blunt ended) was inserted into the Sal I–Sma I restriction sites of pSLFlagBCD-Hd. For BCD-A, an oligonucleotide encoding BCD amino acids 321 to 351 was synthesized flanked by Sal I and Eco RI restriction sites and inserted into the corresponding restriction sites of pSLFlagBCD-Hd (*13*). For generating baculovirus expression plasmids encoding HB-A or HB-Q, fusion proteins composed of the HB zinc finger region (amino acids 185 to 407) and the BCD alanine-rich activation domain (A) or the BCD glutamine-rich activation domain (Q) oligonucleotides flanked by Eco RI restriction sites, encoding the A-domain or the Q-domain, were inserted into the corresponding restriction site of pSLFlagHB-finger (*13*). Proteins were expressed and purified as described (*8*).

11. The reporter plasmids phbCAT-91 and phbCAT-298 have been described (*5*). phbCAT-231X was generated as follows: A 269-bp Hin PI–Bam HI *hb* enhancer-promoter fragment (−154 to +115) derived from the plasmid phbCAT-231 (*5*) was fused to a synthetic oligonucleotide, encompassing the *hb* enhancer from position (−231 to −154), in which two low-affinity BCD DNA binding sites were eliminated by base-pair exchanges at position −220 and −210. The generated enhancer (−231 to +115) substituted the enhancer of phbCAT-231 to generate phbCAT-231X.
12. In vitro transcription with a reconstituted *Drosophila* transcription system was done as described (*8*). Briefly, 25 ng of template were preincubated with activators for 10 min at 4°C and then basal factors were added and the mixture was incubated for 15 min at 20°C. Transcription was started by adding nucleoside triphosphates to a final concentration of 1 mM. Products were detected by primer extension.
13. F. Sauer and R. Tjian, unpublished results.
14. Protein-protein interaction assays were done as described (*6–8*). Briefly, Flag epitope–tagged BCD was immobilized on Flag M2 antibody resin, then incubated with ^{35}S-methionine in vitro expressed TAFs or basal factors. Protein complexes were analyzed by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and visualized by autoradiography.
15. R. O. J. Weinzierl, S. Ruppert, B. D. Dynlacht, N. Tanese, R. Tjian, *EMBO J.* **12**, 5303 (1993).
16. Plasmids encoding GST-BCD fusion proteins were generated by fusing different parts of the BCD cDNA derived from pARBCD [T. Hoey and M. Levine, *Nature* **332**, 858 (1988)] to the GST coding region of the vector pGEX-2TKN (S. Ruppert and R. Tjian, unpublished results). The BCD cDNA fragments were cloned into restriction sites of pGEX-2TKN as follows: (i) GST-BCD (1 to 250), a 750-bp Nde I–Sal I fragment in Nde I–Sal I; (ii) GST-BCD (251 to 495), a blunt-ended 1-kb Sal I–Eco RV fragment into the Sal I–Sma I restriction sites to create pGEX-BCDSE; (iii) GST-BCD (251 to 351), a 300-bp Nde I–Acc I (Acc I blunt ended) fragment from pGEX-BCDSE into the Nde I–Sma I restriction sites; (iv) GST-BCD (251 to 325), a 270-bp Nde I–Rsa I fragment from pGEX-BCDSE into the Nde I–Sma I restriction sites; (v) GST-BCD (251 to 305), a 170-bp Nde I–Hin PI (Hin PI blunt ended) fragment from pGEX-BCDSE into the Nde I–Sma I restriction sites; and (vi) GST-BCD (351 to 495), a 430-bp Acc I–Eco RV fragment into the Bam HI (blunt ended)–Sma I restriction sites.
17. Protein-protein interaction assays were done essentially as described (*6, 7*) with the following modifications: The *Escherichia coli* DH 5α strain was used and protein expression was induced in cultures of these bacteria at an absorbance at 600 nm of 0.2 with 10 μM isopropyl-β-D-thiogalactopyranoside for 2 hours.
18. In vitro transcriptions with recombinant partial TBP-TAF complexes were done as described (*8, 12*) except that the partial TBP-TAF complexes were preincubated with the activator and template at 4°C for 5 min before the remaining basal factors were added.
19. In vitro transcriptions were done as described (*8, 9, 12*) except that 25 ng of the reporter plasmid pBlue*hb*-HBCAT, containing a single HB and BCD DNA binding site, were used (*8*).
20. DNase I footprinting was done as described (*8*) [T. Hoey, B. D. Dynlacht, M. G. Peterson, B. F. Pugh, R. Tjian, *Cell* **61**, 1179 (1990)]. As template we used the 346-bp *hb* enhancer-promoter fragment (−231 to +115) of phbCAT-231X (*11*). Template and activator (or activators) were preincubated at 20°C for 10 min, the partial complexes were added, and the mixture was incubated for an additional 10 min at 20°C. Digestion was initiated by adding 10 mM $MgCl_2$-$CaCl_2$ solution (50 μl) containing DNase I (5 μg/ml; Boehringer) and proceeded for 20 s.
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22. We thank W. Driever for the phbCAT reporter plasmids; C. Nüsslein-Volhard and T. Hoey for various BCD and HB expression plasmids; K. Goodrich for oligonucleotide synthesis.; J.-L. Chen for providing baculovirus expression plasmids; C. Thut for the bacterial expression plasmid pET19bTAF$_{II}$60; all members of the Tjian lab for helpful discussions, especially H. Beckmann, J. A. Goodrich, and E. Wang; and D. Rio, M. Rothe, T. Hoey, H. Beckmann, C. Thut, J. A. Goodrich, S. Albright, and E. Wang for comments on the manuscript. Supported by a European Molecular Biology Organization long-term fellowship (F.S.), a fellowship from the University of Kopenhagen, Denmark (S.K.H.), and in part by an NIH grant (R.T.).

