Int. J. Dev. Biol. 58: 45-50 (2014)
doi: 10.1387/ijdb.130261jb

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

Expression pattern of *zcchc24* during early *Xenopus* development

MARTA VITORINO[1,2], ELIZABETH CORREIA[1,2], ANA-RITA SERRALHEIRO[2], ANA-CATARINA DE-JESUS[1,2], JOSÉ M. INÁCIO[1,2] and JOSÉ A. BELO*[,1,2,3]

[1]Regenerative Medicine Program, Departamento de Ciências Biomédicas e Medicina, Universidade do Algarve, [2] IBB-Institute for Biotechnology and Bioengineering, Centro de Biomedicina Molecular e Estrutural, Universidade do Algarve, Campus de Gambelas, Faro and [3]Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal.

ABSTRACT We report the expression pattern of a novel *Xenopus laevis* gene, *zcchc24*, which encodes a protein containing two zinc finger domains from the zf-CCHC and zf-3CxxC superfamilies. This protein shares >84% amino acid identity with its vertebrate homologues. During *X. laevis* embryonic development, *zcchc24* is expressed at gastrula stages in the dorsal mesoderm, including the cardiac precursors region. During neurula stages, *zcchc24* is expressed as two stripes in the dorsal region, more precisely, in the somitogenic mesoderm until the cardiac mesoderm. At early tailbud stages, *zcchc24* continues to be expressed in these regions, but starts to be expressed in the migrating neural crest. Later, this gene is expressed in the head, branchial arches, heart and somites. The zinc finger domains present in Zcchc24 protein and its dynamic gene expression pattern suggest that Zcchc24 might be involved in the regulation of heart, somites and of branchial arch formation/patterning, namely in the regulation of apoptosis.

KEY WORDS: *zcchc24, zinc finger, heart development, somitogenic mesoderm, neural crest*

The circulatory system is the first one to become functional during vertebrate embryo development, and is composed by the heart, blood cells and vessels. The formation of the heart is a well-conserved process among vertebrate, however, the molecular mechanisms involved in it are not well defined. To address this limitation, a differential screening for genes expressed in the heart precursor cell lineages of chick embryos was performed in our lab (Bento *et al.*, 2011). From the 777 detected genes, 199 were classified as upregulated uncharacterized genes. Among them, it was obtained *ZCCHC24* (*zinc finger domain-containing protein 24*), which predicted amino acid sequence is identical 90.8%, 90.5% and 90.8% to its human, mouse and frog homologs, respectively (Bento *et al.*, 2011).

Bioinformatic analysis showed that *Xenopus laevis zcchc24* (Genbank accession no. KF438010) encodes a 239 amino acids protein, with a predicted molecular mass of 26.99 kDa, and with two zinc finger domains (Fig. 1, http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Zinc fingers are relatively small protein domains that bind to zinc atoms and, normally, contain finger-like protrusions that make contact with their target molecules. They were initially identified as DNA-binding motifs but several studies showed that these domains can bind to DNA, RNA, proteins and lipids (Hall, 2005). Indeed, proteins with zinc finger domains are extremely abundant in eukaryotic genomes but can vary both in structure, as well as in function. More, they are involved in biological functions as diverse as cell growth, differentiation, DNA recognition, RNA packaging, transcriptional activation, regulation of apoptosis, protein folding and assembly, and lipid binding (Laity *et al.*, 2001).

Consequently, there are several superfamilies of zinc finger motifs, which are classified according to its sequence and structure (Krishna *et al.*, 2003). Zcchc24 protein contains two different zinc finger domains: one associated to the zf-CCHC superfamily and the other to the zf-3CxxC superfamily (Fig. 1). A typical example of proteins containing the zf-CCHC domain is the inhibitor of apoptosis (IAP) that has been reported as a regulator of programmed cell death by inhibition of caspases (Krishna *et al.*, 2003). The zf-3CxxC domain is present in several proteins with functions related with modifications in either DNA or chromatin, such as histone H3 lysine 36, and demethylases KDM2A and B (Birke *et al.*, 2002, Blackledge *et al.*, 2010).

Abbreviations used in this paper: ZCCHC24, zinc finger domain-containing protein 24.

***Address correspondence to:** José António Belo. Faculdade de Ciências Médicas da UNL, Edifício CEDOC II, Rua Câmara Pestana nº6, 6-A e 6-B, 1150-082 Lisboa, Portugal. Tel: +351-218-803-102. Fax +351-218-803-010. E-mail: jose.belo@fcm.unl.pt

Accepted: 19 December 2013. *Final, author-corrected PDF published online:* 30 April 2014.

