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Expression patterns of Src-family tyrosine kinases during *Xenopus laevis* development

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ABSTRACT Src family tyrosine kinases (SFKs) play important roles in cell morphology, differentiation, motility and proliferation. Elevated expression and/or specific activity of Src kinases are characteristic for several types of human cancer. However, little information is available about the role and spatio-temporal expression of SFKs in early embryonic development. In this study we characterized, in *Xenopus laevis*, the expression patterns of five SFK genes *src, fyn, yes, lyn* and *laloo* as well as of the *csk* gene, a negative regulator of SFKs, using RT-qPCR and *in situ* hybridisation. We found that transcripts of all SFKs and *csk* were already detectable in one-cell embryos and their levels similarly oscillated during subsequent development. First, after stage 8, the levels of SFK and *csk* mRNAs began to decrease, reached a minimum between stages 10 and 28 and increased again. In the later stages (33-45), the levels of *fyn, yes* and *csk* mRNAs returned to approximately maternal ones, whereas the *src, laloo* and *lyn* mRNA transcripts exceeded, up to about 3.5-6-fold, their maternal levels. *In situ* hybridisation analysis located the SFK and *csk* transcripts in the animal hemisphere of *Xenopus* embryos. Subsequent gastrula stages showed signals in ectodermal cells, mid-neurula stage embryos at neural folds, and the tailbud stages showed strong signals in the brain and neural tube. RT-qPCR concentration profiling along the animal-vegetal axis proved in blastula and gastrula the preferential localisation of *yes, src, lyn* and *csk* transcripts towards the animal pole in a gradient-like manner. In contrast, *laloo* and *fyn* displayed a vegetal pole preference.

KEY WORDS: *Xenopus laevis, quantitative real-time PCR, in situ hybridisation, early development*

Src and Src-family protein-tyrosine kinases (SFKs) are proto-oncogenes and represent one of the nine presently recognised classes of non-receptor tyrosine-kinases (Pellicena and Miller, 2002). As documented in a great many reports the members of the SFK family participate in a variety of signalling pathways that control cell behavior, including differentiation and transformation (for a review see e.g. Blume-Jensen and Hunter, 2001). On the other hand, the role of SFK members in developmental processes has been examined much less extensively. The experiments carried out on mice demonstrated that Src/Fyn and Src/Yes-double knockouts die perinatally (Stein *et al.,* 1994) and Src/Fyn/Yes-triple knockouts at an early stage of embryonic development (Klinghoffer *et al.*, 1999).

In frogs *Xenopus laevis,* Steele and co-workers reported that *src, yes, and fyn* transcripts were already present in the maternal RNA pool (Steele, 1985; Steele *et al.*, 1989, 1990). In contrast to mice, studies on frogs allowed *in vitro* direct and continuous observation and examination of developing embryos from the time of fertilization. We found that overexpression of Src kinase over a certain threshold resulted either in defective gastrulation and death, or in the development of malformed embryos characterized by a depressed level of cadherin and α-, β- and γ-catenins in their tissues (Taka *et al.*, 1998, Jonák, 2000, DvoYákova, 2000). Curiously, SFK downregulation had similar effects. Injection of antisense RNAs against Src, Fyn and Yes led to the failure of *X. laevis* blastopore and neural tube closure, to shortening of the anterior-posterior axis or other defects in embryogenesis (Denoyelle *et al.*, 2001). This demonstrated that the level of Src must be strictly kept within certain boundaries; both too high and

Abbreviations used in this paper: SFK, Src family tyrosine kinase.

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too low level of Src has deleterious effects on the development. Src does not seem to have a role in mesoderm differentiation (Denoyelle *et al.*, 2001), but Fyn and Laloo could induce mesoderm formation in *Xenopus* animal caps assays (Weinstein *et al.*, 2001). *In situ* hybridisation analysis of *Xenopus* embryos demonstrated nervous system-specific expression of *src* mRNA (Collett and Steele, 1992) and *fyn* mRNA (Saito *et al.*, 2001), suggesting that *src* and *fyn* may play a role in elaboration of the nervous system.

RT-PCR analysis of *csk* and *laloo* expression during early *Xenopus* development showed maternally present transcripts, a greatly diminished expression by mid-blastula stages and a rise in expression again in late neurula stages (Song *et al.*, 2001).

The spatial and temporal changes in gene expression are a key mechanism in embryo development. However, as summarized above, such information about SFK members is only fragmentary. Therefore, in the current study, we have utilized a quantitative real-time PCR (RT-qPCR) protocol to examine and compare expression levels of five SFK genes *src, fyn, yes, lyn* and *laloo,* and of *csk,* the negative regulator of SFKs, during the period of early development of *X. laevis*. In addition, we have also detailed the spatio-temporal expression patterns of *csk* and all five SFK genes using the whole-mount *in situ* hybridisation and determined intraembryonal distribution of their transcripts along the animal-vegetal axis by RT-qPCR.

