were used to infect Rat-1 cells expressing the conditional c-Myc allele c-Myc-ERTM (R1MycMER) in the presence of Polybrene (4 μg ml^{-1}).

C-Myc activation. c-Myc was activated by the addition of 100 nM of 4-hydroxytamoxifen to cells that have been serum deprived for 25–28 h (ref. 13).

PKB/Akt assays. *In vitro* kinase assays were performed on immunoprecipitated PKB/Akt proteins[3] using histone H2B as substrate. To this end, PKB/Akt constructs were transfected into R1MycMER using Lipofectamine (Gibco/BRL) and, approximately 48 h later, cells were serum deprived for 24 h before immunoprecipitation with 12CA5 antibody. Endogenous PKB/Akt kinase activation was analysed in immunoprecipitates obtained from serum-deprived cells by using the anti-Rac-CT antibody (Upstate Biotechnology).

ERK2 assays. ERK2 phosphorylation was determined by mobility shift of p42 MAP kinase to its phosphorylated pp42 form. To this end, cells were serum deprived for 48 h, total cell lysates electrophoresed in 15% low *bis*-acrylamide gels, and western immunoblotting performed using anti-ERK2 (Upstate Biotechnology).

Cell death analysis. Flow cytometric analysis for apoptotic DNA fragmentation was performed using Apotag (Oncor Appligene) on cells fixed with 1% paramormaldehyde. Time-lapse video-microscopic analysis was performed as described[6].

Received 21 August 1996; accepted 6 January 1997.

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Acknowledgements. We thank D. Lyon for help with the time-lapse video-microscopy, and D. Davies for the FACS analysis. A.K.-Z. is a fellow of the Kay Kendall Leukaemia Fund. E. Ulrich is a fellow of the F.W.F. (Schrodinger Stipendium). We thank the Bishop Stopford School, Kettering, UK, for a generous donation in memory of Elliot Smith.

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The nuclear receptor homologue Ftz-F1 and the homeodomain protein Ftz are mutually dependent cofactors

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Nuclear hormone receptors and homeodomain proteins are two classes of transcription factor that regulate major developmental processes. Both depend on interactions with other proteins for specificity and activity. The *Drosophila* gene *fushi tarazu* (*ftz*), which encodes a homeodomain protein[1] (Ftz), is required zygotically for the formation of alternate segments in the developing embryo[2]. Here we show that the orphan nuclear receptor αFtz-F1 (ref. 3), which is deposited in the egg during oogenesis[4], is an obligatory cofactor for Ftz. The two proteins interact specifically and directly, both *in vitro* and *in vivo*, through a conserved domain in the Ftz polypeptide. This interaction suggests that other nuclear receptor/homeodomain protein interactions may be important and common in developing organisms.

In a screen for new maternal mutations affecting anteroposterior polarity, we identified two recessive mutations causing a pair-rule phenotype that map near the *Ftz-F1* gene[3] (Fig. 1). Embryos derived from homozygous mutant females lack alternate denticle belts, normally found in segments T2, A1, A3, A5 and A7 (Fig. 1b). Closer examination of the cuticles showed that these deletions are parasegmental in nature (data not shown), as are those of *ftz* (ref. 2) (Fig. 1c).

The *Ftz-F1* gene encodes two protein isoforms, α and β. The α isoform is maternally expressed and evenly distributed in the early embryo[5]. In contrast, the β isoform is zygotically expressed during late embryonic and pupal stages, at which time it is thought to play a role in ecdysone-induced gene expression[4,6]. The two alleles isolated in this study, *Ftz-F1*^{209} and *Ftz-F1*^{282}, produce indistinguishable mutant phenotypes. Two lines of evidence indicate that both alleles specifically compromise maternal expression of the *Ftz-F1* gene. First, northern blots show that α*Ftz-F1* transcripts are not detected in *Ftz-F1*^{282} mutant females, and that a truncated transcript is detected in *Ftz-F1*^{209} mutant females (Fig. 1d). Second, *Ftz-F1*^{209} mutant embryos are rescued by expression of the α*Ftz-F1* transcript under control of the *hsp70* heat-shock promoter (Fig. 1e–h). This experiment was possible because the maternal product is not spatially localized[5]. The finding that, at blastoderm stage, the maternal α*Ftz-F1* gene product is uniformly distributed (data not shown), yet its mutation causes a pair-rule phenotype, indicates a limited requirement for αFtz-F1 in alternate parasegments. Further, it suggests that αFtz-F1 must interact with spatially localized factors.

