ELSEVIER

Mechanisms of Development 87 (1999) 213–216

www.elsevier.com/locate/modo

Gene expression pattern

Gata2 and *Gata3*: novel markers for early embryonic polarity and for non-neural ectoderm in the chick embryo

Guojun Sheng, Claudio D. Stern*

Department of Genetics and Development, Columbia University, 701 West 168th Street #1602, New York, NY 10032, USA

Received 27 April 1999; received in revised form 9 June 1999; accepted 18 June 1999

Abstract

We have investigated in detail the expression patterns of two *Gata* genes, *cGata2* and *cGata3*, during early chick development. In addition to confirming previously described expression of these two genes in developing brain, kidney and blood islands, this study reveals several important novel expression domains during very early stages of development. *cGata2* is expressed in the *area opaca* in pre-primitive streak stages, forming a gradient along the A–P axis (strongest anteriorly). Both genes are expressed strongly in the entire non-neural ectoderm from stage 4+, and neither is expressed in prospective neural plate at any stage. Unlike other previously described non-neural markers, neither gene is expressed in the dorsal neural tube. We also describe dynamic expression of *cGata2* and *cGata3* during eye, ear and gut development.

Keywords: Gata2; Gata3; Epidermis; Non-neural ectoderm; Intermediate mesoderm; Embryonic polarity

1. Results

The vertebrate Gata family of zinc finger transcription factors contains six members, Gata1–6. Gata proteins have been shown to play important roles in hematopoiesis and heart and gut development (Evans and Felsenfeld, 1989; Tsai et al., 1989, 1994; Pevny et al., 1991; Laverriere et al., 1994; Pandolfi et al., 1995; Kuo et al., 1997; Molkentin et al., 1997). More recently, Gata2 and Gata3 have also been suggested to play a role in early embryonic patterning in *Xenopus* and *Zebrafish* (Zon et al., 1991; Neave et al., 1995; Bertwistle et al., 1996; Read et al., 1998; Sykes et al., 1998). To date, the expression of chick Gata2 and Gata3 has been studied in situ only with embryos older than E3.5 (stage 22) (Kornhauser et al., 1994), while their presence during early development has been analyzed only by reverse transcription–polymerase chain reaction (RT-PCR) (Leonard et al., 1993). In this report we have examined in detail, using whole mount in situ hybridization, the expression pattern of *cGata2* and *cGata3* from pre-primitive streak stage X (Eyal-Giladi and Kochav, 1976) to early limb bud stages of chick development.

In pre-primitive streak stages (X–XIII) (Fig. 1A) and early streak stages HH2-3 (Hamburger and Hamilton, 1951) (Fig. 1B), *cGata2* is highly expressed in the anterior *area opaca*. The expression forms a striking gradient along the A–P axis (strongest anteriorly). This expression is restricted to the epiblast (Fig. 1K) and is visible as early as stage X, revealing that the anterior part of the embryo is molecularly distinct even before hypoblast formation begins. Other genes so far described are expressed either in the posterior marginal zone (e.g. *Vg1*) (Shah et al., 1997) or uniformly in the *area opaca* (e.g. *Bmp7*) (Streit et al., 1997). However, one recent study (Pera et al., 1999) reported that the chick *dlx5* gene is expressed in the pre-primitive streak embryo in a pattern similar (but not identical) to that of *cGata2*. One difference is that *dlx5* is expressed in both epiblast and hypoblast. This early expression of *cGata2* disappears abruptly at stage 3+ and a new domain appears at stage 4–4+ in the prospective non-neural ectoderm. This is initially weak and restricted to the anterior region (Fig. 1C), but rapidly intensifies and expands laterally and posteriorly (Fig. 1D). By stage 5, it covers the entire non-neural ectodermal area (Fig. 1E). Although *cGata2* expression is mainly restricted to the epiblast (Fig. 1L), we have also detected weak expression in the lateral mesoderm (Fig. 1L). Expression in non-neural ectoderm persists during neurulation (Fig. 1F–H). From stage 5, *cGata2* is also expressed in the posterior primitive streak, from where extraembryonic mesoderm cells are derived (Schoenwolf et al., 1992; Psychoyos and Stern, 1996). Starting from stage 7, cells in the forming blood islands express *cGata2*

* Corresponding author.