Quantitative real-time PCR

The temporal expression patterns of *src*, *fyn*, *yes*, *lyn*, *laloo* and *csk* mRNAs determined by RT-qPCR are shown in Fig. 1. Previously, we demonstrated that RT-qPCR normalisation of mRNA expression patterns to reference genes such as ODC, GADPH, EF-1α, H4 or L8, widely used in *Xenopus* RT-qPCR experiments, is not particularly suitable, because their levels vary during *Xenopus* development. We found that normalisation to total RNA is more appropriate (Sindelka *et al.*, 2006). Therefore, the mRNA expression profiles of *src*, *fyn*, *yes*, *lyn*, *laloo* and *csk* in stage series were normalised to total RNA of each embryonic stage and to stage one, and are presented in arbitrary units.

All five examined SFK mRNAs as well as the *csk* mRNA were already detectable in *Xenopus* one-cell embryos, indicating their maternal origin. This confirmed the results from Steele´s laboratory (Steele, 1985; Steele *et al.*, 1989, 1990) obtained for *src*, *yes* and *fyn* transcripts as well as for *laloo* and *csk* transcripts described in Song *et al.* (2001). Following fertilization, the levels of all examined mRNAs stayed stable up to about stage 8 (*src, fyn, yes, lyn* and *csk*) except for *laloo*, the level of which increased about 3 times to this stage. Then, the levels of all the transcripts began to decrease to a minimum. It was reached at stages 10.5 (*src*), 10–22 (*fyn*), 16-22 (*yes, laloo, csk*), or 10-16 (*lyn*). The levels of all transcripts then started to rise again obviously as a result of zygotic expression. The *fyn*, *yes* and *csk* mRNA levels roughly returned to maternal levels at around stage 41, 37 and 33, respectively, whereas *src, laloo* and *lyn* mRNAs reached, in more than one of the later stages (33-45), levels about 3.5, 5 and 6-fold higher, respectively, than was their level in the fertilized one-cell embryos. The earliest onset of zygotic expression was detected for *src* mRNA, confirming the results of Collett and Steele (1992). The *laloo* temporal expression profile presented here complements the partial *laloo* expression data previously published by Weinstein *et al.* (1998) and also correlates very well with the RT-PCR expression analysis described in Song *et al.* (2001).

In order to quantitatively compare the expression efficiency of individual SFK and *csk* genes, we normalised their expression levels to that of *lyn* mRNA (Fig. 2). While the separate expression patterns of the examined genes (Fig. 1) particularly highlight similarity in their expression profiles in the course of early development, the *lyn* mRNA normalised patterns mainly visualise quantitative differences among individual SFK in the expression of their genes. The Fig. 2 shows that the levels of *fyn* and *laloo* mRNAs