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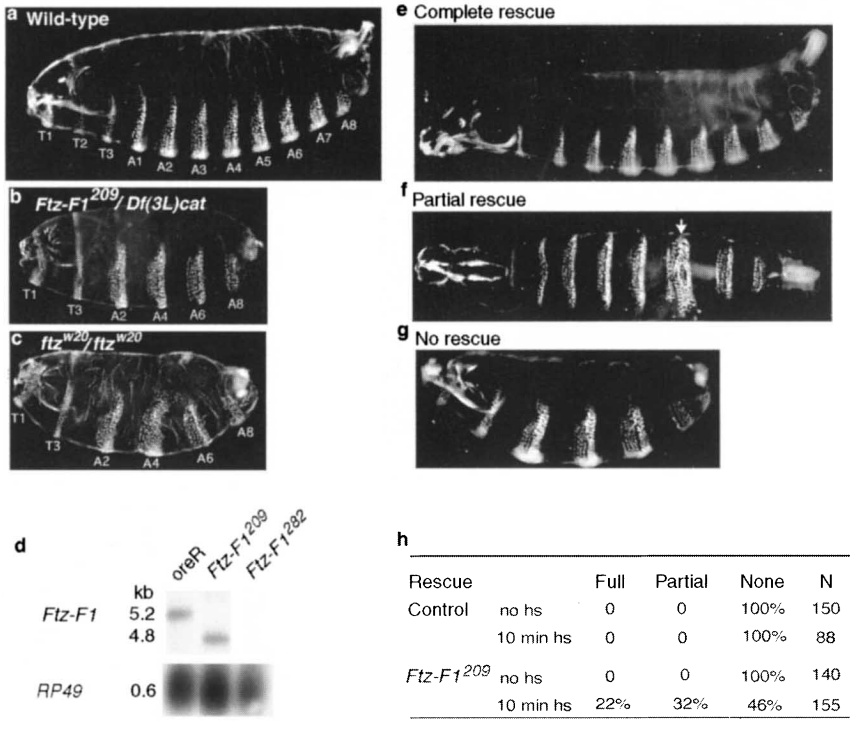
αFtz-F1 was first identified as a protein that binds the *ftz* promoter[7]. It was subsequently shown that αFtz-F1 binding sites are present in two regulatory regions of the *ftz* promoter, the proximal zebra element[7] and the upstream enhancer element[8], and that integrity of these sites is important for proper expression of minimized *ftz–lacZ* reporter genes[5,7–9]. It therefore seemed likely that the *αFtz-F1* phenotype is a consequence of reduced *ftz* expression. Surprisingly, patterns of *ftz* mRNA and protein expression in our *αFtz-F1* mutants are indistinguishable from wild type (Fig. 2a–d). We therefore tested whether other *ftz*-dependent parasegmental markers are correctly expressed. Ftz is required for proper expression of two segment-polarity genes, *engrailed* (*en*) and *wingless* (*wg*), which are each expressed in 14 stripes[10,11] (Fig. 2g, j). Even-numbered *en* stripes are positively regulated by Ftz (ref. 12), whereas even-numbered *wg* stripes are negatively regulated by Ftz (ref. 13). In *Ftz-F1* mutant embryos, as in *ftz* mutant embryos (Fig. 2i, l), Ftz-dependent *en* stripes fail to be expressed (Fig. 2f, h), and *wg* stripes expand (Fig. 2k). Thus αFtz-F1 is required for all Ftz activities tested except that for which it was first identified: regulation of the *ftz* promoter.