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Control of Cell Fate by a Deubiquitinating Enzyme Encoded by the *fat facets* Gene

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Ubiquitin is a highly conserved polypeptide found in all eukaryotes. The major function of ubiquitin is to target proteins for complete or partial degradation by a multisubunit protein complex called the proteasome. Here, the *Drosophila fat facets* gene, which is required for the appropriate determination of particular cells in the fly eye, was shown to encode a ubiquitin-specific protease (Ubp), an enzyme that cleaves ubiquitin from ubiquitin-protein conjugates. The Fat facets protein (FAF) acts as a regulatory Ubp that prevents degradation of its substrate by the proteasome. Flies bearing *fat facets* gene mutations were used to show that a Ubp is cell type– and substrate-specific and a regulator of cell fate decisions in a multicellular organism.

Ubiquitin-mediated protein degradation is an important mechanism for regulating diverse cellular functions in all eukaryotes (*1, 2*). Proteins conjugated to the 76–amino acid ubiquitin (Ub) polypeptide are recognized by the proteasome, a protein degradation complex. Many different enzymes are required to attach Ub to proteins. Ubiquitin-specific proteases (Ubps), in contrast, deubiquitinate proteins, and their role is not as well understood. The Ubps constitute a large protein family (*2–4*) that has been studied mainly in yeast, where they perform a variety of general functions in the Ub-mediated degradation pathway. Some Ubps generate monomeric ubiquitin, either by cleaving polymeric Ub or Ub-protein precursors (*3–5*) or by recycling Ub from partially degraded proteins, a process apparently required to clear the proteasome (*6*). Partly because of the large number of Ubps in yeast, it has been thought that Ubps could also perform specific regulatory functions by deubiquitinating proteins before they reach the proteasome (*2, 4, 7*).

The isolation of several yeast Ubps on the basis of functional assays has revealed that these enzymes are similar to each other primarily in two small regions, the so-called Cys and His domains, centered on a single cysteine (Cys) and two histidine (His) residues thought to be the active site of the protease (*4, 6*) (Fig. 1A). Similar Cys and His domains identify the *Drosophila* Fat facets (FAF) protein as a potential Ubp (*6*) (Fig. 1A). The FAF protein (2691 amino acids long) is required in a cell communication pathway that negatively regulates neural cell determination in the developing compound eye (*8*). The *fat facets* (*faf*) gene is specifically required for eye development, as *faf* null mutants are viable and have abnormal eye morphology (*8*). The most noticeable defect in *faf* mutant eyes is the inclusion of more than the normal complement of eight photoreceptors in each unit eye, or facet (*8*) (Fig. 2, A to H). The appearance of these ectopic photoreceptors is caused by the misdetermination of particular cells that would not normally become neurons (*8*). The only other aberration in *faf* mutant flies is that mutant females lay eggs that never reach cellularization, an early stage of embryogenesis (*8*). Thus, FAF is required in only two tissues of the fly for normal development, the ovary and the eye; it is necessary during eye development for the appropriate cell fate decisions of particular cells. We conducted a series of experiments to determine whether FAF indeed functions as a Ubp.

