type PDGF receptor in 100-mm dishes were incubated at 4°C for 2 hours in the presence or absence of 10 nM PDGF-BB before lysis in RIPA buffer [50 mM NaCl, 50 mM NaF, 30 mM sodium pyrophosphate, 5 mM EDTA, 10 mM tris (pH 7.4), 1% Triton X-100, 1 mM PMSF, 25 μM leupeptin, 10 μM pepstatin A, 0.2 U/ml aprotinin and 1 mM sodium vanadate]. The lysates (0.5 ml) were centrifuged at 38,000 rpm at 4°C for 20 hours in a SW 40.1 rotor through 10 ml of a linear gradient of 5 to 20% sucrose in 20 mM Hepes (pH 7.4), 0.15 M NaCl, 2.5 mM $MgCl_2$, 0.6 mM $MnCl_2$, 10% glycerol, 0.2% Triton X-100, 0.02% NaN_3, 0.5 mM vanadate, 1 mM PMSF, and 10 μM leupeptin. After centrifugation, 0.5-ml fractions were collected, precipitated in methanol, and subjected to SDS-PAGE (7%). Alkaline phosphatase (7S) and catalase (11.3S) were used as sedimentation markers.

26. The cells were lysed in RIPA buffer. Insoluble material was removed by centrifugation at 4°C for 10 min at 13,000*g*. The lysates were incubated with Ab 88 at 4°C for 3 hours and the immune complexes were precipitated by protein A–Sepharose beads (Pharmacia). The immunoprecipitates were washed three times with RIPA buffer and three times with buffer containing 0.5 M LiCl and 0.1 M tris (pH 7.4) before analysis by SDS-PAGE.
27. After overnight incubation of the injected oocytes, healthy oocytes were labeled with [^{35}S]methionine (1 mCi/ml, DuPont–New England Nuclear) in MBS (10 μl per oocyte) for 24 hours at 19°C.
28. We thank P. Lee and D. Johnson for a plasmid containing chicken basic FGF receptor cDNA, M. Kirschner and E. Amaya for discussions, D. Julius for serotonin receptor plasmid, K. Shiokawa and M. Solomon for advice on oocyte handling and injection, and members of our laboratory for reading the manuscript. Supported by NIH grants RO1 HL-32898 and PO1 HL-43821.

25 January 1991; accepted 19 March 1991

FTZ-F1, a Steroid Hormone Receptor–Like Protein Implicated in the Activation of *fushi tarazu*

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The *Drosophila* homeobox segmentation gene *fushi tarazu* (*ftz*) is expressed in a seven-stripe pattern during early embryogenesis. This characteristic pattern is largely specified by the zebra element located immediately upstream of the *ftz* transcriptional start site. The FTZ-F1 protein, one of multiple DNA binding factors that interacts with the zebra element, is implicated in the activation of *ftz* transcription, especially in stripes 1, 2, 3, and 6. An FTZ-F1 complementary DNA has been cloned by recognition site screening of a *Drosophila* expression library. The identity of the *FTZ-F1* complementary DNA clone was confirmed by immunological cross-reaction with antibodies to FTZ-F1 and by sequence analysis of peptides from purified FTZ-F1 protein. The predicted amino acid sequence of FTZ-F1 revealed that the protein is a member of the nuclear hormone receptor superfamily. This finding raises the possibility that a hormonal ligand affects the expression of a homeobox segmentation gene early in embryonic development.

THE PROCESS OF SEGMENTATION IN the *Drosophila* embryo is governed by a hierarchical network of maternal and zygotic genes (*1*). The *fushi tarazu* (*ftz*) gene is a well-studied zygotic segmentation gene and encodes a protein that can function as a transcription factor through the DNA binding specificity of its homeodomain (*2*). Expression of *ftz* in the even-numbered parasegmental primordia of the embryonic blastoderm (the seven-stripe pattern) is crucial for proper development of the corresponding body segments in the *Drosophila* embryo (*3*).

