Labbé, J. C., Dorée, M. and Karsenti, E. (1990). Regulation of microtubule dynamics by cdc2 protein kinase in cell-free extracts of *Xenopus* eggs. *Nature* **343**, 233-238.

Whitfield, W. G. F., Gonzalez, C., Maldonado-Codina, G. and Glover, D. M. (1990). The A- and B-type cyclins of *Drosophila* are accumulated and destroyed in temporally distinct events that define separable phases of the G2-M transition. *EMBO J.* **9**, 2563-2572.

(Received 11 April 1994 - Accepted 10 June 1994)