We first tested whether FAF had Ubp activity in a bacterial assay (*4, 5*) (Table 1).

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The assay tested the ability of FAF to cleave the Ub moiety from the substrate protein Ub-Arg–β-Gal, a fusion of Ub and the *Escherichia coli* protein β-galactosidase (β-Gal) separated by an arginine (Arg) residue. *Escherichia coli* expressing Ub-Arg–β-Gal formed blue colonies in the presence of the chromogenic substrate X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside). A deubiquitinating activity introduced into *E. coli* expressing Ub-Arg–β-Gal would cleave the substrate to Arg–β-Gal, which is an unstable protein (9), and thus white colonies would be produced. *Escherichia coli* expressing both Ub-Arg–β-Gal and a 744–amino acid partial FAF protein that included the Cys and His domains formed white colonies in the presence of X-Gal, indicating that the Ub-Arg bond was cleaved. A single amino acid mutation in FAF that changed the putative active site Cys residue to Ser (see below) destroyed the ability of the FAF protein to cleave Ub-Arg–β-Gal in this assay (Table 1).

To test whether FAF also functions as a Ubp in *Drosophila*, site-directed mutagenesis was used to generate four mutant *faf* genes that encoded proteins with either an altered key Cys residue (Cys^{1677}→Ser) or with changes in one or both of the two conserved His residues (His^{1959}→Arg, His^{1967}→Arg) (Fig. 1A). Mutation of the analogous Cys or His residues in yeast abolishes their Ubps activity (3, 6). Each of the mutant genes ($faf^{Ser1677}$, $faf^{Arg1959}$, $faf^{Arg1967}$, and $faf^{Arg1959,1967}$) was generated in the context of a genomic DNA fragment (Fig. 1A), one copy of which when introduced into the fly genome by *P* element transformation was previously shown to complement the eye defects in a *faf* null mutation, faf^{BX4} (8). Several transformant lines were generated with each mutant *faf* gene, and the ability of each to complement faf^{BX4} in the eye was tested. Although some transformant lines partially complemented faf^{BX4}, we found that the function of each of the four mutant *faf* genes was severely impaired (Figs. 1B and 2, I to L).

The importance of the conserved Cys residue to the function of FAF protein was also tested in an experiment in which wild-type or mutant *faf* complementary DNAs (cDNAs) were expressed from the heat-inducible heat shock protein 70 gene (*hsp70*) promoter (10) in transformant flies (*Hs-faf* or *Hs-*$faf^{Ser1677}$) (Fig. 3). The *hsp70* promoter is active in all cells in the fly in response to heat shock. After heat shock, transformant embryos contained similar amounts of FAF or $FAF^{Ser1677}$ proteins (Fig. 3). When *Hs-faf* or *Hs-*$faf^{Ser1677}$ flies containing the faf^{BX4} mutation were heat-shocked during eye development, the eyes of *Hs-faf* flies were almost completely like wild-type eyes (11). In contrast, the eyes of *Hs-*$faf^{Ser1677}$ flies were indistinguishable from those of faf^{BX4} flies (11).