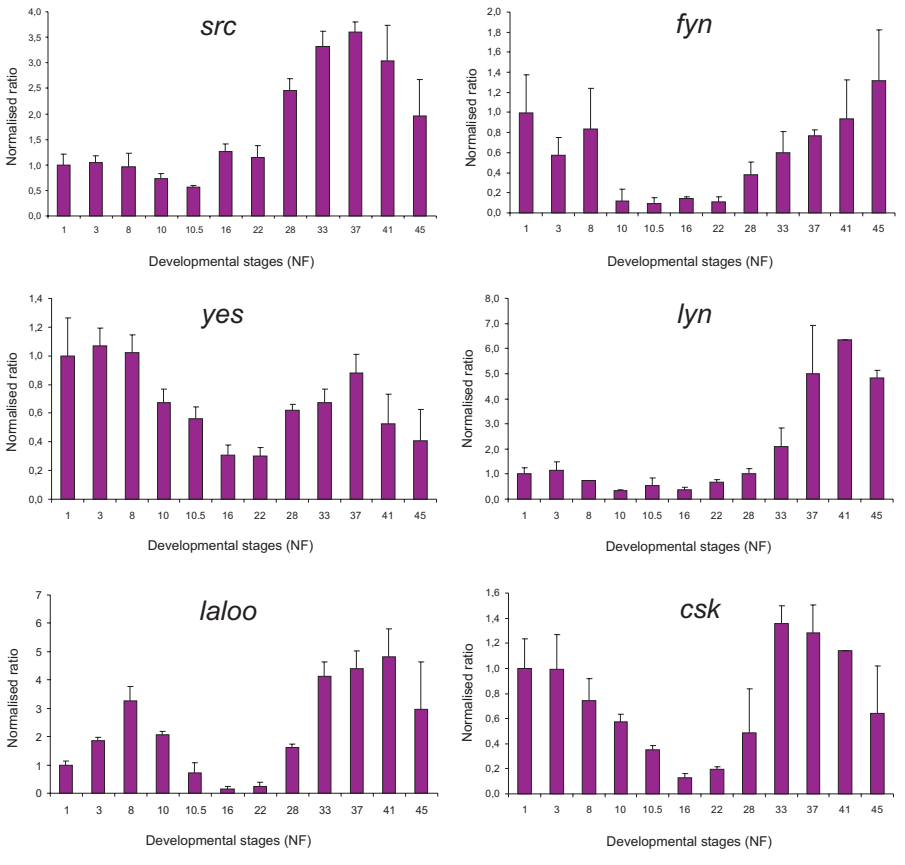

Fig. 1. The mRNA expression profiles of *Xenopus laevis src, fyn, yes, lyn, laloo* and *csk* genes, normalised to total RNA and stage one and expressed in arbitrary units. *The numbers on the vertical axis represent the ratio between the average amount of copies of a mRNA at a particular developmental stage and stage one normalised to the same amount of input RNA (means ± SD, n=6 replicates). The numbers on the horizontal axis represent the* Xenopus *developmental stages determined according to Nieuwkoop and Faber (1967).*

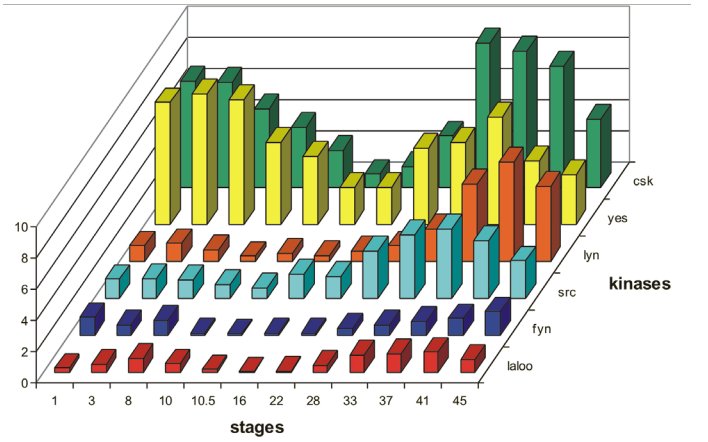

Fig. 2. The mRNA expression profiles of Src family tyrosine kinases (SFKs) and *csk*. *mRNA expression profiles are normalised to total RNA and stage one of* lyn *mRNA and expressed in arbitrary units. The numbers on the vertical axis represent the ratio between the average amount of copies of mRNA at a particular developmental stage and stage one normalised to* lyn *mRNA copy amount. The numbers on the horizontal axis represent the* Xenopus *developmental stages determined according to Nieuwkoop and Faber (1967).*

are kept about 10 times lower throughout early development than are the levels of *yes, lyn* or *src*. Interestingly, the level of *csk* mRNA is maintained higher or approximately comparable with the highest levels of other examined SFK throughout all developmental stages (Fig. 2). This should not be surprising as Csk regulates activity of all SFK members. Indeed, its concentration profile follows most closely that of the most strongly expressed SFK member, the *yes* mRNA.

Whole-mount in situ *hybridisation*

To analyse the spatio-temporal expression patterns of SFKs and *csk*, we performed the whole-mount *in situ* hybridisation on *Xenopus laevis* embryos using digoxigenin-labeled antisense RNA probes. The sense RNA probes were used as negative controls and they did not show any signals. *myoD* digoxigenin-labeled antisense RNA probe was used as a positive control.

Expression of SFKs and *csk* was analysed from the one-cell stage to the tadpole stage 43 (Fig. 3), similarly as in the RT-qPCR experiments. In early cleavage stage embryos, SFKs and *csk* transcripts were detectable in the animal hemispheres but they were absent from the vegetal hemisphere (Fig. 3 A). At gastrula stages, the transcripts were detected only in the ectoderm layer (Fig. 3 B). In mid-neurula stage embryos, expression of SFKs and

