The *Drosophila* Cell Cycle Gene *fizzy* Is Required for Normal Degradation of Cyclins A and B During Mitosis and Has Homology to the *CDC20* Gene of *Saccharomyces cerevisiae*

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Abstract. The *Drosophila* cell cycle gene *fizzy* (*fzy*) is required for normal execution of the metaphase-anaphase transition. We have cloned *fzy*, and confirmed this by P-element mediated germline transformation rescue. Sequence analysis predicts that *fzy* encodes a protein of 526 amino acids, the carboxy half of which has significant homology to the *Saccharomyces cerevisiae* cell cycle gene *CDC20*. A monoclonal antibody against fzy detects a single protein of the expected size, 59 kD, in embryonic extracts. In early embryos fzy is expressed in all proliferating tissues; in late embryos fzy expression declines in a tissue-specific manner correlated with cessation of cell division. During interphase fzy protein is present in the cytoplasm; while in mitosis fzy becomes ubiquitously distributed throughout the cell except for the area occupied by the chromosomes. The metaphase arrest phenotype caused by *fzy* mutations is associated with failure to degrade both mitotic cyclins A and B, and an enrichment of spindle microtubules at the expense of astral microtubules. Our data suggest that *fzy* function is required for normal cell cycle-regulated proteolysis that is necessary for successful progress through mitosis.

When a cell enters mitosis it undergoes a complex series of events involving extensive alterations of cellular structures and functions. Many of the normal processes of living cells, such as transcription, translation, and protein secretion, are temporarily suspended while the cell reorganizes its nucleus, chromosomes, and cytoskeleton to successfully accomplish chromosome segregation. Given the extent and complexity of these reorganizations, it is perhaps surprising to what extent these changes depend on the function of a single protein complex termed maturation promoting factor (MPF)[1] (Masui and Markert, 1971; reviewed by Murray and Hunt, 1993). MPF is a heterodimer composed of a catalytic subunit, the $p34^{cdc2}$ protein kinase, and a regulatory subunit, one of the mitotic cyclins, A or B (Dunphy et al., 1988; Gautier et al., 1988; Draetta et al., 1989: Labbé et al., 1989). Entry into mitosis occurs by activation of MPF (Gould and Nurse, 1989; reviewed by Murray and Kirschner, 1989; Nurse, 1990). MPF activity appears to directly drive the events of early mitosis such as nuclear envelope breakdown, chromosome condensation, and spindle formation by phosphorylating cellular substrates, such as lamins A and B, histone H1, and nucleolin (reviewed by Norbury and Nurse, 1992; Nigg, 1991, 1993). Whereas $p34^{cdc2}$ is required to drive the events of early mitosis, it must subsequently be inactivated to allow the events of late mitosis to proceed; failure to inactivate $p34^{cdc2}$ protein kinase prevents or delays chromosome decondensation, spindle disassembly, and nuclear envelope reformation (Murray et al., 1989; Luca et al., 1991; Gallant and Nigg, 1992; Surana et al., 1993; Holloway et al., 1993).

Inactivation of the cyclin-$p34^{cdc2}$ protein kinase complexes is achieved by degradation of the cyclin subunit by the ubiquitin/26S proteosome-dependent proteolytic pathway (Glotzer et al., 1991; Hershko et al., 1991; Gordon et al., 1993; Ghislain et al., 1993). Cyclin A is degraded during metaphase and cyclin B degradation occurs at approximately the metaphase-anaphase transition in all organisms so far examined (Luca and Ruderman, 1989; Minshull et al., 1990; Whitfield et al., 1990; Hunt et al., 1992). Cyclin B degradation is dependent on the completion of earlier mitotic events, specifically formation of a normal spindle, since treatment with microtubule-destabilizing drugs arrests cells at meta-

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1. *Abbreviations used in this paper*: *cact*, *cactus* gene; CNS, central nervous system; *fzy*, *fizzy* gene or mutant (superscript designates specific allele or allelic combination); fzy, fizzy protein; MPF, maturation promoting factor; PNS, peripheral nervous system; *ry*, rosy.

phase and prevents cyclin B degradation (Whitfield et al., 1990; Hoyt et al., 1991; Li and Murray, 1991). These data, and the central role that cyclin-p34^{cdc2} protein kinase complexes play in regulating mitosis, suggested that cyclin B degradation was the key event that triggered anaphase (Murray and Kirschner, 1989; Ghiara et al., 1991; Glotzer et al., 1991). However, although cyclin-p34^{cdc2} protein kinase complex inactivation is necessary for the completion of other late mitotic events, it has recently been shown that anaphase, the separation and polewards movement of sister chromatids, is initiated by the degradation of some other, as yet unidentified protein(s), by the same ubiquitin/26S proteosome pathway (Holloway et al., 1993; van der Velden and Lohka, 1993). Although the mechanisms by which proteins are ubiquitinated and subsequently degraded are becoming fairly well understood (Jentsch, 1992), we do not yet understand how ubiquitination/degradation is regulated during mitosis or how it is coordinated with the normal progression of mitotic events.

We have previously reported that the *fizzy* (*fzy*) gene of *Drosophila melanogaster* is required for the normal progression through the metaphase-anaphase transition during mitosis (Dawson et al., 1993). Here we show that *fzy* is also required for cyclin A and B degradation during mitosis in *Drosophila* and that *fzy* has significant homology to the *CDC20* gene of *Saccharomyces cerevisiae*, mutation of which also results in mitotic arrest before or during early anaphase in budding yeast (Byers and Goetsch, 1974; Palmer et al., 1989). On the basis of these data, we propose that *fzy* function is required for normal cell cycle-regulated ubiquitin-dependent proteolysis during mitosis.

Materials and Methods

Molecular Biology

General cloning procedures were carried out as described in Maniatis et al. (1982). *fzy* was mapped genetically and molecularly as described in the main text. *fzy* cDNAs were obtained by screening both a 2-14-h embryonic library (a gift from C. Delidakis, University of Crete, Heraklion and R. Fehon, Duke University, Durham, NC) and an ovarian cDNA library (a gift from P. Tolias, Public Health Research Institute of New York) by standard methods. For sequencing, both complete cDNAs and a collection of overlapping restriction fragments from each were subcloned into M13 vectors (Yanish-Perron et al., 1985), and sequenced with Sequenase Reagents (United States Biochemical Corp., Cleveland, OH) using both the universal primer and *fzy*-specific primers. For expression in yeast, a fragment containing the entire *fzy* ORF of cDNA *fzym5* flanked by BglII (5′) and XbaI (3′) sites was generated by PCR amplification using specific primers and cloned into the yeast expression vector pVT102-U (Vernet et al., 1987). This construct was transformed into the haploid yeast strain 405-1-1, genotype *MATa his7 ura3 ade2 cdc20-1* (Sethi et al., 1991) by the method of Chen et al. (1992).

Germline Transformation

Germline transformation was carried out by the method of Spradling and Rubin (1982). A 2.5-kb. Eco47III to EcoR1 fragment, containing the entire *fzy* transcription unit was blunt-ended and cloned into the HpaII site of the transformation vector pCaSpeR2 (Thummel and Pirrotta, 1992). This construct was coinjected with the helper plasmid pπ25.7wcΔ2-3 (Robertson et al., 1988) into w^{118} embryos and w^+ G1 progeny recovered. All inserts tested were able to rescue the lethality of fzy^3/Df(2L)*H60-3*.

Antibody Preparation

A 500-bp fragment encoding amino acids numbers 297-461 of the fzy open reading frame flanked by a native BamH1 site (5′) and an artificial EcoR1 site (3′) was generated by PCR using specific primers and subcloned into the *E. coli* expression vector pGEX-2T (Smith and Johnson, 1988). Upon induction this construct expressed a soluble fusion protein of the expected size, which was purified as described by Frorath et al. (1991). Immunization of mice and hybridoma production, screening, and maintenance was according to the methods described in Harlow and Lane (1988).

Immunoblotting and Immunohistochemistry

For immunoblotting embryo extracts were prepared from staged, dechorionated embryos and exponentially growing yeast cells in Laemmli sample buffer (2% SDS, 10% glycerol, 100 mM DTT, 60 mM Tris, pH 6.8, and 0.001% bromophenol blue), electrophoresed by SDS-PAGE and transferred to nitrocellulose essentially as described in Harlow and Lane (1988). For Westerns, anti-fzy mAb 20.B.9 was used at 1:10 dilution. Detection, using HRP-conjugated goat anti-mouse secondary antibodies (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA), was as described in Johansen et al. (1989).

For whole mount immunohistochemistry of embryos, embryos were prepared and stained essentially according to the protocol of Whitfield et al. (1990) except that 5% normal goat serum was used for blocking. For colchicine and taxol treatment, embryos were permeabilized by the method of Bodmer et al. (1989) and treated with 40 μM colchicine (Sigma Immunochemicals, St. Louis, MO) or 5 μM taxol (Sigma) in cell culture medium for 15-20 min at room temperature. Primary antibodies used were: anti-fzy, mAb 20.B.9 diluted 1:10; anti-tubulin, mAb YL1/2, diluted 1:25 (Sera-Lab, Crawley Down, Sussex, UK); anti-cyclin A and anti-cyclin B polyclonals, diluted 1:500 (Whitfield et al., 1990). Secondary antibodies, diluted between 1:30 to 1:500 were either HRP-, fluorescein- or CY3-conjugated goat anti-mouse, goat anti-rat, or goat anti-rabbit as appropriate (Jackson ImmunoResearch Laboratories, Inc.). For DNA staining, 100 μg/ml RNAse A was included in the incubation with the secondary antibody, and 10 μg/ml propidium iodide included in the first postsecondary antibody wash. Detection of HRP staining was as described in Rothberg et al. (1988). Confocal images were obtained with a Bio-Rad MRC 600 system attached to a Zeiss Axiovert microscope (Bio-Rad Labs., Hercules, CA and Carl Zeiss, Inc., Thornwood, NY). Brightfield images were captured from a Orthoplan 2 (The Leitz Co., Overland Park, KS) microscope, equipped with a Sony 3CCD video camera and Sony DXC-750MD camera control unit directly to a Macintosh IIfx equipped with a NuVista Videographics Card (Truevision, Inc., Indianapolis, IN). All image manipulation was performed on the Macintosh IIfx using the Adobe Photoshop program (Adobe Photosystems, Inc., Mountain View, CA).

