illumination. The *Df(3L)cat* stock was obtained from the Bloomington Stock Center.

Northern blot analysis. Total RNA was isolated from *oreR*, *Ftz-F1*^{209} and *Ftz-F1*^{282} homozygous females, resolved on a 1.5% formaldehyde–agarose gel, and blotted onto nitrocellulose. Hybridization was performed at 55 °C in 50% formamide, 0.25 M $NaPO_4$, pH 7.2, 0.24 M NaCl, 0.1 mM ETDA, 7% SDS. 5′ Antisense riboprobe was made using a 0.9-kb *Sac*I fragment specific for the α cDNA, and subcloned under control of the T3 promoter. Transcription reactions were carried out using a Boehringer Mannheim transcription kit.

Histochemical analysis. Antibody stainings were performed using mouse monoclonal anti-En (ref. 26), mouse monoclonal anti-Wg (provided by S. Cohen), and rabbit anti-Ftz (ref. 27), as previously described[28]. *In situ* hybridization to *ftz* mRNA was as previously described[29].

Affinity chromatography and far western analysis. Ftz affinity chromatography using His-tagged Ftz protein and nickel resins was performed as previously described[15]. Far western analysis was performed as follows. Full-length and homeodomain-deleted (Δ273–303) Ftz polypeptides were expressed in bacteria[15]. *TcFtz* constructs were also expressed in bacteria. Proteins expressed *in vitro* were made using a Promega TNT *in vitro* transcription/translation kit. The N-terminal Ftz polypeptide was truncated at the unique *Sal*I site, and the TcFtz N-terminal polypeptide at the unique *Sac*I site. Blotting of SDS–PAGE gels was as described[30]. To make the αFtz-F1 probe, a full-length *αFtz-F1* cDNA[5], under control of the phage T7 promoter, was expressed *in vitro* as described above, except with ^{35}S-methionine added to label the protein. Blots were incubated with 50 μl labelled αFtz-F1 in 5 ml cocktail (0.1 M NaCl, 20 mM Tris, pH 7.6, 1 mM EDTA, 1 mM DTT, 10% glycerol, and 1% milk powder) for 2 h at 4 °C. The filter was washed in the above buffer for 1.5 h at 4 °C, dried and autoradiographed.

Ectopic-expression studies. P-element vectors expressing *ftz* deletion derivatives under *hsp70* promoter control were generated in the vector pNMT4 (ref. 14). Lines expressing FtzΔ101–150 have been previously described[15]. Embryo collections, heat shocking, *in situ* hybridization and cuticle preparation protocols have all been previously described[14].

Received 24 September; accepted 28 November 1996.

1. Laughon, A. & Scott, M. P. *Nature* **310,** 25–30 (1984).
2. Wakimoto, B. T. & Kaufman, T. C. *Dev. Biol.* **81,** 51–64 (1981).
3. Lavorgna, G., Ueda, H., Clos, J. & Wu, C. *Science* 848–851 (1991).
4. Lavorgna, G., Karim, F., Thummel, C. & Wu, C. *Proc. Natl Acad. Sci. USA* **90,** 3004–3008 (1993).
5. Ohno, C. K. & Petkovich, M. *Mech. Dev.* **40,** 13–24 (1992).
6. Woodard, C., Baehrecke, E. & Thummel, C. *Cell* **79,** 607–615 (1994).
7. Ueda, H., Sonoda, S., Brown, J. L., Scott, M. P. & Wu, C. *Genes Dev.* **4,** 624–635 (1990).
8. Han, W., Yu, Y. & Pick, L. *Mol. Cell. Biol.* **13,** 5549–5559 (1993).
9. Tsai, C. & Gergen, P. *Development* **121,** 453–462 (1995).
10. Kornberg, T., Sidén, I., O'Farrell, P. & Simon, M. *Cell* **40,** 45–53 (1985).
11. Baker, N. E. *EMBO J.* **6,** 1765–1773 (1987).
12. DiNardo, S. & O'Farrell, P. H. *Genes Dev.* **1,** 1212–1225 (1987).
13. Ingham, P. W., Baker, N. E. & Martinez-Arias, A. *Nature* **331,** 73–75 (1988).
14. Fitzpatrick, V. D., Percival-Smith, A., Ingles, C. J. & Krause, H. M. *Nature* **356,** 610–612 (1992).
15. Copeland, J., Nasiadka, A., Dietrich, B. & Krause, H. M. *Nature* **379,** 162–165 (1996).
16. Jost, W., Yu, Y., Pick, L., Preiss, A. & Maier, D. *Wilhelm Roux Arch. Dev. Biol.* **205,** 160–170 (1995).
17. Brown, S. J., Hilgenfeld, R. B. & Denell, R. E. *Proc. Natl Acad. Sci. USA* **91,** 12922–12926 (1994).
18. Dawes, R., Dawson, I., Falciani, F., Tear, G. & Akam, M. *Development* **120,** 1561–1572 (1994).
19. Stewart, J. J., Brown, S. J., Beeman, R. W. & Dennell, R. E. A. *Nature* **350,** 72–74 (1991).
20. Ish-Horowicz, D., Pinchin, S. M., Ingham, P. W. & Gyurkovics, H. G. *Cell* **57,** 223–232 (1989).
21. Struhl, G. *Nature* **318,** 677–680 (1985).
22. Carroll, S. B. & Scott, M. P. *Cell* **45,** 113–126 (1986).
23. Kassis, J. A. *Genes Dev.* **4,** 433–443 (1990).
24. Mangelsdorf, D. J. & Evans, R. M. *Cell* **83,** 841–850 (1996).
25. Erdélyi, M., Michon, A.-M., Guichet, A., Glotzer, J. B. & Ephrussi, A. *Nature* **377,** 524–527 (1995).
26. Patel, N. H. *et al. Cell* **58,** 955–966 (1989).
27. Krause, H. M., Klemenz, R. & Gehring, W. J. *Genes Dev.* **2,** 1021–1036 (1988).
28. Ephrussi, A. & Lehmann, R. *Nature* **358,** 387–392 (1992).
29. Tautz, D. & Pfeifle, C. *Chromosoma* **98,** 81–85 (1989).
30. Staudt, L. M. *et al. Science* **241,** 577–580 (1988).
31. Florence, B., Guichet, A., Ephrussi, A. & Laughon, A. *Development* **124,** 839–847 (1997).
32. Yu, Y. *et al. Nature* (in the press).

