

HOMOG program. Family B is of interest because the mother (III-2) is diagnosed with chronic SMA and the child (IV-1) with acute SMA. Presumably, a disease allele from generation I is transmitted to both carrier parents in generation III, then inherited in double copy by individual IV-1. Consistent with this model, the affected child is homozygous for markers D5S39 and D5S78. The affected mother presumably has one copy of the disease allele in common with the affected child, and one heterologous copy inherited from her father. Thus, this family is consistent with allelic variation among chronic and acute cases of SMA. Alternatively, the affected mother could have transmitted a sporadic dominant mutation for SMA, though this is unusual in cases of childhood-onset SMA^{11,12}.

The nine acute and seven chronic SMA families in this and our previous study¹ provide strong evidence in favour of genetic homogeneity between the two disorders. The combination of linkage data and genetic homogeneity make it very likely that the acute form of SMA maps to chromosomal region 5q. One chronic family (Fig. 2, A) and one acute family (Fig. 2, B) seem to be unlinked to 5q markers, although family A might conceivably provide data in support of linkage when more informative markers are identified. It must be noted that SMA can be confused with clinically overlapping neurological disorders²⁻⁶, and that the subdivision between acute and chronic SMA is arbitrary, with overlap between the groups¹³. It is not yet possible to distinguish whether, or to what extent, the data in Fig. 2 support genetic heterogeneity as opposed to disease misclassification among the SMA family set. These issues should

be resolved as additional families are typed with markers from chromosome 5q, and disease phenotype is carefully scrutinized relative to genotype. The results caution against early expectations for comprehensive prenatal diagnosis. □

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Putative protein kinase product of the *Drosophila* segment-polarity gene *zeste-white3*

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THE metameric pattern of the *Drosophila* embryo is regulated by a combination of maternal and zygotic genes. The segment-polarity class of genes are required for the correct patterning within each segmental unit. Mutations in any one of these genes results in deletions and duplications of parts of each segment¹. The segment-polarity genes act coordinately by means of local cellular interactions to assign and maintain an identity for each cell in the segment, and to establish segment boundaries²⁻⁵. Here we describe the molecular characterization of a novel segment-polarity gene, *zeste-white3* (*zw3*). Embryos derived from germ lines that are homozygous for *zw3* mutations (*zw3* embryos) have phenotypes similar to embryos that are mutant for the segment-polarity gene *naked* (*nkd*)⁶. These embryos lack most of the ventral denticles, which are differentiated structures derived from the most anterior region of each segment. We have isolated the *zw3* gene and compared the structure of one maternal and one zygotic transcript encoded by the gene. The *zw3* gene is unique among the segment-polarity genes so far characterized, in that it encodes proteins that have homology to serine-threonine protein kinases. This indicates that *zw3* may play a part in a signal transduction pathway involved in the establishment of cell identity within each embryonic segment.

The *zw3* locus is located at chromosomal position 3B1, adjacent to the *period* (*per*) gene^{7,8}. To identify the *zw3* gene at the molecular level we isolated overlapping phage encompassing 70 kilobases (kb) of genomic DNA distal to *per*. The breakpoints

of two deficiencies, *Df(1)64j4* and *Df(1)64f1* (ref. 8), define the proximal and distal limits⁶, respectively, of a 55-kb region of DNA within which the *zw3* gene must lie (Fig. 1a and b). Transcriptional analyses of this region revealed several related transcripts ranging in size from 2.5 to 6.3 kb (Fig. 1d). Although any one transcript may extend over as much as 40 kb of genomic DNA, all transcripts are detected by a single small genomic fragment (illustrated by filled bar in Fig. 1a) and are transcribed in the same direction (data not shown). The inversion *zw3*^{b12}, an allele of *zw3* (ref. 8), identifies this transcription unit as the *zw3* locus, as the inversion breakpoint disrupts the genomic DNA from which the transcripts are derived (Fig. 1b and c). Transcripts are detected at all developmental stages, including messenger RNA of maternal origin at 0-1 h after egg laying, and are developmentally regulated (Fig. 1d). Using a probe that is common to all the *zw3* transcripts we detected a uniform pattern of expression throughout the preblastoderm embryo and in all germ layers of the gastrulating embryo (Fig. 2). Expression is at its highest level during the period from fertilization through germ band elongation, and decreases during germ band retraction.