Results

Molecular Cloning of fizzy

The *fzy* gene had been previously localized by deficiency mapping to cytological interval 35F on the left arm of chromosome II (Ashburner et al., 1990). We localized *fzy* more precisely using a new deficiency, Df(2L)*H60-3*, that we identified while screening a collection of P-element lines generated by local hopping of the *cactus*$^{255,rosy(ry)+}$ P-element, which is also located in interval 35F (Tower et al., 1993). One of these lines, *H60-3*, failed to complement all available *fzy* alleles. Further analysis showed that this line carried a chromosomal deficiency uncovering four loci, *cactus* (*cact*), *cornichon*, *fzy*, and *l(2)35Ff* (Fig. 1 *a*). This deficiency, *H60-3*, was presumably generated by imprecise excision of the *cact*$^{255,ry+}$ P-element. The proximal portion of the *cact*$^{255,ry+}$ P-element, encoding the *ry*$^+$ gene, is present on the Df(2L)-*H60-3* chromosome and unaltered relative to its parental chromosome as determined by PCR using P-element and *cact* specific primers (John Tower, personal communication). This observation suggested that the chromosomal segment removed in Df(2L)*H60-3* began in the *cact*$^{255,ry+}$ P-element and extended distally. To confirm the structure of Df(2L)*H60-3* we mapped its breakpoints molecularly. The *cact* gene had been previously cloned (Geisler et al., 1992; Kidd, 1992) and we used the available genomic clones from

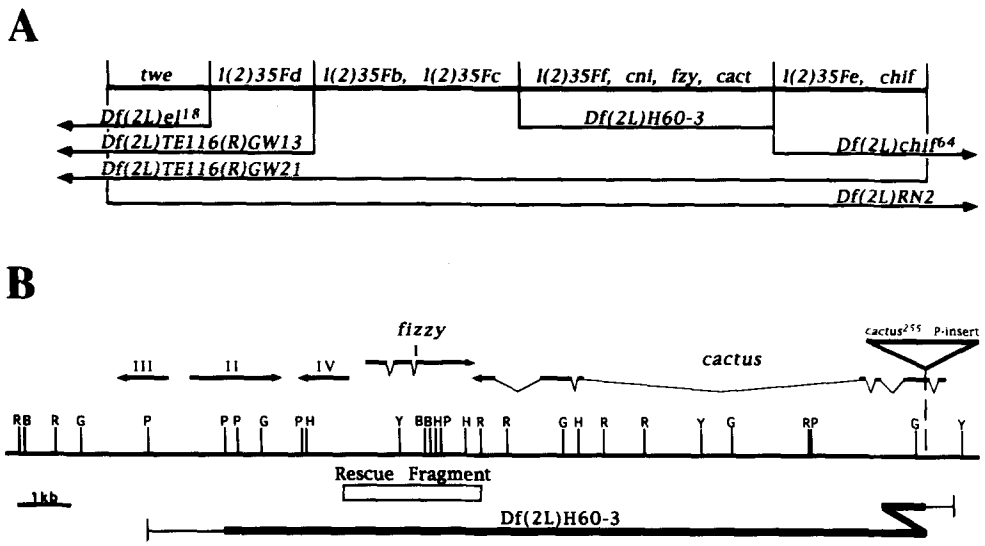


Figure 1. Genetic and molecular maps of the region containing *fzy*. (*A*) Deficiency map of the 35F interval. Complementation groups mapped to the 35F interval are indicated by their abbreviations above the heavy line that represents chromosome II left. The extent of various deficiencies used to map *fzy* are indicated below by solid lines, vertical lines indicate the genetically defined breakpoints of these deficiencies, an arrowhead indicates that the deficiency extends beyond the 35F interval depicted here. This map updates the previously published maps of the 35F interval by Ashburner et al. (1990) and Alphey et al. (1992). (*B*) Molecular map of part of 35F including *cactus* and the region immediately distal. The four transcripts we have mapped are represented by numbered arrows that indicate their relative positions, sizes, and direction of transcription; the positions of introns, where known, are also indicated. The longest *cact* transcript is similarly shown together with the location of the $cact^{255}$ P-element insert (data from Geisler et al., 1992 and Kidd et al., 1992). The heavy black line below the map indicates the extent of sequences removed by Df(2L)*H60-3*, the thinner lines at either end indicate the limits of uncertainty about the precise positions of the endpoints of Df(2L)*H60-3*. The *fzy* rescue fragment is indicated by the open box below the map. Restriction sites are R, Eco RI; B, Bam HI; G, Bgl II; P, Pst I; H, Hind III; and Y, Xho I. Both maps are oriented with proximal to the right and distal to the left.

this region to map the Df(2L)*H60-3* breakpoints by Southern analysis. This analysis placed the proximal breakpoint of Df(2L)*H60-3*, as expected, in the cactus gene ($cact^{255.ry+}$) P-element and the distal breakpoint between 5.0 and 6.5 kb distal to the 3′ end of the *cact* transcription unit (Fig. 1 *b*). Therefore, at least part of the *fzy* gene had to be located in the 6.5 kb immediately distal to *cact*.

To identify transcribed genes within this 6.5-kb interval, we used probes from this region to screen Northern blots of adult and embryonic mRNA (data not shown) and to screen ovarian and embryonic cDNA libraries. By these methods we identified four transcripts, numbered I to IV, deriving either wholly or in part from this 6.5-kb region (Fig. 1 *b*) and obtained cDNAs for each of these. Partial sequence from each of these cDNAs suggested that transcript I was the most likely candidate to correspond to *fzy*. Moreover, transcript I is expressed both maternally and zygotically (data not shown) as expected for *fzy*, which is required both maternally and zygotically for normal embryonic development (Dawson et al., 1993). To confirm that transcript I was indeed *fzy*, we reintroduced a 2.5-kb fragment containing all of transcript I plus adjacent genomic sequences by P-element mediated germline transformation. This fragment was able to rescue to viability otherwise lethal combinations of *fzy* alleles. Although this rescue fragment contains portions of both transcript IV and *cact*, these are noncoding regions from the 5′ and 3′ regions of these transcripts, respectively, and hence unlikely to be contributing to this rescue. We conclude therefore that transcript I is indeed *fzy*.

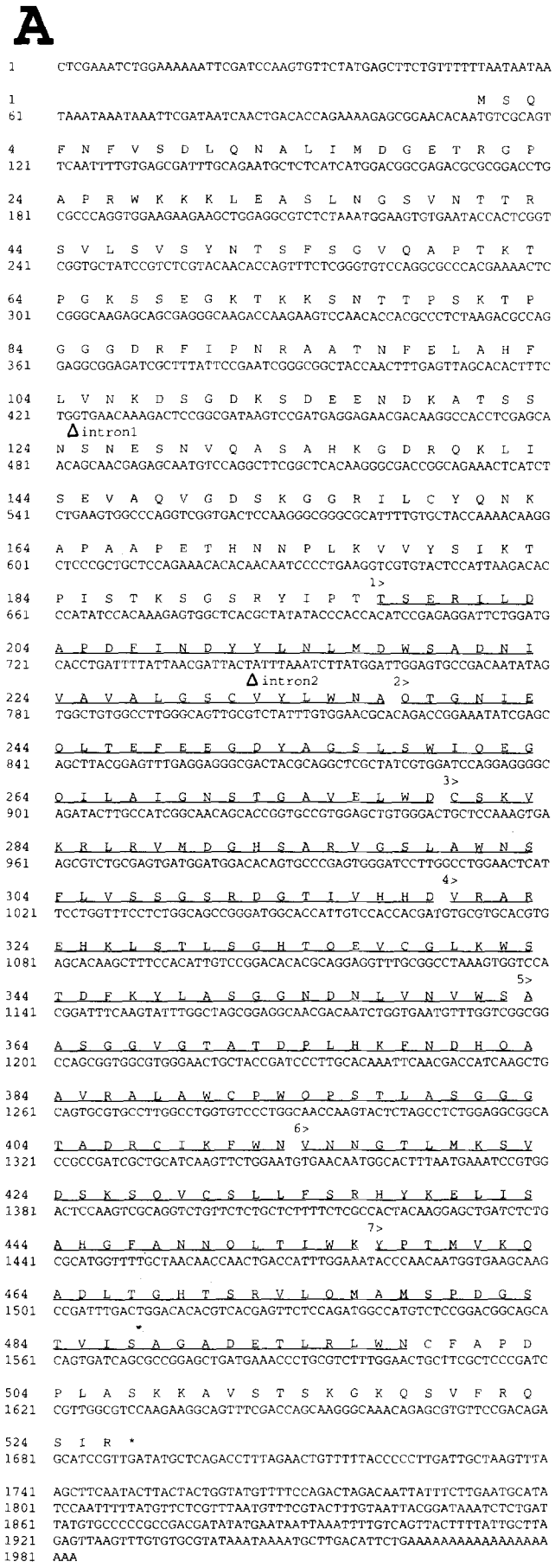
It is worth noting that the genomic organization of transcripts in this 6.5-kb region is somewhat unusual in that the transcription units are extremely densely packed together. The 5′ ends of *fzy* and transcript IV are separated by a maximum of only 260 bp (S. Roth and I. Dawson, unpublished data) and the 3′ noncoding termini of *fzy* and the longest *cact* cDNA actually overlap, on opposite strands, by 70 bp.

fizzy Encodes a Protein Containing WD-40 Repeats That Has Significant Homology to *Saccharomyces cerevisiae CDC20*

We isolated five independent *fzy* cDNAs, three from an ovarian and two from an embryonic cDNA library, all of ~2 kb in length. We sequenced one ovarian and one embryonic cDNA completely, as well as the 5′ and 3′ ends of each of the other cDNAs and the entire 2.5-kb genomic fragment used for the P-element rescue. All three ovarian cDNAs contain identical 5′ ends and terminate within 3 bp of each other immediately 5′ to the polyA tail, suggesting that these represent full-length transcripts. The sequence of the longest of these, cDNA *fzym5*, is given in Fig. 2 *a*. Both embryonic cDNAs are identical to the ovarian ones at the 3′ end but truncated in the predicted noncoding region at the 5′ end, presumably due to premature termination during cDNA synthesis. Sequence from genomic clones show that *fzy* contains two small introns, of 91 and 43 bp, interrupting the 5′ half of the coding region (Fig. 2 *a*).

All five cDNAs contain a single, identical, long open reading frame that can encode a predicted protein of 526 amino acids. The most obvious feature of the deduced amino acid sequence is that just over half (57%) of the predicted fzy protein, from amino acids 196–498 is composed of seven tandemly repeated copies of the WD-40 motif (Simon et al., 1991; van der Voon and Ploegh, 1992).