Acknowledgements. A.G. and J.W.R.C. are first coauthors, and H.M.K. and A.E. are senior coauthors. We thank A. Cyrklaff for cytological localization of P elements; M. Zeidler for the *hs αFtz-F1* transgenic flies; H. Stunnenberg and C. Schulz for the *αFtz-F1*, *hairy*, *runt*, *even-skipped* and *ftz* plasmids; S. Cohen and C. Wu for the Wg, En and Ftz-F1 antibodies; M. Petkovich for αFtz-F1-expressing constructs; H. Ueda, A. Laughon, B. Florence, M. Petkovich and L. Pick for exchange of unpublished data; S. Cohen and F. Stewart for critical reading of the manuscript; and C. Nusslein-Volhard and colleagues for their participation in screening our our female sterile *Drosophila* collection. A.G. was supported by fellowships from the Ministère de la Recherche et du Travail and the EMBL; M.E. by an EMBO long-term fellowship; J.W.R.C. was partly funded by a University of Toronto open fellowship, and D.H. by an NSERC summer student fellowship. The balance of funding in the lab of H.M.K. was provided by the Medical Research Council of Canada. Research in the laboratory of A.E. was funded by the European Molecular Biology Laboratory.

Correspondence and requests for materials should be addressed to H.M.K. (e-mail: h.krause@utoronto.ca) or A.E. (e-mail: ephrussi@stork.Embl-Heidelberg.de).

The nuclear hormone receptor Ftz-F1 is a cofactor for the *Drosophila* homeodomain protein Ftz

Yan Yu*, Willis Li†, Kai Su*, Miyuki Yussa*, Wei Han*, Norbert Perrimon† & Leslie Pick*

** Brookdale Center for Molecular Biology, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, New York 10029, USA*

† Howard Hughes Medical Institute, Department of Genetics, Harvard Medical School, 200 Longwood Avenue, Boston, Massachusetts 02115, USA

Homeobox genes specify cell fate and positional identity in embryos throughout the animal kingdom[1]. Paradoxically, although each has a specific function *in vivo*, the *in vitro* DNA-binding specificities of homeodomain proteins are overlapping and relatively weak. A current model is that homeodomain proteins interact with cofactors that increase specificity *in vivo*[2,3]. Here we use a native binding site for the homeodomain protein Fushi tarazu (Ftz) to isolate Ftz-F1, a protein of the nuclear hormone-receptor superfamily and a new Ftz cofactor. Ftz and Ftz-F1 are present in a complex in *Drosophila* embryos. Ftz-F1 facilitates the binding of Ftz to DNA, allowing interactions with weak-affinity sites at concentrations of Ftz that alone bind only high-affinity sites. Embryos lacking Ftz-F1 display *ftz*-like pair-rule cuticular defects. This phenotype is a result of abnormal *ftz* function because it is expressed but fails to activate downstream target genes. Cooperative interaction between homeodomain proteins and cofactors of different classes may serve as a general mechanism to increase HOX protein specificity and to broaden the range of target sites they regulate.

ftz is a segmentation gene of the pair-rule class located in the Antennapedia (Antp) complex[4]. Although its homeodomain and *in vitro* binding specificity is very similar to other Antp-class proteins[5,6], its role in embryos is unique: loss-of-function *ftz* mutations produce deletions of even-numbered parasegments and ubiquitous expression causes an 'anti-*ftz*' phenotype in which odd-numbered parasegments are missing[7].