ISSN: Online 1696-3547, Print 0214-6282

To analyze the potential function of *X. laevis zcchc24* during early frog embryo development, we examined its expression by whole-mount *in situ* hybridization (WISH).

zcchc24 transcripts were first detected at midblastula stage, in both dorsal and ventral marginal zones of the embryos (Fig. 2 A,A'), showing a ring around the marginal zone or presumptive mesoderm. Then, at the onset of gastrulation, *zcchc24* expression was observed in the dorsal mesoderm of both involuting marginal zone (IMZ), a region immediately above the dorsal blastopore lip, and non-involuting marginal zone (NIMZ), which is the region on the top of IMZ (Fig. 2 B,B').

During gastrulation, and more specifically at stage (st) 11-12, *zcchc24* is expressed in the dorsal mesoderm, in the somitogenic mesoderm, excluding the dorsal midline (Fig. 2 C C',E,E'). *myoD*, whose expression has been described in the same region, was used as a marker of somitogenic mesoderm (Fig. 2 D,F). This gene is involved in the formation of somites, and the knock-down of the MyoD disrupts the correct alignment of muscle fibers (Maguire *et al.*, 2012). At this stage, *zcchc24* is also expressed in two lateral mesoderm stripes around the blastopore that culminate in the dorsal side of the embryo (Fig. 2 C,E). Interestingly, these two lateral mesodermal stripes are correlated with the region in which the heart is originated. As a matter of fact, it has been suggested that, during gastrulation, the precardiac mesoderm migrates in two bilateral heart field located in the anterior lateral mesoderm (Sater and Jacobson, 1989).

From mid (st 11) to the end of gastrulation/beginning of neurulation (st 13), *zcchc24* expression seems to follow the migration of involuting mesoderm along the antero-posterior axis (Fig. 3A). During neurulation, *zcchc24* expression is detected in the anterior and somitogenic mesoderm (Fig. 3 B-B",E), which later will give rise to lateral and neural plate, and the somites, respectively. At these stages, the expression of *zcchc24* decreases progressively from the posterior to the anterior part of the embryo (Fig. 3 B,E). When compared with the early cardiac lineage marker *nkx2.5*, it is possible to observe that anteriorly *zcchc24* expression is adjacent to the cardiac mesoderm, while more posteriorly, *zcchc24* is expressed in the paraxial mesoderm. (Fig. 3 C,D)

Afterwards, at early tailbud stages, *zcchc24* expression is detected in the unsegmented somitogenic mesoderm and in the somites (Fig. 4 A,B). In contrast, *nkx2.5* is predominantly expressed in the differentiating cardiac muscle (Fig. 4C). In addition, when we compare the expression of *zcchc24* with the expression of *twi*, a neural crest marker, we observe that *zcchc24* transcripts are in the migrating neural crest cells (Fig. 4 B,D,E).

At later tailbud stages, *zcchc24* is expressed in the differentiating cardiac muscle, in the presumptive heart region and in the head, excluding the cleft between the branchial arches and cement gland (Fig. 4 F,G). A transverse section of a st 32 embryo showed