Because altering the Cys or His residues hindered FAF activity, we conclude that FAF functions as a Ubp in *Drosophila*. The specificity of the *faf* mutant eye phenotype suggests that FAF is unlikely to perform general functions such as generating Ub monomers or clearing the proteasome of degradation products. It is more likely that FAF antagonizes the proteasome by deubiquitinating a specific regulatory protein targeted for degradation before it reaches the proteasome. To test this hypothesis, we investigated the genetic interactions between *faf* and *l(3)73Ai*, the only *Drosophila* gene encoding a proteasome component for which mutant flies exist (12). Loss-of-function mutations of *l(3)73Ai* are recessive lethal; homozygotes (*l(3)73Ai/l(3)73Ai*) die as young larvae, and heterozygotes (*l(3)73Ai/+*) are wild-type flies (12). The faf^{FO8} allele is a strong mutant allele (8), and the eye defects of faf^{FO8} homozygotes are nearly as severe as those of faf^{BX4} homozygotes. However, in the context of one copy of any of three different mutant alleles of *l(3)73Ai*, the eyes of faf^{FO8} homozygotes (*l(3)73Ai* faf^{FO8}/+ faf^{FO8}) are much more like wild-type eyes (Fig. 2, M and N). That is, reducing the amount of one proteasome component by one-half resulted in strong suppression of the *faf* mutant phenotype, which indicates that FAF activity antagonizes proteasome function.

In yeast, Ubp4 (Doa4) is associated

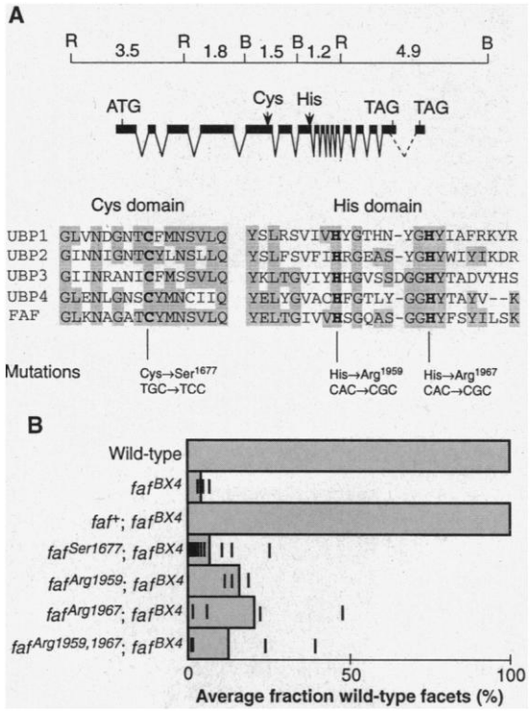

Fig. 1. Effect on *faf* gene function of mutations in putative Ubp active site residues. (**A**) An ~13-kb genomic DNA fragment that contains the *faf* gene, which completely rescues the *faf* mutant phenotype, is shown at the top (8). R and B indicate Eco RI and Bam HI sites, respectively, and the numbers are the approximate sizes of the fragments in kilobases. In the second row, the structure of the *faf* gene within the genomic DNA is shown. Black bars indicate exons, and ATG and TAG are start and stop codons, respectively. There are two forms of the *faf* messenger RNA that encode proteins with slightly different carboxyl termini (8). Cys and His indicate the locations of the Cys and His domains. The bottom rows show the protein sequences of Cys and His domains of yeast Ubp 1 to 4 (4–6, 20). Shaded amino acids indicate exact matches with FAF. There is another class of Ubps that does not share this similarity (21); there is also a variable number of amino acids between the Cys and His domains of different Ubps (3–8). The single-nucleotide changes in the four mutant *faf* genes indicated in (B), which were generated in the context of the ~13-kb fragment of genomic DNA (22), are shown at the bottom. (**B**) The ability of the four mutant *faf* genes to rescue the *faf* mutant eye phenotype is expressed as the average fraction of wild-type facets in transformant lines containing each gene in a faf^{BX4} background. Controls are wild-type (w^{1118}), faf^{BX4}, and faf^{+} transformants containing the wild-type *faf* gene (8). The mutant *faf* genes ($faf^{Ser1677}$, $faf^{Arg1959}$, $faf^{Arg1967}$, and $faf^{Arg1959,1967}$) are described in the text and in (A). Gray bars show the average fraction of wild-type facets in the genotypes indicated at the left, with values for different eyes (faf^{BX4}) or average values for each transformant line indicated by the vertical lines inside and to the right of each bar (23).