The target gene specificity of homeodomain proteins is critically dependent on extrinsic factors, such as interactions with other proteins. Indeed, Ftz can regulate many target genes in the absence of its DNA-binding homeodomain[14,15]. It has been suggested that this activity is mediated by DNA-binding cofactors[14,15]. Our results suggest the possibility that αFtz-F1 may function as one of these cofactors. To examine this possibility, we tested for a direct interaction between Ftz and αFtz-F1 *in vitro*. Nearly all of the αFtz-F1 protein expressed in a reticulocyte lysate system is specifically retained on Ftz micro-affinity columns with no binding to control columns (Fig. 3a). Retention of αFtz-F1 on the Ftz affinity columns is consistently as good as or better than that observed with the previously demonstrated[15] Ftz-interacting protein, Paired (Prd).

Further analysis of the αFtz-F1/Ftz interaction by far western blotting (Fig. 3b) confirmed that the interaction is direct and does not require the Ftz homeodomain. Indeed, the N-terminal third of Ftz is sufficient for a strong interaction (Fig. 3c). By using additional deletion constructs, the interaction domain was narrowed down to residues 101–150 (Fig. 3c). Two regions of the Ftz polypeptide are highly conserved in dipteran Ftz homologues: the homeodomain, and residues within the FtzΔ101–150 deletion. In *Drosophila hydei*, residues corresponding to amino acids 100–133 of Ftz are 100% conserved[16]. In the more distantly related flour beetle (*Tribolium castaneum*) homologue (TcFtz), the only homology, other than the homeodomain, is within this N-terminal region. This homology is more limited, however, consisting of a central LRALLT motif flanked on either side by prolines, adjacent to which are acidic residues on the N-terminal side and basic residues on the C-terminal side[17]. Despite this more limited homology, TcFtz interacts strongly with αFtz-F1 on far western blots, and the N-terminal region is sufficient for this interaction (Fig. 3c). Taken together, these data suggest that the LRALLT domain is the primary contact point for Ftz-F1, and that this domain is sufficiently important to have warranted conservation over >300,000 yr (ref. 17).

It is thought that *ftz* is a rapidly evolving homeobox gene that was first required in insects for development of the central nervous system, and only more recently for segmentation[17,18]. The *Tribolium ftz* homologue may represent an evolutionary *ftz* intermediate, as it is segmentally expressed but has no obvious segmental phenotype[19]. The ability of TcFtz to bind αFtz-F1 suggests that *Tribolium* may also possess a *Ftz-F1* homologue, and that this gene may hold the key to the evolving role of Ftz as a pair-rule protein.