Computer searches of protein sequence databases using the BLASTP program (Altschul et al., 1990) revealed homology between fzy and many other proteins all of which


A
1 CTCGAAATCTGGAAAAAATTCGATCCAAGTGTTCTATGAGCTTCTGTTTTTTAATAATAA
1 M S Q
61 TAAATAAATAAATTCGATAATCAACTGACACCAGAAAAGAGCGGAACACAATGTCGCAGT
4 F N F V S D L Q N A L I M D G E T R G P
121 TCAATTTTGTGAGCGATTTGCAGAATGCTCTCATCATGGACGGCGAGACGCGCGGACCTG
24 A P R W K K K L E A S L N G S V N T T R
181 CGCCCAGGTGGAAGAAGAAGCTGGAGGCGTCTCTAAATGGAAGTGTGAATACCACTCGGT
44 S V L S V S Y N T S F S G V Q A P T K T
241 CGGTGCTATCCGTCTCGTACAACACCAGTTTCTCGGGTGTCCAGGCGCCCACGAAAACTC
64 P G K S S E G K T K K S N T T P S K T P
301 CGGGCAAGAGCAGCGAGGGCAAGACCAAGAAGTCCAACACCACGCCCTCTAAGACGCCAG
84 G G G D R F I P N R A A T N F E L A H F
361 GAGGCGGAGATCGCTTTATTCCGAATCGGGCGGCTACCAACTTTGAGTTAGCACACTTTC
104 L V N K D S G D K S D E E N D K A T S S
421 TGGTGAACAAAGACTCCGGCGATAAGTCCGATGAGGAGAACGACAAGGCCACCTCGAGCA
Δintron1
124 N S N E S N V Q A S A H K G D R Q K L I
481 ACAGCAACGAGAGCAATGTCCAGGCTTCGGCTCACAAGGGCGACCGGCAGAAACTCATCT
144 S E V A Q V G D S K G G R I L C Y Q N K
541 CTGAAGTGGCCCAGGTCGGTGACTCCAAGGGCGGGCGCATTTTGTGCTACCAAAACAAGG
164 A P A A P E T H N N P L K V V Y S I K T
601 CTCCCGCTGCTCCAGAAACACACAACAATCCCCTGAAGGTCGTGTACTCCATTAAGACAC
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184 P I S T K S G S R Y I P T T S E R I L D
661 CCATATCCACAAAGAGTGGCTCACGCTATATACCCACCACATCCGAGAGGATTCTGGATG
204 A P D F I N D Y Y L N L M D W S A D N I
721 CACCTGATTTTATTAACGATTACTATTTAAATCTTATGGATTGGAGTGCCGACAATATAG
Δintron2 2>
224 V A V A L G S C V Y L W N A Q T G N I E
781 TGGCTGTGGCCTTGGGCAGTTGCGTCTATTTGTGGAACGCACAGACCGGAAATATCGAGC
244 Q L T E F E E G D Y A G S L S W I Q E G
841 AGCTTACGGAGTTTGAGGAGGGCGACTACGCAGGCTCGCTATCGTGGATCCAGGAGGGGC
3>
264 Q I L A I G N S T G A V E L W D C S K V
901 AGATACTTGCCATCGGCAACAGCACCGGTGCCGTGGAGCTGTGGGACTGCTCCAAAGTGA
284 K R L R V M D G H S A R V G S L A W N S
961 AGCGTCTGCGAGTGATGGATGGACACAGTGCCCGAGTGGGATCCTTGGCCTGGAACTCAT
4>
304 F L V S S G S R D G T I V H H D V R A R
1021 TCCTGGTTTCCTCTGGCAGCCGGGATGGCACCATTGTCCACCACGATGTGCGTGCACGTG
324 E H K L S T L S G H T Q E V C G L K W S
1081 AGCACAAGCTTTCCACATTGTCCGGACACACGCAGGAGGTTTGCGGCCTAAAGTGGTCCA
5>
344 T D F K Y L A S G G N D N L V N V W S A
1141 CGGATTTCAAGTATTTGGCTAGCGGAGGCAACGACAATCTGGTGAATGTTTGGTCGGCGG
364 A S G G V G T A T D P L H K F N D H Q A
1201 CCAGCGGTGGCGTGGGAACTGCTACCGATCCCTTGCACAAATTCAACGACCATCAAGCTG
384 A V R A L A W C P W Q P S T L A S G G G
1261 CAGTGCGTGCCTTGGCCTGGTGTCCCTGGCAACCAAGTACTCTAGCCTCTGGAGGCGGCA
6>
404 T A D R C I K F W N V N N G T L M K S V
1321 CCGCCGATCGCTGCATCAAGTTCTGGAATGTGAACAATGGCACTTTAATGAAATCCGTGG
424 D S K S Q V C S L L F S R H Y K E L I S
1381 ACTCCAAGTCGCAGGTCTGTTCTCTGCTCTTTTCTCGCCACTACAAGGAGCTGATCTCTG
7>
444 A H G F A N N Q L T I W K Y P T M V K Q
1441 CGCATGGTTTTGCTAACAACCAACTGACCATTTGGAAATACCCAACAATGGTGAAGCAAG
464 A D L T G H T S R V L Q M A M S P D G S
1501 CCGATTTGACTGGACACACGTCACGAGTTCTCCAGATGGCCATGTCTCCGGACGGCAGCA
484 T V I S A G A D E T L R L W N C F A P D
1561 CAGTGATCAGCGCCGGAGCTGATGAAACCCTGCGTCTTTGGAACTGCTTCGCTCCCGATC
504 P L A S K K A V S T S K G K Q S V F R Q
1621 CGTTGGCGTCCAAGAAGGCAGTTTCGACCAGCAAGGGCAAACAGAGCGTGTTCCGACAGA
524 S I R *
1681 GCATCCGTTGATATGCTCAGACCTTTAGAACTGTTTTTACCCCCTTGATTGCTAAGTTTA
1741 AGCTTCAATACTTACTACTGGTATGTTTTCCAGACTAGACAATTATTTCTTGAATGCATA
1801 TCCAATTTTTATGTTCTCGTTTAATGTTTCGTACTTTGTAATTACGGATAAATCTCTGAT
1861 TATGTGCCCCCGCCGACGATATATGAATAATTAAATTTTGTCAGTTACTTTTATTGCTTA
1921 GAGTTAAGTTTGTGTGCGTATAAATAAAATGCTTGACATTCTGAAAAAAAAAAAAAAAAA
1981 AAA
B
Cdc20
fzy
p55
Cdc20
fzy
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Cdc20
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contained WD-40 repeat domains. The best match is between fzy and p55CDC, a mammalian gene that appears to be a cell cycle component (Weinstein et al., 1994), overall fzy and p55CDC are 50% identical and 59% similar (Fig. 2 *b*). The second best match is between fzy and the *Saccharomyces cerevisiae* cell cycle gene product Cdc20 (Sethi et al., 1991), overall they are 32% identical and 46% similar (Fig. 2 *b*). Although the homology between fzy, p55CDC and Cdc20 is most striking within the WD-40 repeat region of these proteins, they also show blocks of conservation outside this region (Fig. 2 *b*). Moreover, the extent of homology between the different WD-40 repeats of fzy, p55CDC and Cdc20 correlated with their position within these proteins: i.e., the first repeat of fzy is most homologous to the first repeat of p55CDC and Cdc20, the second with the second and so on (Fig. 2 *b*). These comparisons suggest that *fzy*, *p55CDC* and *CDC20* may represent a family of orthologous genes, within the WD-40 repeat superfamily, which are involved in cell cycle regulation.

To test if *fzy* was functionally as well as structurally homologous to *CDC20* we placed the *fzy* coding sequence from cDNA *fzy*m5 in a yeast expression vector and transformed this into the temperature-sensitive *cdc20-1* strain. However, we were unable to rescue the temperature sensitive lethality of the *cdc20-1* mutant with this *fzy* construct even though we know it is expressed, and produces full-length fzy protein. Moreover, wild-type *CDC20* expressed from the same vector under the same conditions will rescue (Dawson, I. and B. Rockmill, unpublished results and Fig. 3 *a*).

Expression of fizzy Protein

To examine the distribution of *fzy* protein during development and mitosis we generated mouse monoclonal antibodies against a fzy fusion protein. One of these antibodies, mAb 20.B.9, recognizes a single protein of ~59 kD on Western blots of *Drosophila* embryonic extracts (Fig. 3 *a*, lane *1*). This is in agreement with the expected molecular mass of 59 kD for fzy predicted by sequence analysis. To demonstrate that this 59-kD band is indeed fzy protein we used mAb 20.B.9 to probe a Western blot of extracts from yeast cells, which either carried or did not carry the fzy expression construct. This antibody detects a 59-kD band in the extract from the cells containing the fzy expression construct but nothing in the control extract from untransformed yeast (Fig. 3 *a*, lanes *2* and *3*), demonstrating both that the 59-kD band seen on Western blots is fzy and the specificity of the mAb 20.B.9 antibody for fzy.

To test the specificity of this antibody for use in whole mount staining of embryos we compared the staining of embryos homozygous for Df(2L)*H60-3*, which completely removes the *fzy* coding sequence, with their phenotypically wild-type sibs. Up to stage 10 mAb 20.B.9 shows homogeneous staining of all embryos in the population regardless of their genotype (Fig. 3 *b*). Failure to detect obvious differ-

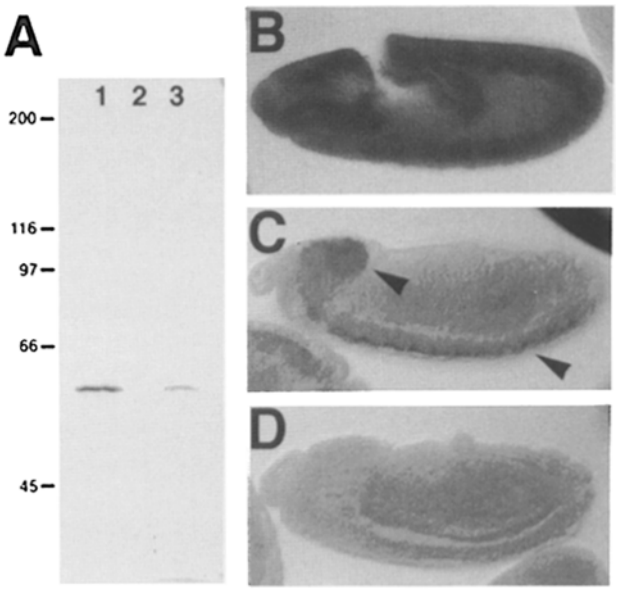


Figure 3. Specificity of anti-fzy antibody MAb 20.B.9. (*A*) Western blot of extracts of *D. melanogaster* embryos (lane *1*), *S. cerevisiae* (lane 2) and *S. cerevisiae* transformed with a *fzy* expression construct (lane *3*) probed with anti-fzy MAb 20.B.9. The antibody detects a single band of ~59 kD in the fly extract and the extract from yeast transformed with *fzy*, but nothing in the extract of untransformed yeast. (*B*) Stage 10 embryo (genotype +/+, +/*H60-3* or *H60-3*/*H60-3*, (*C*) stage 14 wild-type embryo (genotype +/+ or +/*H60-3* and (*D*) stage 14 *H60-3* embryo stained with anti-fzy MAb 20.B.9. In the stage 10 embryo there is intense staining throughout the germband but by stage 14 staining in wild-type embryos staining is largely confined to the neuroblasts on the surface of the CNS and brain lobes (*arrowheads*), which are the major population of mitotically active cells at this stage of development. In contrast, in the stage 14 *H603* homozygous embryo, at a similar plane of focus as the wild-type embryo in (*C*) there is no detectable staining of the CNS and brain lobes. The three embryos shown in *B–D* are from the same preparation, were stained in the same reaction and the images were captured and processed identically: anterior is to the left and dorsal uppermost in these panels.

