

The cofactor Extradenticle is a homeodomain protein²⁴, which interacts with several HOX proteins to increase DNA-binding affinity and influence binding-site selectivity³. 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 organisms²⁵. 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 described¹¹. 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 described¹¹. 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 ³²P-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 described²⁷ 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' technique²⁸. Briefly, progeny of 1(3)03649 FRT³¹/TM3, Sb females were crossed with FLP²²/Y; P(ovo^{D1})³¹ FRT³¹/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 FLP²²; 1(3)03649 FRT³¹/P(ovo^{D1}) FRT³¹, mated with 1(3)03649 FRT³¹/TM3, Sb males were analysed. For Southern blot analysis, genomic DNA was digested with *Eco*RI, *Hind*III 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).

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CORRECTIONS

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

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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

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

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