0925-4773/99/$ - see front matter
PII: S0925-4773(99)00150-1

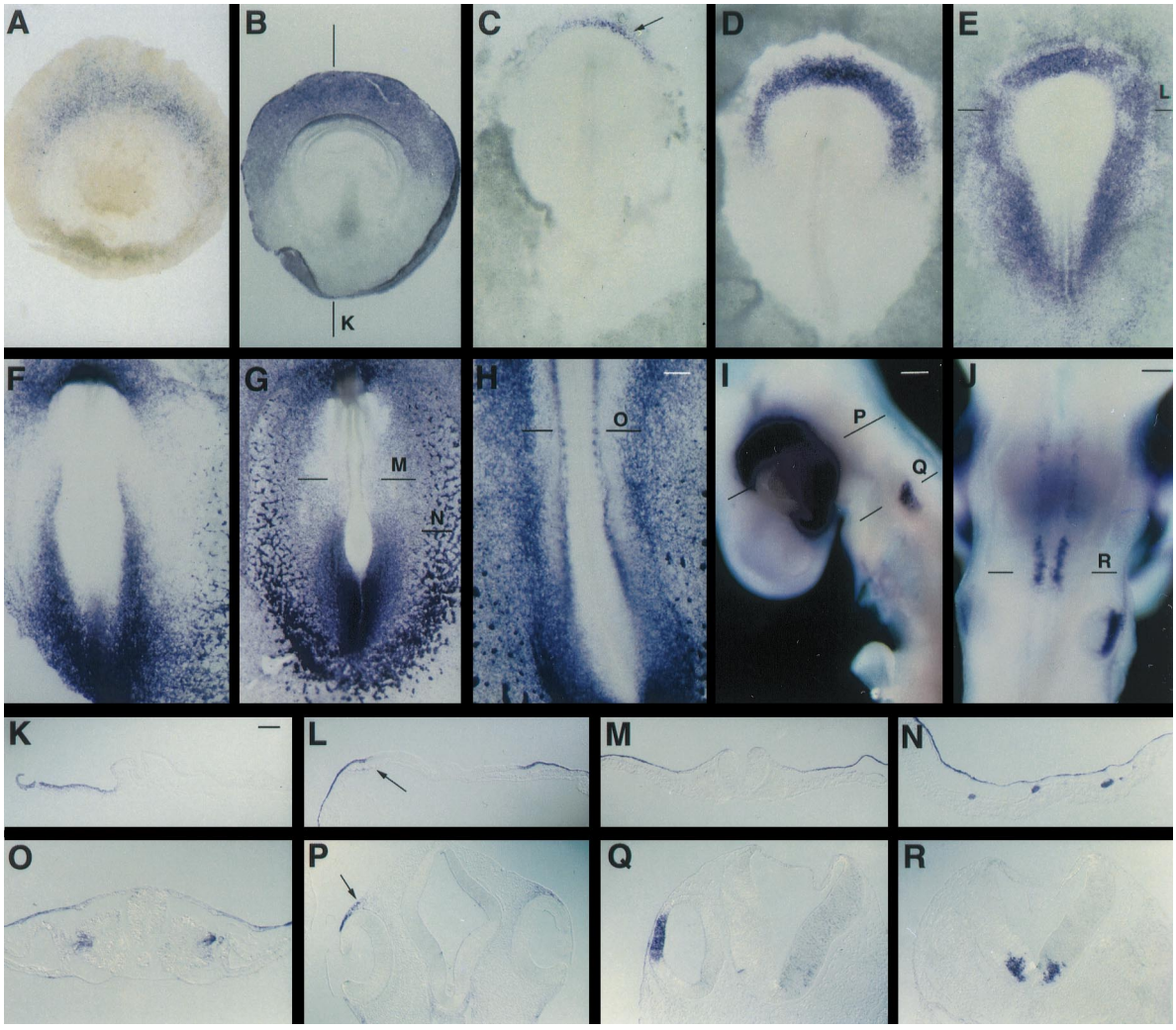

Fig. 1. Expression of *cGata2*. Panels (A–J) show the expression in the whole embryo and (K–R) are paraffin sections (10 μm) as indicated. All embryos are viewed from the dorsal side unless specified and are oriented with anterior to the top. (A) Pre-primitive streak stage XI embryo. (B) Stage 3. (C) Stage 4+. The arrow indicates the initial weak expression in the anterior non-neural region. (D) Stage 5−. (E) Stage 5. (F) Stage 7. Note expression in the forming blood islands. (G) Stage 8. (H) Stage 13. Note the expression in the lateral portion of the somites. Scale bar = 250 μm. (I) Stage 18 (left view). Note expression in the otic vesicle and in mesenchymal cells surrounding the optic cup. Scale bar = 100 μm. (J) Dorsal view of a stage 18 embryo, showing expression in the hindbrain, including strong expression in rhombomere 4. Scale bar = 100 μm. (K) Parasagittal section of the embryo in B (anterior to the left). Note that only the epiblast is stained. (L) Section of the embryo in (E). The arrow indicates the weak expression in the lateral mesoderm. (M) Section of the embryo in (G), showing expression in the non-neural ectoderm. (N) Section of the embryo in (G), showing expression in the blood islands. (O) Section of the embryo in (H). Note the expression in the lateral portion of the somite. (P) Section of the embryo in (I) at the eye level. The arrow indicates the mesenchymal expression of *cGata2*. (Q) Section of the embryo in (I) at the otic level, showing expression in the otic vesicle. (R) Section of the embryo in (J). at the level of rhombomere 4, showing ventral expression. For panels (K–R), the scale bar (shown in K) is 100 μm.