Expression of *ftz* is controlled primarily at the level of transcription. Sequences that confer the seven-stripe pattern of expression have been localized to ~600 bp of DNA upstream of the *ftz* structural gene (the zebra element) (*4*). Among several transacting factors that bind directly to the zebra element, the FTZ-F1 protein has been implicated as a positive regulator of *ftz* transcription (*5*). Transformed embryos that carry a zebra element–*lacZ* construct mutated at a FTZ-F1 binding site show a pronounced decrease of β-galactosidase activity in the anterior three *ftz* stripes and stripe six, in addition to an overall decrease in activity (*5, 6*).

We purified FTZ-F1 to homogeneity from *Drosophila* embryos and studied the properties of FTZ-F1 protein in detail (*5*). The DNA binding activity of FTZ-F1 is detectable in early (1.5- to 4-hour) embryo extracts, coincident with the expression of *ftz*. An electrophoretically altered form of FTZ-F1 can also be detected at a second, later phase of embryogenesis (after 13 hours). This late activity may be related to the subsequent repression of *ftz* gene or to the regulation of other genes.

In this report, we present the cloning and sequence analysis of *FTZ-F1*. In order to clone *FTZ-F1*, we screened a 0- to 16-hour embryo cDNA expression library with a concatenated FTZ-F1 DNA binding site. Out of 8×10^6 plaques, one interacted with the wild-type FTZ-F1 recognition sequence, but not with a sequence mutated at nucleotides important for FTZ-F1 binding (*5*) (Fig. 1A). Recombinant protein extracted from this clone showed specific binding to the wild-type FTZ-F1 recognition sequence, as analyzed by an electrophoretic mobility shift assay (Fig. 1B, lanes 1 to 4). The recombinant and the natural FTZ-F1 proteins also displayed identical contacts with the recognition sequence, as shown by a methylation interference assay (Fig. 1C). We further tested the recombinant protein for cross-reaction with antibodies to purified FTZ-F1. The antiserum, which inhibits binding of the early and late embryo FTZ-F1 protein to DNA (*5*), inhibited the binding of the recombinant protein (Fig. 1B, lanes 5 to 7).

We isolated overlapping cDNAs for the early form of FTZ-F1 by screening early embryo cDNA libraries with the initial *FTZ-F1* cDNA clone. Sequence analysis of the cDNA clones revealed a continuous open reading frame (ORF) of 1043 amino acids (Fig. 2). The predicted molecular size of the early form of FTZ-F1 is 110 kD, greater than the 95-kD size of late embryo FTZ-F1 as measured by SDS–polyacrylamide gel electrophoresis (*5*). The size difference could be due to modification, anomalous electrophoretic mobility, or intrinsic differences between early and late FTZ-F1 proteins (*5*). Additional evidence that FTZ-F1 is encoded by this ORF was obtained by microsequencing six tryptic peptides derived from FTZ-F1 protein purified to homogeneity from late stage (12- to 24-hour) embryos (2 kg). The sequences of the FTZ-F1 peptides are found without discrepancy in the ORF (underlined residues in Fig. 2). The correspondence of the six peptides derived from late embryo FTZ-F1 with the predicted amino acid sequence of early embryo FTZ-F1 implies that the early and late FTZ-F1 proteins are similar.

A search of the protein sequence database revealed sequence similarity between FTZ-F1 and members of the nuclear hormone receptor superfamily (*7*). The conserved regions include the DNA binding domain, which bears two potential Cys_2-Cys_2 zinc finger motifs and the ligand binding domain of the nuclear receptor superfamily (Fig. 3A). The linear separation between these two domains is greater for FTZ-F1 than for other nuclear receptors. The putative DNA binding domain (region I) of FTZ-F1 is well conserved across the nuclear receptor superfamily, showing identity with all 20 invariant amino acids (*7*). Nonetheless, FTZ-F1 is somewhat distinct from the two major classes of nuclear receptors, which bind to either the glucocorticoid or the estrogen-thyroid hormone response ele-