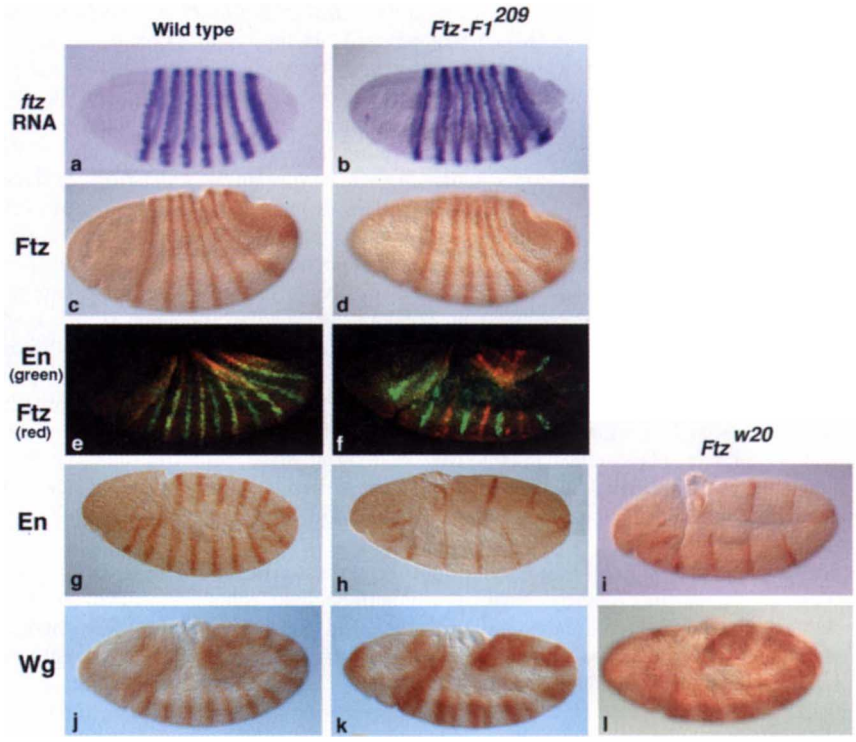


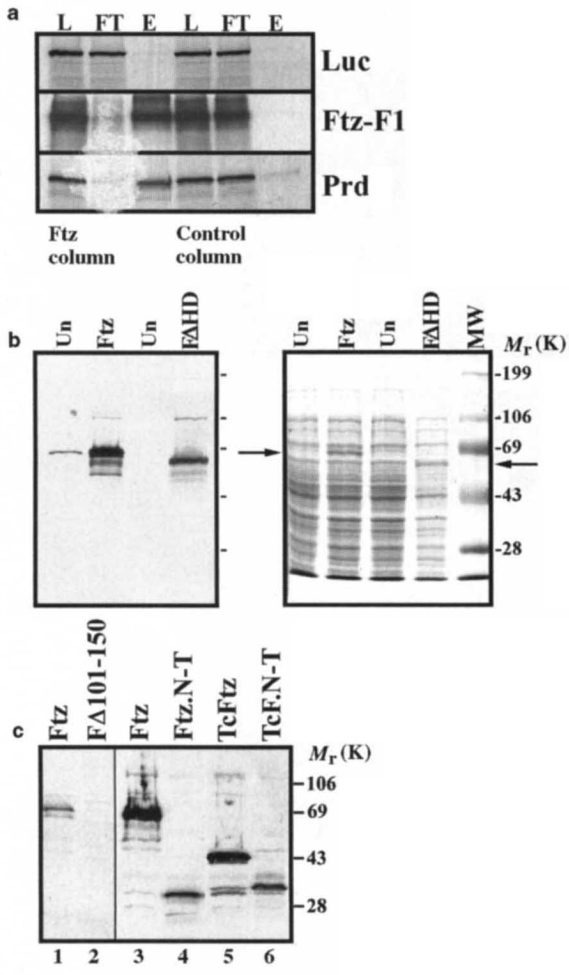
h

Rescue		Full	Partial	None	N
Control	no hs	0	0	100%	150
	10 min hs	0	0	100%	88
Ftz-F1^{209}	no hs	0	0	100%	140
	10 min hs	22%	32%	46%	155

Figure 1 Mutation of the *ftz-F1* α isoform leads to a maternal-effect, pair-rule phenotype that is indistinguishable from that of *ftz*. **a**, Wild-type (w^{118}) embryos. *ftz-F1*^{209}*/Df(3L)cat* (**b**) and *ftz*^{w20}*/ftz*^{w20} (**c**) embryos have indistinguishable cuticular phenotypes in which segments T2, A1, A3, A5, A7 are deleted. **d**, Northern blot containing RNA isolated from adult *oreR* (wild type), *Ftz-F1*^{209}, and *Ftz-F1*^{282} homozygous females. Hybridization with a 5′ probe specific for the α isoform reveals a single 5.2-kb transcript in wild-type females. This transcript corresponds to the *αFtz-F1* mRNA[4]. In *Ftz-F1*^{209}, a shorter transcript (4.8 kb) is present, whereas in *Ftz-F1*^{282} no transcript is detected. A probe specific for ribosomal protein 49 (RP49) serves as loading control. **e**–**h**, Zygotic rescue of the segmentation defects of *Ftz-F1*^{209} by a heat-shock (*hs*) transgene *hs αFtz-F1*. **f**, The white arrow in **f** indicates the remaining fused segments A5–A6 in the partially rescued embryo.