To understand the molecular basis of Ftz function *in vivo*, we developed a modification of the yeast two-hybrid system to identify cofactors that modulate its transcriptional activity (Y.Y., J. Hirsch and L.P., manuscript in preparation). This screen used a native Ftz-target element from the upstream regulatory region of the *ftz* gene itself[5,8,9]. The *ftz* proximal enhancer is required to establish and maintain the *ftz* seven stripes[10]. A core 323-base-pair proximal enhancer (323-bp fPE; Fig. 1a) contains five native binding sites for Ftz protein that mediate autoregulation[5,11]. The 323-bp fPE was fused upstream of the yeast *HIS3* gene and integrated into the yeast genome. This reporter gene was expressed at low levels in yeast cells, allowing growth in low concentrations of 3-aminotriazole (3-AT; Fig. 1b). Expression of Ftz did not significantly increase reporter gene expression, enabling Ftz-interacting proteins to be isolated by growth selection in high concentrations of 3-AT. The native Ftz-target element facilitated the isolation of cofactors whose interactions with Ftz protein require their binding to DNA. One complementary DNA was isolated that supported only limited growth in 25 mM 3-AT in the absence of Ftz but robust growth when Ftz was present. At 50 mM 3-AT, little growth was detected without Ftz, but cells grew avidly when Ftz was also expressed (Fig. 1b). This cDNA encodes the full-length open reading frame of the α-form of the nuclear hormone receptor Ftz-F1 (refs 12, 13), originally identified as a DNA-binding protein that interacts with the *ftz* zebra[12] and

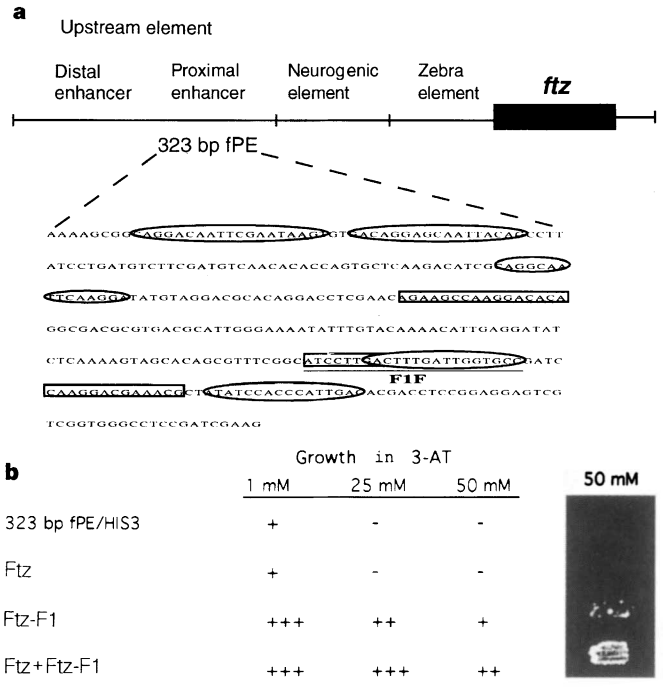

Figure 1 Ftz-F1 was isolated as a Ftz cofactor in a yeast interaction screen. **a**, the *ftz* gene (top); regulatory elements are indicated[29]. A core region of the proximal enhancer, the 323-bp *ftz* proximal enhancer (323 bp fPE, positions 2,167–2,490) directs expression in seven *ftz*-like stripes *in vivo* through a heterologous promoter[11] and contains five binding sites for Ftz protein (ovals); one is a high-affinity, three are of medium affinity and one is a low-affinity site[5], and there are three binding sites for Ftz-F1 protein (rectangles)[11]. One of the Ftz-F1 sites abuts a medium-affinity FTZ site (underlined, F1F). **b**, The growth of cells carrying the 323-bp fPE-HIS3 reporter gene in the absence of histidine and in the presence of increasing concentrations of the competitive inhibitor 3-AT. Ftz protein did not significantly stimulate growth; Ftz-F1 supported growth in 25 mM 3-AT and allowed for minimal growth in 50 mM 3-AT. Ftz and Ftz-F1 together strongly activated reporter gene expression, resulting in growth in 50 mM 3-AT.