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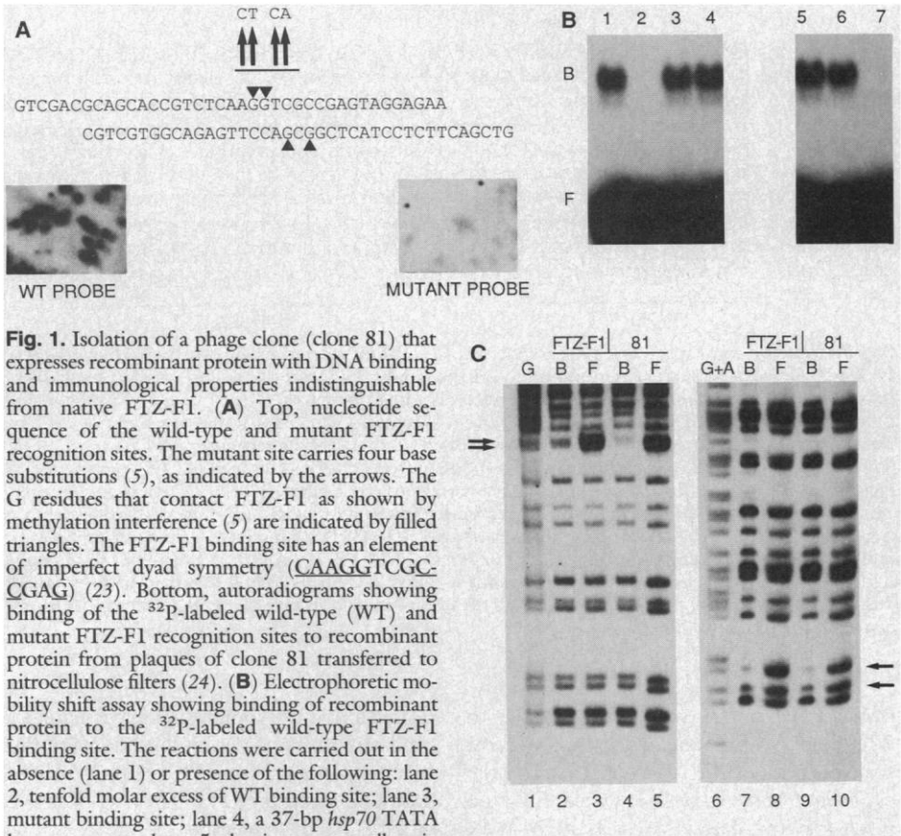

Fig. 1. Isolation of a phage clone (clone 81) that expresses recombinant protein with DNA binding and immunological properties indistinguishable from native FTZ-F1. (**A**) Top, nucleotide sequence of the wild-type and mutant FTZ-F1 recognition sites. The mutant site carries four base substitutions (*5*), as indicated by the arrows. The G residues that contact FTZ-F1 as shown by methylation interference (*5*) are indicated by filled triangles. The FTZ-F1 binding site has an element of imperfect dyad symmetry (CAAGGTCGCCGAG) (*23*). Bottom, autoradiograms showing binding of the ^{32}P-labeled wild-type (WT) and mutant FTZ-F1 recognition sites to recombinant protein from plaques of clone 81 transferred to nitrocellulose filters (*24*). (**B**) Electrophoretic mobility shift assay showing binding of recombinant protein to the ^{32}P-labeled wild-type FTZ-F1 binding site. The reactions were carried out in the absence (lane 1) or presence of the following: lane 2, tenfold molar excess of WT binding site; lane 3, mutant binding site; lane 4, a 37-bp *hsp70* TATA box sequence; lane 5, bovine serum albumin (1%); lane 6, a 1:10 dilution of preimmune mouse serum (1 μl); lane 7, a 1:10 dilution of antiserum to FTZ-F1 (1 μl) (*5*). The positions of free (F) and bound (B) DNA are noted. (**C**) Methylation interference analysis of recombinant and native FTZ-F1 protein. The autoradiogram shows chemical cleavage at sites of G methylation on the coding strand (lanes 2 to 5) and noncoding strand (lanes 7 to 10) of a Dde I–Nru I fragment (positions −362 to −249 of *ftz*). Arrows indicate the fragments depleted from the overall cleavage pattern in the lanes that represent DNA bound to protein (B); the same fragments are enriched in the lanes that represent free DNA (F). Lanes marked G and G + A show chemical cleavage at those residues.