Figure 2 *Ftz-F1 affects en* and *wg*, but not *ftz*, expression. **a**, **c**, **e**, **g**, **j**, Wild-type embryos. **b**, **d**, **f**, **h**, **k**, Embryos from *Ftz-F1*^{209}*/Df(3L)cat* females, referred to as *Ftz-F1*^{209} embryos. **i**, **l**, Homozygous *ftz*^{W20} embryos. **a**, **b**, *ftz* mRNA expression in stage-5 embryos. The pattern of expression of *ftz* mRNA in *Ftz-F1*^{209} embryos (**b**), is indistinguishable from that in wild-type embryos (**a**). **c**, **d**, Ftz protein expression in stage-6 embryos. As is the case for *ftz* mRNA, the pattern of Ftz protein expression in *Ftz-F1*^{209} (**d**) embryos is indistinguishable from that of wild-type embryos (**c**). **e**, **f**, Double-labelling revealing En protein (green) and Ftz protein (red) expression patterns. In *Ftz-F1*^{209} embryos (**f**), En is absent from the stripes expressing Ftz, indicating that correctly expressed Ftz protein cannot activate *en* in the absence of Ftz-F1. **g**–**i**, En protein expression in stage-9 embryos. In wild-type embryos (**g**), En is expressed in 14 stripes, whereas in *Ftz-F1*^{209} (**h**) and in *ftz*^{w20} embryos (**i**), En is expressed in 7 stripes. **j**–**l**, Wg protein expression in stage-8 embryos. In wild-type embryos (**j**), Wg is expressed as 14 stripes, but in *Ftz-F1*^{209} embryos (**k**), Wg is expressed as 7 broad stripes, as in *ftz*^{w20} embryos (**l**). The late expression pattern of *runt* is also affected in *Ftz-F1*, as in *ftz*^{w20} embryos (data not shown). Expression of *even-skipped* and *hairy* is not affected in *Ftz-F1*^{209} mutants (data not shown).





To test whether αFtz-F1 and Ftz interact directly *in vivo*, we tested the ability of an ectopically expressed Ftz polypeptide, missing residues 101–150, to regulate αFtz-F1-dependent target genes. When expressed under control of a heat-shock promoter, ubiquitous Ftz expression in blastoderm embryos represses alternate *wg* stripes, broadens alternate *en* stripes, and broadens endogenous *ftz* stripes[20] (Fig. 4). This results in an 'anti-*ftz*' pair-rule phenotype in which *ftz*-independent parasegments are deleted[21] (Fig. 4n). These effects do not require the Ftz homeodomain, nor do they depend on the endogenous *ftz* gene[14,15].

Deletion of amino acids 101–150 disrupts all but one of the Ftz activities described above (Fig. 4). FtzΔ101–150 cannot repress *wg*,

Figure 3 Ftz and αFtz-F1 interact directly. **a**, Ftz affinity chromatography. Reticulocyte lysate-translated Luciferase (Luc), αFtz-F1 and Prd proteins were passed over Ftz (left) or control (right) affinity columns. Equivalent portions of the ^{35}S-labelled load (L), flow-through (FT), and eluate (E) fractions were subjected to SDS–PAGE and autoradiography. Luciferase, used as a negative control, flows through both the Ftz and control columns. In contrast, αFtz-F1 is specifically retained on the Ftz affinity column and quantitatively recovered in the eluate. Prd, a previously identified Ftz-interacting[15] protein, also binds specifically to the Ftz affinity column, but with lower efficiency. All other proteins within the reticulocyte lysate flowed through the column (ref. 15 and data not shown). **b**, Far western analysis. Autoradiogram (left) and corresponding Coomassie blue stain (right) show uninduced (Un) and induced bacterial lysates containing Ftz or FtzΔHD[14] subjected to SDS–PAGE. Left, proteins were transferred from the gel to nitrocellulose. Right, gel was stained with Coomassie blue to reveal total proteins. αFtz-F1 bound specifically to the induced Ftz and FtzΔHD proteins marked with arrows on the Coomassie-stained gel. MW, molecular weight markers. **c**, Far western with deleted Ftz polypeptides probed with ^{35}S-labelled αFtz-F1. Lanes 1 and 2 contain full-length and N-terminally deleted (residues 101–150) Ftz polypeptides expressed *in vitro*. Lanes 3–6 contain *Drosophila* and *Tribolium* Ftz polypeptides expressed in bacteria: lane 3, full-length Ftz; lane 4, Ftz 1–171; lane 5, TcFtz; lane 6, TcFtz 1–197. All Ftz proteins except FtzΔ101–150 bound well to the αFtz-F1 probe.

