Expression of zebrafish GATA 3 (gta3) during gastrulation and neurulation suggests a role in the specification of cell fate

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Expression of zebrafish GATA 3 (*gta3*) during gastrulation and neurulation suggests a role in the specification of cell fate

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Abstract

In order to understand the role of the transcription factor GATA 3 in vertebrate development, we have examined its expression and some aspects of its regulation during gastrulation and neurulation in the zebrafish. The complete coding sequence of the cDNA encoding the zebrafish GATA 3 homologue, termed *gta3*, is described. Analysis of expression patterns by in situ hybridisation shows the gene to be expressed during gastrulation in the ventral region of the embryo which includes tissue fated to form the non-neural ectoderm. By the end of gastrulation, there is a clear border to the *gta3* expression domain that is close to the edge of the neural plate. Subsequently, *gta3* expresses in the pronephric duct and in defined regions of the central nervous system which include specific cells in each segment of the spinal cord and nuclei in the brain. Double labelling embryos with a probe for *gta3* and antibodies which identify differentiated neurons suggest that *gta3* is dynamically expressed during the early differentiation phase of a subset of neurons but not in the terminal phase. Analysis of *gta3* expression in dorsalised embryos and in *cyc* and *spt* mutant embryos indicates that the neural expression of the gene is subject to control by signals from the mesoderm, including both the notochord and the somites, which influence the segmental organisation of expression in the spinal cord.

Keywords: GATA genes; Transcription factors; Gastrulation; Cell fate; Blood cells; Neurons; Development

1. Introduction

Many of the patterning events which underlie the generation of tissue diversity in the vertebrate embryo occur during gastrulation. One approach to studying the molecular basis of the spatial control of cell differentiation is to isolate and characterise nuclear regulatory proteins which are expressed during these early developmental stages. A recently described group of DNA binding proteins, the GATA family of transcription factors (Yamamoto et al. 1990; Orkin 1992), are attracting increasing attention as potential modulators of differential gene expression during differentiation of particular cell types. Members of this family recognise and bind to a consensus DNA sequence [T/A(GATA)A/G] from which they derive their name; proteins of each family member contain zinc finger DNA-binding domains which are very highly conserved. GATA 1–3 were initially shown to play a role in the control of differentiation of blood cell lineages. For example, mice which are homozygous for mutations in GATA 1 or GATA 2 genes lack specific sets of blood cells (Pevny et al., 1991; Tsai et al., 1994). GATA 3 was first characterised as a T-cell-specific transcription factor which activates transcription of T cell receptor genes (Ho et al., 1991; Ko et al., 1991). However, a number of lines of evidence now indicate that members of the GATA family play a more diverse role in the control of cell differentiation, affecting expression of genes in a broader array of cell types from early stages of development. Thus, although GATA 1 appears to be restricted to various blood cell lineages and testis (Martin et al., 1990; Ito et al., 1993), analysis of GATA 2 and 3 transcripts indicates that the genes are expressed during gastrula stages in *Xenopus* and that GATA 2 is a maternal message (Zon et al., 1991; Walmsley et al., 1994).

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Both genes are also transcribed in the CNS in *Xenopus* (Zon et al., 1991) and in chick (Engel et al., 1992). In addition, there are at least a further three GATA genes, GATA 4–6, which are predominantly expressed in the embryonic heart and gut (Arceci et al., 1993; Kelley et al., 1993; Laverriere et al., 1994).

Zebrafish provide an ideal model system to study gene expression patterns during early stages of development when they are transparent. In addition, the zebrafish is a newly established vertebrate system for the study of developmental genetics (Mullins et al. 1994) and a number of interesting mutants have been described which show phenotypes during the gastrula and early neurula stages, which are relevant to the analysis of the control of GATA-3 expression. In this paper, we describe the sequence for the zebrafish *gta3* cDNA and present a detailed description of the expression pattern of the gene up to 52 h of development. This analysis shows *gta3* transcripts to be present in the late blastula and subsequently in the ventral regions of the gastrula. Expression then occurs in a limited number of tissues including the pronephric duct and in specific populations of neurons in the developing CNS. Double labelling experiments indicate that neural expression of *gta3* occurs in the early phases of differentiation of specific subpopulations of neurons. Analysis of *gta3* expression in two mutants which affect specific events during gastrulation and neurulation, and in embryos which have been dorsalised by treatment with lithium, gives some initial insights into the mechanisms leading to the localised expression of *gta3* in the embryo.

2. Results

2.1. Characterisation of zebrafish GATA 3 (gta3)

In order to isolate GATA family members expressed in the zebrafish during early development, a neurula stage library was screened with the conserved metal binding finger region of the GATA family (Zon et al., 1991). A partial cDNA was isolated and used to rescreen the same library and to isolate two clones which between them contained the complete coding region as well as flanking untranslated sequences (Fig. 1A). At both the DNA and predicted amino acid level, the sequence was most closely related to GATA 3 cDNAs from other organisms (Fig. 1B) and we propose that it encodes the zebrafish homologue of GATA 3 (*gta3*).

The composite cDNA formed from the two overlapping clones isolated is 2576-base pairs long, including a polyA tail of 19 bases. The presence of a consensus polyadenylation signal 40 bases upstream of the polyA tail implies that this represents the true 3′ end of the mRNA and does not result from the oligo dT priming on internal oligo dA stretches which we found in a number of other clones. The first ATG in the cDNA begins an open reading frame of only 4 amino acids, however, the flanking sequences of this codon are predicted to be unfavourable to translation initiation (Kozak, 1987). The second ATG begins a 438 amino acid open reading frame encoding a predicted protein with high similarity to other vertebrate GATA 3 proteins. This ATG is in a much more favourable context for translational initiation (A at −3, G at +4; Kozak 1987), while the presence of in-frame upstream terminator codons indicates that this ATG corresponds to the initiator of translation of this open reading frame. The protein encoded is very similar in size to other vertebrate GATA 3 proteins (predicted M.W. 47.6kD) and contains the highly conserved C4 metal-binding domains characteristic of the GATA protein family. The protein sequence inside and outside this region places it within the GATA 3 subfamily (Fig. 1B).

2.2. Expression during gastrulation

In situ hybridisation of a range of blastula stages with a *gta3* probe shows that it is not present as a maternal (Fig. 2A) mRNA but that the message appears at about 4 hours post fertilisation (hpf) (Fig. 2B), soon after the mid blastula transition (MBT — Kane et al., 1992; Kane and Kimmel 1993). By 50% epiboly, the onset of gastrulation, *gta3* transcripts do not appear to be localised to an axis of the embryo but are confined to the yolk syncitial layer (YSL) and some of the outer most deep cell blastomeres (Fig. 2C). By 75% epiboly, expression is localised to a segment of the blastula (Fig. 2D). This region increases in size until the end of epiboly by which time the pattern of expression is a domain with an edge resembling the stitching of a baseball (Fig. 2E and F). To establish the axial location of the *gta3* expression domain, we double labelled embryos with anti-ntl antibody which reveals the dorsally located notochord. Such embryos show that *gta3* expression is localised to the ventral half, a region fated to become non-neural ectoderm (Kimmel et al., 1990, data not shown). This conclusion is reinforced by examining embryos double labelled by in situ hybridisation with *gta3* and *pax2* probes. At 10 hpf (95% epiboly), *pax2* is expressed in a stripe in the neural plate in the region of the presumptive midbrain, as well as in the pronephric duct (Krauss et al., 1991). The *pax2* midbrain stripe lies in the region of the embryo not expressing *gta3*, confirming the latter to be in ventral, non-neural tissue. In addition, there is a gap of a few cell diameters between the *gta3* boundary and the dorsal edge of the *pax2* stripe in the neural plate (Fig. 2G and H). If *pax2* expresses to the dorsal extreme of the neural plate at this stage, as it does in the eventual neural keel (Krauss et al., 1991), the boundary of *gta3* may lie at the outer edge of the more laterally positioned neural crest. Once gastrulation is complete, expression within cells of the ventral epiblast is down regulated.