strongly (Fig. 1F,G,N). After neurulation, the non-neural ectodermal expression of *cGata2* fades gradually and new expression is detected in the lateral portion of the somites (Fig. 1H,O), the lateral epithelium of the otic vesicle (Fig. 1I,Q), mesenchymal cells surrounding the optic cup (Fig. 1I,P) and ventral hindbrain (Fig. 1J,R).

Unlike *cGata2*, we did not detect *cGata3* expression in pre-primitive streak or early streak stage embryos. *cGata3* starts to be expressed at stage 4–4+, in a pattern very similar to that of *cGata2*, in the area surrounding the prospective neural ectoderm (Fig. 2A), and quickly expands posteriorly (Fig. 2B). Expression of *cGata3* at these stages is only observed in the epiblast (Fig. 2I) and persists throughout neurulation (Fig. 2C,D,J). However, unlike other non-neural markers (e.g. *Bmp4*, *msx1*) (Liem et al., 1995), neither *cGata3* nor *cGata2* is detectable in the dorsal neural tube (Figs. 2J,M and 1M,O). Unlike *cGata2*, *cGata3* expression is barely detectable in blood islands. By stage 8, strong expression is seen in the developing foregut (Fig. 2D,J), and later becomes restricted to the ventral region (Fig. 2L). After stage 10, new expression domains appear in the developing eye, including both prospective lens ectoderm and optic vesicle (Fig. 2E,K), and in the otic region (Fig. 2E,L). *cGata3* expression in the eye then weakens and is