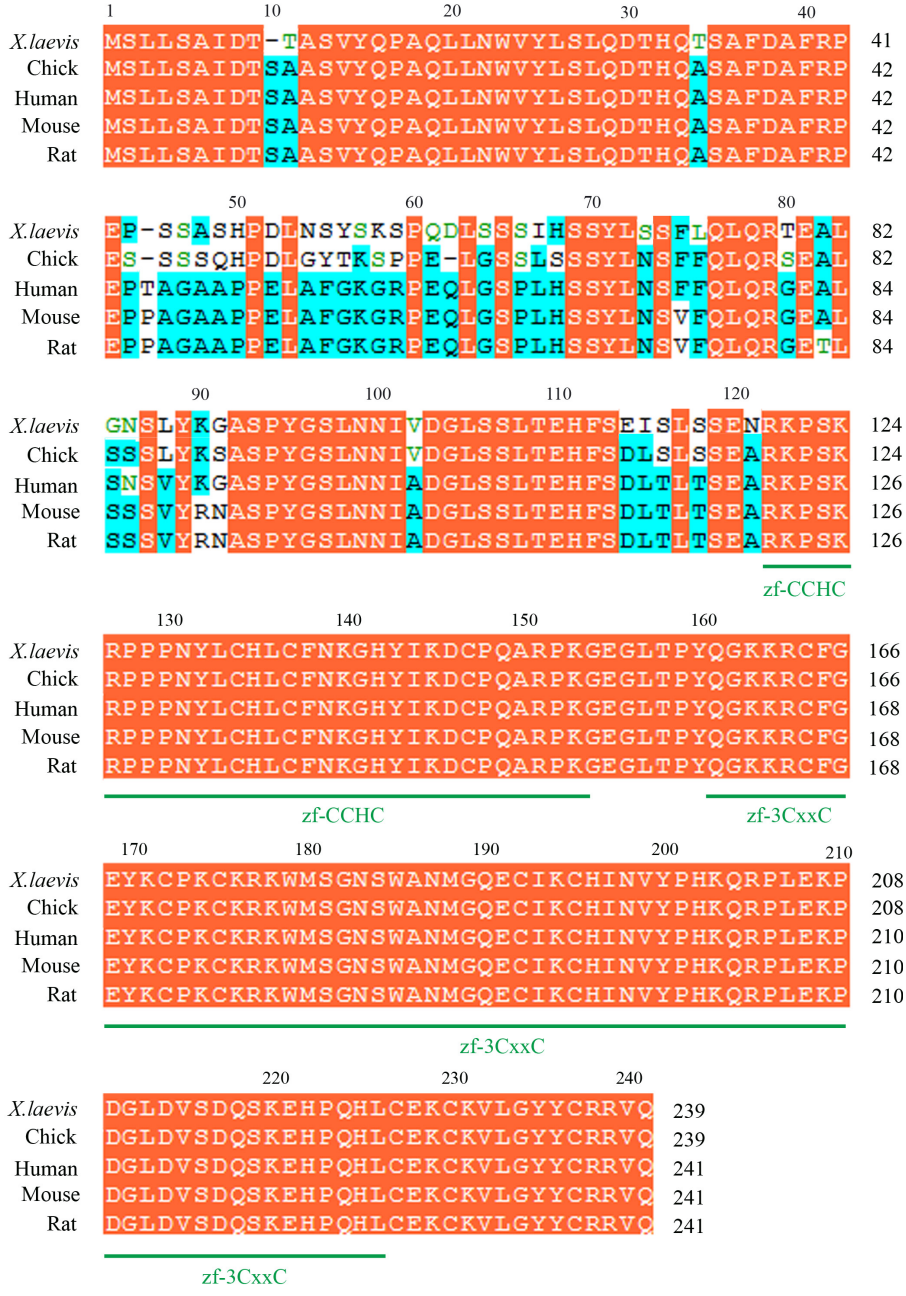

Fig. 1. Sequence alignment of Zcchc24 family members. *Comparison of the predicted amino acid sequence of* X. laevis *Zcchc24 with its chick, human, mouse and rat homologs.* X. laevis *Zcchc24 (Genbank accession no. KF438010) shares 90.8% of identity with chick ZCCHC24 (XP_421599), 86.3% with human ZCCHC24 (NP_699198), 84.6% with mouse ZCCHC24 (NP_001094903), and 84.2% with ZCCHC24 rat (NP_001101864) homologs. Conserved residues are shaded in orange while identical amino acids among some of the sequences are shaded in light blue. The absence of residues at the corresponding region is indicated by dashes. Both zf-CCHC and zf-3CxxC zinc finger domains are displayed in green.*