Table 1. Bacterial assay for deubiquitinating activity of FAF. The assay is described in the text and in detail elsewhere (4). pQE30 is an expression vector and served as a negative control. pRB173 expresses the yeast Ubp2 protein (4) and served as a positive control. pQE-FAF produces a wild-type partial FAF protein, and pQE-$FAF^{Ser1677}$ produces a mutant partial FAF protein (29). In the presence of Ub-Arg–β-Gal, white colonies indicative of Ubp activity were observed only when Ubp2 (pRB173) or wild-type FAF (pQE-FAF) was also present.

Plasmid	Colony color
pUb-Arg–β-Gal	Blue
pQE30	White
pRB173	White
pQE-FAF	White
pQE-$FAF^{Ser1677}$	White
pUb-Arg–β-Gal + pQE30	Blue
pUb-Arg–β-Gal + pRB173	White
pUb-Arg–β-Gal + pQE-FAF	White
pUb-Arg–β-Gal + pQE-$FAF^{Ser1677}$	Blue

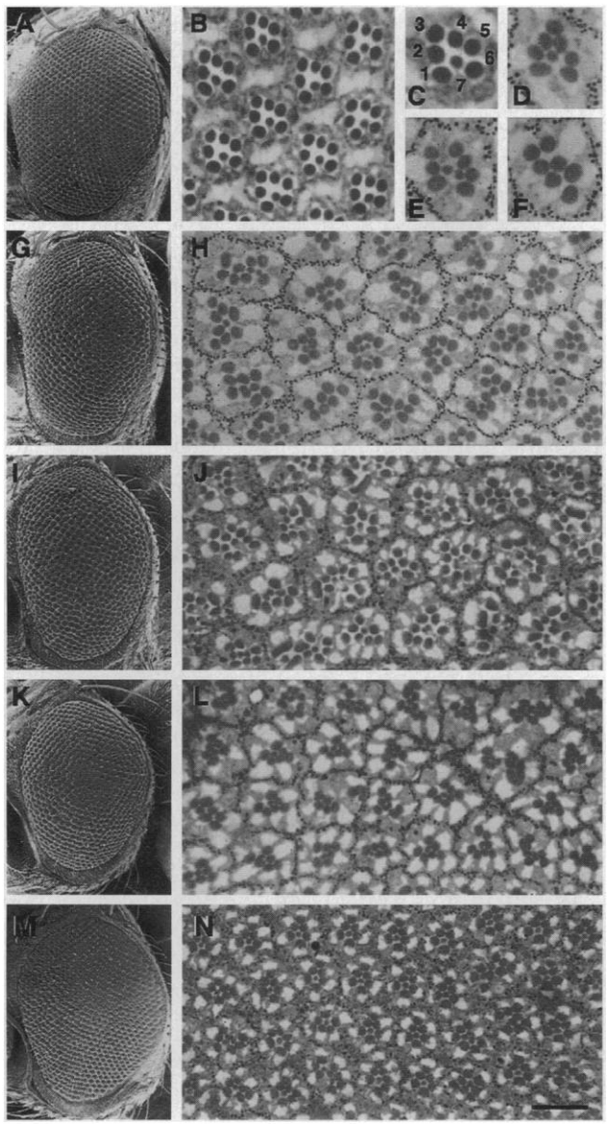

Fig. 2. Eye phenotypes of flies with mutant *faf* genes and suppression by *l(3)73Ai*. Scanning electron micrographs (A, G, I, K, and M) and apical tangential sections (B to F, H, J, L, and N) of eyes are shown (*24*). (**A** and **B**) Wild-type eye. Compound eyes are composed of about 800 identical facets, each of which has eight photoreceptors (R cells) surrounded by a hexagonal lattice of accessory cells (*25*). Each R cell is identified on the basis of its position within a trapezoid. R1 to R6 are "outer" R cells, and R7 and R8 are "inner" R cells, with R8 lying beneath R7. (**C**) R1 to R7 are visible in apical sections. (**D** to **H**) faf^{BX4} eyes. The outer surface of the eye (G) is less regular than that of the wild type because of underlying retinal defects (H). Enlargements of individual faf^{BX4} facets (D to F) show the three defects observed: extra outer photoreceptors (one extra in this case) (D), extra R7-like R cells (one extra R7 and also an extra outer R cell) (E), and R7 missing (also an extra outer cell) (F). (**I** and **J**) $faf^{Arg1959,1967}$; faf^{BX4} eyes. The eyes resemble faf^{BX4} as the particular P element containing the mutant *faf* gene appears not to complement the mutation even partially (see Fig. 1B). (**K** and **L**) $faf^{Arg1967}$; faf^{BX4} eyes. This particular line rescues the faf^{BX4} eye phenotype most effectively (see Fig. 1B). (**M** and **N**) $l(3)73Ai^{1}$ faf^{FO8}/+ faf^{FO8} eyes. $l(3)73Ai^{1}$/+ suppresses faf^{FO8}/faf^{FO8} 10-fold (*26*). The scale bar in (N) pertains to all panels and represents 20 μm in (B), (H), (J), (L), and (N), 12 μm in (C) to (F), and 160 μm in (A), (G), (I), (K), and (M).