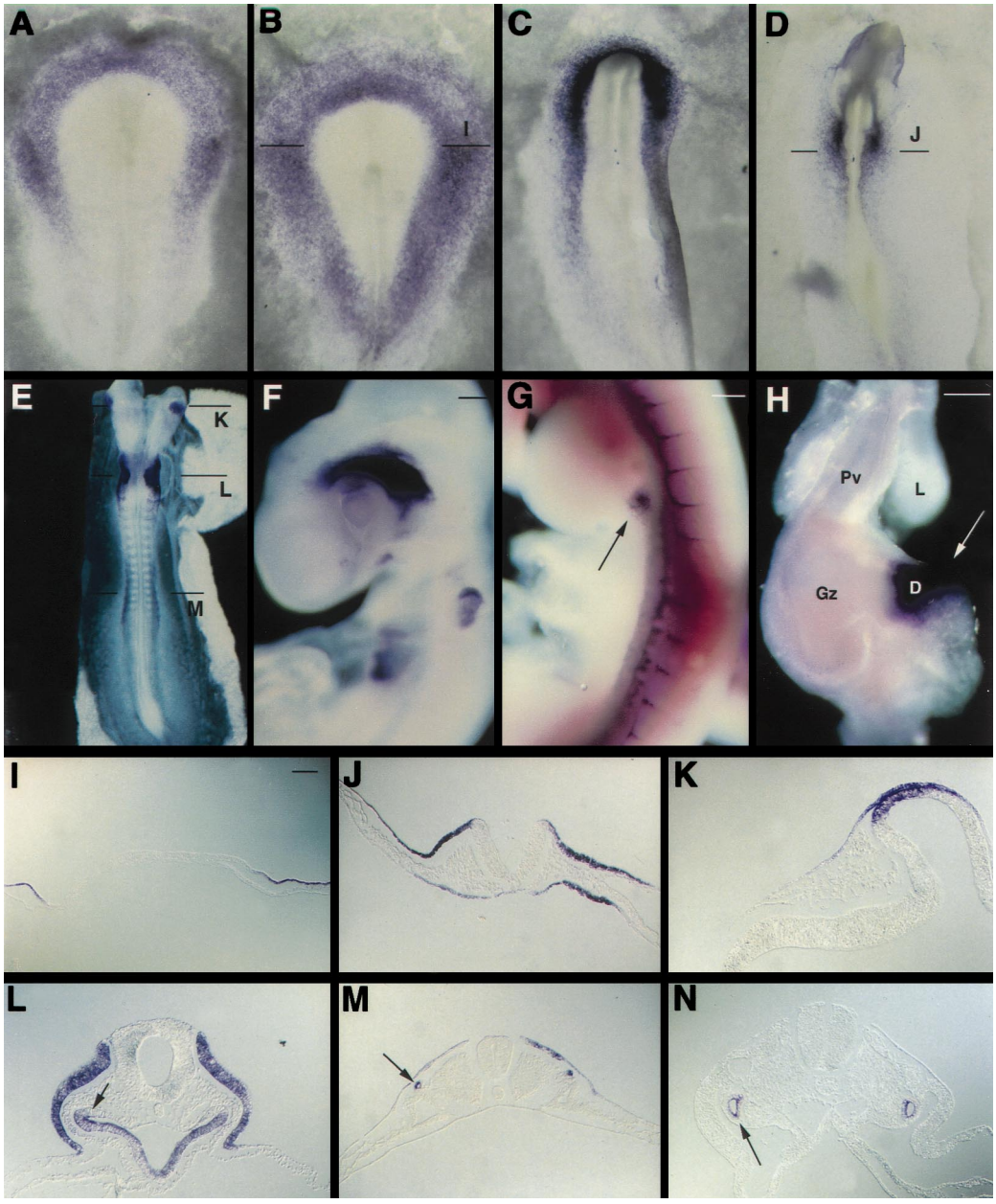

Fig. 2. Expression pattern of *cGata3*. Panels (A–H) show the expression in the whole embryo and (I–N) are paraffin sections (10 μm) as indicated. All embryos are viewed from the dorsal side unless specified and oriented with anterior to the top. (A) Stage 4+ embryo. (B) Stage 5. (C) Stage 7−. (D) Stage 8. (E) Stage 11. Note the expression in the eye, otic region and intermediate mesoderm. (F) Stage 18 (left view), showing expression in mesenchymal cells surrounding the dorsal part of the optic cup, otic vesicle and branchial arches. Scale bar = 100 μm. (G) Stage 22 (left view). The arrow indicates the posterior–proximal expression in the developing wing. Also note the expression in the intersomitic vessels and mesonephros. The staining visible anterior to the wing corresponds to a portion of the gut, better seen in (H). Scale bar = 100 μm. (H) Gut dissected from a stage-28 embryo, showing expression in the proximal duodenum. L, lung; Pv, proventriculus; Gz, gizzard; D, duodenum. Scale bar = 150 μm. (I) Section of the embryo in (B). (J) Section of the embryo in (D). (K) Section of the embryo in (E) at the optic level (dorsal to the left). Note that both the lens ectoderm and prospective neural retina express *cGata3*. (L) Section of the embryo in (E) at the otic level. The arrow indicates the dorsal limit of expression in the foregut. (M) Section of the embryo in (E). The arrow shows expression in the intermediate mesoderm. (N) Section of the embryo in (F) at posterior trunk level. The arrow indicates the expression in the mesonephric duct. For panels (I–N), the scale bar (shown in I) is 100 μm.