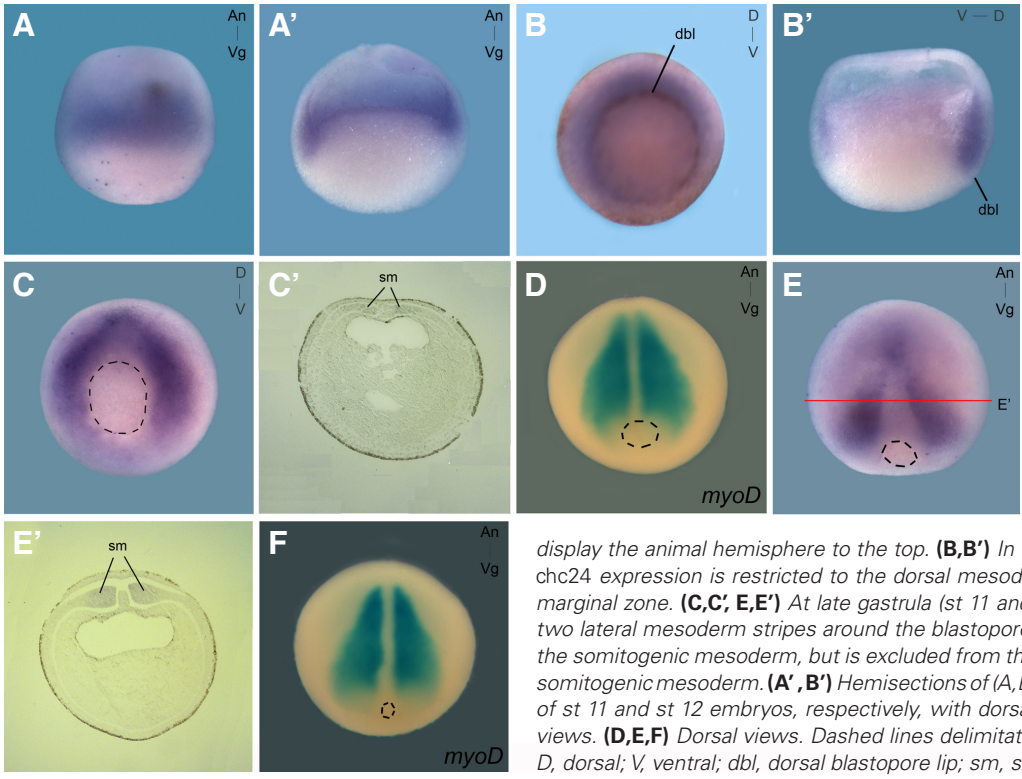

Fig. 2. *zcchc24* expression from blastula to gastrula stages. *Whole mount* in situ *hybridization using DIG labelled* zcchc24 **(A-C', E,E')** *or fluorescein labelled* myoD **(D,F)** *was performed on embryos from blastula to gastrula stages.* **(A,A')** *At blastula stages,* zcchc24 *is expressed as a ring around the marginal zone in both dorsal and ventral sides. The embryos display the animal hemisphere to the top.* **(B,B')** *In the beginning of gastrulation (st 10.5),* zcchc24 *expression is restricted to the dorsal mesoderm in both involuting and non-involuting marginal zone.* **(C,C', E,E')** *At late gastrula (st 11 and 12),* zcchc24 *transcripts are detected in two lateral mesoderm stripes around the blastopore that culminate in the dorsal mesoderm, the somitogenic mesoderm, but is excluded from the midline.* **(D,F)** myoD *is expressed in the somitogenic mesoderm.* **(A' ,B')** *Hemisections of (A,B), respectively.* **(C', E')** *Transverse sections of st 11 and st 12 embryos, respectively, with dorsal side displayed to the top.* **(B,C)** *Vegetal views.* **(D,E,F)** *Dorsal views. Dashed lines delimitate the blastopore. An, animal; Vg, vegetal; D, dorsal; V, ventral; dbl, dorsal blastopore lip; sm, somitogenic mesoderm.*

that *zcchc24* transcripts are clearly detected in heart region, more precisely, in endocardium, myocardium and pericardium, and in branchial arch mesenchyme (Fig. 4G'). The expression of *zcchc24* (Fig. 4G) in the heart is similar to the expression of *nkx2.5* in this region (Fig. 4 H,I). More, when *zcchc24* and *twi* expressions are compared, it becomes obvious that both genes are expressed in the branchial arches (Fig. 4 J,K).

According to our data, the expression pattern of *Xenopus zcchc24* is in part similar to the expression pattern of its chick homolog. Here, we show that, early in development, *Xenopus zcchc24* is expressed in the dorsal mesoderm, in which the cardiac mesoderm is initially formed, and later is expressed in the head, heart and somites, like its chick homolog at Hamburger and Hamilton stage 10 (HH10; Fig. 5). This suggests that these homologs might have a role in heart formation, both in *Xenopus* and chick. Moreover, it demonstrates that *zcchc24* expression pattern was conserved during evolution.