Figure 2. Sequence of *fzy* and comparison between fzy, p55CDC, and CDC20. (*A*) Complete sequence of the longest *fzy* cDNA (cDNAm5). The locations of the two introns, between positions 422–423 and positions 744–745, are indicated by Δ below the DNA sequence. The deduced amino acid sequence of the longest open reading frame is shown above, beginning at the first possible methionine at nucleotide 111. The seven WD-40 repeats in the carboxy half of the deduced protein are underlined, the number of each repeat is indicated above its first animo acid. The cDNA sequence of *fzy* has been submitted to the EMBL/Genbank databases under accession number U22419. (*B*) Comparison of the deduced fzy, p55CDC and CDC20 proteins, aligned using the Geneworks Version 2.2 program; identical residues are boxed and shaded, conservative substitutions (I=L=V=M, K=R, S=T, D=E=N=Q, F=Y, and A=G) are shaded, and – indicates gaps introduced to improve the alignment. The WD-40 domains are underlined. The human p55CDC sequence is from Weinstein et al. (1994) and our unpublished data. The original published *CDC20* sequence contained an error introducing a frameshift near the carboxy terminus of the deduced Cdc20 protein: the above CDC20 sequence is the revised and corrected version (D. Burke, personal communication).

ences in staining patterns between wild-type and homozygous Df(2L)*H60-3* embryos up to this point is not unexpected as genetic evidence indicates that maternally supplied *fzy*$^+$ product perdures until approximately this stage. However, by stage 14, when Df(2L)*H60-3* embryos can be unambiguously identified morphologically by their *fzy*$^-$ phenotype and the maternally supplied *fzy*$^+$ product has been depleted, phenotypically wild-type embryos show specific staining that is absent in their mutant sibs (Figs. 3, *c* and *d*).

Having confirmed the specificity of mAb 20.B.9 for fzy, we have used it to examine the distribution of fzy protein during embryonic development. In newly fertilized eggs and very early embryos maternally supplied fzy appears to be relatively homogeneously distributed throughout the embryo (data not shown). As the nuclear density increases during stage 2 fzy staining becomes more pronounced in the energids, the cytoplasmic islands associated with the nuclei, than in the surrounding yolk (Fig. 4 *a*). By stage 5 when the majority of nuclei have migrated to the embryonic periphery and cellularization is occurring most of the fzy staining is also present in the cortical cytoplasm at the embryonic periphery. There is no fzy staining associated with the vitellophages that remain in the yolk (Fig. 4 *b*); the vitellophages do not divide again (Campos-Ortega and Hartenstein, 1985; Smith and Orr-Weaver, 1991). These differences in fzy staining during early development presumably reflect changes in the distribution of maternally supplied protein as they occur before high levels of zygotic transcription occur (Edgar and Schubiger, 1986). From stage 5 to 10 fzy is uniformly expressed throughout the cellular regions of the embryo (Fig.

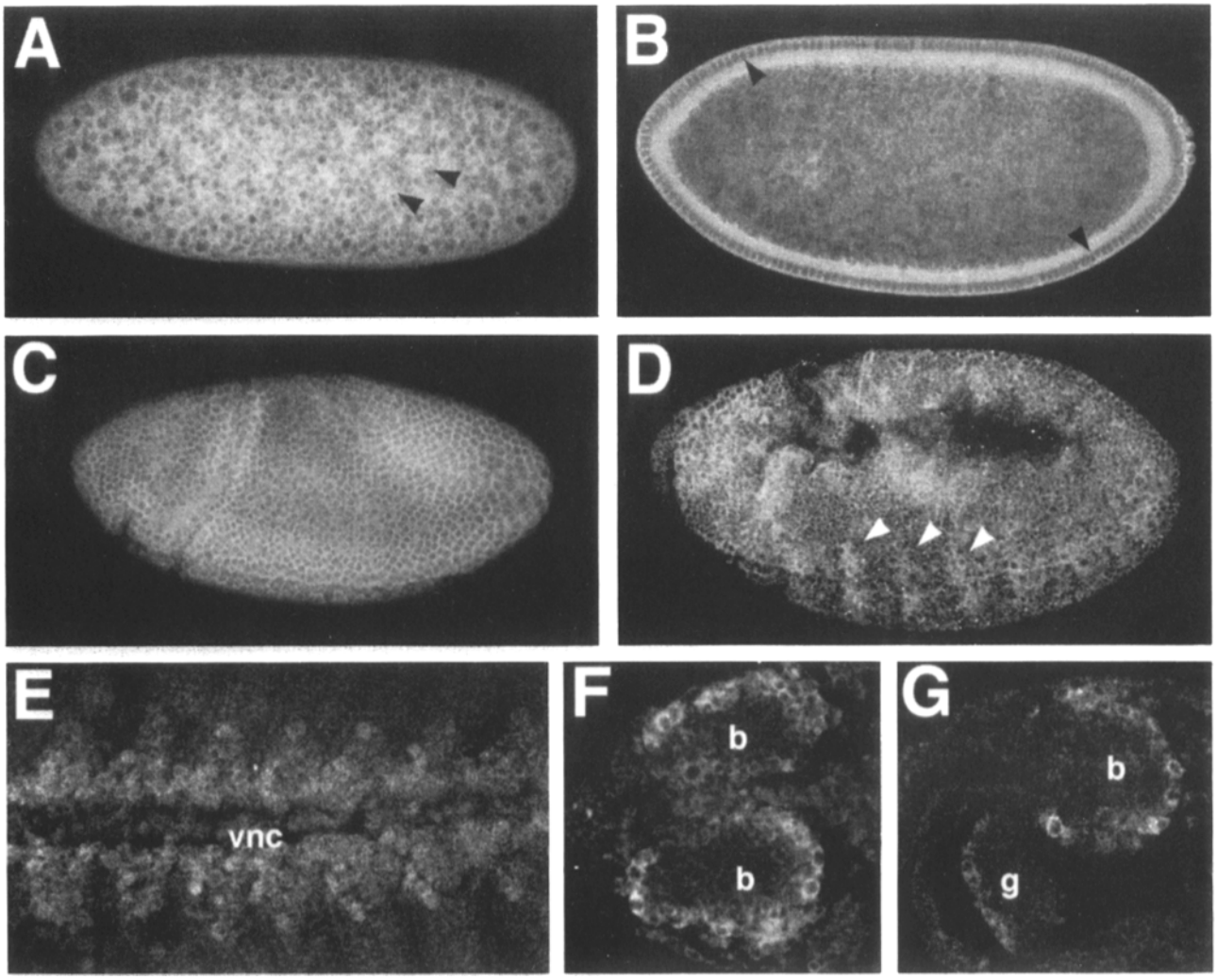


Figure 4. Confocal images of fzy expression during embryonic development. Anterior is to the left in all panels. (*A*) A late stage 2 embryo showing maternally supplied fzy protein concentrated in the energids, or cytoplasmic islands, associated with the nuclei (*arrowheads*). (*B*) An early stage 5 embryo. The bulk of maternally supplied fzy protein remains associated with the cytoplasm around the nuclei (*arrowheads*) and is now found at the embryonic periphery. (*C*) A stage 7 embryo. fzy is expressed in all cells at this stage. (*D*) A stage 11 embryo. By this stage fzy expression is declining in some cells but remains more strongly expressed in others. Analysis of this and other stage 11 embryos shows that the groups of strongly expressing cells indicated (*arrowheads*) are the precursors of the CNS, which are still actively dividing at this stage, whereas in the epidermis cell division is almost complete and fzy expression is declining. (*E*) Ventral, (*F*) dorsal, and (*G*) lateral views of stage 14 embryos. fzy expression has almost disappeared from most tissues but is still present in the neuroblasts and ganglion mother cells at the edges of the ventral nerve cord and brain lobes. *vnc*, ventral nerve cord; *b*, brain lobe; *g*, gnathal ganglion.

4 *c*). During these stages fzy is also present in cells of the amnioserosa, which do not undergo further division (Foe, 1989), which we presume reflects the perdurance of maternally supplied protein (data not shown). Only fairly late in embryogenesis, from stage 11 onwards, do noticeable differences in intensity of fzy staining become apparent (Fig. 4 *d*). These changes appear to correlate with cell division patterns: in tissues where cell division is ceasing, such as the epidermis and mesoderm, fzy staining gradually declines, whereas in the remaining actively dividing tissues such as the neuroblasts and ganglion mother cells of the central nervous system (CNS; Prokop and Technau, 1991; Smith and Orr-Weaver, 1991) fzy is still expressed strongly. This correlation between continued high fzy expression and mitotic activity is most marked in later stages where fzy is expressed exclusively in the few remaining actively dividing cells, the neuroblasts and ganglion mother cells of the CNS (Fig. 4, *e–g*).

We have examined the subcellular distribution of fzy during mitosis. The nuclear divisions of the precellular blastoderm stage embryos, because of their synchrony and the superficial and single-layered arrangement of nuclei are the easiest to examine. In interphase of these divisions, before entry into mitosis, fzy is primarily or exclusively cytoplasmic (Fig. 5 *a*). While we see some weak nuclear staining this is much less intense than the cytoplasmic staining and we cannot be certain whether this represents background from the detection methods used or whether this accurately reflects the distribution of fzy protein. During prophase fzy remains primarily cytoplasmic but the level of nuclear staining increases; in addition, the boundary between nuclear and cytoplasmic staining becomes much less distinct (Fig. 5 *b*). By prometaphase/metaphase fzy staining is ubiquitous, though the intensity of staining is significantly less in the region occupied by the chromosomes themselves than in the adjacent areas (Fig. 5 *c*). During anaphase, the exclusion of fzy from the region occupied by the DNA becomes more pronounced (Fig. 5 *d*) and by telophase, as the nuclear envelope is reformed, fzy staining once again becomes cytoplasmic (Fig. 5 *e*). This same alteration in fzy distribution during mitosis, i.e., from cytoplasmic to ubiquitous, except over the DNA itself, to cytoplasmic again, also occurs during the later cellular divisions with the same timing relative to mitotic progression (Fig. 5 *f*).

fizzy Mutations Prevent Normal Degradation of Cyclins A and B and Lead to Excess Accumulation of Spindle Microtubules

During the cellular mitoses of wild-type embryos cyclin A degradation occurs during metaphase whereas cyclin B degradation occurs at the metaphase–anaphase transition (Whitfield et al., 1990). We used polyclonal antisera specific for either cyclin A or cyclin B (Whitfield et al., 1990) to assay cyclin degradation in *fzy*⁻ embryos. In the dorsal epidermal region of wild-type embryos by stage 14 most cells have ceased dividing and consequently few cells stain positively for either cyclin A or B (Fig. 6, *a* and *b*). In contrast, in the same region of stage 14 *fzy*⁻ embryos many more cyclin A and cyclin B positive cells are present and many of these cells contain metaphase figures (Fig. 6, *c* and *d*). We have previously shown that in *fzy*⁻ embryos the peripheral nervous system (PNS) precursors underlying the dorsal epidermis arrest in metaphase (Dawson et al., 1993). Based on this observation, the pattern and the subepidermal position of the cyclin positive cells in the dorsal epidermal region of the *fzy*⁻ embryos we conclude that these are metaphase-arrested PNS precursors. Similar results were observed in the metaphase-arrested cells of the cephalic and ventral epidermis of *fzy*⁻ embryos. Higher magnification views of such metaphase-arrested cells in the epidermis of *fzy*⁻ embryos show almost all stain positively for cyclin A and most stain positively for cyclin B (Fig. 6, *e* and *f*). Thus the metaphase arrest phenotype caused by *fzy*⁻ mutations is usually accompanied by failure to degrade both mitotic cyclins A and B.