replaced by strong expression in mesenchymal cells surrounding the optic cup (Fig. 2F), as with *cGata2* (Fig. 1I). An interesting difference is that *cGata3* is only expressed in dorsal mesenchymal cells (Fig. 2F) while *cGata2* is expressed in both dorsal and ventral cells (Fig. 1I). We also detected *cGata3* in a small posterior-proximal region of the developing limbs (both fore- and hindlimbs) (Fig. 2G), in the intersomitic vessels (Fig. 2G) and in a restricted, proximal portion of duodenum (Fig. 2H). In addition, consistent with previous observations (George et al.,

1994; Neave et al., 1995), *cGata3* is also expressed in the intermediate mesoderm (Fig. 2M), and later in the mesonephric duct (Fig. 2N) and kidney (not shown), in the branchial arches (Fig. 2F) and brain (not shown).

2. Materials and methods

A fragment of 749 bp of the 3′ UTR of *cGata2* (Ko et al., 1991) and a fragment of 700 bp of *cGata3* (containing the 5′ UTR and the first 205 amino acids, excluding the zinc finger domain) (Ishihara et al., 1995) were generated by PCR from a stage 12–15 cDNA library (kind gift of Dr D. Wilkinson) with the primers: *cGata2*, 5′CCTGACGACCAAGAG3′ and 5′CATACTGCGGCTACAG3′; *cGata3*, 5′GACTCTGCACAGCCGT3′ and 5′CCATGCTGCTCCTAG3′.

The PCR fragments were cloned in the pGEM-T vector (Promega) and used for making DIG labeled RNA probes. White Leghorn chick eggs (Spafas, Preston, CT) were incubated at 38°C to the appropriate stages. The embryos were then fixed in 4% paraformaldehyde in PBS with 2 mM EGTA and processed for in situ hybridization as described previously (Stern, 1998).

Acknowledgements

This study was funded by the National Institutes of Health (HD31942 and GM53456 to C.D.S. G.S. was supported by NIH Training Grant MH15174).

References

Bertwistle, D., Walmsley, M.E., Read, E.M., Pizzey, J.A., Patient, R.K., 1996. GATA factors and the origins of adult and embryonic blood in Xenopus: responses to retinoic acid. Mech. Dev. 57, 199–214.

Evans, T., Felsenfeld, G., 1989. The erythroid-specific transcription factor Eryf1: a new finger protein. Cell 58, 877–885.

Eyal-Giladi, H., Kochav, S., 1976. From cleavage to primitive streak formation: a complementary normal table and a new look at the first stages of the development of the chick. I. General morphology. Dev. Biol. 49, 321–337.

George, K.M., Leonard, M.W., Roth, M.E., Lieuw, K.H., Kioussis, D., Grosveld, F., Engel, J.D., 1994. Embryonic expression and cloning of the murine GATA-3 gene. Development 120, 2673–2686.

Hamburger, V., Hamilton, H.L., 1951. A series of normal stages in the development of the chick embryo. J. Morphol. 88, 49–92.

Ishihara, H., Engel, J.D., Yamamoto, M., 1995. Structure and regulation of the chicken GATA-3 gene. J. Biochem. (Tokyo) 117, 499–508.

Ko, L.J., Yamamoto, M., Leonard, M.W., George, K.M., Ting, P., Engel, J.D., 1991. Murine and human T-lymphocyte GATA-3 factors mediate transcription through a cis-regulatory element within the human T-cell receptor delta gene enhancer. Mol. Cell Biol. 11, 2778–2784.

Kornhauser, J.M., Leonard, M.W., Yamamoto, M., LaVail, J.H., Mayo, K.E., Engel, J.D., 1994. Temporal and spatial changes in GATA transcription factor expression are coincident with development of the chicken optic tectum. Mol. Brain Res. 23, 100–110.

Kuo, C.T., Morrisey, E.E., Anandappa, R., Sigrist, K., Lu, M.M., Parmacek, M.S., Soudais, C., Leiden, J.M., 1997. GATA4 transcription factor is required for ventral morphogenesis and heart tube formation. Genes Dev. 11, 1048–1060.

Laverriere, A.C., MacNeill, C., Mueller, C., Poelmann, R.E., Burch, J.B., Evans, T., 1994. GATA-4/5/6, a subfamily of three transcription factors transcribed in developing heart and gut. J. Biol. Chem. 269, 23177–23184.