Fig. 2. The predicted amino acid sequence of FTZ-F1 (*25*). The sequence of six tryptic peptides of FTZ-F1 are underlined. For protein sequencing, ~10 μg of FTZ-F1 protein was purified from ~2 kg of *Drosophila* embryos (Oregon R) essentially as described (*5*). The protein was digested with trypsin and peptides were separated by reversed-phase liquid chromatography and microsequenced as described (*26*). Abbreviations for the amino acid residues are A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. The DNA sequence corresponding to the FTZ-F1 ORF has been submitted to GenBank (accession number M63711).

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          10                  30                  50                  70
MDTFNVPMLAESSNTNYATEATSNHHHLQHQHQQQHSHQQQQQQQQQLLMPHHHKDQMLAAGSSPMLPFYSHLQLQQKDA
          90                 110                 130                 150
TATIGPAAAAAAVEAATTSANADNFSSLQTIDASQLDGGISLSGLCDRFFVASPNPHSNSNMTLMGTATAATTTTTNNNN
         170                 190                 210                 230
NNNTNNNNNNNNVEAKTVRPSNGNSVIIESVTMPSFANILFPTHRSANECIDPALLQKNPQNPNGNNSSIIVPPVEYHQLK
         250                 270                 290                 310
PLEVNSSTSVSTSNFLSSTTAQLLDFEVQVGKDDGHISTTTTTGPGSGSASGSGSGSGSGSGSIASTIGTATPTTTTSMS
         330                 350                 370                 390
NTANPTRSSLHSIEELAASSCAPRAASPNSNHTSSASTTPQQQQQQQHHMQSGNHSGSNLSSDDESMSEDEPGLEIDDNG
         410                 430                 450                 470
GYQDTTSSHSQQSGGGGGGGGGNLLNGSSGGSSAGGGYMLLPQAASSSGNNGNPNAGHMSSGSVGNGSGGAGNGGAGGNS
         490                 510                 530                 550
GPGNPMGGTSATPGHGGEVIDPKHLPEELCPVCGDKVSGYHYGLLTCESCKGFFKRTVQNKKVYTCVAERSCHIDKTQRK
         570                 590                 610                 630
RCPYCRFQKCLEVGMKLEAVRADRMRGGRNKFGPMYKRDRARKLQVMRQRQLALQALRNSMGPDIKPTPISPGYQQAYPN
         650                 670                 690                 710
MNIKQEIQIPQVSSLTQSPDSSPSPIAIALGQVNASTGGVIATPMNAGTGGSGGGGLNGPSSVGNGNSSNGSSNGNNSS
         730                 750                 770                 790
TGNGTSGGGGGNNAGGGGGGTNSNDGLHRNGGNDSSSCHEAGIGSLQNTADSKLCPDSGTHPSSTADALIEPLRVSPMIR
         810                 830                 850                 870
EFVQSIDDREWQTQLFALLQKQTYNQVEVDLFELLMCKVLDQNLFSQVDWARNTVFFKDLKVDDQMKLLQHSWSDMLVLD
         890                 910                 930                 950
HLHHRIHNGLPDETQLNNGQVFNLMSLGLLGVPQPGDYFNELQNKLQDLKFDMGDYVCMKFLILLNPSVRGIVNRKTVSE
         970                1010                1030
GHDNVQAALLDYTLTCYPSVNDKFRGLVNILPEIHAMAVRGEDHLITCTPSTVPAVRPPKRCSWRCCTPSARDRGRENVT
RNT
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ments, depending on three key amino acids that confer recognition specificity (*8*) (Fig. 3B, arrows). In FTZ-F1, the recognition amino acids (CESCKG) represent a hybrid: the first and third residues are like residues of the estrogen-thyroid hormone receptor subfamily (CEGCKA/G), while the second residue matches the glucocorticoid receptor subfamily (CGSCKV). As might be expected, the sequences of four FTZ-F1 binding sites found in *ftz* are distinct from both the glucocorticoid and estrogen response elements (*5, 7*), and FTZ-F1 does not bind to these elements in an electrophoretic mobility shift assay (*9*). Because vertebrate hormone response elements show perfect or imperfect dyad symmetry separated by a short variable spacer, we reexamined the sequence of the FTZ-F1 binding sites. A similar symmetry can be discerned from the FTZ-F1 binding sites on the *ftz* gene (legend to Fig. 1A).