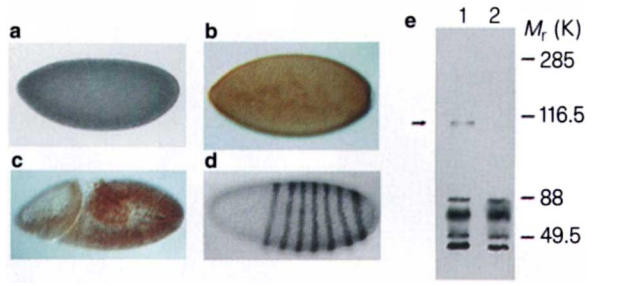

Figure 2 Ftz and Ftz-F1 are coexpressed in developing embryos and from a complex *in vivo*. **a**, Ftz-F1 was detected in unfertilized eggs; **b**, at the cellular blastoderm stage, Ftz-F1 was found in all somatic nuclei but not in the pole cells; **c**, expression remains ubiquitous through germ-band extension. **d**, Expression of Ftz in seven stripes delimits the regions of potential interaction between the two proteins. **e**, Co-immunoprecipitation of Ftz-F1 with Ftz from *Drosophila* nuclear extracts; western blot is shown. Lanes: 1, nuclear extract precipitated with purified anti-Ftz antibody; 2, nuclear extract precipitated with preimmune serum. Arrow indicates the position of Ftz-F1. The four smaller species present in both lanes are nonspecific.

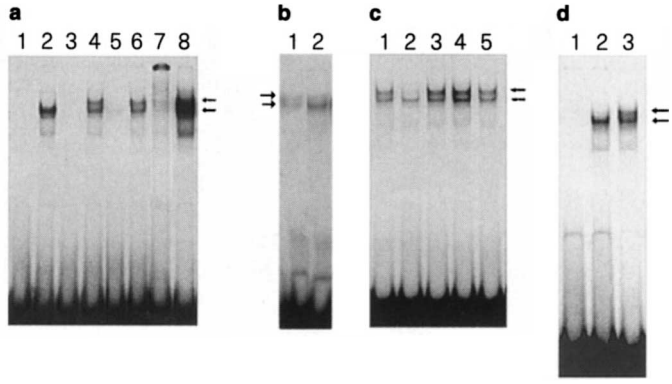

Figure 3 Ftz-F1 facilitates the binding of Ftz to its target DNA. **a**, The binding of Ftz to DNA in the absence and presence of Ftz-F1 was compared in a gel retardation assay. Lanes: 1, control extract; 2, 3 μg Ftz-F1; 3, 3 μg Ftz; 4–8, both Ftz and Ftz-F1 proteins. Note the formation of a Ftz/Ftz-F1/DNA ternary complex in lane 4. Binding was inhibited by a 50-fold molar excess of specific competitor (F1F site, lane 5) but not an unrelated oligonucleotide (Slp1 binding site, lane 6). Both the Ftz-F1/DNA complex and the ternary complex were supershifted by anti-Ftz-F1 antibody (lane 7) but not preimmune serum (lane 8). **b**, Anti-FTZ antibody (lane 2) abolished formation of the ternary complex but not the Ftz-F1/DNA binary complex (control, lane 1). **c**, The presence of Ftz in the ternary complex was verified by immunoprecipitation with anti-Ftz antibody (lane 2) compared with preimmune serum (lane 3) or protein A–Sepharose-coated beads alone (lane 4). The ternary complex (lanes 1 and 5, top arrow) was selectively inhibited by anti-Ftz antibody. **d**, Formation of the ternary complex requires both Ftz and Ftz-F1 binding sites. When the core Ftz-F1 binding site was changed from AGGA to ATAG, Ftz-F1 did not bind to DNA and no ternary complex was formed (lane 1). Mutation of the Ftz binding site from ATTG to CGAT had no effect on Ftz-F1 binding to DNA, but the ternary complex was not detected (lane 2). A control reaction with wild-type F1F DNA is shown in lane 3.

upstream elements[11]. As the *ftz-f1* cDNA we isolated was not fused to the Gal4 activation domain, Ftz-F1 is a potent transcription activator in yeast cells, as it is in mammalian and *Drosophila* cells[14,15]. Our findings suggest that Ftz-F1 not only interacts with DNA but also cooperates with Ftz protein to activate transcription.

Ftz-F1 protein was detected in unfertilized eggs (Fig. 2a), suggesting that it is maternally deposited, and at higher levels during cellularization and germ band extension in all somatic nuclei (Fig. 2b, c). Ftz is expressed in seven stripes that surround the embryo at the cellular blastoderm stage (Fig. 2d). Thus, Ftz and Ftz-F1 proteins are co-expressed in the nuclei of all cells where Ftz is expressed. To test whether Ftz and Ftz-F1 are associated in *Drosophila* embryos, *Drosophila* embryo nuclear extract was immunoprecipitated with anti-Ftz antibodies. Western blot analysis with anti-Ftz-F1 antibody revealed a single 110K band, corresponding to Ftz-F1 (Fig. 2e, lane 1). This band was not detected after immunoprecipitation with preimmune serum (Fig. 2e, lane 2). This suggests that Ftz and Ftz-F1 proteins are complexed *in vivo*.