2.3. Expression in the pronephric duct

We assessed the position of the boundary of the ex-

A

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AACGGACGGACTTTGTAAAAACAAATTGTCTTGTTTAATTTCAGGTGATCGGAAGTAAAA 60

AAAAATCGAACCAGTGGATTAAACAAAGAAGGAGTTTGGACTTTTTTTGAACTTTCGTGT 120

TGTGTGTATCGGTGAGTGTATGAACGTTTGTTAAAAAAATAAACGATGGAAGTAAGTCCG 180
                                                M  E  V  S  P   5

GAGCAGCACCGTTGGGTGACCCATCACACGGTCGGCCAGCATCCTGAGACACATCACCCA 240
E  Q  H  R  W  V  T  H  H  T  V  G  Q  H  P  E  T  H  H  P   25

GGACTGGGACACTCCTACATGGACCCGTCGCAGTATCAACTTGCGGAAGATGTGGATGTA 300
G  L  G  H  S  Y  M  D  P  S  Q  Y  Q  L  A  E  D  V  D  V   45

TTGTTTAATATCGACGGACAGAGCAACCATCCGTACTACGGGAACCCGGTTCGGGCCGTG 360
L  F  N  I  D  G  Q  S  N  H  P  Y  Y  G  N  P  V  R  A  V   65

CAGAGATACCCACCGCCACCTCACAGTAGTCAGATGTGTCGCCCATCGTTGCTGCACGGC 420
Q  R  Y  P  P  P  P  H  S  S  Q  M  C  R  P  S  L  L  H  G   85

TCTCTTCCTTGGCTCGATGGAGGCAAATCTATCGGCCCTCACCACAGTACCTCCCCCTGG 480
S  L  P  W  L  D  G  G  K  S  I  G  P  H  H  S  T  S  P  W   105

AACCTTGGGCCTTTCCCCAAGACTTCCCTCCATCACAGTTCCCCTGGGCCTCTGTCCGTC 540
N  L  G  P  F  P  K  T  S  L  H  H  S  S  P  G  P  L  S  V   125

TATCCTCCAGCTTCTTCCTCCTCGCTGTCCGCTGGCCACTCCAGCCCACATCTCTTCACC 600
Y  P  P  A  S  S  S  S  L  S  A  G  H  S  S  P  H  L  F  T   145

TTCCCCCCAACTCCACCCAAAGATGTGTCCCCTGACCCTGCAATTTCTACGTCAGGTTCG 660
F  P  P  T  P  P  K  D  V  S  P  D  P  A  I  S  T  S  G  S   165

GGCTCATCTGTTCGACAGGAAGACAAAGAGTGCATTAAGTACCAGGTGTCTCTGGCAGAA 720
G  S  S  V  R  Q  E  D  K  E  C  I  K  Y  Q  V  S  L  A  E   185

AGCATGAAGCTGGATTCGGCTCATAGTCGGAGCATGGCCTCAATCGGAGCGGGCGCGTCC 780
S  M  K  L  D  S  A  H  S  R  S  M  A  S  I  G  A  G  A  S   205

TCCGCGCACCACCCCATCGCCACTTACCCTTCCTATGTACCCGATTATGGGCCGGGGCTC 840
S  A  H  H  P  I  A  T  Y  P  S  Y  V  P  D  Y  G  P  G  L   225

TTCCCACCAAGTAGCCTGATAGGTGGGTCCTCTTCTAGTTATGGTTCCAAAACGAGACCA 900
F  P  P  S  S  L  I  G  G  S  S  S  S  Y  G  S  K  T  R  P   245

AAAACAAGGTCCTCTTCAGAGGGTAGAGAATGTGTAAACTGCGGGGCCACCTCGACTCCT 960
K  T  R  S  S  S  E  G  R  E  C  V  N  C  G  A  T  S  T  P   265

CTGTGGCGGAGGGATGGCACCGGTCACTATTTGTGTAACGCCTGCGGACTTTACCACAAG 1020
L  W  R  R  D  G  T  G  H  Y  L  C  N  A  C  G  L  Y  H  K   285

ATGAACGGACAGAACCGACCCCTCATCAAGCCCAAACGGAGGCTGTCTGCTGCCAGACGA 1080
M  N  G  Q  N  R  P  L  I  K  P  K  R  R  L  S  A  A  R  R   305

GCTGGGACCTCATGCGCAAACTGTCAAACGACCACGACGACACTGTGGCGGAGAAATGCC 1140
A  G  T  S  C  A  N  C  Q  T  T  T  T  L  W  R  R  N  A   325

AACGGCGACCCTGTCTGCAATGCCTGCGGCCTGTATTACAAATTACACAATATCAACCGA 1200
N  G  D  P  V  C  N  A  C  G  L  Y  Y  K  L  H  N  I  N  R   345

CCGCTCACCATGAAGAAGGAGGGCATCCAGACCCGGAACAGGAAAATGTCCAGCAAGTCC 1260
P  L  T  M  K  K  E  G  I  Q  T  R  N  R  K  M  S  S  K  S   365
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AAGAAGAGCAAAAAGTCCCATGACAGCATGGAGGATTTCTCCAAAAGCTTGATGGAAAAG 1320
K  K  S  K  K  S  H  D  S  M  E  D  F  S  K  S  L  M  E  K   385

AACAGTTCCTTCAGCCCGGCCGCCCTGTCGCGTCACATGACCTCGTTCCCTCCCTTTTCG 1380
N  S  S  F  S  P  A  A  L  S  R  H  M  T  S  F  P  P  F  S   405

CACTCGGGCCACATGCTCACCACACCCACCCCAATGCACCCCTCCTCTAGCCTGCCCTTC 1440
H  S  G  H  M  L  T  T  P  T  P  M  H  P  S  S  S  L  P  F   425

GCCTCACACCACCCATCTAGTATGGTGACGGCGATGGGCTAAAGCGACGGCCCTGCTACC 1500
A  S  H  H  P  S  S  M  V  T  A  M  G  *                     438

TCCAATCTCCCACCGGTGGACATCTCAAAGCACCGTTAACCTTGAAGCCTGTCACCCGCC 1560
GCAACCCTCCAGCTCCCTCACGCGCTACATGGCCCCCCCTTTGATACCTGTCCCGCTTAA 1620
AACCCTTATTTCCCCCGTATTACAAGGAAAGAGAACAGACAATGACTTTCTTCATTGCCC 1680
CTTCAACCTTGAAGCCTCGCACTGATGTCAACCTTCATTTGTGTACAAATGCCATCTTTC 1740
CTGTGGGCATCGAGGCACAAACATTTACCATACTTTAAAGTAACCTTCTCTAACTCCAAA 1800
ACCCCTTCTTCCTCTTGATCGCATCTCCTCTACTTCATCCCCCAACCTGTGAGACACGAG 1860
GAATCGAGCTGTGAATGATAACAGGAGATGAACTCTGCATATATTTGTAAAAAAAAATGT 1920
CTGAAATACATTTAATGAAGACTTTTTAAAAGAGCGAAAAGGCGAGGACGGGAAATGCCC 1980
GCGGGCAGCTATTGATAAGAAGGGTACGACTTAAACCTGCAAGGTGGAATGACTCTGTCA 2040
CGATTTCTATCCGAAAAGTAGGAGGTGAGATGTAGGGAGAGGAAACCTAGAATGGAAATG 2100
GTGTGTAATGCATTAAAACTCGTAGTCAGGTCTGTCTTGCAGACTTCCAGCTACTCTTCT 2160
GGATGTGTTATAGGTCTTGGGCAATTGGTTTGTGTTCACGTACACATACACATACGCACA 2220
TGCACACACACACACACATACATGCACACACACGCACGCACACACACTCTCTCACATATA 2280
CACCAGAAAGACTTCTTTGGGCCCAGGTTATATGCATTGTGAAAAAAAGTTCCTGTATTT 2340
GTTATTTGTATGTATAATTCAGAGTACCAAAAATACGAAAGAAACAAAGATGTAGAATTA 2400
TTTCTTTATGTAATGCAGACTGAATGGTTGTACAAATTTAATAATCACTAGTGTAAAAAG 2460
AAGACTTGCTTTTTTGTTGCGTAATTTTTTTTTCCCTCTTTGAAAATAATAAACTAGTTT 2520
AATCTCTGTTGACATCATACAAGAATGCCACGCCAAGAAAAAAAAAAAAAAAAAAA      2576
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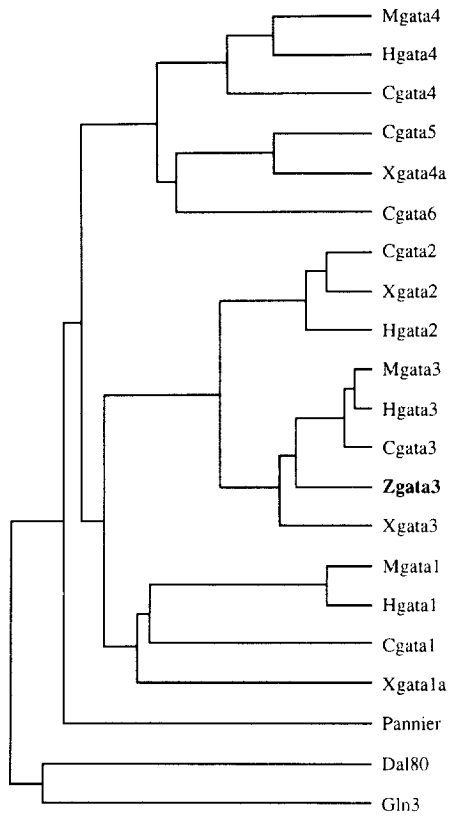
B