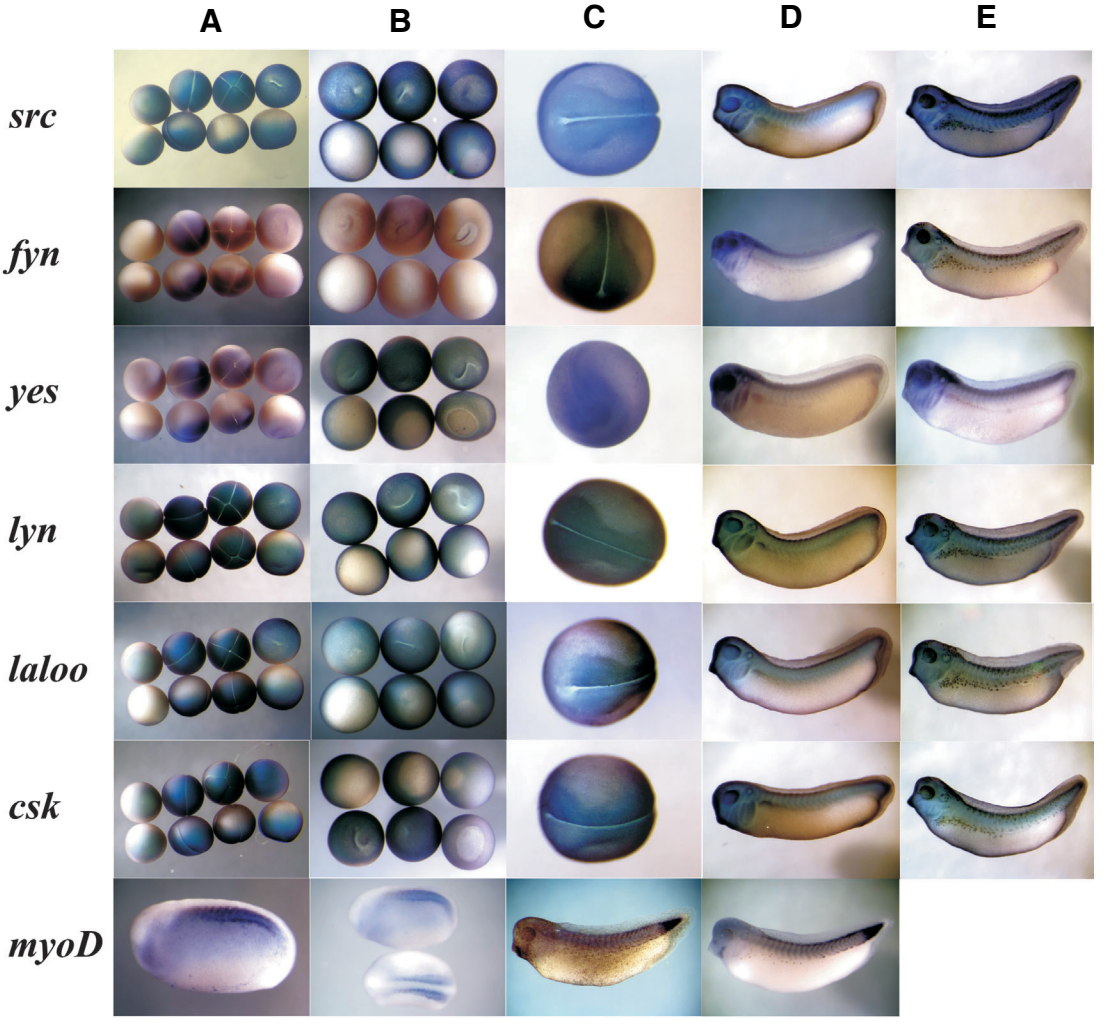

Fig. 3. The spatial and temporal expression patterns of *src, fyn, yes, lyn, laloo* and *csk* mRNAs during *Xenopus laevis* development. (A) *Animal (upper row) and lateral (lower row) view of embryonic stages 1, 2, 3 and 8 (blastula) showing enhanced signals in the animal hemisphere.* **(B)** *Animal (upper row) and vegetal (lower row) view of gastrula stages (10, 10.5, 11) showing no signal in endodermal cells.* **(C)** *Anterior and dorsal view of mid-neurula (stage 16) embryo. SFKs and* csk *are expressed at the neural folds from anterior to caudal end, but absent from the dorsal midline.* **(D,E)** *Lateral view of tailbud (stage 28, 31) embryos. Expression of SFKs and* csk *persists in the developing brain, neural tube, eye, branchial arches and otic vesicle. Bottom panels depict the expression pattern of* myoD *used as a positive control. First two panels show lateral and dorsal view of stage 23 embryos and the other two panels show lateral view of stage 31 embryos with the expression pattern of* myoD.