Since *CDC20* has been proposed to regulate microtubule behavior (Sethi et al., 1991), we also examined the effects of treatment with either colchicine, a microtubule-destabilizing agent, or taxol, a microtubule-stabilizing drug, on mitotic cyclin degradation during the postblastoderm divisions of *Drosophila* embryos. Treatment with either drug results in a pseudometaphase arrest in which many mitotic cells with condensed chromatin are present but no anaphase or telophase figures are seen (Figs. 6, *g* and *h* and 7, *a* and *d*). In agreement with Whitfield et al. (1990), who analyzed the effects of colchicine on cyclin degradation during mitosis of larval neuroblasts, we find that during the postblastoderm divisions colchicine-treated pseudometaphase-arrested cells degrade cyclin A but not cyclin B (Fig. 6, *g* and *h*). Taxol has a similar effect on mitotic cyclin degradation as colchicine, specifically taxol-treated pseudometaphase-arrested cells are readily able to degrade cyclin A (Fig. 7, *b* and *c*) but do not degrade cyclin B (Fig. 7, *e* and *f*).

One of the phenotypes of the *cdc20-1* mutation of budding yeast is an increase in the amount of tubulin incorporated into spindle microtubules when *cdc20-1* cells are arrested in mitosis at the restrictive temperature (Sethi et al., 1991). We have used an anti-tubulin antibody to examine spindle morphology in embryos from fzy^6/fzy^7 mothers, fzy^{mat} embryos. Such fzy^{mat} embryos lack sufficient maternally supplied *fzy* product, do not develop beyond the 2nd or 3rd nuclear division and their nuclei arrest at the metaphase–anaphase transition (Dawson et al., 1993). Most of the spindles of the metaphase arrested nuclei in fzy^{mat} embryos clearly contain an excess of microtubules as compared with the spindles of control, wild-type embryos at the same stage of mitosis and in the same division cycle (Fig. 8, *a* and *b*). In addition, whereas in control embryos astral microtubules as well as spindle microtubules can be seen to radiate out from the centrosomes (Fig. 8 *a*), in the fzy^{mat} embryos all the microtubules emanating from the centrosomes are incorporated into the spindle (Fig. 8 *b*). Although most spindles in fzy^{mat} embryos exhibit this excess of microtubules, the degree to which this occurs if somewhat variable and there are occasional spindles in fzy^{mat} embryos that are indistinguishable from those of the wild-type controls. Similarly, in the ventral epidermis of *fzy*⁻ embryos we see some metaphase-arrested cells that appear to contain excess spindle compared to the spindles of mitotic figures in the wild-type sibs present in the same preparation, again the degree to which this occurs is quite variable (data not shown).

Discussion

In this paper we report the molecular characterization of the

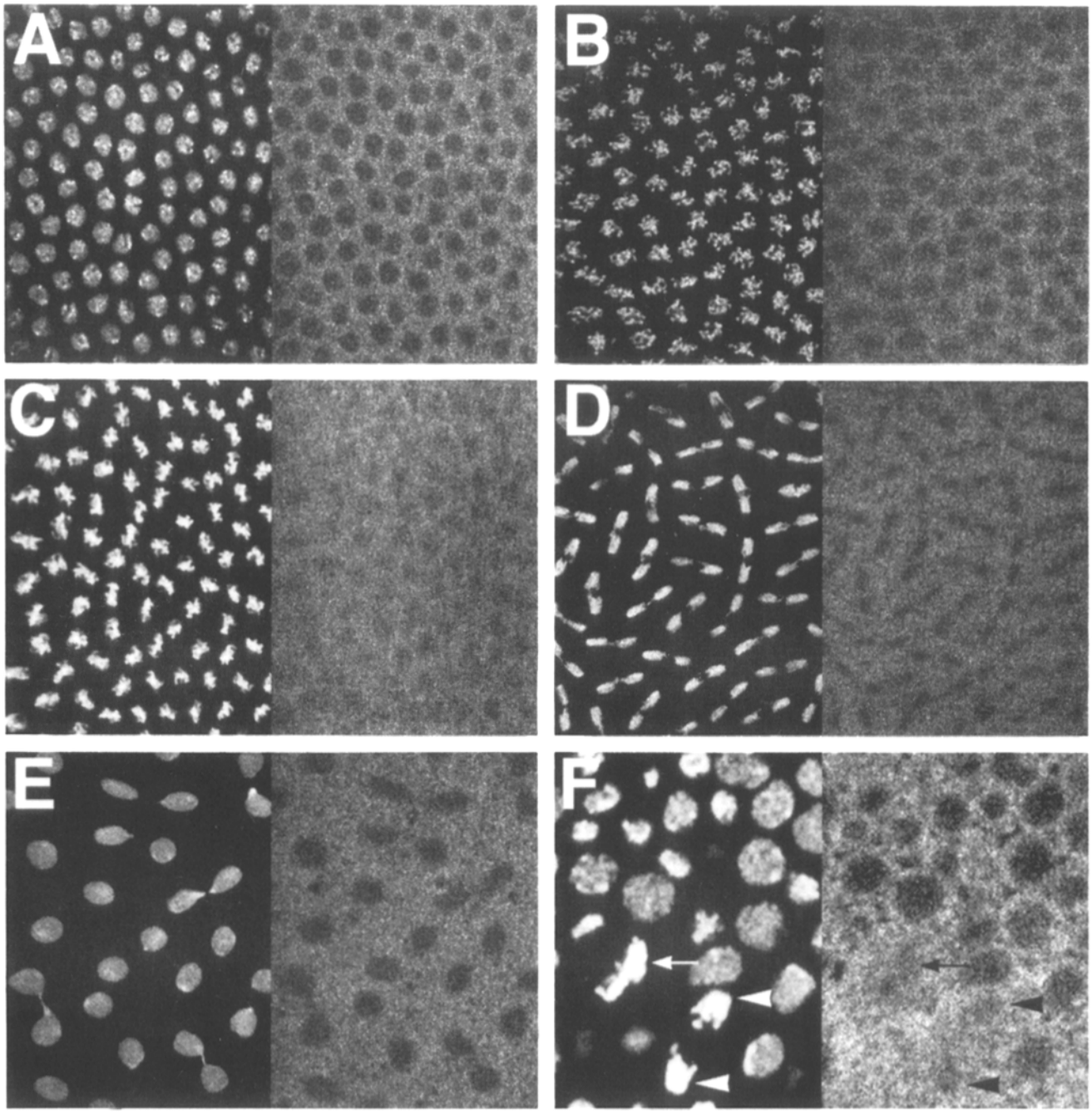
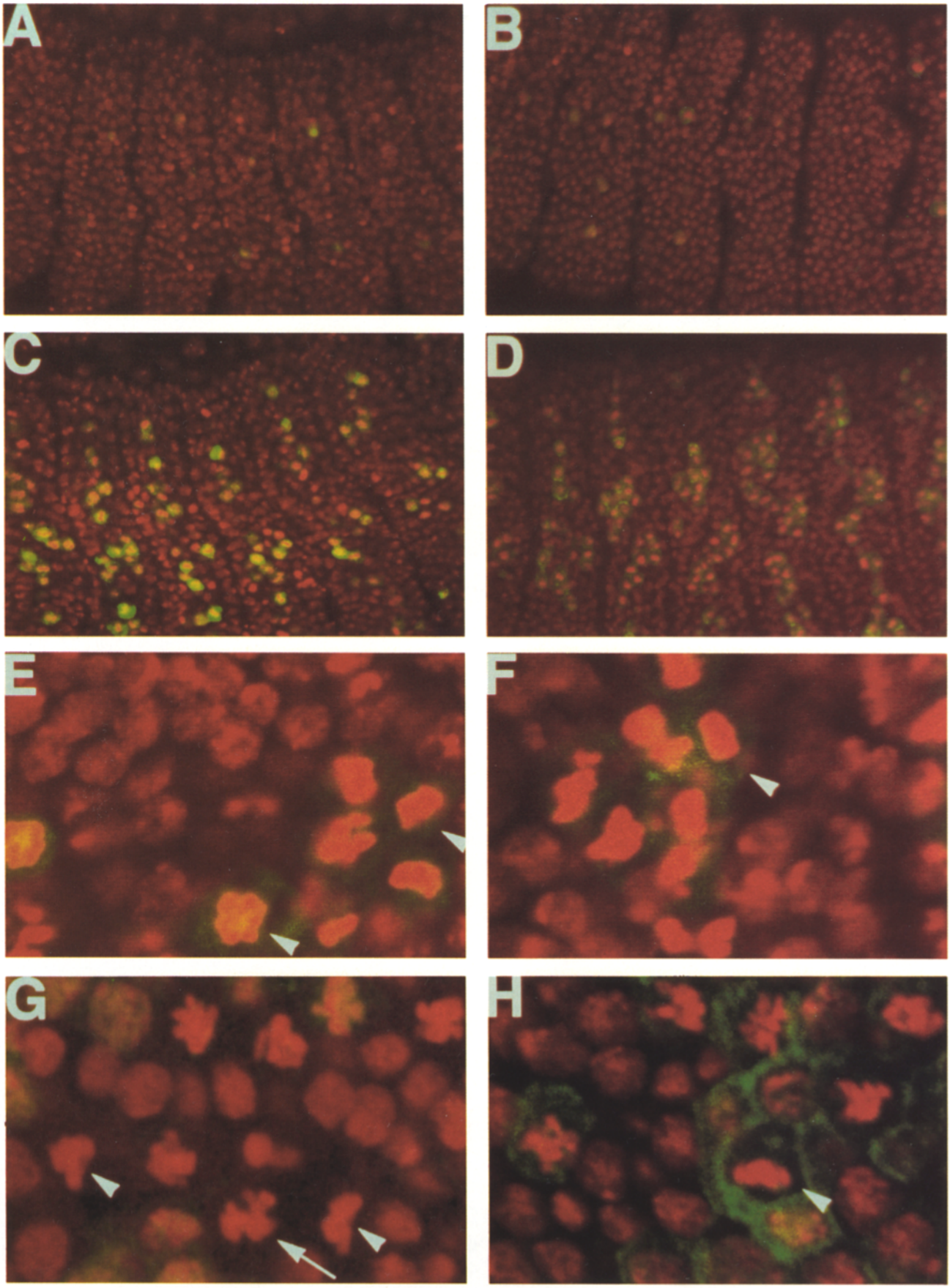


Figure 5. Confocal images of fzy expression during mitosis. In each panel the same field of nuclei/cells is shown stained for DNA (*left*) and fzy (*right*). *A–E* are from precellular blastoderm embryos during cycles 10–13. (*A*) A field of interphase nuclei between mitoses. fzy is primarily cytoplasmic. (*B*) Prophase nuclei. fzy is still mainly cytoplasmic but the boundary between the nuclear and cytoplasmic staining is less distinct. (*C*) Prometaphase/metaphase nuclei. The DNA has condensed to from recognizably individual chromosomes, and fzy is ubiquitously distributed throughout the formerly nuclear and cytoplasmic compartments but fzy staining is less intense in the areas containing the chromosomes. (*D*) Anaphase nuclei. fzy remains generally distributed except over the chromosomes. (*E*) Interphase nuclei just after telophase. fzy staining is again primarily cytoplasmic. (*F*) A field of nuclei at various stages of the cell cycle from a stage 8 embryo. In interphase cells fzy is cytoplasmic, but in the early anaphase (*small arrow*) and late anaphase (*arrowheads*) cells fzy is generally distributed though staining is again less intense over the DNA.