pression domain relative to the differentiation of lateral mesodermal structures from the end of epiboly up to 24 hpf. Sections of embryos between these time points are shown in Fig. 3. By 10 hpf the boundary of expression is sharp (Fig. 3A); however, we cannot determine whether cells at the boundary are limited to epiblast (future ectoderm) or hypoblast (future mesoderm and endoderm). By 13 h, there is an obvious band of *gta3* positive cells within the mesoderm which correspond in position to cells in the intermediate (nephrogenic) mesoderm (Fig. 3B). These localised regions of expression become more defined by 16 hpf (Fig. 3C) and by 24 hpf it is clear that these local regions of expression are in the pronephric ducts (Fig. 3D). As the expression within the duct becomes more established the earlier boundary of expression becomes less distinct. Expression within the pronephric duct is restricted to the caudal third of the tube (Fig. 5A). Consistently, a small cluster of *gta3* expressing cells was found at more rostral levels of the duct (Fig. 5A). Pronephric expression was still present at 52h, the latest stage studied.

2.4. gta 3 is expressed in specific neurons of the central nervous system

During neurulation, *gta3* displays a third pattern of expression following the domains previously described during gastrulation and the formation of the pronephros. The first detectable expression in the central nervous system occurs by 15 hpf in cells of the spinal cord. Expressing cells are segmentally arranged in small groups within the ventral half of the spinal cord. There appears to be an association between the location of the first expressing cells within the neural tube and the boundaries between forming somites (Fig. 5A). Spinal *gta3* expression begins in the trunk region and spreads more caudally as the tail bud develops. Expressing cells are found within the ventral half of the spinal cord (Fig. 4A); however, within each segment the pattern of cells is not identical (Fig. 5A and B). From 36 hpf, the level of expression in spinal cord cells decreases but remains detectable at a lower level.

The first rostrally located *gta3* expressing cells are located in the ventral midbrain, in a group of cells in the position of the nucleus of the medial longitudinal fasciculus (nMLF), 20 hpf (Fig. 4B–D). Expression in cells of the hindbrain occurs by 28 hpf in a broadly metameric pattern (Fig. 4E). Subsequently, expression occurs more broadly in the ventral midbrain and diencephalon (Fig. 4E) and by 40 hpf in cells of the optic tectum (Fig. 4F). Transcripts are also detected within files of mesenchymal cells around the eye (Fig. 4F) and in the ventral region of the otic vesicle. By 52 hpf, expression in the hindbrain is restricted to medially located cells (Fig. 4G), some of which maybe neurons of the raphe nuclei which occupy this location in other fish (see, for example, Meek and Joosten 1989).

To assess the timing of expression of *gta3* in differentiating cells, hybridised specimens were labelled with antibodies to HNK-1. This antibody was used to reveal differentiated neurons and axon tracts (Wilson et al., 1990). The relationship between *gta3* expression and neural differentiation is particularly clear in the region of the forming nMLF (Fig. 4B–D). By 24 hpf, *gta3* cells within this region are located away from the ventricular surface and, therefore, are probably post mitotic (Fig. 4B). Antibody stained embryos show that differentiated neurons within then MLF do not express *gta3* despite these cells being intermixed (Fig. 4C and D). This observation suggests that cells destined to be neurons of the MLF nucleus express *gta3* transiently during their differentiation, but by the time that they are immunoreactive for HNK-1 they no longer express the gene.

2.5. Ectopic expression of gta3 occurs in dorsalised embryos

Inductive signals from the notochord contribute to the patterning of the ventral regions of the spinal cord (Jessell and Dodd 1992; Yamada et al. 1993). In order to establish if the transcriptional regulation of *gta3* in the spinal cord is regulated by the signalling pathway initiated by the notochord, we analysed expression of *gta3* in lithium-treated embryos which possessed ectopic

Fig. 1. (A) Zebrafish *gta3* sequence. Composite *gta3* cDNA sequence derived from two overlapping cDNA clones. The predicted translation product of the major open reading frame is shown. This begins at the second ATG of the cDNA, which lies in a favourable context for translational initiation (Kozak, 1987 and see Results). The metal co-ordinating ('Zinc finger') DNA binding domain conserved amongst the GATA family is shown underlined. The 5′ ATGs and the polyadenylation signal (AATAAA) sequence are shown in bold. (B) Dendrogram of pairwise clustering of GATA proteins generated by the program Pileup (Genetics Computer Group, 1991). The alignment included human (H), mouse (M), chicken (C) and *Xenopus* (X) GATA proteins, the two-Zn^{2+}-finger *Drosophila* GATA protein, *pannier* (also known as dGATAa — Ramain et al., 1993; Winick et al., 1993), as well as zebrafish GATA 3. An outgroup was provided by the one-finger GATA-binding proteins DAL80 and GLN3 of *S. cerevisiae*. (Minehart and Magasanik 1991; Cunningham and Cooper 1991). This dendrogram is not a phylogenetic reconstruction. The horizontal branch lengths are, however, proportional to the similarity between the protein sequences (calculated using a modified Dayhoff matrix (Schwartz and Dayhoff 1979; Gribskov and Burgess 1986).The gene that is the subject of this study clearly clusters with GATA 3 of other vertebrates, showing that it is likely to encode zebrafish GATA 3. The alternative possibility, that it is a highly diverged GATA 2, is precluded by the fact that we (A.R. unpublished data) and others (L. Zon pers. comm.) have cloned the zebrafish homologue of GATA 2. In addition, this clustering is in agreement with the suggestion of Laverriere et al., (1994) that GATA 1/2/3 and 4/5/6 form distinct subfamilies, and that Xenopus GATA 4 is not the homologue of human, mouse and chicken GATA 4s and should be renamed GATA 5