Using the genomic fragment that is common to all of the *zw3* transcripts we have isolated complementary DNAs from both ovarian⁹ and embryonic 9-12 h¹⁰ cDNA libraries. We have determined the nucleotide sequence of the largest ovarian cDNA (1,257 bp) and the largest embryonic cDNA (3,660 bp). The ovarian cDNA corresponds to the 2.5-kb maternal mRNA, and the embryonic cDNA is assumed to correspond to a transcript that is first detected at 6 h of embryonic development and that persists to the adult stage.

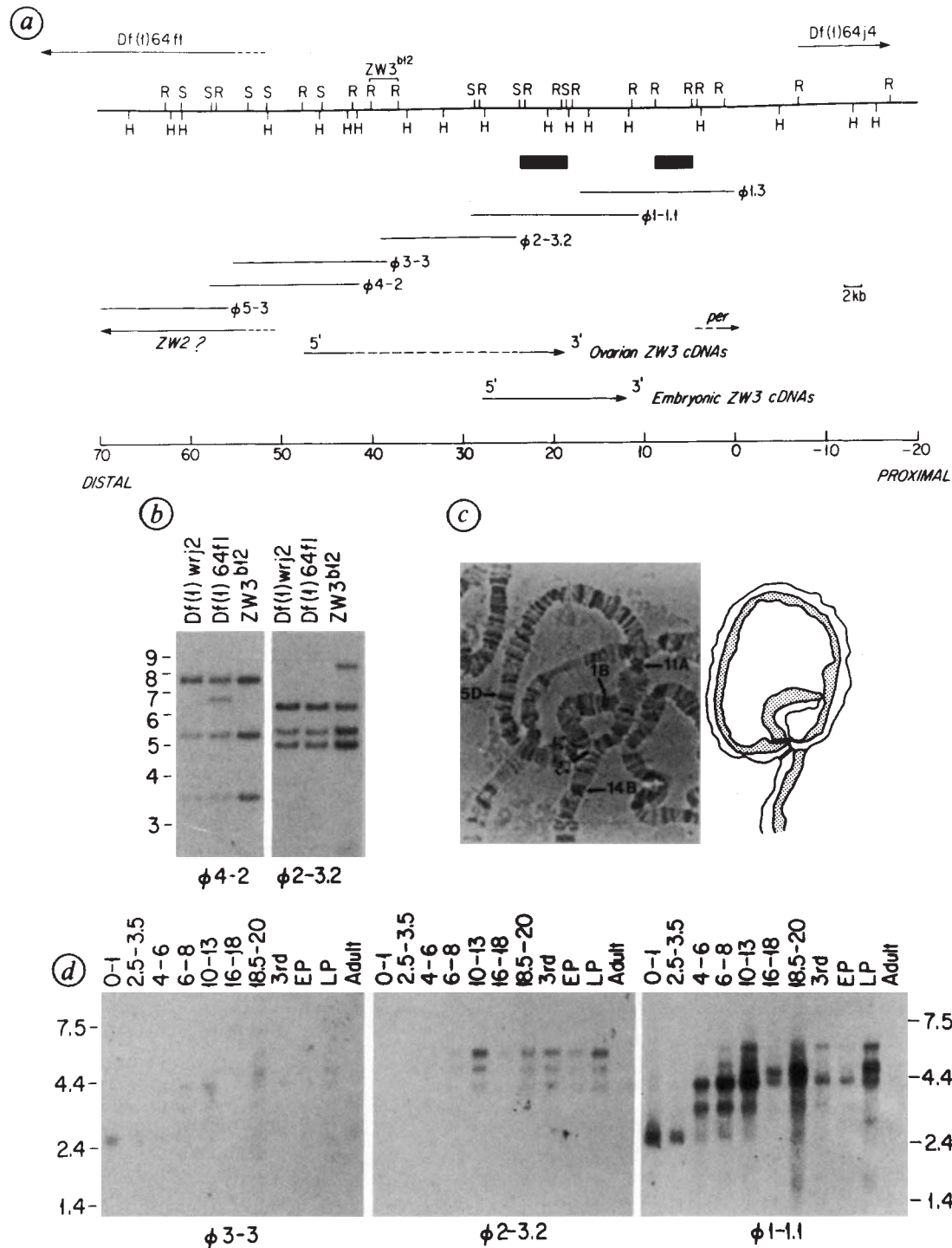
The embryonic cDNA contains a putative open reading frame of 2,199 nucleotides beginning at the ATG at position 378 (Fig. 3b). The context of this initiation codon is not a particularly good match to the *Drosophila* consensus for translation start sites ((C/A)AA(C/A)AUG) (ref. 11); there is another in-frame initiation codon at position 507, which is closer to the consensus. The open reading frame is followed by a 3' untranslated region of 1,057 nucleotides. Several embryonic cDNAs have poly(A)