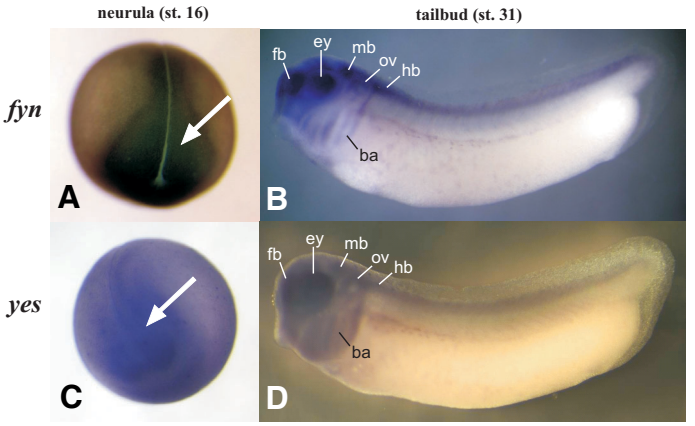

Fig. 4. Detailed expression patterns of *Xenopus fyn* and *yes* genes. *The* fyn *gene expression patterns* **(A,B)**. *Anterior view* **(A)** *of* Xenopus *embryo at neurula stage. The arrow shows the expression of the* fyn *gene in neural folds. Lateral view* **(B)** *of a tailbud (stage 31) embryo.* Fyn *expression persists in the developing brain, neural tube, eye, branchial arches and otic vesicle. The* yes *gene expression pattern* **(C,D)**. *Anterior view* **(C)** *of* Xenopus *embryo at neurula stage. The arrow shows the expression of* yes *gene in neural folds. Lateral view* **(D)** *of embryo at stage 31. The arrows show the major expression of* yes *gene in the developing brain, neural tube, eye, branchial arches and otic vesicle. hb; hindbrain, mb; midbrain, fb; forebrain, ba; branchial arches, ey; eye, ov; otic vesicle.*

csk was detected at neural folds (Fig. 3 C).

At stages 28 and 31 (Fig. 3 D, E), strong signals were found in the brain region of the neural tube and the expression of SFKs and *csk* also appeared in the eyes, branchial arches and otic vesicles. *Fyn* (Fig. 4 B) and *yes* (Fig. 4 D) expression at tailbud stage persisted in the forebrain, midbrain, hindbrain, neural tube, eyes, otic vesicles and branchial arches. The expression of SFKs and *csk* became ubiquitous from tadpole stage 41 (not shown).

We conclude that SFKs as well as their negative regulator *csk* are in *Xenopus* expressed maternally, their levels oscillate; decline around stage 10.5 and resume again in later developmental stages. Quantitatively, the levels of *yes*, *lyn* and *src* mRNAs detected in early embryos are several times higher than those of *fyn* and *laloo* and comparable with the level of *csk* mRNA. Expression of SFKs and *csk* is transiently nervous system-specific as documented in the whole-mount *in situ* hybridisation experiments.

Detailed RT-qPCR analysis of blastula and gastrula stage

To verify, by RT-qPCR analysis, the early ectodermal pattern of some SFKs and *csk* expression described above, we determined concentration profiles of transcripts of all kinases along the animal-vegetal (A/V) axis in blastula and gastrula stage embryos. Blastula embryos (stage 8.5) were dissected into three portions (animal pole, marginal zone, and vegetal pole), gastrula embryos (stage 11) into four portions (animal pole, dorsal and ventral marginal zone and vegetal pole; see scheme in Fig. 5) and the portions were subjected to RT-qPCR analysis. The amount of transcripts in each section was expressed in per cent of the overall content of individual transcripts present in the whole embryo.

This RT-qPCR "intraembryonal" analysis detected majority of *yes*, *src*, *lyn* and *csk* transcripts in the animal pole portion, less in the marginal zone portion and still less in the vegetal pole portion of the embryos. *Yes*, *src*, *lyn* and *csk* transcripts formed concentration gradients along the embryonic *X. laevis* animal-vegetal axis (Fig. 5). Approximately 50% of transcripts of these kinases were present in the animal pole portion, approximately 30-40% in the marginal zone portion and less than 20% of transcripts were present in vegetal pole portion of blastula stage embryos (Fig. 5 A). Very similar concentration ratios among the animal, marginal and vegetal transcripts of *yes*, *src*, *lyn* and *csk* were also detected in gastrula stage embryos (Fig. 5 B). No significant differences in expression levels of these kinases in dorsal and ventral marginal zones were detected. Altogether, these findings are in good agreement with the data obtained by *in situ* hybridisation (Fig. 3 A, B) and y*es*, *src*, *lyn*