The 323-bp fPE contains five Ftz binding sites and three Ftz-F1 binding sites. One Ftz-F1 site is adjacent to a medium-affinity Ftz binding site (Fig. 1a). These sites are present in the *Drosophila hydei ftz* upstream element[16], suggesting that they have a conserved function. A 27-bp oligonucleotide spanning this region (Ftz-F1/Ftz composite site, abbreviated to F1F; Fig. 1) interacted with Ftz-F1, generating a single band in gel retardation assays (Fig. 3a, bottom arrow in lane 2). Ftz alone (Fig. 3a, lane 3) did not bind F1F DNA under these conditions, although it bound strongly to a high-affinity site at the same concentration (not shown). When both Ftz and Ftz-F1 were included in binding reactions, a more slowly migrating complex formed (Fig. 3a, top arrow, lane 4). The formation of both complexes was specific; a 50-fold molar excess of unlabelled F1F DNA competed for binding (lane 5), but an unrelated oligonucleotide did not (lane 6). This suggested that the slowly migrating complex formed as a result of the specific binding of both Ftz-F1 and Ftz to F1F DNA.

Inclusion of anti-Ftz-F1 antibodies abolished formation of the Ftz-F1/DNA complex and of the more slowly migrating species, whereas preimmune serum had no effect (Fig. 3a, lanes 7 and 8),

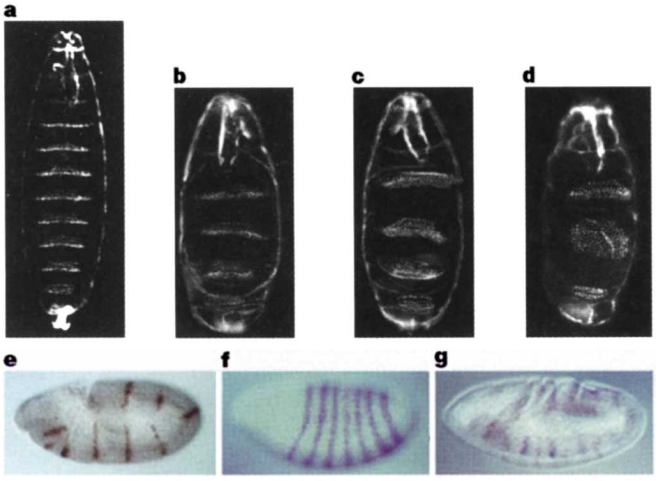

Figure 4 Mutations in *fts-f1* cause *ftz*-like pair-rule defects and alter Ftz function. Cuticle preparations of first instar larva are shown (**a**–**d**). **a**, Wild type, **b**, ftz^{w20} mutant, and **c**, *ftz-f1* mutant, all displaying *ftz*-like pair-rule defects; **d**, *ftz-f1* mutant displaying a more extreme *ftz* phenotype typical of ftz^{9H34} mutants. **e**, Every other En stripe, detected with an anti-En monoclonal antibody, was missing in *ftz-f1* mutants. **f**, The expression pattern of *ftz* RNA, shown at the blastoderm stage and detected with digoxygenin labelled RNA probes, was normal in *ftz-f1* mutant embryos. **g**, The missing En stripes correspond to the Ftz-dependent stripes, such that the remaining stripes (brown) alternate with correctly positioned *ftz* stripes (blue).

indicating that both complexes contain Ftz-F1 protein. Addition of anti-Ftz antibodies abolished formation of the upper complex only (Fig. 3b). To confirm the presence of Ftz in the slowly migrating complex, it was first removed from bacterial preparations by immunoprecipitation. Formation of the slowly migrating complex was selectively reduced (Fig. 3c, lane 2) whereas mock depletion with preimmune serum (lane 3) or protein-A–Sepharose alone (lane 4) had no effect. Together these results show that the slowly migrating complex is a ternary complex containing Ftz-F1, Ftz and DNA. More important, they demonstrate that Ftz-F1 protein dramatically boosts the binding of Ftz to DNA: the ternary Ftz-F1/Ftz/DNA complex formed at concentrations of Ftz that alone did not bind the same oligonucleotide. Ftz alone did not bind to F1F over the 50-fold concentration range tested here, but the ternary complex was detectable at the lowest concentrations of Ftz tested. Therefore the apparent affinity of Ftz for DNA was increased a minimum of 50-fold by Ftz-F1 protein.

To determine whether both proteins interact directly with DNA in the ternary complex, binding was assayed by using oligonucleotides carrying point mutations in either the Ftz-F1 or Ftz binding site within F1F. Mutation of the Ftz-F1 binding site abolished interaction of Ftz-F1 with the oligonucleotide and also abolished formation of the ternary complex (Fig. 3d, lane 1). Mutation of the Ftz binding site had no effect on the formation of a Ftz-F1/DNA binary complex, but the ternary complex was not detected (Fig. 3d, lane 2). Together these results indicate that Ftz and Ftz-F1 bind cooperatively to DNA.