tracts at their 3' end, preceded by the sequence AAATTA which is a variant of the canonical polyadenylation sequence, AATAAA (ref. 12).

The ovarian cDNA contains an open reading frame of 867 nucleotides beginning with the ATG at position 391 and continuing to the end of the clone (Fig. 3a). This cDNA is not

full-length and lacks sequence from the 3' end of the transcript that it represents. The sequence flanking the initiation codon (TACG) is consistent with the *Drosophila* consensus at the highly conserved second position¹². The ovarian and embryonic cDNAs are identical in a region of protein coding sequence starting at position 476 in the ovarian cDNA and position 1,162

FIG. 1 The molecular organization of the *zw3* locus. **a**, Restriction map and the positions of the deficiencies *Df(1)64j4* (ref. 7) and *Df(1)64f1*, and the inversion *zw3^{bl2}* are shown. The lines above the molecular map indicate where the genomic DNA is present in the deficiencies, the stippled portions indicate uncertainty in the position of the breakpoint. The hatched bar represents the probe used to initiate the phage walk and the filled bar represents the probe used to isolate cDNAs. R, *EcoRI*; H, *HindIII*; S, *SalI*. **b**, DNA from heterozygous flies (*Df(1)64f1/FM7* and *zw3^{bl2}/FM7*) was digested with *HindIII* and probed with the recombinant phage 4-2 and 2-3.2. Alterations were detected with several restriction enzymes and this data was used to map the breakpoints (data not shown). *Df(1)wrj2* (ref. 8) is a large deficiency that removes DNA in the region of the genome represented by the phage walk. **c**, *In situ* hybridization to polytene chromosomes from 3rd instar larvae of the genotype, *zw3^{bl2}/yf*, using the recombinant phage 2-3.2 as a probe. This phage hybridizes to genomic DNA on either side of the inversion, *zw3^{bl2}*, which extends from 3B1 to 12F (ref. 8). Open arrows indicate the position of the hybridization and the thin black arrows indicate cytological positions on the chromosome. The stippled region in the panel on the right represents the *yf* chromosome. **d**, Northern-blot analysis of the *zw3* locus. A northern blot containing mRNA from different developmental stages was probed with three overlapping phage that cover the *zw3* locus. The embryonic stages represented are described in hours after egg laying (AEL), the other stages are third instar larvae (3rd), 0–24 h pupae (EP), 96–120 h pupae (LP) and adult.



METHODS. Overlapping phage were isolated from a phage EMBL3 *Drosophila* genomic library²². Sixteen cDNAs were isolated from a LamdaZap (Stratagene) cDNA library made to ovarian RNA⁹ and the longest was sequenced. Embryonic cDNAs were isolated from a λ gt11 library made from mRNA isolated from 9–12 h embryos¹⁰. Eight embryonic cDNAs were isolated and analysed and the longest was chosen for sequencing. Restriction maps have been determined for all of the cDNAs and the ends of all of them have

been sequenced (data not shown). Southern transfer, northern transfer and all hybridizations were performed using standard techniques (Final washes were 0.2 \times SSC, 0.1% SDS at 65 $^{\circ}$ C)²³. Chromosome squashes for the *in situ* hybridization to polytene chromosomes were as described²⁴, with minor modifications. The recombinant phage 2–3.2 was labelled by nick translation with biotinylated dUTP (ref. 25) and hybridization was detected with the Detek-1-HRP kit (Enzo Diagnostic Inc.).