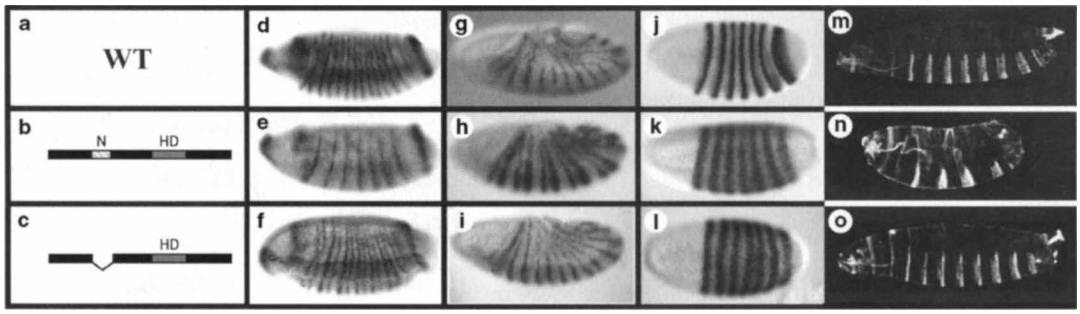


Figure 4 Deletion of the αFtz-F1 interaction domain disrupts αFtz-F1-dependent Ftz activities. The left hand column (**a**–**c**) indicates Ftz constructs expressed upon heat shock and responsible for the corresponding patterns in the panels to the right. **a**, No construct expressed (WT); **b**, full-length Ftz; **c**, FtzΔ101–150. Adjacent columns, from left to right, show resulting *wg* (**d**–**f**), *en* (**g**–**i**) and *ftz* (**j**–**l**) expression patterns. The right-hand column (**k**–**m**) shows final cuticular phenotypes. Note that deletion of residues 101–150 disrupts the ability of Ftz to repress even-numbered *wg* stripes, to broaden even-numbered *en* stripes, and to generate an anti-*ftz* cuticular phenotype, but not to broaden stripes of endogenous *ftz*.

activate *en* or generate an anti-*ftz* cuticular phenotype, although it is still capable of broadening endogenous *ftz* stripes. Similar results were obtained by expressing a full-length Ftz polypeptide in an *αFtz-F1* mutant background (data not shown). Thus removal of the αFtz-F1 interaction domain from the Ftz polypeptide results in the same loss of Ftz activities as removal of αFtz-F1.

Previous studies indicated that Ftz-mediated repression of *wg* requires the pair-rule protein Prd, and that this requirement involves a direct interaction between the two proteins[15]. The expanded pattern of *wg* expression in *Ftz-F1* mutants is not due to a defect in Prd expression, as the pattern of expression of Prd in *Ftz-F1* mutant embryos is indistinguishable from the wild-type pattern (data not shown). Thus Ftz-mediated repression of *wg* appears to require both Prd and αFtz-F1. This interaction could involve either simultaneous or competitive interactions amongst the three proteins, as Prd also contacts residues 101–150 of Ftz (ref. 15). Unlike Ftz-F1, however, Prd requires additional contact points on the Ftz polypeptide for a strong interaction (J.W.R.C. and H.M.K., manuscript in preparation).

Prd may be a cofactor of Ftz or Ftz-F1 that is required for target genes that are repressed by Ftz, because Prd is required for Ftz-dependent *wg* repression, but not for Ftz-dependent activation of *en* (ref. 12) or for *ftz* auto-regulation[22]. For the latter two genes, no Ftz cofactors had previously been identified. In the case of *en* regulation, recent studies[31] support our findings that Ftz and αFtz-F1 are both required, and suggest a likely *cis*-acting target element. Binding sites in the first *en* intron, required for early *en* expression[23], were shown to contain binding sites for both Ftz and αFtz-F1; the two proteins bind to these sites *in vitro* in a cooperative fashion. In the embryo, expression of a minimal reporter gene containing these sites requires the presence of both Ftz and αFtz-F1, and both DNA binding sites.