Figure 6. Confocal pseudocolour images of cyclin A and B expression in wild-type, *fzy* and colchicine-treated embryos. In all panels cyclins are green and DNA is red. (*A*) Cyclin A and (*B*) cyclin B expression in the dorsal epidermal region of wild-type stage 14 embryos. (*C*) Cyclin A and (*D*) cyclin B expression in the dorsal epidermal region of stage 14 *fzy*$^-$ embryos. Many more cells express both cyclins in the mutant embryo compared to the wild type; judging from the position of these cells and analysis of PNS development in *fzy* embryos (Dawson et al., 1993) these are PNS precursors that have arrested in mitosis and retained high cyclin expression. (*E*) Cyclin A and (*F*)



cyclin B expression in high magnification views of mitotically arrested cells in the ventral/cephalic epidermis fzy embryos. It is clear that most metaphase arrested cells retain high levels of cyclin A and cyclin B expression (*arrowheads*). (*G*) Cyclin A and (*H*) cyclin B expression in colchicine-treated stage 10/11 wild-type embryos. Colchicine treatment results in pseudometaphase arrest and prevents cyclin B degradation but not cyclin A degradation in such pseudometaphase-arrested cells (*arrowheads*). Indeed, cyclin A levels in colchicine-treated embryos appear to decline with a normal temporal profile and have declined substantially by prometaphase (*arrow*).

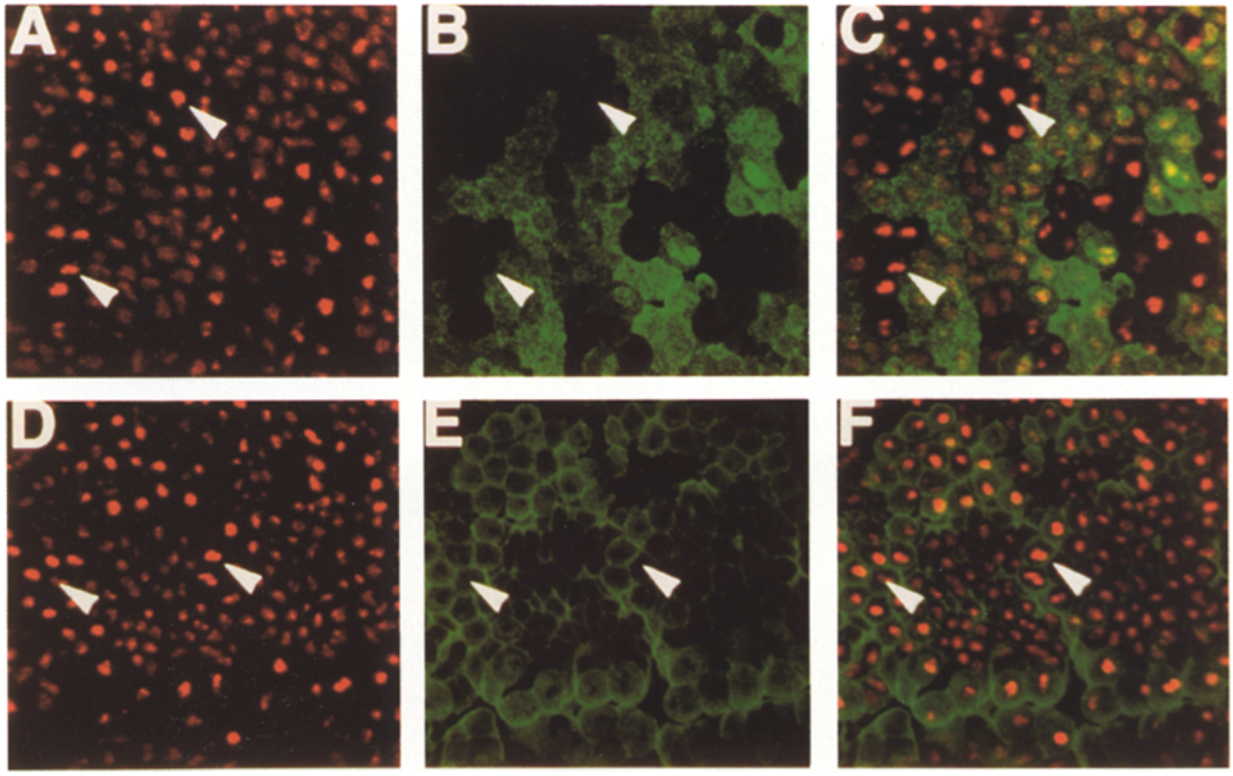


Figure 7. Confocal images of cyclin expression in taxol-treated embryos. Cyclin is green, DNA is red. *A*–C show DNA, cyclin A and the merged image, respectively, for the same field of cells; *D*–F show DNA, cyclin B and the merged image of a second field of cells from a different embryo. After taxol treatment numerous cells contain condensed chromatin, visible as groups of more intensely staining nuclei (*arrowheads*) in *A* and *D*. Normarski views of these same fields of cells show that those with condensed chromatin are rounded up and protruding from the apical surface of the embryo confirming that these are mitotic cells (data not shown). The lack of anaphase figures indicates that these cells are arrested in pseudometaphase. Almost all such pseudometaphase-arrested cells degrade cyclin A; comparison of *A*–C shows that the cells contain condensed chromatin correspond to "holes" in the cyclin A staining (*arrowheads* point to the same nuclei in each panel). Similar comparison of *D–F* show that almost none of the taxol arrested pseudometaphase cells degrade cyclin B.

fizzy locus of *Drosophila* and further analysis of the metaphase arrest phenotype caused by *fzy* mutations.

We have molecularly localized *fzy* to a small 2.5-kb genomic fragment immediately distal to *cactus* in the 35Ff interval of the second chromosome, which we demonstrate by P-element mediated germline transformation rescue. This genomic fragment containing only one complete transcription unit that we conclude therefore encodes *fzy*. The deduced amino acid sequence of *fzy* shares significant homology with the *S. cerevisiae* cell cycle gene *CDC20* and the mammalian *p55^{CDC}* gene. This homology between fzy and p55^{CDC} extends throughout the protein, whilst that between fzy and Cdc20 is largely confined to the WD-40 repeat domains in the carboxy halves of each protein. However, the homology between fzy and Cdc20 is more significant than just the fact that both are members of the large family of WD-40 repeat containing genes. The WD-40 repeats of fzy, p55^{CDC}, and Cdc20 are more similar to each other than any are to any other member of this family. Moreover, individual WD-40 repeats in fzy and p55^{CDC} are most similar to their corresponding repeat in Cdc20. The overall similarity of the WD-40 domains of these proteins, and particularly the correlation between individual repeats at the equivalent position in each gene having the highest similarity, suggests that *fzy*, *p55^{CDC}*, and *CDC20* are homologous genes.

Furthermore, loss of function mutations in both *fzy* and *CDC20* results in a similar phenotype: both cause mitotic arrest that is associated with excess accumulation of tubulin into the spindle microtubules. This similarity of the *cdc20-1* and *fzy* mutant phenotypes, together with the homology shared by these gene products, strongly suggests that *fzy* and *CDC20* perform equivalent functions during mitosis in their respective organisms. Frequently homologous cell cycle genes from diverse organisms are sufficiently well conserved that they can functionally substitute for each other (Lee and Nurse, 1987; Jiminez et al., 1990; Lehner and O'Farrell, 1990). We attempted to test if *fzy* was functionally, as well as structurally, homologous to *CDC20* by assaying if *fzy* could rescue the temperature-sensitive lethality of the *cdc20-1* mutation. However, we were unable to obtain any evidence of rescue (Dawson, I. and B. Rockmill, unpublished data). This could imply that *fzy* and *CDC20* are not in fact functionally homologous. Alternatively, it could imply that *fzy* and *CDC20* have diverged from each other to such an extent that *fzy* cannot substitute for *CDC20* in this heterologous system. While we cannot distinguish between these two interpretations at present, given the similarity between both the sequence and the mutant phenotypes of *fzy* and *CDC20*, we think the latter possibility is more likely.

In early embryos fzy appears to be uniformly expressed

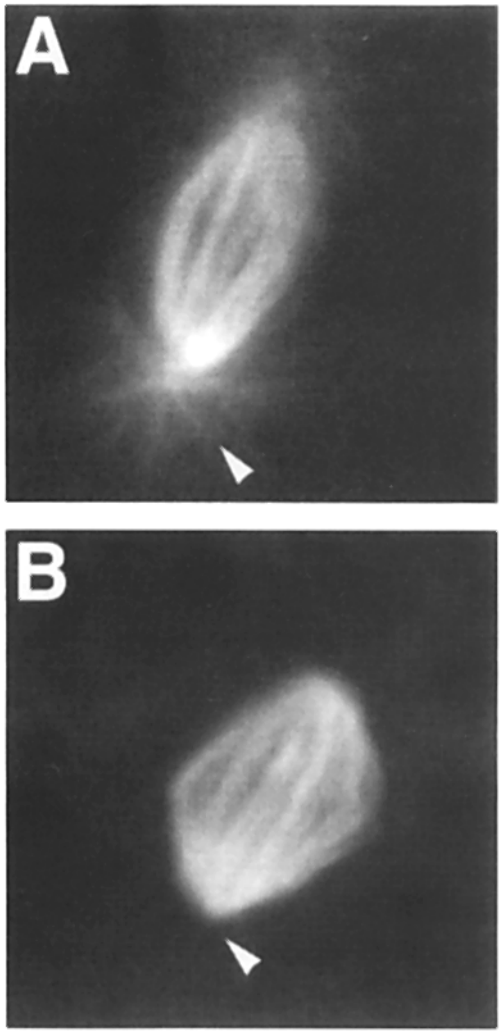


Figure 8. Confocal images of the mitotic spindle in wild-type and fzy^{mat} embryos visualized with the anti-tubulin antibody mAb YL1/2. (*A*) A metaphase nucleus at mitosis 2 in a wild-type embryo; astral microtubules (*arrowhead*) are clearly present in addition to the spindle microtubules. (*B*) A nucleus arrested at metaphase during mitosis 2 in a fzy^{mat} embryo. There are excess spindle microtubules present in the fzy^{mat} nucleus compared to wild-type nucleus. In addition, no astral microtubules are present in this nucleus (*arrowhead*), suggesting that all the microtubules emanating from the centrosomes have been incorporated into the mitotic spindle.

throughout the cellular regions. Generalized fzy expression declines from stage 10 onwards, in a pattern that correlates with the completion of embryonic cell divisions. Notably however, from stage 11 onwards, fzy expression is absent from those cells that have entered endocycle, rounds of DNA synthesis without mitotic division that lead to polyploidization (Smith and Orr-Weaver, 1991). This distribution of fzy protein that we see during embryogenesis is in complete agreement with what we expected based on the our previous analysis of the *fzy* embryonic phenotype (Dawson et al., 1993): that is, that *fzy* is maternally supplied and the maternally supplied product perdures until at least stage 10; that *fzy* would be required by all dividing cells; and that *fzy* would not be required by cells that are not undergoing division.