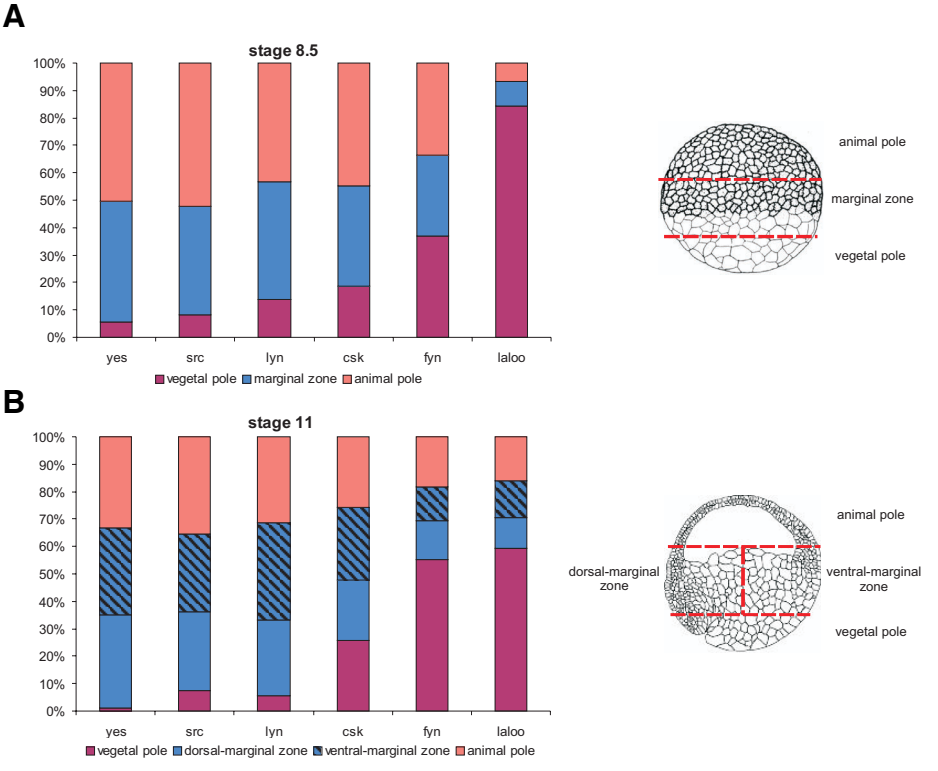

Fig. 5. "Intraembryonal" expression analysis of *Xenopus* Src-family tyrosine kinases *(SFK)* and *csk* mRNAs at blastula and gastrula stages by RT-qPCR. *Blastula (8.5) stage* **(A)** *and gastrula (11) stage* **(B)** *embryos were dissected into three (animal, marginal, vegetal) and four (animal, dorsal-marginal, ventral-marginal, vegetal), respectively, sections along the animal-vegetal axis as indicated and analysed as described in Materials and Methods.*

and *csk* could be considered as "animal genes".

On the other hand, expression patterns of *fyn* and *laloo* were found to be different. The amount of *fyn* transcripts was distributed almost identically among all three A/V sections of blastula stage embryos (Fig 5 A), and at gastrula stage it increased towards the vegetal pole (Fig. 5 B). The *laloo* mRNA expression pattern turned out to be quite opposite to that of *yes*, *src*, *lyn* and *csk* mRNAs. More than 80% of *laloo* transcripts at the blastula stage and almost 60% at the gastrula stage were found to be located in the vegetal pole portion and could be considered as a "vegetal gene". Dorsal and ventral marginal zones of gastrula did not differ in expression of either *fyn* or *laloo* (5 B).

The expression profile of *laloo* transcripts determined by RT-qPCR was not consistent with the *in situ* hybridisation results (Fig. 3 A, B). Possible reason could be a weaker penetration of RNA probes to cells of vegetal pole of *Xenopus* embryos as well as a lower concentration of both *fyn* and *laloo* transcripts in embryos in comparison to all other kinases examined here (Fig. 2). We believe that RT-qPCR can measure intraembryonal mRNA gradients with greater resolution and sensitivity than traditional *in situ* hybridisation. Compare also with RT-qPCR "tomography" carried out on *X. leavis* oocytes (Sindelka *et al.,* 2008).

Materials and Methods

Embryos and explants

Xenopus laevis embryos were obtained by *in vitro* fertilization and staged according to the Nieuwkoop and Faber tables (Nieuwkoop and Faber, 1967). Embryos were dejellied in 2% cysteine (Sigma) (pH 8) and then cultured in 0.1xNAM (Slack and Forman, 1980).

To analyse the distribution of mRNA transcripts along the animal-vegetal axis in blastula (8.5) and gastrula (11) stage *Xenopus* embryos they were manually dissected into three (animal, marginal, vegetal) and four, respectively, portions (see Fig. 5 for details) and subjected to real-time RT–qPCR expression analysis of SFKs and *csk*. The mRNA expression levels were normalised to total RNA and *gapdh* expression level.

RNA probes

Digoxygenin sense and antisense transcripts were synthesized using SP6/T7 RNA labelling kit (Roche) following the manufacturer's instructions.