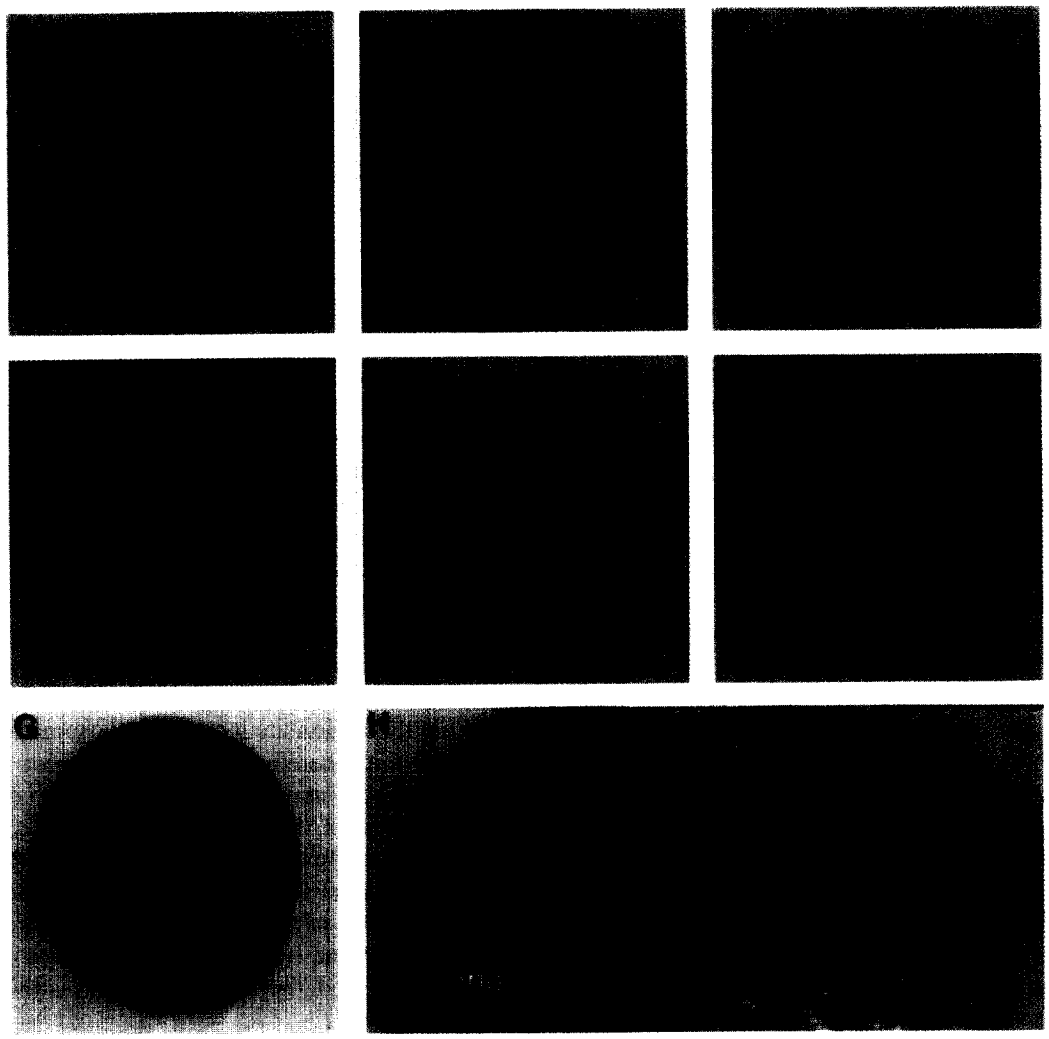


Fig. 2. Expression of *gta3* during gastrulation. Lateral views are shown and dorsal is right except in (F) which is an animal pole view. (A) Eight-cell embryos showed no specific staining (y: yolk). (B) By 4 hpf the blastula shows low levels of *gta3* transcripts throughout the embryo. (C) At 50% epiboly *gta3* is expressed close to the epibolising edge. At this stage *gta3* is still broadly expressed and is evident in the yolk syncitial layer (arrows). (D) As epiboly continues, *gta3* expression becomes localised to the ventral side of the embryo, clearly seen by 75% epiboly. The domain of *gta3* positive cells does not include the germ ring; the epibolising edge is marked by the arrows. (E) 95% epiboly. The domain of *gta3* expression increases in size during epiboly until the yolk plug (arrow) closes. (F) The expression is clearly restricted to the ventral side and a clear boundary, marked by the arrows has emerged which is seen clearly in a view from the animal pole. (G) The border of *gta3* expression was analysed with respect to the expression of the neural marker *pax2*. At 95% epiboly, *pax2* expresses in a stripe of cells extending out towards the edge of the neural plate (np) in the region of the presumptive midbrain/hindbrain border. (H). At high power, a clear gap can be seen between *gta3* cells in the non-neural ectoderm (nne) on the ventral side and *pax2* cells within the neural plate (np). Scale bar is 100 μm.

notochords (Stachel et al. 1993). Following lithium treatment, embryos exhibiting radialised phenotypes were selected at 24-26 hpf. In such embryos, *gta3* expression is present in cells in induced neural tissue overlying the ectopic notochords (Fig. 4H).

2.6. *gta3* expression varies in mutant embryos

gta3 expression was analysed and compared in mutant embryos in order to begin an assessment of the possible interactions involved in controlling its expression in neuronal and mesodermal cells. *gta3* expression was analysed in two well-characterised mutant lines *cyc* ^{b16} (*cyclops*) and *spt* ^{b104} (*spadetail*). In each case, the position and number of expressing cells per 100 μm rostrocaudal section of the cord was recorded from whole mounted embryos in camera lucida drawings. This allowed a direct statistical comparison of the number of *gta3*-expressing cells to be made with wildtype, as well as giving a clear indication of any alteration in relative position of these cells within the cord. Photographs of

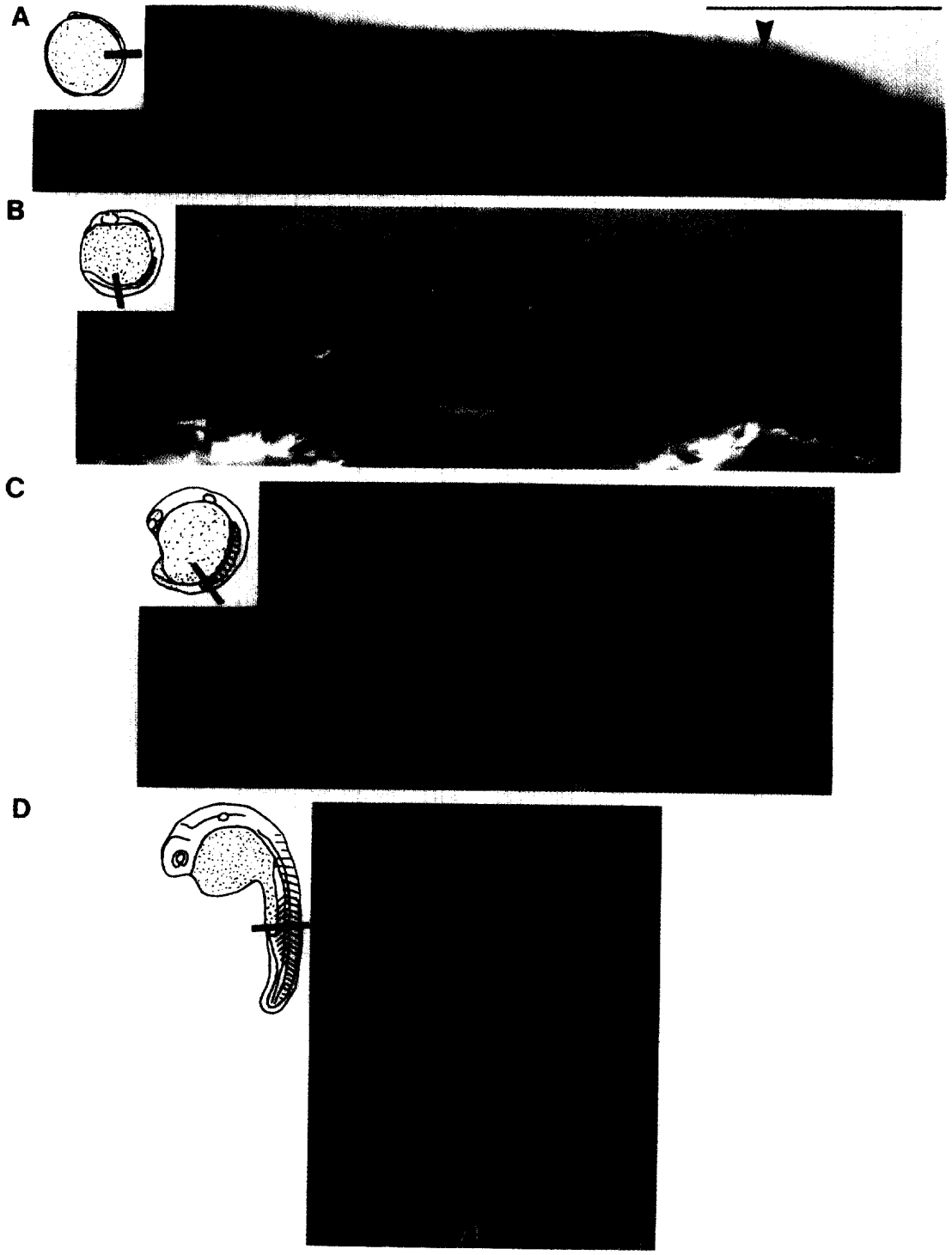


Fig. 3. *gta3* expression was analysed during the differentiation of the intermediate mesoderm into the pronephric duct. Manual transverse sections of the embryo were used except for the 10 μm wax section in (D). Dorsal is top and the cartoons to the left hand slide depict where the section was taken. (A) 100% epiboly, high power view showing the right half of the neural plate (np). Ventral non-neural ectoderm (nne) *gta3* expression is right and the neural plate (np) is to the left. The boundary between them is shown by the arrow. The expression appears in the epiblast (e) and the hypoblast (h). (B) At 13 hpf, mesodermal cells expressing *gta3* appear in a localised region (arrow), which is the first sign of expression in the intermediate mesoderm. This is separate from the more lateral non-neural ectoderm (nne) expression. (C) By 16 hpf the neural keel (nk), notochord (n) and somites (s) are clearly visible, with high levels of *gta3* expression in the formed tubes of the pronephric ducts (pd) shown by the arrow. (D) A 24 hpf embryo showing the pronephric ducts (arrow) running down the lateral side of the embryo above the yolk tube (yt). Expression in the neural tube (nt) can also be seen. Scale bars (for A and B–D) are 100 μm.