In a large screen for autosomal zygotic-lethal mutations associated with maternal-effect embryonic defects, we identified a P-element-induced mutation, 1(3)03649, which exhibits a pair-rule maternal effect phenotype[17] (Fig. 4). 1(3)03649 mutant animals derived from heterozygous mothers die during larval stages. However, all embryos, irrespective of paternal contribution, derived from homozygous 1(3)03649 germline clones die during embryogenesis and display *ftz*-like pair-rule phenotypes (Fig. 4c, d). 1(3)03649 was mapped by the Berkeley *Drosophila* genome project to 75D4-5. Because the *ftz-f1* gene mapped to 75C1-D8 (ref. 13), we examined whether the P-insertion associated with 1(3)03649 disrupted the *ftz-f1* gene. Southern analysis revealed alterations in restriction fragments in the *ftz-f1* coding region in 1(3)03649 mutants. We further tested whether the phenotype could be rescued by zygotic expression of a *ftz-f1* cDNA under the control of a heat-shock promoter. The maternal-effect phenotype was partially rescued following a 20-min heat shock, and completely rescued following a one-hour heat shock (not shown).

If Ftz-F1 functions *in vivo* as a Ftz cofactor to regulate downstream target genes, *ftz*-like phenotypes would result from loss of Ftz function rather than a change in the expression pattern of the *ftz* gene itself. *ftz* is required for the establishment of alternate *en* stripes. Whereas En protein is detected in fourteen stripes in wild-type embryos, only seven En stripes were detected in *ftz-f1* mutant embryos (Fig. 4e), as in *ftz* mutant embryos. In contrast, *ftz* stripes were correctly positioned and expressed at the cellular blastoderm stage in *ftz-f1* mutants (Fig. 4f) and stripes remained strong through the stage at which *en* expression is initiated. *ftz* expression was visualized by *in situ* hybridization and embryos were double-stained with anti-En antibodies (Fig. 4g). As expected, the seven remaining En strips were present in alternate parasegment primordia where *ftz* was not expressed. Thus, the Ftz-dependent En stripes were not detectable in *ftz-f1* mutant embryos, in support of the idea that Ftz-F1 acts as a Ftz cofactor *in vivo*, because Ftz is present but does not activate *en* expression or direct the development of alternate parasegments in the absence of wild-type Ftz-F1.

Mutations in the *ftz-f1* gene mimic loss-of-function *ftz* mutations. Thus, despite the fact that it is expressed ubiquitously in early embryos, loss of maternal Ftz-F1 function results in *ftz*-like pair-rule defects. This suggests that the primary—if not exclusive—function of Ftz-F1 through the cellular blastoderm stage is to serve as a Ftz cofactor. The requirements for Ftz-F1 during later stages of development are unknown. Ftz-F1 binds several sites in *ftz* regulatory regions, including the zebra element[12] and the upstream element[11], a region of which was used in our yeast screen (Fig. 1). Mutations in Ftz-F1 binding sites in the zebra element suggested a role in activating *ftz* stripes[12]. Similarly, mutations of the three Ftz-F1 binding sites in the 323-bp fPE abolished expression of a *ftz/lacZ* fusion gene in embryos[18], so it was surprising that *ftz* was expressed in *ftz-f1* mutants. However, Ftz-F1 may still play a role at late stages in *ftz* autoregulation. First, *ftz* stripes decay slightly earlier in *ftz-f1* mutants than in wild-type embryos during germ-band extension, although this effect is subtle. Second, the autoregulatory phase is more clearly observed when enhancer fragments are fused to a basal heterologous promoter[5,9,10]. Expression of 323-bp proximal enhancer/*lac Z* fusion genes dramatically decreased in *ftz-f1* mutants, supporting a role for Ftz-F1 in *ftz* autoregulation (data not shown). Finally, the role of Ftz-F1 in regulating *ftz* expression may be masked because of redundancy. We found that three other *Drosophila* proteins interact with Ftz-F1 binding sites *in vitro*; at least two activate transcription through the 323-bp fPE *in vitro* and are present in early embryos[11,18]. Therefore, in *ftz-f1* mutant embryos, any one of these proteins may be sufficient to activate *ftz* transcription. This is consistent with our previous observations that a high degree of redundancy is built into the *ftz* regulatory network[10].

Genetic analysis shows that Ftz-F1 is required for wild-type function of the *ftz* gene. Ectopic expression defined a 'homeodomain-independent' function for Ftz[19–21] which may also be explained by interactions with Ftz-F1 (see accompanying manuscript[22]). In contrast, gene rescue experiments using the endogenous *ftz* promoter demonstrated a strict requirement for the homeodomain[23] and we have found that DNA binding is necessary for cooperative Ftz/Ftz-F1 interactions. This indicates that Ftz and Ftz-F1 bind target DNA cooperatively in the wild-type situation, whereas regions of Ftz outside the homeodomain are sufficient to mediate weaker interactions with Ftz-F1 under conditions of ectopic expression.