We have not observed any cell cycle-related periodicity to fzy expression during embryonic development. That is not surprising as we know that maternally supplied product persists through many divisions. These observations suggest that if *fzy* activity is cell cycle regulated this is likely to occur at the protein level by post translational modifications. It should be noted however, that the postblastoderm cell cycles are fairly rapid, between 1 to 2 h, and do not have a G1 phase (Campos-Ortega and Hartenstein, 1985; Foe, 1989; Edgar and O'Farrell, 1990); potentially, in the longer cycles during larval development, which do have G1, regulation of *fzy* expression or activity could be more complicated.

We have examined fzy distribution during mitosis and found that fzy is ubiquitously distributed throughout the cell, though the region occupied by the chromosomes themselves always stains less intensely. This general distribution of fzy protein that we observe throughout the cell during mitosis suggests that fzy's role during mitosis is not specifically associated with any of the specialized structural components of the mitotic apparatus. That in turn suggests that *fzy* may provide some global, presumably regulatory rather than physical or structural, function that is required throughout the cell for progress through mitosis.

Analysis of *cdc20* mutations in *S. cerevisiae* has shown that *CDC20* function is required for a number of processes, such as progress into anaphase (Byers and Goetsch, 1974; Palmer et al., 1989), accurate chromosome segregation during mitosis (Hartwell and Smith, 1985), and nuclear fusion during mating (Sethi et al., 1991). It has been suggested that *CDC20* may function to regulate microtubule behavior during these events; specifically, since mitotically arrested *cdc20-1* cells accumulate excess spindle, it has been suggested that *CDC20* normally functions to destabilize microtubules (Sethi et al., 1991).

Our data for *fzy* suggest an alternative explanation for the phenotypes we observe. Firstly, we find no evidence that fzy distribution is associated with that of microtubules (Fig. 5). Secondly, cyclin degradation occurs by a ubiquitin-dependent proteolysis pathway (Glotzer et al., 1991; Hershko et al., 1991). Recently, it has been demonstrated that sister chromatid separation, and hence the initiation of anaphase, also depends upon ubiquitin-dependent proteolysis of some other as yet unidentified protein or proteins (Holloway et al., 1993; van der Velden and Lohka, 1993). We have shown previously that *fzy* activity is required for the metaphase–anaphase transition (Dawson et al., 1993) and in this report that *fzy* activity is required for normal cyclin A and B degradation. These data suggest therefore that *fzy* function may be necessary to promote the ubiquitin-dependent proteolytic events that occur during mitosis. Such a function is consistent with the phenotypes we observe in *fzy* mutant embryos, namely failure to degrade cyclins A and B and to initiate anaphase. Furthermore such a function is also consistent with the excess accumulation of spindle microtubules in the nuclei of fzy^{mat} embryos. In this case the excess spindle accumulation would be attributable to both metaphase arrest, caused by a failure to degrade the required protein(s) to initiate anaphase, coupled with failure to degrade cyclins A and B, resulting in continued high MPF kinase activity, which could be responsible for promoting continued spindle formation.

It is harder to explain all the *fzy* phenotypes if one postu-

lates that *fzy* functions by regulating microtubule behavior. Lack of such a function could obviously lead to an excess accumulation of spindle microtubules as we observed. The metaphase–anaphase transition is a checkpoint control point during mitosis, at which the cell is able to monitor the integrity of its spindle before proceeding to inactivate MPF and initiate chromosome separation (reviewed by Murray, 1992). Hence, a defect in spindle function could lead to both metaphase arrest and failure to degrade cyclin B, as we observe with either colchicine or taxol treatment. However, it is harder to reconcile *fzy* having a microtubule regulatory function with the observed failure to degrade cyclin A in *fzy* mutants. While regulation of cyclin B degradation is clearly dependent on the formation of a normal spindle that of cyclin A is apparently not, since cyclin A degradation in *Drosophila* embryos is insensitive to either microtubule disruption by colchicine (Whitfield et al., 1990; Fig. 6 *g*) or microtubule stabilization by taxol (Fig. 7). Thus, the failure to degrade cyclin A in *fzy* embryos therefore is inconsistent with the hypothesis that *fzy* functions to regulate microtubule behavior. In particular, the negligible effect of taxol treatment on cyclin A degradation (Fig. 7) argues strongly against *fzy*, and by extension *CDC20*, functioning to destabilize microtubules during mitosis. On balance therefore, the failure to degrade cyclin A, along with the failure to degrade cyclin B and the failure of sister chromatid separation that occurs in *fzy* embryos, all of which are dependent on cell cycle-regulated ubiquitin-dependent proteolysis, suggests that it is this process that is primarily disrupted in *fzy* embryos. We propose therefore that *fzy* function is required for normal cell cycle-regulated ubiquitin-dependent proteolysis to occur during mitosis.

If this hypothesis is correct, then our data implies that proteolysis of cyclins and/or other proteins during mitosis is required for the progress of the *Drosophila* cell cycle, as does analysis of the *three rows* mutant (Philp et al., 1993) and as is the case in other organisms (Murray et al., 1989; Holloway et al., 1993; Surana et al., 1993). That in turn has implications for understanding the regulations of the earliest preblastoderm nuclear cycles, progression of which requires maternally supplied *fzy* (Dawson et al., 1993) but where fluctuations in cyclin protein levels or MPF kinase activity are not observed (Maldonado-Codina and Glover, 1992; Edgar et al., 1994). Recent detailed analysis of the preblastoderm cycles has however, led to the hypothesis that degradation of only an undetectably small portion of the available cyclin pool, perhaps associated with a specific subcellular compartment, might be what drives the earliest preblastoderm cycles (Edgar et al., 1994). Our data and hypothesis about the function of *fzy* are consistent with this idea.

If this is the case then this raises the question of how *fzy* could function in such a process. We do not at present know how *fzy* functions biochemically. Sequence analysis of *fzy* has shown that more than half of the protein is composed of seven tandemly repeated copies of a degenerate protein motif, the WD-40 repeat (Simon et al., 1991; van der Voorn and Ploegh, 1992). This repeat is found in many proteins with very diverse functions; unfortunately therefore, the presence of WD-40 repeats in fzy does not provide any immediate clues to its likely cellular function. However, it has been suggested that WD-40 repeats are involved in binding to other proteins (Goebl and Yanagida, 1991). It is possible therefore, that fzy could potentially bind to and regulate the activity of one or more other proteins. Whether *fzy* functions in such a manner remains to be determined, and if it does, then understanding the molecular basis of *fzy* function during mitosis will require identifying and analyzing the functions of any such partners.

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References

Alphey, L., J., Jiminez, H. White-Cooper, I. A. Dawson, P. Nurse, and D. M. Glover. 1992. *twine*, a *cdc25* homolog that functions in the male and female germline of *Drosophila*. *Cell.* 69:977–988.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403–410.

Ashburner, M., P. Thomson, J. Roote, P. F. Lasko, Y. Grau, M. El Messal, S. Roth, and P. Simpson. 1990. The genetics of a small autosomal region of *Drosophila melanogaster* containing the structural gene for alcohol dehydrogenase. VII. Characterization of the region around the *snail* and *cactus* loci. *Genetics.* 126:679–694.

Bodmer, R., R. Carretto, and Y. N. Jan. 1989. Neurogenesis of the peripheral nervous system in *Drosophila* embryos: DNA replication patterns and cell lineages. *Neuron.* 3:21–32.

Byers, B., and L. Goetsch. 1974. Duplication of spindle plaques and integration of the yeast cell cycle. *Cold Spring Harbor Symp. Quant. Biol.* 38:123–131.

Campos-Ortega, J. A., and V. Hartenstein. 1985. The Embryonic Development of *Drosophila melanogaster*. Springer-Verlag, Berlin Heidelberg. 1–227.

Chen, C., B.-C. Yang, and T.-T. Kuo. 1992. One-step transformation of yeast in stationary phase. *Current Genetics.* 21:83–84.

Dawson, I. A., S. Roth, M. Akam, and S. Artavanis-Tsakonas. 1993. Mutations of the *fizzy* locus cause metaphase arrest in *Drosophila melanogaster* embryos. *Development (Camb.).* 117:359–376.

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

Dunphy, W. G., L. Brizuela, D. Beach, and J. W. Newport. 1988. The *Xenopus cdc2* protein is a component of MPF, a cytoplasmic regulator of mitosis. *Cell.* 54:423–431.

Edgar, B. A., and G. Schubiger. 1986. Parameters controlling transcriptional activation during early *Drosophila* development. *Cell.* 44:871–877.

Edgar, B. A., and P. H. O'Farrell. 1990. The three postblastoderm cell cycles of *Drosophila* embryogenesis are regulated in G2 by *string*. *Cell.* 62: 469–480.

Edgar, B. A., and P. H. O'Farrell. 1989. Genetic control of cell division patterns in the *Drosophila* embryo. *Cell.* 57:177–187.

Edgar, B. A., F. Sprenger, R. J. Duronio, P. Leopold, and P. H. O'Farrell. 1994. Distinct molecular mechanisms regulate cell cycle timing at successive stages of *Drosophila* embryogenesis. *Genes & Dev.* 8:440–452.

Foe, V. E. 1989. Mitotic domains reveal early commitment of cells in *Drosophila* embryos. *Development (Camb.).* 107:1–25.

Frorath, B., M. Scanarini, H. J. Netter, C. C. Abney, B. Liedvogel, H. J. Lakomek, and W. Northemann. 1991. Cloning and expression of antigenic epitopes of the human 68-kDa (U1) ribonucleoprotein antigen in *Escherichia coli*. *Biotechniques.* 11:364–371.