The *ftz* enhancer is perhaps the best-characterized target of Ftz activity, and contains αFtz-F1 binding sites[7,8]. Recently, Yu *et al.*[32] screened in yeast for Ftz-interacting proteins, using portions of the *ftz* enhancer, and isolated αFtz-F1. The two proteins bind cooperatively to the sites used in their screen. These data support our findings of a direct interaction between Ftz and Ftz-F1, but are surprising in that *αFtz-F1* mutations have no obvious effects on *ftz* expression during the stages we examined. Also, we find that Ftz expressed ectopically is still capable of broadening endogenous *ftz* stripes in an *αFtz-F1* mutant background (data not shown). A possible explanation for these apparent discrepancies is that our *αFtz-F1* mutations affect only a subset of αFtz-F1 activities. However, this possibility is not consistent with our genetic and molecular analyses; rather, our results suggest two alternatives. The first is that sequences in the *ftz* promoter bound by αFtz-F1 *in vitro* are not occupied by αFtz-F1 *in vivo*. The second is that these sites are occupied by αFtz-F1 *in vivo*, but that the contribution of these complexes, in the context of the whole *ftz* promoter, is not essential, owing to the redundant action of other cofactors. Regardless of the explanation, the ability of Ftz to autoregulate in the absence of its homeodomain, and in the absence of either Prd or αFtz-F1, indicates that there are additional Ftz cofactors yet to be identified.

In summary, αFtz-F1 is a maternally provided cofactor required for Ftz-mediated regulation of the *en* and *wg* genes. Indeed, the two proteins seem to be mutually dependent cofactors for all processes tested except *ftz* autoregulation. The ability of Ftz to act in the absence of its homeodomain suggests that αFtz-F1 may be important for recruitment of the Ftz polypeptide to specific DNA sequences. In turn, Ftz may influence the transcriptional activity of Ftz-F1. Ftz-F1 is an orphan nuclear receptor, as no ligand has yet been identified[24]. Nevertheless, it is tempting to speculate that other nuclear receptors, for which ligands have been identified, might also form complexes with homeodomain proteins. This would provide a plausible mechanism by which hormones could, through their nuclear receptors, modulate the activity of homeodomain proteins. Conversely, interactions of this sort might allow homeodomain proteins to modulate the activity or target range of nuclear hormone receptors. □

Methods

Isolation of *Ftz-F1* alleles. We carried out a P-element mutagenesis[25], and screened for maternal-effect lethal mutations affecting the anteroposterior polarity of embryos. Of these mutations, two had indistinguishable pair-rule phenotypes, and after further analysis were designated as *Ftz-F1*^{209} and *Ftz-F1*^{282}. Both alleles are homozygous viable and maternal-effect lethal. The P-element inserts map to the cytological loci 75D and 67D, respectively. Both fail to complement an overlapping deficiency at 75D *(Df(3L)cat)* but complement a deficiency uncovering 67D, demonstrating that the mutations responsible for the phenotype lie in the 75D locus. *Ftz-F1*^{282} exhibits the same pair-rule phenotype as *Ftz-F1*^{209} (data not shown). Embryos from *Ftz-F1*^{209}/*Df(3L)cat* trans-heterozygous females and those from *Ftz-F1*^{209}/*Ftz-F1*^{209} homozygous females (data not shown) also exhibit indistinguishable phenotypes. The P element in *Ftz-F1*^{209} was remobilized, generating wild-type, maternal-effect lethal, as well as homozygous lethal, excisions. The defects observed in embryos from females homozygous for *Ftz-F1*^{209} and *Ftz-F1*^{282} cannot be rescued by wild-type sperm (data not shown). In addition, homozygous mutant embryos with zygotic lethal excisions exhibit no segmental defects. Hence the pair-rule phenotype observed is strictly maternal.

Zygotic rescue of *Ftz-F1*^{209}. *Ftz-F1*^{209}/*Df(3L)cat* females were crossed with males homozygous for *hs αFtz-F1*, or with wild-type males (control). Embryos 2 h old were heat-shocked for either 0 or 10 min, and allowed to develop for 24 h before preparation of cuticles[20]. Cuticles were observed under dark-field

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.

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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.

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

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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