The cofactor Extradenticle is a homeodomain protein[24], which interacts with several HOX proteins to increase DNA-binding affinity and influence binding-site selectivity[3]. Thus, interactions of HOX proteins with different cofactors will generate different HOX–cofactor complexes that regulate distinct sets of downstream genes. The ability of homeodomain proteins to interact with proteins of the nuclear hormone receptor superfamily increases the interaction combinations that can modulate HOX protein activity in the embryo. Additional control may be provided by binding of Ftz-F1 to an as-yet unidentified ligand. Unlike the *ftz* gene itself, *ftz-f1* is conserved in higher organisms[25]. The identification of Ftz-F1 as a homeodomain cofactor in *Drosophila* suggests that mammalian Ftz-F1 may have a similar function in regulating target-gene expression in higher organisms. □

Methods

Ftz-F1 was isolated in a yeast double-interaction screen, to be described elsewhere (Y.Y., J. Hirsch and L.P., manuscript in preparation). Immunolocalization of Ftz-F1 was done using standard methods with a rabbit polyclonal antibody provided by C. Wu. For co-immunoprecipitation, affinity-purified rabbit polyclonal anti-Ftz antibody was used. Immunoprecipitation was done with protein A–Sepharose beads (Pharmacia) preincubated with either 10 μg of preimmune serum or purified anti-Ftz antibody. *Drosophila* nuclear extract was prepared from 0–9 h-staged embryos as described[11]. For western analysis, a 1:1,000 dilution of anti-Ftz-F1 antibody was used. Following incubation with biotin-coupled anti-rabbit IgG, bands were visualized using an ABC Elite kit (Vector Labs). Gel retardation assays were carried out as described[11]. Binding reactions (25 μl) containing 25 mM HEPES, pH 7.8, 0.5 mM EDTA, 0.5 mM DTT, 10% glycerol, 1 μg poly(dI·dC) and ~10 fmol ^{32}P-labelled F1F oligonucleotide, and protein, were incubated on ice for 1 h. Reactions were analysed by electrophoresis through 4% non-denaturing polyacrylamide gels using 0.5 × TBE as running buffer. Ftz and Ftz-F1 proteins were synthesized in bacteria with the T7 system, using a pGEMF1 (ref. 26) and pJC20FTZ-F1 (gift from C. Wu) expression plasmids. The *ftz-f1* expression plasmid encodes a protein lacking 200 amino acids at the N terminus and the *ftz* expression plasmid encodes a full-length protein with three amino acids inserted after the initiator methionine. Proteins were prepared as described[27] by denaturation with 4M guanidine-HCl, followed by extensive dialysis to facilitate renaturation. Germline clones homozygous for 1(3)03649 were generated using the 'autosomal FLP-DFS' technique[28]. Briefly, progeny of 1(3)03649 FRT^{3L}/TM3, Sb females were crossed with FLP^{22}/Y; P(ovo^{D1})^{3L} FRT^{3L}/TM3, Sb. Females were allowed to lay eggs for 1 d in glass vials and their progeny were heat-shocked twice for 2 h at 37 °C in a circulating water bath over a period of two days, when they reached late L2 to L3 larval stages. Subsequently, embryos derived from females of genotype FLP^{22}; 1(3)03649 FRT^{3L}/P(ovo^{D1}) FRT^{3L} mated with 1(3)03649 FRT^{3L}/TM3, Sb males were analysed. For Southern blot analysis, genomic DNA was digested with *Eco*RI, *Hin*dIII or *Sac*I. Control genomic DNA was from 1(3)04556/TM3, Sb, a P-element lethal mutation induced on the same parental chromosome as 1(3)03649, but at a different chromosomal location. Zygotic rescue of the maternal effect segmentation phenotype associated with 1(3)03649 was achieved as follows. Embryos derived from females with 1(3)03649 germline clones were crossed to males homozygous for either an *hsp70-ftz-f1* (experimental) or an *hsp70-ftz-f1β/DHR39* (control) transgene (gift from M. Petkovich). The *ftz-f1β/DHR39* gene encodes an orphan receptor that is very similar in structure to Ftz-F1 (refs 14, 15). Embryos of 1.5–2.5 h after egg-laying were heat-shocked by submerging the egg-collection plates, sealed with parafilm, into a 37 °C water bath for 20 or 60 min. The *hsp70–ftz-f1*, but not the *ftz-f1β/DHR39*, transgene was able partially to rescue ($n = 8/29$ embryos) the maternal pair-rule phenotypes associated with 1(3)03649 after 20 min heat shock. Partially rescued embryos showed more than 4 denticle bands. Rescue was complete after 60 min heat shock (of 50 embryos examined, 20 were rescued to various extents, of which 3 appeared to be wild type).

Received 24 September; accepted 18 December 1996.