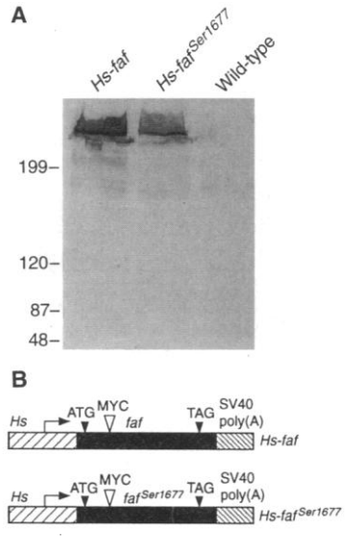

Fig. 3. Expression of FAF and $FAF^{Ser1677}$ from the *hsp70* promoter in *Drosophila*. (**A**) Immunoblot of proteins produced after heat shock in *Drosophila* embryo transformants containing each of the constructs indicated. FAF protein was detected with an antibody to the 11–amino acid Myc epitope (*27*). Proteins of the expected size (~300 kD) were detected in the embryos transformed with the *Hs-faf* and $Hs\text{-}faf^{Ser1677}$ genes but not in the wild-type (nontransformed) flies. Thus, both FAF and $FAF^{Ser1677}$ proteins are stable in *Drosophila* embryos. (**B**) Two hybrid genes from which wild-type or mutant FAF proteins were produced in response to heat shock (*28*). The striped bar at left indicates DNA sequences of the *hsp70* promoter, including the transcription start site (arrow) and 5′ untranslated sequences. The black bar indicates wild-type *faf* or mutant $faf^{Ser1677}$ cDNA sequences, including some 5′ and 3′ untranslated sequences. ATG and TAG are the start and stop of translation, respectively. MYC indicates the insertion of a Myc epitope tag (*27*). The striped region at the right indicates polyadenylation [poly(A)] sequences from SV40.

with the proteasome (*2*) and is thought to clear the proteasome of multi-Ub chains conjugated to peptides after protein degradation (*6*). Dominant-negative mutations are produced by overexpression of a Ubp4 protein with altered Cys residues presumably as a result of poisoning of the proteasome by a nonfunctional Ubp (*6*). We did not observe a negative effect in transformant flies bearing multiple copies of $faf^{Ser1677}$ or $Hs\text{-}faf^{Ser1677}$ genes in a wild-type background or even in a faf^{FO8}/faf^{BX3} background, which is sensitive to a slight decrease in FAF activity (*13*). These results suggest that unlike Ubp4, FAF does not associate with the proteasome, which is consistent with the role for FAF that we propose.

The *faf* mutation provided the opportunity to observe the consequences of the loss of one particular Ubp in a multicellular organism. The *faf* mutant phenotype reveals that a Ubp has cell-type and substrate specificity. The Ub-mediated protein degradation pathway regulates diverse cellular functions, including the cell cycle (*14*), transcription (*15*), major histocompatibility complex antigen processing (*16*), and axon guidance (*17*). Here, we have demonstrated a role for the Ub pathway in controlling cell fate.