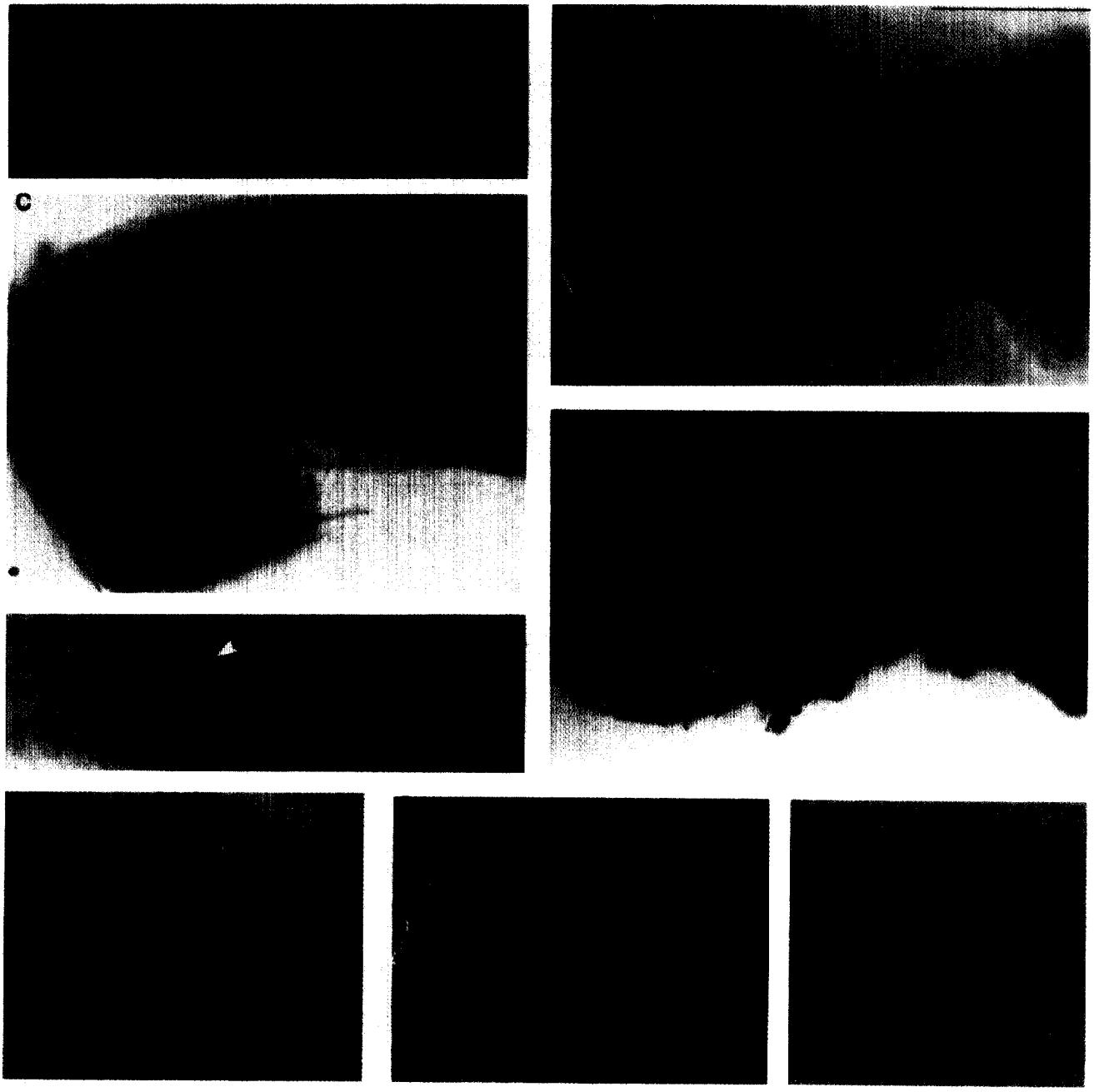


Fig. 4. Expression of *gta3* in the CNS. Views are lateral with anterior left and dorsal top except in (B) which is a dorsal view. (F and G) manual transverse sections. (A) Lateral view of a 24 hpf spinal cord showing the position of *gta3* positive neurons in the ventral half of the spinal cord. Notochord, n. (B) *gta3* is expressed in cells emanating from the ventricular surface of the midline (ml) towards the site of the nucleus of the MLF (nmlf) at 24 hpf. (C,D) A 24 hpf embryo labelled with *gta3* by in situ hybridisation and HNK-1 antibody. *gta3* is blue (white arrow) in the cytoplasm and the antibody label is brown (black arrow). HNK-1 labels the cell bodies, axons and the golgi apparatus, which appear as dark regions in the labelled cells. (D) Shows a high power view of the group of MLF cells shown in (C). These do not contain *gta3* cells which coexpress the HNK1 epitope. (E) At 36 hpf, *gta3* expression is spread throughout nuclei of the mid and hindbrain (hb). (F) Transverse sections at the level with the lens (l) of the eye shows *gta3* expression in the dorsal tectum (te) and ventral tegmentum (tg) at 48 hpf. (G) At 52 hpf, this expression continues further back in the hindbraln past the level of the otic vesicle (ov) where expression is localised to medial cells which may be those of the raphe nucleus. (H) Embryos treated with lithium chloride are hyper-dorsalised with the resulting multiple axes evident at 24 hpf. *gta3* expression occurs among cells along the axes which is similar to its expression along the spinal cord at that time. Scale bar is 100 μm.

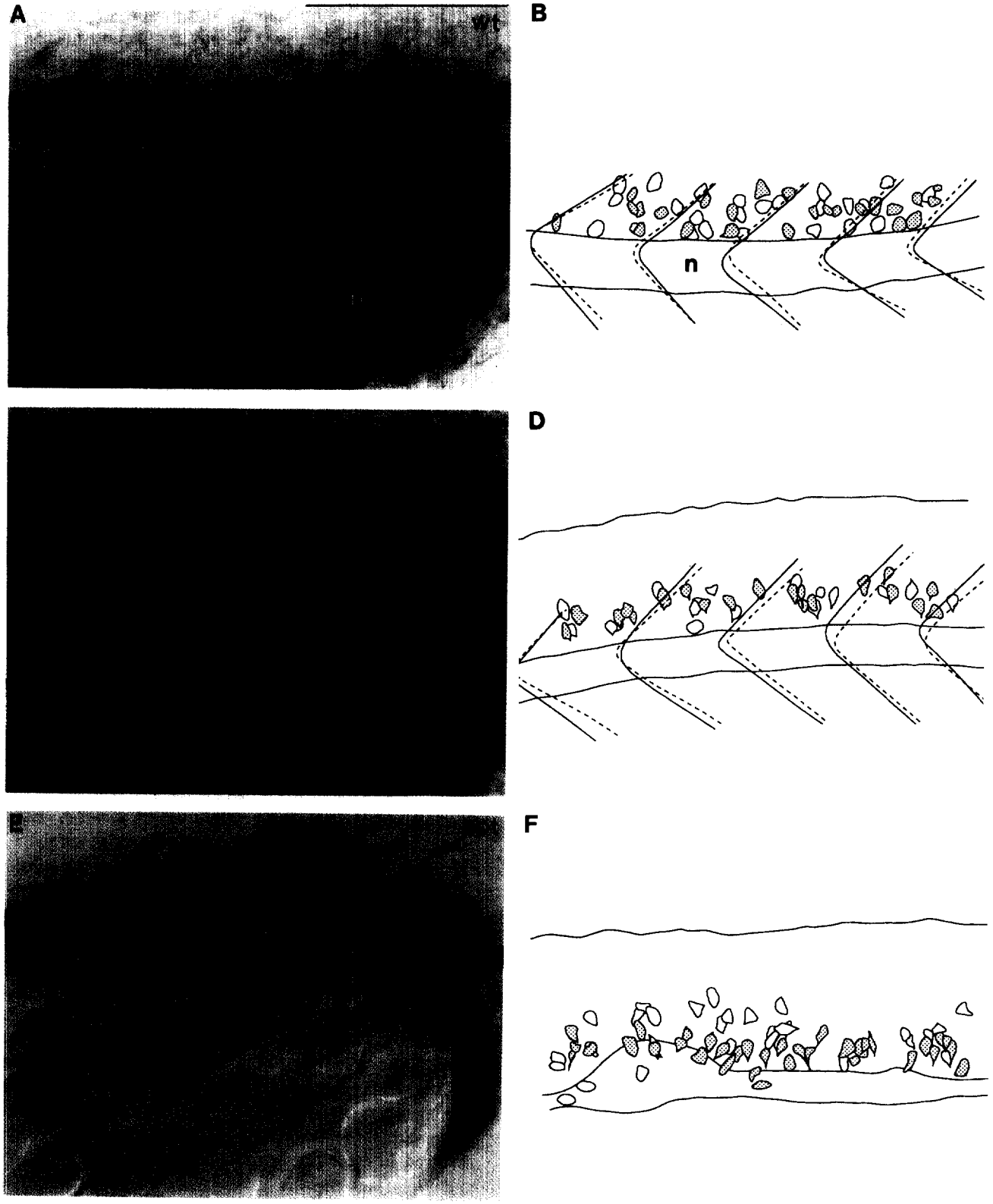


Fig. 5. *gta3* expression varies in the spinal cord and pronephric duct (pd) of mutant embryos. Anterior is left with dorsal at the top of wild type (A and B), homozygous *cyc* (C and D) and *spt* (E and F) embryos at 24 hpf. Photographs (A, C and E) and camera lucida drawings (B, D and F) are of the trunk region. *gta3*-positive cells were drawn and hatching indicates cells from the contralateral side. The notochord (n) and somite boundaries are also shown. Wild type embryos (A and B) show *gta3* cells throughout the spinal cord adjacent to the formed somites. *cyc* embryos (D and E) show fewer *gta3* cells in the same region (see text) but the organisation of expressing cells appears normal. *spt* embryos (E and F) have a similar number of cells to control but their organisation within the spinal cord is disrupted. In all the embryos, there is no difference in the expression of *gta3* in the pronephric duct (pd). Yolk tube, yt. Scale bar is 100 μm.