The COOH-terminal half of the FTZ-F1 sequence shows amino acid sequence similarity to the ligand binding domain of hormone receptors. Sequence alignment identified two core regions (regions II and III) (*10*) that are well conserved across the nuclear receptor superfamily (Fig. 3A). The FTZ-F1 sequence in these regions is most similar to the human COUP (*10*) transcription factor [also called erb-A related 3, EAR 3 (*11*) (44% identity)], erb-A related 2, EAR 2 (*11*) (40%), and the *Drosophila* neural determination protein Sevenup (*12*) (40%). It was least similar to the human estrogen receptor (*13*) (23%), human retinoic acid receptor (*14*) (22%), and human thyroid receptor (*15*) (21%). In many instances, the similarity between FTZ-F1 and members of the receptor superfamily also extend beyond the core-conserved regions in the ligand binding domain. The sequence conservation in the ligand binding domain suggests that FTZ-F1 is a receptor for a hormonal ligand in *Drosophila*, but the nature of this ligand remains to be discovered. In addition, a stretch of 12 amino acids near the COOH-terminus of FTZ-F1 (Lys^{791} to Ile^{802}) shows 58% similarity/33% identity to COOH-terminal residues (Arg^{507} to Ile^{518}) of the mouse estrogen receptor (*16*). These residues are important for dimerization and high affinity DNA binding of the mouse receptor (*16*).

The *FTZ-F1* gene was mapped by in situ hybridization to a unique cytological locus (75 CD) on the salivary gland polytene chromosome (Fig. 4A). Southern (DNA) blot analysis indicated that *FTZ-F1* is a single copy gene (Fig. 4B). The proximity of *FTZ-F1* to another hormone receptor–like sequence E75 at chromosomal locus 75 B (*17*) raises the possibility that a complex of