Several zinc finger proteins with a role in heart development

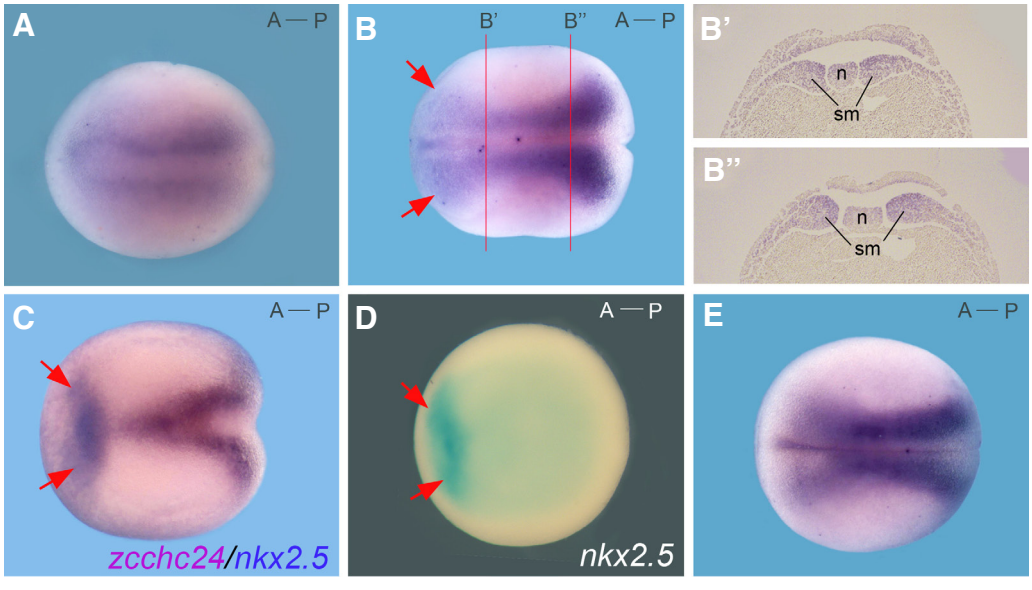

Fig. 3. *zcchc24* expression during neurula stages. *Whole mount* in situ *hybridization using DIG labelled* zcchc24 **(A-B", E)** *or fluorescein labelled* nkx2.5 **(D)** *and double whole mount* in situ *hybridization with DIG labelled* zcchc24 *and fluorescein labelled* nkx2.5 **(C)** *were performed on embryos at neurula stages.* **(A)** *At late gastrula/early neurula (st 13),* zcchc24 *is expressed as two stripes in the dorsal side of the embryo along the antero-posterior axis but is excluded from the notochord.* **(B-B", E)** *During neurula stages,* zcchc24 *is expressed in anterior and somitogenic mesoderm, decreasing the expression from the posterior to the anterior region. Comparison between the expression of* zcchc24 *and* nkx2.5 **(B,C,D)** *shows that the most anterior expression of* zcchc24 *is adjacent to the cardiac mesoderm.* **(A,B, C-E)** *Embryos in a dorsal view.* **(B', B")** *Transversal sections of st 15 embryos with the dorsal region displayed to the top. Red arrows indicate the heart precursor region. A, anterior; P, posterior; n, notochord; sm, somitogenic mesoderm.*