the mid trunk region of representative embryos and drawings from corresponding positions are shown in Fig. 5, wildtype embryos appearing in Fig. 5A and B.

2.6.1. cyc ^{b16} Alteration of *gta3* expression in homozygous *cyc* mutant embryos can be explained in either of two ways. (i) *gta3* regulation in spinal cord is dependent upon signals from the floor plate which is lacking in mutant embryos (Hatta et al., 1991) or (ii) *cyc* is acting autonomously on *gta3*-expressing cells. The differences in the mutant embryos (Fig. 5C and D) can best be seen by comparing them with the wild type embryo (Fig. 5A and B). The results show that the segmental pattern of expression is maintained, however, comparison with wildtype embryos shows a statistically significant reduction in the number of cells per 100 μm which are *gta3* positive (wt, 29.6 +/− 1.4; *cyc*, 20 +/− 3.03 [$n = 6$, $P = 0.1$]).

2.6.2. spt ^{b104} In homozygous *spt* embryos, cells fated to become trunk mesoderm migrate incorrectly, heading towards the tail during gastrulation; consequently, posterior ventral structures, including trunk and tail somites, the pronephros and the blood, develop abnormally (Kimmel et al., 1989). In the spinal cord of mutants, *gta3*-expressing cells are present but they have lost the segmental arrangement evident in normal embryos (compare Fig. 5E and F to 5A and B). Analysis of numbers of expressing cells indicated that there was no significant difference in the *spt* embryos as compared to controls (wt, 29.6 +/− 1.4; *spt*, 37.8 +/− 8.4, $n = 6$). Consistent with the observation that the pronephric duct is often malformed in spadetail mutants, *gta3* expression was also variable in this region (Fig. 5E) but it was seldom abolished.

3. Discussion

We have isolated a cDNA encoding the zebrafish homologue of GATA 3 (termed *gta3*). This is shown by the sequence similarity at both the nucleic acid and protein level. Clustering of pairwise comparisons within the GATA family (Fig. 1B) shows that the protein encoded by the cDNA lies within the GATA 3 subfamily. Probes derived from this cDNA have been used to investigate the appearance of *gta3* mRNA in normal and mutant zebrafish embryos in order to gain insights into the normal role of this gene and the mechanisms which control its spatially localised pattern of expression.

The initial pattern of *gta3* expression in zebrafish blastulae and gastrulae shows that the gene is regulated spatially, a feature characteristic of many regulatory genes involved in the patterning process. The transcripts are initially localised to a single outer layer of deep cell blastomeres and to the YSL. Although the function of the YSL is unknown, it has been implicated in the control of gastrulation movements, particularly epiboly (Trinkaus 1984, 1992; Warga and Kimmel 1990; Strahle and Jesuthesan 1993) and in the patterning process (Long 1983). To date, *gta3* is only the second nuclear regulatory gene to be shown to be expressed there, the other being *snail* (Thisse et al., 1993). During gastrulation, *gta3* transcripts became more restricted and are located in blastomeres fated to form ventral, non-neural ectoderm (Kimmel et al., 1990). This expression domain is similar to that for GATA 2 at this stage in both zebrafish (Rodaway et al., unpublished) and *Xenopus* (Walmsley et al., 1994). In *Xenopus*, this early GATA 2 expression domain is influenced by the distribution of dorsalising signals such as activin and noggin which down-regulate GATA 2 transcription in animal cap experiments and in the mesoderm. It is possible, therefore, that the more spatially restricted expression pattern seen for *gta3* reflects the activity of mesodermal and neural inducing agents prior to and during gastrulation. During gastrulation, the *gta3* expression domain rapidly expands to give a negative outline to the region of the embryo which includes the neural plate. This domain, which has the appearance of a baseball, has a clear edge which is positioned several cell diameters lateral to the lateral margin of the *pax2* expression domain in the presumptive midbrain. Subsequently, in the differentiating neural tube, *pax2* expression includes the dorsal roof plate, but not the neural crest (Krauss et al., 1991; Puschel et al., 1992). It is possible, therefore, that the gap of several cell diameters at the margin of the *gta3* boundary represents cells of the presumptive neural crest. This expression pattern suggests that *gta3* may play a role in determining the non-neuronal phenotype within the epiblast. This notion is consistent with the role of one of the GATA genes recently identified in *Drosophila* (Ramain et al. 1993) called *pannier*. This gene is normally expressed in a spatially regulated manner in imaginal discs and null alleles cause an overproduction of peripheral neurons. The normal function of the *pannier* gene is to negatively regulate the expression of *achaete-scute* genes (Ramain et al. 1993) which are normally required for the differentiation of the neural phenotype in flies (Campuzano and Modelell 1992; Campos-Ortega 1993). Homologues of *achaete-scute* genes in vertebrates, called *ash* genes, have been identified in rat and *Xenopus* (Johnson et al. 1990; Ferreiro et al. 1992; Turner and Weintraub 1994). In both species, members of the *ash* class are expressed in the neural plate and in *Xenopus Xash 3* has been shown to be involved in establishing the neural phenotype (Turner and Weintraub 1994).

The likelihood that the boundary of *gta3* expression has some functional significance in patterning is further strengthened by the observation that expression of other regulatory genes, such as zebrafish *dlx3* anteriorly (Akimenko et al., 1994) and *pax2* posteriorly (Krauss et al., 1991, Puschel et al., 1992) reveal a similar boundary. Once differentiation of cells begins within these regions

of the embryo, the gene is expressed in particular cell types such as neurons in the spinal cord and brain and in cells of the pronephric wall. *gta3* may be functioning both during the early patterning process and later during the differentiation of precise cell types; it may be, therefore, that the protein is regulating the transcription of different genes in the two phases of development.

gta3-expressing cells emerge in the central nervous system, in the brain and the spinal cord, between 15 and 20 hours. In the cord, cells with *gta3* transcripts are organised into a distinct pattern which has a clear segmental arrangement, yet, in each segmental group the exact position of labelled cells varies. In the light of other observations described below, our interpretation of this result, which is illustrated in Figs. 4A and 5A and B, is that *gta3* is expressed in a subset of ventrally located neuronal cell types and that the gene is transiently expressed by many such cells during their differentiation, a point we shall return to below. The ventral location of this subgroup of neurons indicates that it is likely to include some or all of the primary motor neurons (Myers et al., 1986; Eisen, 1992) and a subset of interneurons, at least some of which have been previously described (Bernhardt et al., 1990). We are currently trying to establish precisely the identity of the members of the *gta3* subgroup of identified neurons.