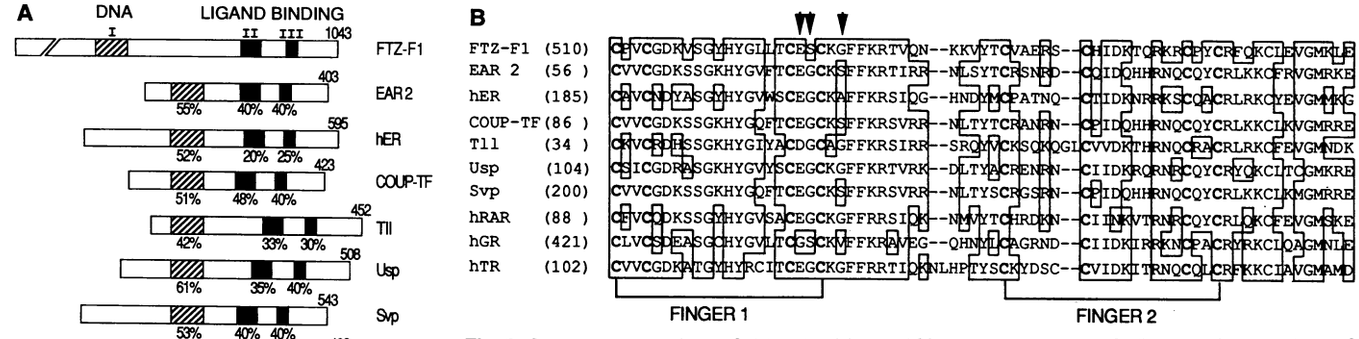

Fig. 3. Sequence comparison of FTZ-F1 with steroid hormone receptors. (**A**) Structural comparison of FTZ-F1 with members of the steroid hormone superfamily. Alignment of amino acids on the basis of maximum similarity identifies regions in the DNA binding domain (I, striped box) and in the ligand binding domain (II, III, shaded boxes); the percent identity to FTZ-F1 is noted below the boxes. The number of residues in each polypeptide is indicated at the COOH terminus. Designations are as follows: erb-A related 2, EAR 2 (*11*); human estrogen receptor, hER (*13*); human COUP transcription factor, COUP (*10, 11*); *Drosophila* gap segmentation protein Tailless, Tll (*20*); *Drosophila* Ultraspiracle, Usp (*19*); *Drosophila* Sevenup, Svp (*12*); human retinoic acid receptor, hRAR (*14*); human glucocorticoid receptor, hGR (*27*); human thyroid receptor, hTR (*15*). Reference and numbering, except for Tll, Svp, and Usp, are as in Swissprot Databank Version 15.0. (**B**) Protein sequence comparison between FTZ-F1 and members of the steroid receptor superfamily in the DNA binding domain. Identical or similar (E, D; F, W, Y; I, L, V, M; T, S) residues are boxed if they occur in at least five out of ten of the aligned positions. The position of the first residue is noted in brackets. Arrows denote the three amino acids postulated to confer recognition specificity (see text). The alignment of the two Zn fingers on the linear sequence is taken from the solution structures of the DNA binding domains of the glucocorticoid and estrogen receptors (*28*).

hormone receptor–like genes may lie in cytological region 75 of the *Drosophila* chromosome (*18*).

We analyzed the expression of *FTZ-F1* transcripts by Northern (RNA) blot analysis of RNAs isolated from staged embryos (Fig. 4C). A *FTZ-F1* RNA of 5.2 kb likely to be

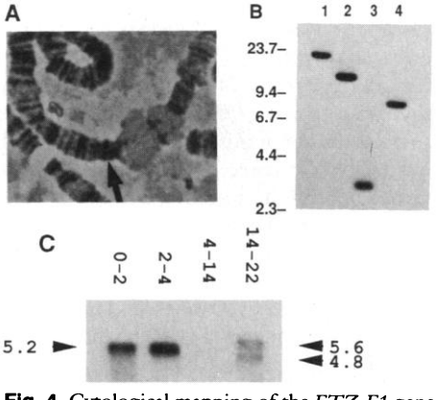

Fig. 4. Cytological mapping of the *FTZ-F1* gene and developmental expression of *FTZ-F1* RNA. (**A**) In situ hybridization of a digoxigenin-substituted Acc I–Acc I fragment (*FTZ-F1* DNA sequence coordinates 1628 to 2543) to polytene chromosomes that shows hybridization to the 75 CD region (arrow). (**B**) Southern blot analysis of *FTZ-F1*. Genomic DNA from *Drosophila* embryos was digested with Sal I, Bam HI, Hind III, and Eco RI (lanes 1 to 4, respectively). The DNA blot was probed with a 500-bp 3′-terminal fragment of the *FTZ-F1* cDNA. (**C**) Northern (RNA) blot analysis of *FTZ-F1* transcripts in staged embryo RNAs. Poly(A)$^+$ RNA (1 μg) was loaded onto each lane. The blot was probed with a *FTZ-F1* fragment (DNA sequence coordinates 2283 to 3215). In situ hybridization, Southern, and Northern blotting followed standard procedures (*29*).

of maternal origin was present in 0- to 2-hour and 2- to 4-hour embryos, consistent with the period of FTZ-F1 activity and the expression of *ftz* in early embryos. *FTZ-F1* RNA was not detectable in 4- to 14-hour embryos, but reappeared in 14- to 22-hour embryos. These late RNA species are slightly different in size (5.6 and 4.8 kb) from the early *FTZ-F1* RNA, suggesting that they are modified at the transcriptional or post-transcriptional level. The appearance of early and late *FTZ-F1* transcripts during embryogenesis is correlated with the developmental pattern of FTZ-F1 DNA binding activity, which is first detectable in early embryos, absent in mid-stage embryos, and present again (with a different electrophoretic mobility) in late-stage embryos (*5*). The reappearance of *FTZ-F1* RNA and protein activity at a time when *ftz* is silent suggests that *FTZ-F1* has a function distinct from the activation of *ftz*. Both the maternal contribution and the potential functional pleiotropy suggest how *FTZ-F1* could have been missed by genetic screens for zygotic lethal mutations that cause specific defects in the segmented pattern of the embryo cuticle.