Xenopus src, *fyn*, *yes*, *lyn*, *laloo*, *csk* coding sequences were cloned into the pCS2- vector (kind gift of D. Turner) after PCR amplification using following primer pairs:

src sense, 5'-ccgctcgagatgggtgccactaaaagtaag-3'
antisense, 5'-gctctagattaaaggttgtccccaggc-3'
fyn sense, 5'-ggaattccatgggctgtgtgcaatgcaag-3'
antisense, 5'-ccgctcgagttacaggttgtctccaggctg-3'
yes sense, 5'-cgggatcctcatgggctgtataaaaagtaagg-3'
antisense, 5'-ggaattccttatagattgtccccaggctggt-3'
lyn sense, 5'-cgggatcctcatgggatgtataaaatcaaaaac-3'
antisense, 5'-ggaattccctaaggctgttgttgatattg-3'
laloo sense, 5'-ggaattccatgggctgcatcaagtcaaag-3'
antisense, 5'-ccgctcgagttaaggttgtgcctggtactg-3'
csk sense, 5'-ccgctcgagccatgtcggtggtacaggccc-3'
antisense, 5'-gctctagatcagtgatacagttccttggc-3'.

Primers were designed using the published sequences for *src* BC110764, *fyn* X52188, *yes* X14377, *lyn* AB003358, *laloo* AF081803 and *csk* AF052430.

For antisense RNA, the pCS2- vectors were linearized with BamHI (*fyn*, *yes*, *lyn*, *laloo*), HindIII (*src*) or ApaI (*csk*). For sense RNA (negative control experiments), the pCS2- vectors were linearized with NotI. Both antisense- and sense-digoxigenin-labeled RNA probes were obtained using T7 or SP6 RNA polymerase (Roche). RNA probes were purified with ProbeQuant G-50 Micro Columns (Amersham) and checked by agarose gel electrophoresis. Synthesized control sense probes gave no staining after whole-mount *in situ* hybridisation (results not shown).

Whole-mount in situ *hybridisation and sectioning*

Whole-mount *in situ* hybridisation was performed according to the standard protocol (Harland, 1991). The antisense *src*, *fyn*, *yes*, *lyn*, *laloo*, and *csk* probes were designed to hybridise specifically with their unique N-terminal region.

RNA isolation and cDNA preparation

RNA was isolated from *Xenopus* tissue using the Trizol (Invitrogen) or TriReagent (Sigma) method of extraction. A cDNA pool was generated from total cellular RNA by using random oligonucleotides and MMLV reverse transcriptase as previously described (Sindelka *et al.*, 2006).

Quantitative real-time RT-qPCR

The reaction was accomplished in a total volume of 25 μl. The reaction mixture contained 2 μl of the cDNA template, 0.2 mM dNTPs, 240 nM forward and reverse primers, 1 U Taq polymerase (Promega), 2.5 μl of supplied 10x buffer, 2 mM $MgCl_2$, 2.5 μl of 10,000-fold diluted SYBRGreen solution (Molecular Probes) and 0.25 μl of 50 nM Fluorescein solution (Bio-Rad). The reactions were measured in iCycler (Bio-Rad) with cycling conditions: 95°C for 5 min, 40 cycles at 95°C for 15 sec and 60°C for 60 sec. Serially diluted PCR fragments (standards), identical with those amplified in the real-time PCR experiment, were prepared to obtain calibration curves. Reaction efficiencies determined from calibration curves for each set of primers were between 85 and 100%. The Cts (threshold cycles) of the samples and standards were analysed with Microsoft Excel program and the number of amplified cDNA copies as PCR products from particular stages of development were determined from calibration curves. The average deviation between Cts in parallel experiments did not exceed about 5% for all tested genes and stages. The expression profiles were derived from three independent *X. laevis* serial experiments. Specificity of every amplification reaction was verified by melting curve analysis and gel electrophoresis.

Primers used for real-time PCR were designed by using the Beacon Designer 2.00 program (Premier Biosoft International). Primers used are as follows:

src sense, 5'-gcgactgattgaggacaatgagta-3'
antisense, 5'-aggagaattccaaaagaccagaca-3'
fyn sense, 5'-gccaggcaccatgtctccag-3'
antisense, 5'-ctcctcagacaccacagcgtag-3'
yes sense, 5'-caccaacaccagtcccttaccc-3'
antisense, 5'-atcttgcttcccaccagtcacc-3'
lyn sense, 5'-atccagcttctcgtacaccaag-3'
antisense, 5'-tccaccattctccatgctcttc-3'
laloo sense, 5'-tctaagcaccccagagagga-3'
antisense, 5'-ccgctcgagctttagcaggagatggtccc-3'
csk sense, 5'-ggcaagctgagcattgacgaag-3'
antisense, 5'-gcggctactgttccctccatc-3'
gapdh sense, 5'-gccgtgtatgtggtggaatct-3'
antisense, 5'-aagttgtcgttgatgacctttgc-3'.