Leonard, M.W., Lim, K.C., Engel, J.D., 1993. Expression of the chicken GATA factor family during early erythroid development and differentiation. Development 119, 519–531.

Liem Jr., K.F., Tremml, G., Roelink, H., Jessell, T.M., 1995. Dorsal differentiation of neural plate cells induced by BMP-mediated signals from epidermal ectoderm. Cell 82, 969–979.

Molkentin, J.D., Lin, Q., Duncan, S.A., Olson, E.N., 1997. Requirement of the transcription factor GATA4 for heart tube formation and ventral morphogenesis. Genes Dev. 11, 1061–1072.

Neave, B., Rodaway, A., Wilson, S.W., Patient, R., Holder, N., 1995. Expression of zebrafish GATA 3 (gta3) during gastrulation and neurulation suggests a role in the specification of cell fate. Mech. Dev. 51, 169–182.

Pandolfi, P.P., Roth, M.E., Karis, A., Leonard, M.W., Dzierzak, E., Grosveld, F.G., Engel, J.D., Lindenbaum, M.H., 1995. Targeted disruption of the GATA3 gene causes severe abnormalities in the nervous system and in fetal liver haematopoiesis. Nat. Genet. 11, 40–44.

Pera, E., Stein, S., Kessel, M., 1999. Ectodermal patterning in the avian embryo: epidermis versus neural plate. Development 126, 63–73.

Pevny, L., Simon, M.C., Robertson, E., Klein, W.H., Tsai, S.F., D'Agati, V., Orkin, S.H., Costantini, F., 1991. Erythroid differentiation in chimaeric mice blocked by a targeted mutation in the gene for transcription factor GATA-1. Nature 349, 257–260.

Psychoyos, D., Stern, C.D., 1996. Fates and migratory routes of primitive streak cells in the chick embryo. Development 122, 1523–1534.

Read, E.M., Rodaway, A.R., Neave, B., Brandon, N., Holder, N., Patient, R.K., Walmsley, M.E., 1998. Evidence for non-axial A/P patterning in the nonneural ectoderm of Xenopus and zebrafish pregastrula embryos. Int. J. Dev. Biol. 42, 763–774.

Schoenwolf, G.C., Garcia-Martinez, V., Dias, M.S., 1992. Mesoderm movement and fate during avian gastrulation and neurulation. Dev. Dyn. 193, 235–248.

Shah, S.B., Skromne, I., Hume, C.R., Kessler, D.S., Lee, K.J., Stern, C.D., Dodd, J., 1997. Misexpression of chick Vg1 in the marginal zone induces primitive streak formation. Development 124, 5127–5138.

Stern, C.D., 1998. Detection of multiple gene products simultaneously by in situ hybridization and immunohistochemistry in whole mounts of avian embryos. Curr. Top. Dev. Biol. 36, 223–243.

Streit, A., Sockanathan, S., Perez, L., Rex, M., Scotting, P.J., Sharpe, P.T., Lovell-Badge, R., Stern, C.D., 1997. Preventing the loss of competence for neural induction: HGF/SF, L5 and Sox-2. Development 124, 1191–1202.

Sykes, T.G., Rodaway, A.R., Walmsley, M.E., Patient, R.K., 1998. Suppression of GATA factor activity causes axis duplication in Xenopus. Development 125, 4595–4605.

Tsai, S.F., Martin, D.I., Zon, L.I., D'Andrea, A.D., Wong, G.G., Orkin, S.H., 1989. Cloning of cDNA for the major DNA-binding protein of the erythroid lineage through expression in mammalian cells. Nature 339, 446–451.

Tsai, F.Y., Keller, G., Kuo, F.C., Weiss, M., Chen, J., Rosenblatt, M., Alt, F.W., Orkin, S.H., 1994. An early haematopoietic defect in mice lacking the transcription factor GATA-2. Nature 371, 221–226.

Zon, L.I., Mather, C., Burgess, S., Bolce, M.E., Harland, R.M., Orkin, S.H., 1991. Expression of GATA-binding proteins during embryonic development in *Xenopus laevis*. Proc. Natl. Acad. Sci. USA 88, 10642–10646.