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amounts of certain other gene products are reduced by 50% (J. A. Fischer-Vize, unpublished data). One *Hs-faf*^{Ser1677} line was tested for dominant-negative activity in a *faf*^{FO8}/*faf*^{BX3} background by heat shocking transformant larvae as described (*11*), but no increase in eye roughness was ever observed.

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22. Site-directed mutagenesis was performed with oligonucleotides and single-stranded DNA templates (subclones of an ~0.7-kb Hpa I–Kpn I genomic DNA fragment containing the Cys domain and an ~2.0-kb Kpn I–Sph I fragment containing the His domain) by means of standard techniques (*18*). To ensure that no other mutations were introduced, we determined the entire DNA sequence of each mutagenized fragment before reconstituting the ~13-kb *faf* genomic DNA fragment. Oligonucleotides (Integrated DNA Technologies Inc., Coralville, IA) used for the mutagenesis (mutagenic residues are in bold) were 5′-CAGGTGCCACTT**C**CTACATGAATTC-3′ (Cys^{1677}→Ser), 5′-GCCTGTCCGCTGC**G**CACCACAATGC-3′ (His^{1959}→Arg), and 5′-TAACTGAAGTAG**C**GACCGCCGGAAG-3′ (His^{1967}→Arg). The mutant *faf* genes were cloned as Not I fragments into the P element transformation vector Casper3 (*19*), and P element transformants were generated in the strain *w*^{1118} as described [A. C. Spradling, in Drosophila: *A Practical Approach*, D. B. Roberts, Ed. (IRL Press, Oxford, 1986), pp. 175–197].
23. The average fraction of wild-type facets for each transformant line was calculated by counting 300 to 1300 facets in two to six eyes for each line. The variation between eyes within a given genotype was significant only for the *faf*^{Arg1959} and *faf*^{Arg1967} lines. All of the transformant lines contained two copies of the P element except for two *faf*^{Ser1677} lines and one *faf*^{Arg1967} line, which contained one copy each (these gave nonexceptional results). In all but one case, all three *faf* mutant facet phenotypes (Fig. 2, D to F) were present in the lines containing the mutant *faf* gene, indicating that the sole function of FAF in the eye is as a Ubp. Some transformant lines partially rescue the *faf*^{BX4} eye phenotype, probably because Ser and Arg can substitute functionally (although poorly) for Cys and His, respectively. Thus, rescue likely occurs in the lines that express the most mutant FAF protein. In all cases, female transformants remained sterile in a *faf*^{BX4} background.
24. Sectioning and photography were performed as described [A. Tomlinson and D. F. Ready, *Dev. Biol.* **123**, 264 (1987)]. For scanning electron micrographs (SEMs), flies were dehydrated in ethanol, critical-point-dried in carbon dioxide, sputter-coated, and photographed with a Philips 515 scanning electron microscope.
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26. Suppression was quantitated by calculating the fraction of wild-type facets in the eyes of *faf*^{FO8}/*faf*^{FO8} and *l(3)73Ai*^{1} *faf*^{FO8}/ + *faf*^{FO8} flies: 495 facets in sections of two *faf*^{FO8}/*faf*^{FO8} eyes and 981 facets in four *l(3)73Ai*^{1} *faf*^{FO8}/ + *faf*^{FO8} eyes were counted, and 6.5% versus 65% wild-type facets were found, respectively. Similar results were obtained with two other alleles of *l(3)73Ai* [*l(3)73Ai*^{1rv10e} and *l(3)73Ai*^{1rv22e}; unpublished alleles obtained from J. Belote].
27. Embryos were collected on plates for 20 hours; the plates were put in a 37°C incubator for 1.5 hours and then at room temperature for 4 to 5 hours. Embryo extracts were then prepared as described [S. Misra and D. Rio, *Cell* **62**, 269 (1990)], quantitated with the Bio-Rad protein assay, and 200 μg of protein per lane was run on a 5% SDS acrylamide gel. Electrophoresis and transfer to nitrocellulose were according to standard procedures (*18*). The blot was developed with a monoclonal antibody to the Myc epitope (cell supernatant from 9E10.2 cells; American Type Culture Collection, Rockville, MD) and alkaline phosphatase–conjugated secondary antibody to mouse immunoglobulin G (Vector Laboratories, Burlingame, CA) with an alkaline phosphatase substrate kit (Vector Laboratories).
28. The wild-type *faf* cDNA was assembled by joining cDNAs 3-2 and 7-3 (*8*) and thus encoded the form of FAF protein that uses the most 5′ TAG (Fig. 1A). Before assembly, annealed oligonucleotides (Integrated DNA Technologies) containing the Myc epitope [P. A. Kolodziej and R. A. Young, *Methods Enzymol.* **194**, 508 (1994)] within Xho I ends (5′-TCGAGGATCCCCCCCGAGCAGAAGCTGATCTCCGAGGAGGACCTGAAC-3′ and 5′-TCGAGTTCAGGTCCTCCTCGGAGATCAGCTTCTGCTCGGGGGGGATCC-3′) were inserted into an Xho I site in the *faf* cDNA, which resulted in the addition of 16 amino acids (RTPPEQKLISEEDLNS) (*20*) between Ser^{53} and Ser^{54} in FAF (*8*). The assembled wild-type *faf* cDNA was cloned as an Asc I fragment into pBShsp- (N2-A)pA, a modified form of pBShsp that contains heat shock promoter sequences (*10*) and the simian virus 40 (SV40) polyadenylation site from pC4βgal [C. S. Thummel, A. M. Boulet, H. D. Lipshitz, *Gene* **74**, 543 (1988)] separated by an Asc I site. The entire hybrid gene was then cloned into the P-element transformation vector Casper3 (*19*) as a Not I fragment. The *Hs-fafSer*^{1677} gene was constructed the same way except that a 0.7-kb Hpa I–Kpn I fragment of genomic DNA containing the Ser^{1677} mutation was substituted for the wild-type cDNA fragment.
29. A 2.2-kb Bam HI–Sph I fragment of wild-type or mutant *faf* cDNA (see Fig. 3) was subcloned into pQE30 (Qiagen, Inc., Chatsworth, CA) to produce the pQE-FAF or pQE-FAF^{Ser1677}, respectively. Isopropyl-β-D-thiogalactopyranoside induction produced an abundant protein of the expected size (84 kD) from each plasmid.
30. We thank J. Belote for the *l(3)73Ai* alleles, J. Mendenhall and the University of Texas, Austin, Cell Research Institute for the SEMs, S. Olivelle and the University of Texas, Austin, Hybridoma Center for preparing the anti-Myc supernatant, J. Loera for fly culture media, G. Gage and J. Young for preparation of Figs. 2 and 3, M. Simon for pBShsp, and M. Hochstrasser and everyone in our laboratories for stimulating discussions. Supported by grants from the NIH and the Molecular Biology Institute at the University of Texas, Austin (J.A.F.-V.), by a grant from the Australian Capital Territory Cancer Society (R.T.B.), and by an Australian Research Council QEII Fellowship (R.T.B.).