Gallant, P., and E. A. Nigg. 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.

Gautier, J., C. Norbury, M. Lohka, P. Nurse, and J. Maller. 1988. Purified maturation-promoting factor contains the product of a *Xenopus* homolog of

the fission yeast cell cycle control gene *cdc2*$^+$. *Cell.* 54:433–439.
Geisler, R., A. Bergmann, Y. Hiromi, and C. Nüsslein-Volhard. 1992. *cactus*, a gene involved in dorsoventral pattern formation of *Drosophila*, is related to the IκB gene family of vertebrates. *Cell.* 71:613–621.
Ghiara, J. B., H. E. Richardson, K. Sugimoto, M. Henze, D. J. Lew, C. Wittenberg, and S. I. Reed. 1991. A cyclin B homolog in *S. cerevisiae: Chronic* activation of the cdc28 protein kinase by cyclin prevents exit from mitosis. *Cell.* 65:163–174.
Ghislain, M., A. Udvardy, and C. Mann. 1993. *S. cerevisiae* 26S protease mutants arrest cell division in G2/metaphase. *Nature (Lond.).* 366:358–362.
Glotzer, M., A. W. Murray, and M. W. Kirschner. 1991. Cyclin is degraded by the ubiquitin pathway. *Nature (Lond.).* 349:132–138.
Goebl, M., and M. Yanagida. 1991. The TPR snap helix: a novel protein repeat motif from mitosis to transcription. *Trends Biochem. Sci.* 16:173–177.
Gordon, C., G. McGurk, P. Dillon, C. Rosen, and N. D. Hastie. 1993. Defective mitosis due to a mutation in the gene for a fission yeast 26S protease subunit. *Nature (Lond.).* 366:355–357.
Gould, K. L., and P. Nurse. 1989. Tyrosine phosphorylation of the fission yeast cdc2$^+$ protein kinase regulates entry into mitosis. *Nature (Lond.).* 342: 39–45.
Harlow, E., and D. Lane. 1988. Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 139–282.
Hartwell, L. H., and D. Smith. 1985. Altered fidelity of mitotic chromosome transmission in cell cycle mutants of *S. cerevisiae. Genetics.* 110:381–395.
Hershko, A., D. Ganoth, J. Pehrson, R. E. Palazzo, and L. H. Cohen. 1991. Methylated ubiquitin inhibits cyclin degradation in clam embryo extracts. *J. Biol. Chem.* 266:16376–16379.
Holloway, S. L., M. Glotzer, R. W. King, and A. W. Murray. 1993. Anaphase is initiated by proteolysis rather than by the inactivation of maturation-promoting factor. *Cell.* 73:1393–1402.
Hoyt, M. A., L. Totis, and B. T. Roberts. 1991. *S. cerevisiae* genes required for cell cycle arrestin response to loss of microtubule function. *Cell.* 66: 507–517.
Hunt, T., F. C. Luca, and J. V. Ruderman. 1992. The requirements for protein synthesis and degradation, and the control of destruction of cyclins A and B in the meiotic and mitotic cell cycles of the clam embryo. *J. Cell Biol.* 116:707–724.
Jentsch, S. 1992. Ubiquitin-dependent protein degradation: a cellular perspective. *Trends Cell Biol.* 2:98–103.
Jimenez, J., L. Alphey, P. Nurse, and D. M. Glover. 1990. Complementation of fission yeast *cdc2*^{ts} and *cdc25*^{ts} mutants identifies two cell cycle genes from *Drosophila*: a *cdc2* homolog and *string. EMBO (Eur. Mol. Biol. Organ.) J.* 9:3565–3571.
Johansen, K. M., R. G. Fehon, and S. Artavanis-Tsakonas. 1989. The *Notch* gene product is a glycoprotein expressed on the cell surface of both epidermal and neural precursor cells during *Drosophgila* development *J. Cell Biol.* 109:2427–2440.
Kidd, S. 1992. Characterization of the *Drosophila cactus* locus and analysis of interactions between cactus and dorsal proteins. *Cell.* 71:623–635.
Labbé, J. C., J.-P. Capony, D. Caput, J. C. Cavadore, J.-M. Derancourt, M. Kaghad, M. Lelias, A. Picard, and M. Dorée. 1989. MPF from starfish oocytes at the first meiotic metaphase is a heterodimer containing one molecule of cdc2 and one molecule of cyclin B. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:3053–3058.
Lee, M. G., and P. Nurse. 1987. Complementation used to clone a human homolog of the fission yeast cell cycle control gene *cdc2. Nature (Lond.).* 327:31–35.
Lehner, C. F., and P. H. O'Farrell. 1990. *Drosophila cdc2* homologues: a functional homolog is coexpressed with a cognate variant. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:3573–3581.
Li, R., and A. W. Murray. 1991. Feedback control in mitosis of budding yeast. *Cell.* 66:519–531.
Luca, F. C., and J. V. Ruderman. 1989. Control of programmed cyclin destruction in a cell-free system. *J. Cell Biol.* 109:1895–1909.
Luca, F. C., E. K. Shibuya, C. E. Dohrmann, and J. V. Ruderman. 1991. Both cyclin AΔ60 and BΔ97 are stable and arrest cells in M-phase, but only cyclin BΔ97 turns on cyclin destruction. *EMBO (Eur. Mol. Biol. Organ.) J.* 10: 4311–4320.
Maldonado-Codina, G., and D. M. Glover. 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.
Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Masui, Y., and C. L. Markert. 1971. Cytoplasmic control of nuclear behavior during meiotic maturation of frog oocytes. *J. Exp. Zool.* 177:129–145.
Minshull, J., R. Golstyn, C. S. Hill, and T. Hunt. 1990. The A- and B-type cyclin associated cdc2 kinases in *Xenopus* turn on and off at different times in the cell cycle. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:2865–2875.
Murray, A. W. 1992. Creative blocks: cell-cycle checkpoints and feedback controls. *Nature (Lond.).* 359:599–604.
Murray, A. W., and M. W. Kirschner. 1989. Dominos and clocks: the union of two views of the cell cycle. *Science (Wash. DC).* 246:614–621.
Murray, A. W., and T. Hunt. 1993. The Cell Cycle. W. H. Freeman and Co., NY.
Murray, A. W., M. J. Solomon, and M. W. Kirschner. 1989. The role of cyclin synthesis and degradation in the control of maturation promoting factor activity. *Nature (Lond.).* 339:503–508.
Nigg, E. A. 1991. The substrates of the cdc2 kinase. *Semin. Cell Biol.* 2: 261–270.
Nigg, E. A. 1993. Targets of cyclin-dependent protein kinases. *Curr. Opin. Cell Biol.* 5:187–193.
Norbury, C., and P. Nurse. 1992. Animal cell cycles and their control. *Annu. Rev. Biochem.* 61:441–470.
Nurse, P. 1990. Universal control mechanism regulating onset of M-phase. *Nature (Lond.).* 344:503–508.
Palmer, R. E., M. Koval, and D. Koshland. 1989. The dynamics of chromosome movement in the budding yeast *Saccharomyces cerevisiae. J. Cell Biol.* 109:3355–3366.
Philp, A. V., J. M. Axton, R. D. C. Saunders, and D. M. Glover. 1993. Mutations in the *Drosophila melanogaster* gene *three rows* permit aspects of mitosis to continue in the absence of chromatid segregation. *J. Cell Sci.* 106:87–98.
Prokop, A., and G. M. Technau. 1991. The origin of postembryonic neuroblasts in the ventral nerve cord of *Drosophila melanogaster. Development.* 111:79–88.
Robertson, H. M., C. R. Preston, R. W. Phillis, D. Johnson-Schlitz, W. K. Benz, and W. R. Engels. 1988. A stable genomic source of P element transposase in *Drosophila. Genetics.* 118:461–470.
Rothberg, J. M., D. A. Hartley, Z. Walther, and S. Artavanis-Tsakonas. 1988. *slit*:an EGF-homologous locus of *D. melanogaster* involved in the development of the embryonic central nervous system. *Cell.* 55:1047–1059.
Sethi, N., M. C. Monteagudo, D. Koshland, E. Hogan, and D.J. Burke. 1991. The *CDC20* gene product of *Saccharomyces cerevisiae*, a β-transducin homolog, is required for a subset of microtubule-dependent processes. *Mol. Cell. Biol.* 11:5592–5606.
Simon, M. I., M. P. Strathmann, and N. Gautam. 1991. Diversity of G proteins in signal transduction. *Science (Wash. DC).* 252:802–808.
Smith, D. B., and K. S. Johnson. 1988. Single step purification of polypeptides expressed in *Escherichia coli* as a fusion with glutathione S-transferase. *Gene (Amst.).* 67:31–40.
Smith, A. V., and T. L. Orr-Weaver. 1991.The regulation of the cell cycle during *Drosophila* embryogenesis: the transition to polyteny. *Development (Camb.).* 112:997–1008.
Spradling, A., and G. Rubin. 1982. Transposition of cloned P elements into germ line chromosomes. *Science (Wash. DC).* 218:341–347.
Surana, U., A. Amon, C. Dowzer, J. McGrew, B. Byers, and K. Nasmyth. 1993. Destruction of the CDC28/CLB mitotic kinase is not required for the metaphase to anaphase transition in budding yeast. *EMBO (Eur. Mol. Biol. Organ.) J.* 12:1969–1978.
Thummel, C. S., and V. Pirrotta. 1992. New pCaSpeR P element vectors. *Drosoph. Inform. Serv.* 71:150.
Tower, J., G. Karpen, N. Craig, and A. C. Spradling. 1993. Preferential transposition of *Drosophila* P elements to nearby chromosomal sites. *Genetics.* 133:347–359.
van der Velden, H. M. W., and M. J. Lohka. 1993. Mitotic arrest caused by the amino terminus of *Xenopus* cyclin B. *Mol. Cell Biol.* 13:1480–1488.
van der Voon, L., and H. L. Ploegh. 1992. The WD-40 repeat. *FEBS (Fed. Eur. Biochem. Soc.).* 307:131–134.
Vernet, T., D. Dignard, and D. Y. Thomas. 1987. A family of yeast expression vectors containing the phage f1 intergenic region. *Gene (Amst.).* 52:225–233.
Weinstein, J., F. W. Jacobsen, J. Hsu-Chen, T. Wu, and L. G. Baum. 1994. A novel mammalian protein, p55CDC, present in dividing cells is associated with protein kinase activity and has homology to the *Saccharomyces cerevisiae* cell division cycle proteins Cdc20 and Cdc4. *Mol. Cell. Biol.* 14:3350–3363.
Whitfield, W. G. F., C. Gonzalez, G. Maldonado-Codina, and D. M. Glover. 1990. The A- and B-type cyclins of *Drosophila* are accumulated and destroyed in temporally distinct events that define separate phases of the G2-M transition. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:2563–2572.
Yanish-Perron, C., J. Vieira, and J. Messing. 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequence of the M13mp18 and pUC19 vectors. *Gene (Amst.).* 33:103–119.