Developmental regulators in *Drosophila* known to be related to the nuclear receptor superfamily include the retinoid X receptor–related protein XR2C, the product of the *ultraspiracle* locus (*19*) (also known as chorion gene transcription factor CF-1 or the 2C receptor protein), the neural determination protein Sevenup (*12*), and ecdysone-inducible proteins E75-A, E75-B (*17*), and the gap segmentation protein Tailless (*20*). In addition, the gap segmentation protein Knirps, and related proteins Knirps-related and Embryonic gonad have sequence similarity to the DNA binding domain, but lack the canonical ligand binding domain of hormone receptors (*21*). The identification of FTZ-F1 as a member of the hormone receptor superfamily with sequence similarity in both the DNA binding and ligand binding domains suggests that undiscovered hormonal ligands may contribute to the unusual spatial effects of FTZ-F1 on *ftz* expression and directly affect the patterning of the early *Drosophila* embryo. The observation that a putative activator of a homeobox segmentation gene is a member of the nuclear hormone receptor family further reveals an intersection of these two families of developmental regulators in *Drosophila* that is reminiscent of the activation of homeobox genes by the hormone retinoic acid in human embryonal carcinoma cells (*22*). Finally, the demonstration that *FTZ-F1* does not correspond to a previously identified gene that affects segmentation highlights the necessity to employ molecular and biochemical methods in addition to genetic screens in order to identify all the genes important for early pattern formation.

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23. The five contiguous nucleotides CAAGG that are invariant for all four FTZ-F1 binding sites in *fiz* (consensus $P_yCAAGGP_yCP_uCCP_u$) (*5*) make up the first half of the dyad, separated by three nucleotides from the second half-site, which is mismatched at two out of five positions. The FTZ-F1 binding sequence is shifted upstream of the GGTCRC motif that is recognized by many hormone receptors.
24. A random-primed, 0- to 16-hour embryo cDNA library in λgt11 was probed with a concatenated, synthetic FTZ-F1 DNA binding site (positions −267 to −299 of *fiz* terminated by two Sal I sites) labeled with ^{32}P by nick-translation. Colonies [8×10^6 plaque-forming units (pfu)] were screened at a density of 2×10^4 pfu per plate, as described [C. Vinson, K. L. LaMarco, P. F. Johnson, W. H. Landschulz, S. L. McKnight, *Genes Dev.* **2**, 801 (1988)], except that the duplicate filter was left on the plate for 10 hours and the binding buffer was supplemented with heat-denatured salmon sperm DNA (5 μg/ml). Plaque-purified clone 81 was plated and screened with ^{32}P-labeled wild-type and mutant FTZ-F1 binding sites. Synthesis of the recombinant protein was induced and the protein was extracted from a lysogen of clone 81 as described [M. Miyamoto *et al.*, *Cell* **54**, 903 (1988)], except that the lysis buffer was supplemented with leupeptin (5 μg/ml), pepstatin (5 μg/ml), and aprotinin (0.75 μg/ml). Electrophoretic mobility shift and methylation interference analyses were performed as described (*5*) except that the gel matrix was agarose (1%). For the methylation interference study, we used a 16- to 19-hour embryo nuclear extract (lanes 2 and 7) as a source of native FTZ-F1 protein.
25. The 1043–amino acid ORF was reconstructed from two overlapping cDNA clones isolated from a 0- to 4-hour embryo cDNA library [N. H. Brown and F. C. Kafatos, *J. Mol. Biol.* **203**, 425 (1988)] and a 2- to 4-hour embryo cDNA library (*9*), respectively. The first methionine in the ORF is presumed to be initiating. DNA sequencing was by the dideoxynucleotide method and data were assembled with the UWGCG sequence analysis programs [J. Devereux, P. Haeberli, O. Smithies, *Nucleic Acids Res.* **12**, 387 (1984)]. Like many developmental genes, *FTZ-F1* contains the *opa* repeat sequence $(CAG)_x$ [K. A. Wharton, B Yedvobnick, V. G. Finnerty, S. Artavanis-Tsakonas, *Cell* **40**, 55 (1985)], which is translated into stretches of Q (residues 39 to 47 and 361 to 367).
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30. We thank C. Klee for microsequencing FTZ-F1 peptides; B. Hovemann, N. Brown, and F. C. Kafatos for cDNA expression libraries; J. Kennison for confirming the cytological map position of *FTZ-F1*; B. Davis for managing the NIH *Drosophila* colony; and J. Kennison, J. Kassis, A. Oro, and the reviewers for helpful comments and suggestions. Supported by the National Cancer Institute Intramural Research Program and by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan and the Joint Studies Program of the Graduate University for Advanced Studies.