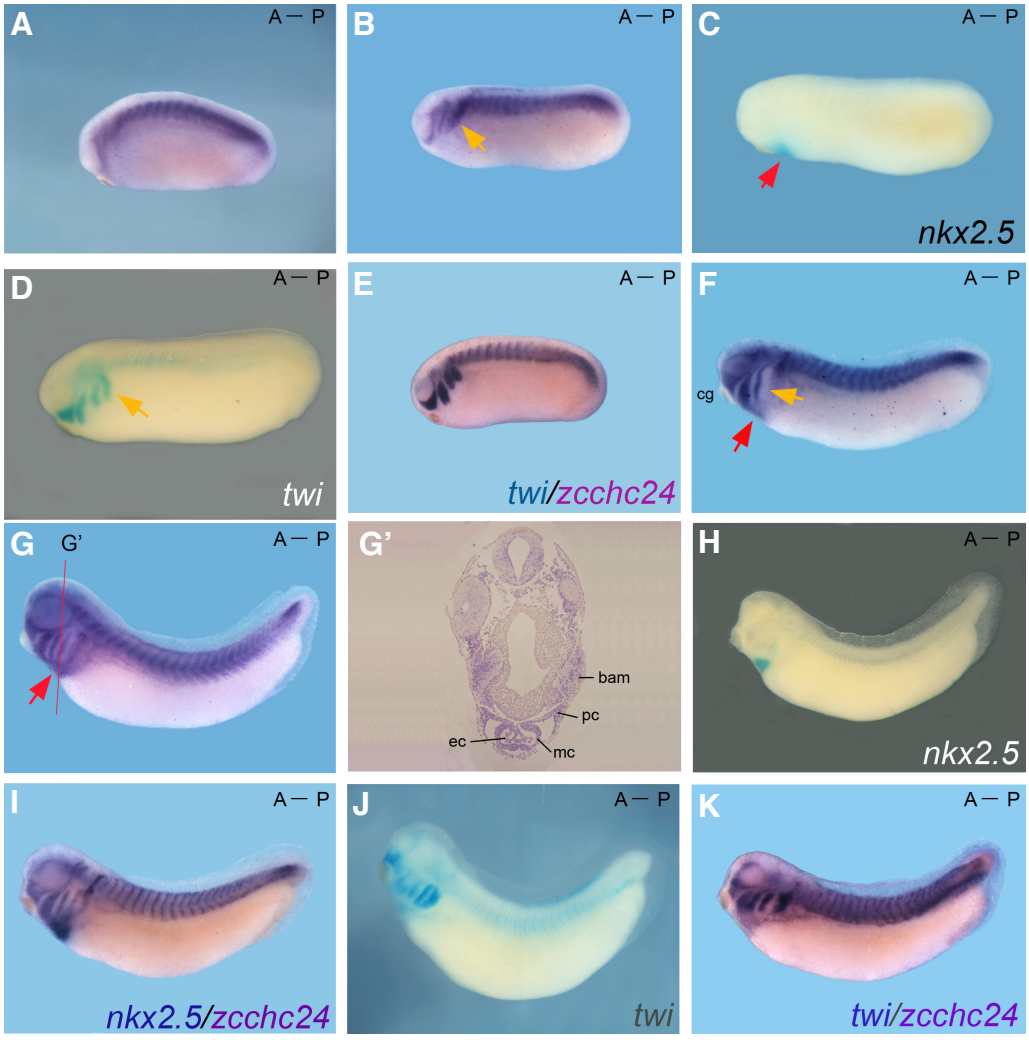

Fig. 4. Expression pattern of *zcchc24* during tailbud stages. *Whole mount* in situ *hybridization using DIG labelled* zcchc24 **(A,B,F-G')**, *fluorescein labelled* nkx2.5 **(C,H)** *or fluorescein labelled* twi **(D,J)**, *and double whole mount* in situ *hybridization using DIG labelled* zcchc24 *and fluorescein labelled* twi **(E,K)** *or DIG labelled* zcchc24 *and fluorescein labelled* nkx2.5 *(I) were performed on embryos at tailbud stages.* **(A-E)** *At early tailbud stages,* zcchc24 *is strongly expressed in the unsegmented somitogenic mesoderm, somites, and migrating neural crest but is absent in the region of differentiating cardiac muscle.* **(F)** *Later, at st 28,* zcchc24 *transcripts start to be detected in the forming heart, and in the entire head except the region between the branchial arches.* **(G-K)** *This expression is maintained throughout tailbud development, being* zcchc24 *expressed in the somites, branchial arches, head and heart. All the embryos are displayed in a lateral view with the anterior region to the left.* **(G')** *Transversal section of the heart region of a st 32 embryo. The orange arrows indicate the neural crest/branchial arches, and the red arrows indicate heart-forming region/heart. A, anterior; P, posterior; ec, endocardium; pc, pericardium; mc, myocardium; bam, branchial arch mesenchyme; cg, cement gland.*

have been described. The GATA zinc finger-containing transcription factors are a family of proteins that have been implicated in regulation of gene expression in the heart development (Haworth *et al.*, 2008). It was demonstrated in zebrafish that GATA5 is necessary for the production of the correct number of myocardial precursors and for the correct expression of several cardiac genes including *nkx2.5*. Indeed, the overexpression of *GATA5* induces contractile heart-like tissue (Reiter *et al.*, 1999). GATA 4 is another GATA family zinc finger that is also implicated in heart and liver development. It was shown that the knock-down of this transcript affects heart and liver primordia following their specification (Haworth *et al.*, 2008, Holtzinger and Evans, 2005). In addition, after heart specification, GATA4 interacts with another GATA family member, GATA6, during its action in the development of heart in mouse, *Xenopus* and zebrafish (Holtzinger and Evans, 2005, Peterkin *et al.*, 2003, Zhao *et al.*, 2005). Therefore, since *zcchc24* is expressed in heart precursor cells and later in the heart (Fig. 6), like GATA family genes, it is tempt fate to extrapolate a role for *zcchc24* in the formation of the heart.

Nevertheless, *zcchc24* is also highly expressed in somitogenic mesoderm and later in the somites. Sev-

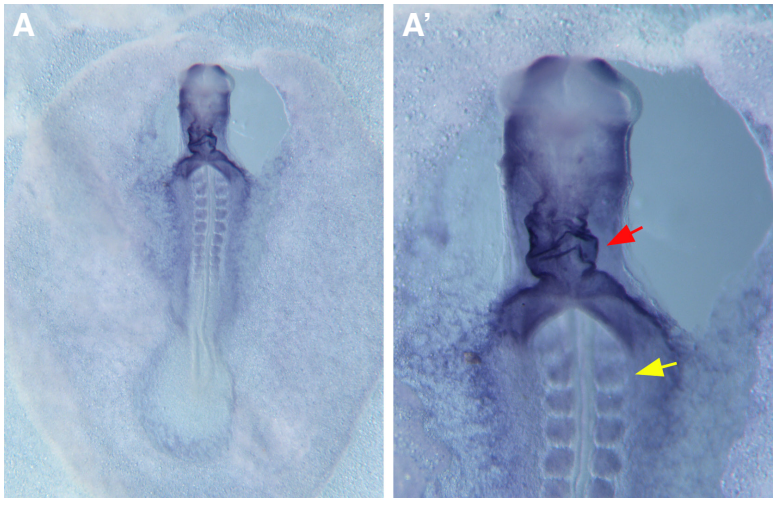

Fig. 5. In chicken embryos, *ZCCHC24* is expressed in the heart and somites. *Whole mount* in situ *hybridization using DIG labelled* ZCCHC24 *in chick embryos at HH10.* **(A,A')** *At this stage,* ZCCHC24 *is expressed in the heart (red arrow), somites (yellow arrow) and in head mesenchyme. (A') Magnification of (A).*