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References

BLUME-JENSEN, P., HUNTER, T. (2001). Oncogenic kinase signaling. *Nature* 411: 355-365.

COLLETT, J.W., STEELE, R.E. (1992). Identification and developmental expression of Src+ mRNAs in *Xenopus laevis*. *Dev. Biol.* 152: 194-198.

DENOYELLE, M., VALLES, A.M., LENTZ, D., THIERY, J.P., BOYER, B. (2001). Mesoderm-independent regulation of gastrulation movements by the src tyrosine kinase in *Xenopus* embryo. *Differentiation* 69: 38-48.

DVORAKOVA, K., HABROVA, V., TAKAC, M., JONAK, J. (2000). Depression in the level of cadherin and alpha-, beta-, gamma-catenins in transgenic *Xenopus laevis* highly expressing c-Src. *Folia Biol.* (Praha) 46: 3-9.

HARLAND, R.M. (1991). *In situ* hybridization: an improved whole-mount method for *Xenopus* embryos. *Methods Cell Biol.* 36: 685-695.

JONAK, J. (2000). Sperm-mediated preparation of transgenic *Xenopus laevis* and transmission of transgenic DNA to the next generation. *Mol. Reprod. Dev.* 56: 298-300.

KLINGHOFFER, R.A., SACHSENMAIER, C., COOPER, J.A., SORIANO, P. (1999). Src family kinases are required for integrin but not PDGFR signal transduction. *EMBO J.* 18: 2459-2471.

NIEUWKOOP, P.D. and FABER, J. (1967). Normal Table of *Xenopus laevis* (Daudin). Amsterdam: North-Holland.

PELLICENA, P., MILLER, W.T. (2002). Coupling kinase activation to substrate recognition in SRC-family tyrosine kinases. *Front. Biosci.* 7: d256-267.

SAITO, R., FUJITA, N., NAGATA, S. (2001). Overexpression of Fyn tyrosine kinase causes abnormal development of primary sensory neurons in *Xenopus laevis* embryos. *Dev. Growth Differ.* 43: 229-238.

SINDELKA, R., FERJENTSIK, Z., JONÁK, J. (2006). Developmental expression profiles of *Xenopus laevis* reference genes. *Dev. Dyn.* 235: 754-758.

SINDELKA, R., JONÁK, J., HANDS, R. BUSTIN, S. and KUBISTA, M. (2006). Intracellular expression profiles measured by real-time PCR tomography in the Xenopus laevis oocyte. *Nucleic Acids Res.* 36: 387-392.

SLACK, J.M., FORMAN, D. (1980). An interaction between dorsal and ventral regions of the marginal zone in early amphibian embryos. *J. Embryol. Exp. Morphol.* 56: 283-299.

SONG, Y., COHLER, A.N., WEINSTEIN, D.C. (2001). Regulation of Laloo by the *Xenopus* C-terminal Src kinase (Xcsk) during early vertebrate development. *Oncogene* 20: 5210-5214.

STEELE, R.E. (1985). Two divergent cellular src genes are expressed in *Xenopus laevis*. *Nucleic Acids Res.* 13: 1747-1761.

STEELE, R.E., DENG, J.C., GHOSN, C.R., FERO, J.B. (1990). Structure and expression of fyn genes in *Xenopus laevis*. *Oncogene* 5: 369-376.

STEELE, R.E., IRWIN, M.Y., KNUDSEN, C.L., COLLETT, J.W., FERO, J.B. (1989). The yes proto-oncogene is present in amphibians and contributes to the maternal RNA pool in the oocyte. *Oncogene Res.* 4: 223-233.

STEIN, P.L., VOGEL, H., SORIANO, P. (1994). Combined deficiencies of Src, Fyn, and Yes tyrosine kinases in mutant mice. *Genes Dev.* 8: 1999-2007.

Takac, M., HABROVA, V., MACHA, J., CESKOVA, N., JONAK, J. (1998). Development of transgenic *Xenopus laevis* with a high C-src gene expression. *Mol. Reprod. Dev.* 50: 410-419.

WEINSTEIN, D.C., HEMMATI-BRIVANLOU, A.A. (2001). Src family kinase function during early *Xenopus* development. *Dev. Dyn.* 220: 163-168.

WEINSTEIN, D.C., MARDEN, J., CARNEVALI, F., HEMMATI-BRIVANLOU, A. (1998). FGF-mediated mesoderm induction involves the Src-family kinase Laloo. *Nature* 394: 904-8. Erratum in: Nature (1998) 395: 921.