25 January 1991; accepted 20 March 1991

Ca^{2+} Permeability of KA-AMPA–Gated Glutamate Receptor Channels Depends on Subunit Composition

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NMDA (*N*-methyl-D-aspartate) receptors and non-NMDA receptors represent the two major classes of ion channel–linked glutamate receptors. Unlike the NMDA receptor channels, non-NMDA receptor channels have usually been thought to conduct monovalent cations only. Non-NMDA receptor ion channels that can be gated by kainic acid (KA) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) are formed by the glutamate receptor subunits GluR1, GluR2, and GluR3. These subunits were expressed in various combinations in *Xenopus* oocytes so that their permeability to divalent cations could be studied. At physiological resting potentials, KA and AMPA elicited inward calcium currents in oocytes expressing GluR1, GluR3, and GluR1 plus GluR3. In contrast, oocytes expressing GluR1 plus GluR2 or GluR3 plus GluR2 showed no such permeability. Thus, in neurons expressing certain KA-AMPA receptor subunits, glutamate may trigger calcium-dependent intracellular events by activating non-NMDA receptors.

THE GLUTAMATE-GATED ION CHANnels include those receptors activated by NMDA, KA, and AMPA (*1, 2*). The conductance mechanism of these ionotropic glutamate receptor (GluR) subtypes is cationic (*3*): NMDA receptor channels are permeable to monovalent cations and Ca^{2+} (*4–7*) whereas non-NMDA receptor channels have negligible permeability to divalent cations (*1, 8*). Many of the important physiological and pathological functions of NMDA receptors, for example in synaptic plasticity, learning, excitotoxic cell death, and neurological disorders (*9–11*), are, at least in part, consequences of the Ca^{2+} permeability of their integral ion channels. Although it is generally maintained that Ca^{2+} permeability of non-NMDA receptor channels is very low or absent (*4, 5, 12*), some KA-gated channels have been reported to be permeable to Ca^{2+} (*13, 14*), making this a controversial issue.

To test the permeability properties for divalent cations of the channels formed by different combinations of the KA-AMPA receptor subunits GluR1, GluR2, and GluR3 (*15, 17–19*), we expressed these subunits in various combinations in *Xenopus* oocytes that had been injected with cRNA (RNA transcribed in vitro from the cDNA clones). The ion channels were activated with KA, the most efficacious agonist at these receptors (*15, 17–19*), and ion permeability properties were analyzed under voltage clamp in test buffers of different ionic composition (*20*). In normal Ringer solution, the inward current through all combinations of GluR ion channels was carried mainly by an inward Na^+ flow because elimination of Na^+ from the external medium almost completely abolished the inward currents, whereas elimination of Cl^- neither changed the amplitudes nor shifted the reversal potentials (Fig. 1) (*21*). The outward currents seen at positive membrane potentials, which presumably indicate outward flow of K^+, remained unchanged when the external ions were exchanged. These findings indicate that, in normal Ringer, the inward current through GluR channels is carried mainly by Na^+ and K^+.

To test the GluR channels for calcium permeability, we used a Na^+,K^+-free medi-

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