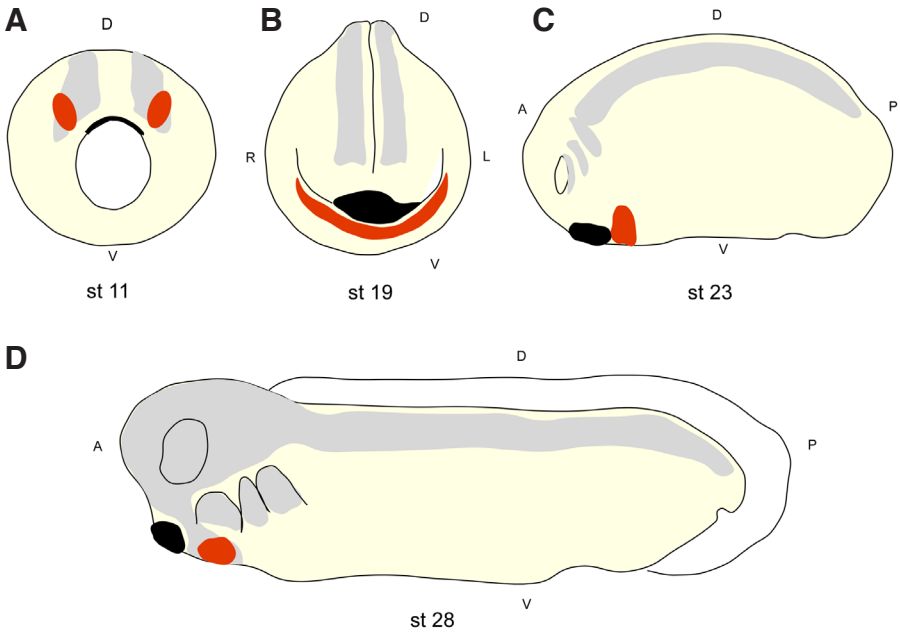

Fig. 6. Schematic representation of the localization of heart precursors and *zcchc24* expression during *X. laevis* embryonic development. *Red areas represent the heart precursors and the gray areas represent the expression of* zcchc24. **(A)** *Vegetal view,* **(B)** *anterior view,* **(C,D)** *lateral views with the anterior region to the left. A, anterior; P, posterior; D, dorsal; V, ventral; R, right; L, left.*

eral zinc fingers proteins like Gli, Gli3 and Gli4 members of the Hedgehog (Shh) signaling pathway have been implicated in somite formation. Gli-type proteins function as transcriptional repressors that respond to Shh signals, and control the expression of Shh-responsive genes such as *myf5*, the muscle master regulator (Hui and Angers, 2011). Therefore, we think that Zcchc24 putative role in the regulation of the proper somite segmentation could not be excluded.

The expression of *zcchc24* in the migrating neural crest and later in the branchial arches suggests that *zcchc24* might have a function during the development and/or migration of this tissue. Curiously, several zinc finger proteins of the Snail family have been associated to the neural crest formation (del Barrio and Nieto, 2002, Nieto *et al.*, 1994). For example, it was reported that *slug* and *snail*, two members of this family, are important for neural crest specification and migration. In *Xenopus* embryos or animal caps, the overexpression of *snail* is able to induce the expression of *slug* among other neural crest markers such as *foxD3, twi* and *ets1*. On the other hand, *slug* is not able to induce these neural crest markers, however, gain-of-function studies performed in chick showed that *slug* overexpression increases cranial neural crest production, and its loss-of-function inhibits neural crest migration (Aybar *et al.*, 2003, del Barrio and Nieto, 2002, Nieto *et al.*, 1994).

Taken together, our results showed that *zcchc24* is expressed mainly in three different precursors/structures: cardiac precursors/heart, somitogenic mesoderm/somites, and neural crest/branchial arches. Curiously, several proteins of the zf-CCHC superfamily of zinc finger containing proteins were described to have a role on cell death inhibition. Moreover, the proper formation of the heart, branchial arches and somites requires none or a low level of apoptosis. High levels of apoptosis in these three structures have been reported to be responsible for defects (Graham *et al.*, 1996, Kang and Izumo, 2000, Sanders and Parker., 2001). These observations indicate that Zcchc24 might be particularly important for the regulation of migration and/or apoptosis. Nevertheless, further genetic and biochemical analysis must be performed to clarify the role of *zcchc24* during *X. laevis* embryonic development.

Materials and Methods

Xenopus *embryo manipulations*

Xenopus eggs were obtained from females injected with 300 IU of human chorionic gonadotropin (Sigma) and were fertilized *in vitro*. Eggs were dejellied with 2% cysteine hydrochloride, pH 8.0. Embryos were grown in 0.1X MBS-H (1X MBS-H = 88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO3, 0.82 mM MgSO4, 0.41 mM CaCl2, 0.33 mM Ca(NO3)2, 10 mM HEPES, pH 7.4, 10 μg/mL streptomycin sulphate and 10 μg/mL penicillin) and staged according to Nieuwkoop and Faber (1967).

Cloning of partial coding sequence of zcchc24

Since the coding sequence (CDS) of *zcchc24* was not available in stock centers, to obtain it, a partial coding sequence was isolated by RT-PCR. With this purpose, total RNA from stage 20 of *Xenopus laevis* embryos was isolated using trizol reagent according to the manufacturer's instruction. To perform the RT-PCR, first strand cDNA was synthesized using oligoDT hexamers as primers and *zcchc24* CDS was amplified using a specific pair of primers (Forward 5'-CCATCCACTCCAGCTATCTGAGCA-3'; Reverse 5´-TTACTGAACACGGCGGCAGTAGTAGC-3'). The PCR product was cloned into pGEM®-T Easy and sequenced to confirm for correct DNA sequence.