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Regulation of the pituitary-specific homeobox gene *GHF1* by cell-autonomous and environmental cues

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HOMEODOMAIN proteins^{1,2} function in determination of mating type in yeast³, segmentation in fruit flies⁴ and cell-type specific gene expression in mammals⁵. In *Drosophila*, expression of homeobox genes is controlled by cell-autonomous interactions between regulatory proteins and environmental cues^{6–8}. Similar controls may operate during mammalian limb development^{9,10} and frog embryogenesis^{11,12}. But, the exact way in which expression of homeodomain proteins is regulated in these systems is not clear and requires biochemical analysis of homeobox gene transcription. We now describe such an analysis of the *GHF1* gene, which encodes a mammalian homeodomain protein specifying expression of the growth hormone (GH) gene in anterior pituitary somatotrophs^{13–15}. *GHF1* is transcribed in a highly restricted manner and the presence of GHF1 protein is correlated both temporally and spatially with activation of the *GH* gene during pituitary development¹⁶. Analysis of the *GHF1* promoter indicates that transcription is also controlled by cell-autonomous interactions involving positive autoregulation by GHF1, and environmental cues that modulate the intracellular level of cyclic AMP and thereby the activity of cAMP response element binding protein (CREB)¹⁷, a ubiquitous transactivator that binds to the *GHF1* promoter.

We examined the regulation of *GHF1* promoter activity by protein factors in cell lines derived from a GH-expressing anterior pituitary tumour (GH3 or GC cells) compared with L cells which do not express GH and can eliminate GH and *GHF1* expression on fusion to GH3 cells¹⁸. *GHF1* was isolated from a rat genomic library¹⁹ and its start site of transcription was

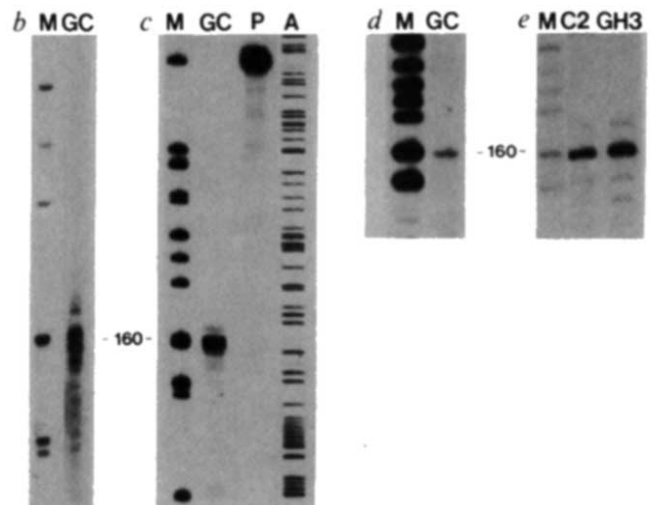
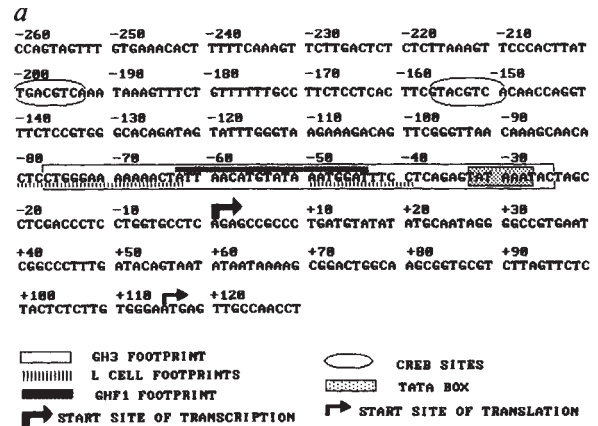