1. McGinnis, W. & Krumlauf, R. *Cell* **68,** 283–302 (1992).
2. Hayashi, S. & Scott, M. P. *Cell* **63,** 883–894 (1990).
3. Mann, R. S. & Chan, S.-K. *Trends Genet.* **12,** 258–262 (1996).
4. Wakimoto, B. T., Turner, F. R. & Kaufman, T. C. *Dev. Biol.* **102,** 147–172 (1984).
5. Pick, L. Scheir, A., Affolter, M., Schmidt-Glenewinke. T. & Gehring, W. J. *Genes Dev.* **4,** 1224–1239 (1990).
6. Laughon, A. *Biochemistry* **30,** 11357–11367 (1991).
7. Struhl, G. *Nature* **318,** 677–680 (1985).
8. Hiromi, Y. & Gehring, W. J. *Cell* **50,** 963–974 (1987).
9. Schier, A. F. & Gehring, W. J. *Nature* **356,** 804–807 (1992).
10. Yu, Y. & Pick, L. *Mech. Dev.* **50,** 163–175 (1995).
11. Han, W., Yu, Y., Altan, N. & Pick, L. *Mol. Cell. Biol.* **13,** 5549–5559 (1993).
12. Ueda, H., Sonoda, S., Brown, J. L., Scott, M. P. & Wu, C. *Genes Dev.* **4,** 624–635 (1990).
13. Lavorgna, G., Ueda, H., Clos, J. & Wu, C. *Science* **252,** 848–851 (1991).
14. Ohno, C., Ueda, H. & Petkovich, M. *Mol. Cell. Biol.* **14,** 3166–3175 (1994).
15. Ayer, S. *et al. Nucl. Acids Res.* **21,** 1619–1627 (1993).
16. Maier, D., Preiss, A. & Powell, J. R. *EMBO J.* **9,** 3957–3966 (1990).
17. Perrimon, N. A., Lanjuin, C., Arnold, E. & Noll, E. *Genetics* **144,** 1681–1692 (1996).
18. Han, W., Yu, Y., Kohanski, R. A. & Pick, L. *J. Biol. Chem.* (submitted).
19. Fitzpatrick, V. D. & Ingles, C. J. *Nature* **337,** 666–668 (1989).
20. Hyduk, D. & Percival-Smith, A. *Genetics* **142,** 481–492 (1996).
21. Copeland, W. R., Nasiadka, A., Dietrich, B. H. & Krause, H. M. *Nature* **379,** 162–165 (1996).
22. Guichet, A. *et al. Nature* (**this issue**).
23. Furukubo-Takunaga, K. *et al. Genes Dev.* **6,** 1082–1096 (1992).
24. Rauskolb, C., Peifer, M. & Wieschaus, E. *Cell* **74,** 1101–1112 (1993).
25. Ikeda, Y. *et al. Mol. Endocrinol.* **7,** 852–860 (1993).
26. Krause, H. M., Klemenz, R. & Gehring, W. J. *Genes Dev.* **2,** 1021–1036 (1988).
27. Treisman, J., Gonczy, P., Vashishtha, M., Harris, E. & Desplan, C. *Cell* **59,** 553–562 (1989).
28. Chou, T.-B. & Perrimon, N. *Genetics* **144,** 1673–1679 (1996).
29. Hiromi, Y., Kuroiwa, A. & Gehring, W. J. *Cell* **43,** 603–613 (1985).

Acknowledgements. We thank R. Kohanski and D. Sassoon for comments on the manuscript; A. Guichet, A. Ephrussi and H. Krause for sharing information before publication; C. Wu for Ftz-F1 antibodies and cDNAs; I. Duncan and S. DiNardo for Ftz and En monoclonal antibodies; S. Elledge for the λACT vector; and M. Petkovich for the heat-shock lines. This is publication 212 from BCMB. N.P. is an Associate Investigator of the Howard Hughes Medical Institute. This work was supported by a grant from the NIH to L.P. L.P. was a recipient of an Irma T. Hirschl career scientist award.

Correspondence and requests for materials should be addressed to L.P. (e-mail: PICK@msvax.mssm.edu).

CORRECTIONS

Structural basis for the binding of a globular antifreeze protein on ice

Zongchao Jia, Carl I. DeLuca, Heman Chao & Peter L. Davies

Nature **384,** 285–288 (1996)

In the legend to Fig. 1b, the orientation of AFP should have been compared to that in Figs 1a, 2b; also, in Fig. 2a legend, the rotation of the planar amide group should have been 180° (not 18° as published). □

Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents

Ravi G. Kurumbail, Anna M. Stevens, James K. Gierse, Joseph J. McDonald, Roderick A. Stegeman, Jina Y. Pak, Daniel Gildehaus, Julie M. Miyashiro, Thomas D. Penning, Karen Seibert, Peter C. Isakson & William C. Stallings

Nature **384,** 644–648 (1996)

We omitted to cite an earlier report on (human) cyclooxygenase-2 structure by C. Luong *et al.*[1]. □

1. Luong, C., Miller, A., Barnett, J., Chow, J., Ramesha, C. & Browner, M. F. Flexibility of the NSAID binding site in the structure of human cyclooxygenase-2. *Nature Struct. Biol.* **3,** 927–933 (1996).