Analysis of *gta3*-expressing cells in lithium-treated embryos and in the *cyc* and *spt* mutant fish allows us to begin to elucidate some of the signalling pathways which lead to a sub-population of neural cells transcribing the gene. When given to embryos prior to the mid-blastula transition, lithium causes dorsalisation of the embryo, one manifestation of which is the formation during gastrulation of multiple notochords and overlying neural tissue (Stachel et al., 1993). Our analysis shows that in such embryos, *gta3*-expressing cells are found in this ectopic neural tissue (Fig. 4H), indicating that the pathway leading to the induction of *gta3* transcription in some spinal cord (and probably brain) cells is triggered by the initial inductive signal from the notochord (Yamada et al., 1993). Furthermore, the maintenance of *gta3* expressing cells in the ventral region of the spinal cord in *cyc* embryos (Fig. 5C and D), which lack a floorplate, indicates that a floorplate-derived signal, which is thought to influence the character of ventral neuron types, is not necessary to induce *gta3* expression. It is likely, because *cyc* embryos have a normal notochord (Hatta et al., 1991), that ventral spinal cord patterning signals are supplied by this structure (Jessell and Dodd 1992). The conclusion that a signal from the notochord can pattern the ventral spinal cord is consistent with the findings of other studies which have assessed the differentiation of cells in the spinal cord of *cyc* embryos. These studies have shown that primary motor neurons (Eisen 1992; Bernhardt et al., 1992) and specific classes of interneurons (Bernhardt et al., 1990) differentiate normally; in addition, we have recently observed that a pair of ventral spinal cord cells in each segment which express the receptor tyrosine kinase gene *rtk2* differentiate normally in such mutants (Xu and Holder unpublished results). Thus it is clear that the majority of neuronal cells are not affected by absence of the floor plate. However, there is a class of spinal interneurons, the Kolmer-Agduhr (KA) cells, which have been observed not to form in normal numbers in *cyc* embryos (Bernhardt et al., 1992). It is possible, therefore, that some of the *gta3* expressing cells in each segment are KA interneurons and that the reduced numbers of *gta3* expressing cells in *cyc* could in part be explained by the reduction in KA cells.

In contrast to *cyc* embryos, evidence from the analysis of primary motorneurons in *spt* mutants indicates that the segmental organisation of neurons in the trunk region is lost (Eisen and Pike, 1991). A similar interpretation may explain two other observations; that spinal cord cells which express the nuclear protein islet 1 (Inoue et al. 1994) or the membrane receptor *rtk2* in *spt* mutants (Xu and Holder, unpublished results) show abnormal segmental organisation. Analysis of *gta3*-expressing cells in the spinal cord of *spt* embryos would support this conclusion as the pattern of these cells is very disorganised. Thus, we can conclude that the somites, which are abnormally formed in the trunk and tail region of *spt* mutant embryos, normally influence the spatial arrangement of *gta3*-expressing cells in the spinal cord

The pattern of *gta3*-expressing cells in each cord segment suggests that the gene is only transcribed transiently during the differentiation process of individual cells within the subgroup. Evidence in support of this possibility comes from assessment of embryos double stained for *gta3* and HNK-1, used in this case to identify neurons which have formed an axon and which have, therefore, fully differentiated. The relationship of cells expressing the two markers was most clearly visible in the forming *mlf* nucleus in the midbrain and, although *gta3*-expressing cells were clearly present in the nucleus, no double-labelled cells were seen, suggesting that the neurons of the *mlf* nucleus down regulate *gta3* before they finally differentiate. If this is the case, *gta3* is expressed in neurons transiently following their birth at the ventricular zone. As a result of this conclusion, it must be a possibility that *gta3* is expressed transiently during the differentiation of the other cell types in the developing CNS in which transcripts are found; it is this possibility that leads us to conclude that the *gta3*-expressing neurons in the spinal cord are members of a subgroup in each segment, the slight variation in location of expressing cells from segment to segment reflecting the position of each cell as it differentiates (Fig. 4A). The overall temporal pattern of *gta3*-expressing cells is also consistent with this general conclusion; thus,

from 36 hpf *gta3* is down regulated in all spinal cord cells. The possibility is that all of the neurons in the classes expressing the gene have differentiated by this time. More rostrally, *gta3*-expressing cells are, by this stage, located in later differentiating regions, such as the raphe system in the hindbrain and the tectum in the midbrain. It is interesting in this regard that the GATA 3 gene in chick is also expressed in the midbrain tectum (Engel et al., 1992; Kornhauser et al., 1994). Down regulation of *gta3* in cells prior to their final differentiation means that it is not as yet possible to prove that expressing cells are neurons; in principle, they could be glial cells. However, this is unlikely because the patterns of expressing cells are very localised and correspond to known neuronal nuclei in the brain and to a limited group of cells in the spinal cord. There has been no prior description of glial cells which have such characteristic localised features of differentiation.

Finally, with the strong indication that the first three GATA family members are functionally involved in controlling differentiation in specific lineages of the blood system (Orkin 1992), we were interested in examining a possible link between *gta3* expression and haematopoiesis in the zebrafish. The current study provides no direct evidence for such a mechanistic association, however, there are two points worthy of further discussion. Firstly, *gta3* is expressed in cells in the distal third of the pronephric duct. Al-Adhami and Kunz (1977) have argued, based on histological criteria in zebrafish, that initially the pronephros (from the 8th to the 12th day) and subsequently the mesonephros (from the 30th day) are sites of generation of erythrocyte, granulocyte and lymphocyte lineages. It is possible, therefore, that *gta3* expressing cells in the pronephric duct, which are evident from as early as 13h, subsequently give rise to blood cells in the pronephros and mesonephros. Secondly, this interpretation is consistent with a recent finding by Dzierzak and her colleagues (unpublished results) that the early blood stem cell marker Sca 1 is first expressed in cells of the mesonephric wall in the mouse embryo. The study of Al-Adhami and Kunz (1977) additionally demonstrates that the first blood cells are formed in large part from a region of mesoderm they refer to as the intermediate cell mass, which lies lateral to the somites but which is separate from the pronephric duct. This region of the embryo may correspond to an area in the mouse embryo which includes the aorta and intermediate mesoderm which generates the first blood stem cells (Medvinsky et al. 1993). This source of blood cells would, therefore, be separate from the later site in the pronephric duct and the mesonephros. It is most likely, therefore, that the loss of blood observed in the *spt* mutant embryos results from an effect on the intermediate cell mass. Although the *gta3* expressing cells in the pronephric duct are also affected in *spt* embryos, the effects on blood would not be seen until several days later when the kidney is active in haematopoiesis (Al-Adhami and Kunz 1977). In summary, our conclusion is that if the *gta3* positive cells in the pronephric duct are involved in haematopoiesis in the zebrafish, it is in the generation of the definitive (later phased and kidney derived) rather than the primitive (early phased and intermediate cell mass derived) blood lineages. This is consistent with the finding that, compared to the related genes GATAs 1 and 2, GATA 3 is involved in definitive haematopoiesis in other vertebrates (Yamamoto et al., 1990; Briegel et al., 1993; Leonard et al., 1993).

4. Experimental Procedures

4.1. Maintenance of fish

Breeding fish were maintained at 28.5°C on a 14-h light/10-h dark cycle. Embryos were collected by natural spawning. Homozygotes for the recessive lethal cyclops (*cyc*-1, b16) (Hatta et al. 1991) and spadetail (*spt*-1, b104rl) (Kimmel et al. 1989) mutations were bred from heterozygotes. Embryos were raised in 10% Hanks saline with 0.2 mM Phenylthiocarbamide (Vischer 1989) at 28.5°C and staged according to 'The Zebrafish Book' (Westerfield 1989). For lithium treatment, embryos between the stage of 64 to 1024 cells were exposed to 0.3 M LiCl in 10% Hanks for 10 min at 28.5°C (Stachel et al. 1993).

4.2. Nomenclature

The recently accepted nomenclature for the naming of genes in the zebrafish is to use three letter designations in lower case followed by a number if the gene is a member of a family (Westerfield 1993). The gene name is given in italics and the protein product in normal case. The GATA family of genes have traditionally been described as four letter designations with capitals. We have decided to adhere to the nomenclature for the organism rather than the gene to maintain consistency with the rapidly growing list of genes and cDNAs isolated in the zebrafish. This usage is particularly important for eventual chromosome mapping purposes. The zebrafish GATA 3 homologue is therefore referred to as *gta3*.

4.3. cDNA cloning and sequencing of zebrafish GATA 3

Between 2.5 and 5.0 x 10^5 recombinant phage from a zebrafish neurula-stage cDNA library (David Grunwald) were screened with the zinc finger domains of *Xenopus laevis* GATA 2a (884–1263bp) (Zon et al., 1991). Pre-hybridisation was in 5 × Denhardts, 6 × SSC, 0.1% SDS, 50 μg/ml sonicated salmon sperm, 10 μg/ml poly A at 55°C and hybridised at 55°C overnight. Post-hybridisation washes were performed in 0.1% SDS, 2× SSC at room temperature for 5 × 5 min, then once at 55°C for 90 min. The stringency was in-

creased to 58°C for 60 min. From a tertiary screen, positive Bluescript phagemids were rescued from λZAP II and a 1.6-kb partial cDNA containing the 3′ end of the coding sequence and the whole of the 3′ non-coding sequence of zebrafish GATA 3 was identified. This was used to rescreen the same library at high stringency (65°C, 0.1 × SSC) and obtained an overlapping cDNA which contained the whole of the coding sequence and flanking non-coding sequences. Both strands were sequenced using the Sequenase Version 2.0 kit and synthetic oligonucleotide primers.