FIG. 1 Identification of the rat *GHF1* promoter. **a**, Nucleotide sequence of the *GHF1* promoter region. The locations of the TATA box, CREB binding sites, a GHF1 binding site and the GH3 and L cell-specific footprints are indicated. **b** and **c**, RNase mapping. Total GC cell RNA was hybridized to 780- or 310-nucleotide (nt) *GHF1* antisense riboprobes complementary to positions -560 to +160 (**b**) or -90 to +160 (**c**), digested with RNase and the protected products were separated on a sequencing gel and visualized by autoradiography. The predominant 160-nt protected fragment corresponds to transcripts initiated 27 bp downstream of the TATA box indicated in **a**. M, relative molecular mass (M_r) size markers; P, undigested probe; A-A, specific sequence ladder. **d**, Primer extension analysis of total GC cell RNA with a *GHF1* specific primer. Primer extended products were separated on a sequencing gel and visualized by autoradiography. **e**, Primer extension analysis of *GHF1* RNA transcribed *in vitro*. The p2.0GHF1-BS template was incubated with either C2 or GH3 whole cell extracts to generate *in vitro* synthesized *GHF1* RNA. The same *GHF1* primer used in **d** was used to analyse the RNA synthesized *in vitro*.

METHODS. Plaque-forming units (1×10^6) of a rat genomic library¹⁹ were

screened with a *GHF1* cDNA probe¹³. Duplicate nitrocellulose filters were washed stringently (50 °C, in $0.1 \times$ SSC, 50% formamide) and positive phages were rescreened with a *GHF1* cDNA 5'-specific probe. Of 16 positive clones, three hybridized to the 5' probe and the largest was subsequently subcloned into Bluescript (BS, Stratagene) as *EcoRI-EcoRV*, *EcoRV-EcoRV* and *EcoRV-SalI* fragments. Only the 2.0 kb *EcoRI-EcoRV* subclone (p2.0GHF1-BS) hybridized to northern blots of GC cell RNA (data not shown). The complete nucleotide sequence of this fragment was determined on both strands as previously described³². For RNase protection 20 μ g total RNA extracted from GC cells was resuspended in 20 μ l hybridization buffer in the presence of 1×10^6 c.p.m. of the different riboprobes³². Probes were synthesized from p2.0GHF1-BS using the T7 RNA polymerase as described by Stratagene. The 780-nt probe was generated by linearizing with *SacI* (-560), whereas the 310-nt probe was generated by linearizing with *HpaI* (-90). Both riboprobes end at the *EcoRI* site at position +160 of *GHF1* followed by 60 nt of Bluescript sequences. Hybridization was for 5 min at 80 °C and overnight at 45 °C. Hybrids were digested as described³² and protected RNA fragments were separated on a 6% polyacrylamide, 7M urea sequencing gel, and visualized by autoradiography. For primer extension analysis, 20 μ g total GC cell RNA was hybridized with 20 fmol³²P-labelled *GHF1*-specific primer (5'-GAATTCAGAGGTATAAAGGTATCTGCCGACCTGAAA-GGTTGGAAC-3') in 20 μ l of 250 mM KCl, 10 mM Tris buffer pH 7.5, 1 mM EDTA at 55 °C for 1 h. Primer extension was performed as described¹⁸. For *in vitro* analysis whole cell extracts were prepared as described¹⁸. Subclone p2.0GHF1-BS (100 ng) was incubated with 75 μ g of C2 or GH3 whole cell extracts for 15 min on ice in 30 μ l of 50 mM HEPES buffer, pH 7.9, 100 mM KCl, 12 mM $MgCl_2$, 1 mM DTT, 1 mM EDTA, 20% glycerol and 10 U of Promega RNasin. Transcription was initiated by addition of 20 μ l of 1 mM (each) rNTP and 5% polyvinylalcohol. After 1 h at 30 °C, reactions were stopped by addition of 150 μ l transfer RNA stop mix (200 mM NaCl, 20 mM EDTA, 1% SDS, 5 μ g tRNA) and analysed by primer extension as described above with the *GHF1*-specific primer.