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Precise Spatial Positioning of Chromosomes During Prometaphase: Evidence for Chromosomal Order

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The relative locations of several chromosomes within wheel-shaped prometaphase chromosome rosettes of human fibroblasts and HeLa cells were determined with fluorescence hybridization. Homologs were consistently positioned on opposite sides of the rosette, which suggests that chromosomes are separated into two haploid sets, each derived from one parent. The relative locations of chromosomes on the rosette were mapped by dual hybridizations. The data suggest that the chromosome orders within the two haploid sets are antiparallel. This chromosome arrangement in human cells appears to be both independent of cell type– and species-specific and may influence chromosome topology throughout the cell cycle.

Studies with fluorescence in situ hybridization (FISH) have increased our understanding of the structural and functional organization of the cell nucleus (*1*, *2*). At interphase, individual chromosomes occupy compact, discrete territories. The specific structure and location of these chromosome territories may be specific for both cell type and stage of the cell cycle (*3*). For example, in cells of the human and mouse central nervous system, specific chromosomal domains are organized in a reproducible manner (*4*). In human fibroblasts, chromosome 8 centromeric regions are distributed nonrandomly, and the distribution changes during the cell cycle, such that the chromosomes are situated peripherally with arms extending toward the nuclear interior at G_1 but are more centrally located with arms

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