4.4. Whole mount in situ hybridisation

The protocol was that described by Thisse et al., (1993). The digoxygenin labelled RNA probe concentration was 100–200 ng/ml and was hybridised at 65°C. Ethanol dehydrated embryos were embedded in wax and 10 μm sections were cut. Manually sectioned embryos were cut with a tungsten needle. Embryos were cleared in methanol and mounted in 70% glycerol for photography.

4.5. Immunohistochemistry

The method was that of Holder and Hill (1991) but 0.4% PBTx was used. The antibodies used were polyclonal anti-Ntl (Schulte-Merker et al. 1992) (rabbit IgG, 1 in 1000), monoclonal HNK-1 (mouse IgM, 1 in 20).

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References

Akimenko, A-E., Ekker, W., Wegner, J., Lin, W. and Westerfield, M. (1994) J. Neurosci. 14, 3475–3486.

Al-Adhami, M.A. and Kunz, Y.W. (1977) Dev. Growth Differ. 19, 171–179.

Arceci, R.J., King, A.A., Simon, M.C., Orkin, S.H. and Wilson, D.B. (1993) Mol. Cell Biol. 13, 2235–2246.

Bernhardt, R.R., Chitnis, A.B., Lindamer, L. and Kuwada, J.Y. (1990) J. Comp. Neurol. 302, 603–616.

Bernhardt, R.R., Patel, C.K., Wilson, S.W. and Kuwada, J.H. (1992) J. Comp. Neurol. 326, 263–272.

Briegel, K., Lim, K-C., Plank, C., Beug, H., Engel, J. and Zenke, M. (1993) Genes Dev. 7, 1097–1109.

Campos-Ortega, J. (1993) J. Neurobiol. 24, 1305–1327.

Campuzano, S. and Modelell, J. (1992) Trends Genet. 8, 202–208.

Cunningham, T.S. and Cooper, T.G. (1991) Mol. Cell. Biol. 11, 6205–6215.

Eisen, J.S. (1992) Neuron 8, 231–240.

Eisen, J.S. and Pike, S.H. (1991) Neuron 6, 767–776.

Engel, J.D., Beug, H., LaVail, J.H., Zenke, M.W., Mayo, K., Leonard, M.W., Foley, K.P., Yang, Z., Kornhauser, J.M., Ko, L.J., Lim, K., George, K.M. and Breigel, K. (1992) J. Cell Science (suppl.) 16, 21–31.

Ferreiro, B., Skogland, P., Bailey, A., Dorsky, R. and Harris, W. (1992) Mech. Dev. 40, 25–36.

Genetics Computer Group. (1991) Program Manual for the GCG Package, Version 7, April 1991, 575 Science Drive, Madison, Wisconsin, USA 53711.

Gribskov, M. and Burgess, R.R. (1986) Nucleic Acids Res. 14, 6745–6763.

Hatta, K., Kimmel, C.B., Ho, R.K. and Walker, C. (1991) Nature 350, 339–341.

Ho, I.-C., Vorhees, P., Marin, N., Oakley, B.K., Tsai, S., Orkin, S.H. and Leiden, J.M. (1991) EMBO J. 10, 1187–1192.

Holder, N. and Hill, J. (1991) Development 113, 1159–1170.

Inoue, A., Takahashi, M., Hatta, K., Hotta, Y. and Okamoto, H. (1994) Dev. Dyn. 199, 1–11.

Jessell, T.M. and Dodd, J. (1992) Harvey Lect. 86, 87–128.

Johnson, J., Birren, S. and Anderson, D. (1990) Nature, 346, 858–861

Kane, D.A. and Kimmel, C.B. (1993) Development 119, 447–456.

Kane, D.A., Warga, R.M. and Kimmel C.B. Nature 360, 735–737.

Kelley, C., Blumberg, H., Zon, L.I. and Evans, T. (1993) Development 118, 817–827.

Kimmel, C.B., Warga, R.M. and Schilling, T.F. (1990) Development 108, 581–594.

Kimmel, C.B., Kane, D.A., Walker, C., Warga, R.M. and Rothman, M.B. (1989) Nature 337, 359–362.

Ko, L., Yamamoto, M., Leonard, M.W., George, K.M., Ting, G.P. and Engel, J.D. (1991) Mol. Cell Biol. 1, 2778–2784.

Kornhauser, J.M., Leonard, M.W., Yamamoto, M., LaVail, J.H., Mayo, K.E. and Engel, J.D. (1994) Mol. Brain Res. 23, 100–110.

Kozak, M. (1987) Nucleic Acid Res. 15, 8125–8148.

Krausse, S., Johansen, T., Korzh, V. and Fjose, A. (1991) Development 113, 1193–1206.

Laverriere, A.C.C., MacNeill, C., Mueller, R.E., Poelmann, J.B.E., Burch, and Evans, T. (1994) J. Biol. Chem. 269, 23177–23184.

Leonard, M., Lim, K. and Engel, J.D. (1993) Development 119, 519–531.

Long, W.L. (1983) J. Exp. Zool. 228, 91–97.

Martin, D.I.K., Zon, L.I., Mutter, G. and Orkin, S.H. (1990) Nature 344, 444–449.

Meek, J. and Joosten, H. (1989) J. Comp. Neurol. 281, 206–224.

Medvinsky, A.L., Samoylina, N.L., Muller, A.M. and Dzierzak, E.A. (1993) Nature 364, 64–67.

Minehart, P.L. and Magasanik, B. (1991) Mol. Cell. Biol. 11, 6216–6228.

Mullins. M.C., Hammerschmidt, M., Haffter, P. and Nusslein-Volhard, C. (1994) Curr. Biol. 4, 189–202.

Myers, P.Z., Eisen, J.S. and Westerfield, M. (1986) J. Neurosci. 6, 2278–2289.

Orkin, S.H. (1992) Blood 80, 575–581.

Pevny, L., Simon, M.C., Robertson, E., Klein, W.H., Tsai, S.-F., D'Agati, V., Orkin, S.H. and Costantini, F. (1991) Nature 349, 257–260.

Puschel, A., Westerfield, M. and Dressler, G. (1992) Mech. Dev. 38, 197–208.

Ramain, P., Heitzler, P., Haenlin, M. and Simpson, P. (1993) Development 119, 1277–1291.

Schutte-Merker, S., Ho, R.K., Herrmann, B.G. and Nüsslein-Volhard, C., (1992) Development 116, 1021–1032.

Stachel, S.E., Grunwald, D.J. and Myers, P.Z. (1993) Development 117, 1261–1274.
Strahle, U. and Jesuthasan, S. (1993) Development 119, 909–919.
Schwartz, R.M. and Dayhoff, M.O. (1979) In Dayhoff, M.O. (ed.), Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington D.C., pp. 353–358.
Thisse, C., Thisse, B., Schilling, T.F. and Postlethwait, J.H. (1993) Development 119, 1203–1215.
Turner, D.L. and Weintraub, H. (1994) Genes Dev. 8, 1434–1447.
Trinkaus, J.P. (1984) Am. Zool. 24, 673–688.
Trinkaus, J.P. (1992) Development (Suppl.) 75–80.
Tsai, F-Y., Keller, G., Kuo, F., Weiss, M., Chen, J., Rosenblatt, M., Alt, F.W. and Orkin, S. (1994) Nature 371, 221–226.
Vischer, H.A. (1989) Brain Behav. Evol. 33, 205–222.
Walmsley, M.E., Guille, M.J., Bertwistle, D., Smith, J.C., Pizzey, J.A. and Patient, R.K. (1994) Development 120, 2519–2529.
Warga, R. and Kimmel, C.B. (1990) Development 108, 569–580.
Westerfield, D. (1989) The Zebrafish Book, University of Oregon Press.
Wilson, S.W., Ross, L., Parrett, T. and Easter, S. (1990) Development 108, 121–145.
Winick, J., Abel, T., Leonard, M.W., Michelson, A.M., Chardon-Loriaux, I., Holmgren, R.A., Maniatis, T. and Engel, J.D. (1993) Development 119, 1055–1065.
Yamada, T., Pfaff, S.L., Edlund, T. and Jessell, T.M. (1993) Cell 73, 673–686.
Yamamoto, M., Ko, L., Leonard, M., Beug, H., Orkin, S. and Engel, J. (1990) Genes Dev. 4, 1650–1662.
Zon, L.I., Mather, C., Burgess. S., Bolce, M.E., Harland, R.M. and Orkin, S.H. (1991) Proc. Natl. Acad. Sci. USA 88, 10642–10646.