Whole mount in situ hybridization and histology

Single and double whole mount *in situ* hybridization and anti-sense probes preparation was carried out as previously described (Belo *et al.*, 1997). To generate the fluorescein labelled *nkx2.5, myoD* and *twi* anti-sense RNA probes, plasmids containing fragments of these genes were linearized using XbaI, HindIII and EcoRI, respectively, and transcribed using T7 RNA polymerase. To synthesize *zcchc24* DIG labelled probe, a plasmid containing *zcchc24* fragment was linearized using SalI enzyme and transcribed using T7 RNA polymerase. Probes were purified using quick Spin Mini RNA columns (Roche). Hybridized RNAs were detected with alkaline phosphatase conjugated anti-DIG-antibody (Roche) and with alkaline phosphatase conjugated anti-Fluorescein-antibody (Roche) and developed with BM purple (Roche) or BCIP (Roche). Stained embryos were bleached by illumination in 1% H_2O_2, 4% formamide and 0.5X SSC pH7.0. Embryos were photographed under bright light using a MicroPublisher 5.0 RTV camera coupled with a Leica MZ16FA stereoscope or in a Zeiss Sterio Lumar V12 Stereomicroscope coupled with an Axiocam MRC.

For histology, after *in situ* hybridization, the *Xenopus* embryos were fixed overnight at 4°C in 4% PFA, embedded in paraffin, and sectioned in a Zeiss microtome. Sections were observed in a Zeiss Z2 microscope and photographed with a Zeiss AxioCam ICc3 camera.

Acknowledgements

M Vitorino and J M Inácio are recipients of a F.C.T. Post-Doc fellowship with reference SFRH/BPD/65923/2009 and SFRH/BPD/41081/2007,

respectively. Elizabeth Correia is recipient of a F.C.T B.I. fellowship (PTDC/SAU-BID/114902/2009). This work has been supported by research grants from F.C.T. and from IBB/CBME, LA to J.A. Belo in the frame of Project refº Pest-OE/EQB/LA0023/2013.

References

AYBAR, M.J., NIETO, M.A. and MAYOR, R. (2003). Snail precedes Slug in the genetic cascade required for the specification and migration of the *Xenopus* neural crest. *Development* 130: 483-494.

BELO, J., BOUWMEESTER, T., LEYNS, L., NKERTESZ, GALLO, M., FOLLETTIE, M. and ROBERTIS, E.D. (1997). Cerberus-like is a secreted factor with neutralizing activity expressed in the anterior primitive endoderm of the mouse gastrula. Mech Dev. *Mech Dev* 68: 45-57.

BENTO, M., CORREIA, E., TAVARES, A.T., BECKER, J.D. and BELO, J.A. (2011). Identification of differentially expressed genes in the heart precursor cells of the chick embryo. *Gene Expression Patt.* 11: 437-447.

BIRKE, M., SCHREINER, S., GARCÍA-CUÉLLAR, M.-P., MAHR, K., TITGEMEYER, F. and SLANY, R.K. (2002). The MT domain of the proto-oncoprotein MLL binds to CpG-containing DNA and discriminates against methylation. *Nucleic Acids Res.* 30: 958-965.

BLACKLEDGE, N.P., ZHOU, J.C., TOLSTORUKOV, M.Y., FARCAS, A.M., PARK, P.J. and KLOSE, R.J. (2010). CpG Islands Recruit a Histone H3 Lysine 36 Demethylase. *Molec. Cell* 38: 179-190.

DEL BARRIO, M.G. and NIETO, M.A. (2002). Overexpression of Snail family members highlights their ability to promote chick neural crest formation. *Development* 129: 1583-1593.

GRAHAM, A., KOENTGES, G. and LUMSDEN, A. (1996). Neural Crest Apoptosis and the Establishment of Craniofacial Pattern: An Honorable Death. *Molec. Cell Neurosci.* 8: 76-83.

HALL, T.M. (2005). Multiple modes of RNA recognition by zinc finger proteins. *Curr. Op. Struct. Biol.* 15: 367-373.

HAWORTH, K., KOTECHA, S., MOHUN, T. and LATINKIC, B. (2008). GATA4 and GATA5 are essential for heart and liver development in *Xenopus* embryos. *BMC Dev. Biol.* 8: 74.

HOLTZINGER, A. and EVANS, T. (2005). Gata4 regulates the formation of multiple organs. *Development* 132: 4005-4014.

HUI, C.-C. and ANGERS, S. (2011). Gli Proteins in Development and Disease. *Annu. Rev. Cell. Dev. Biol.* 27: 513-537.

KANG, P.M. and IZUMO, S. (2000). Apoptosis and Heart Failure: A Critical Review of the Literature. *Circ. Res.* 86: 1107-1113.

KRISHNA, S.S., MAJUMDAR, I. and GRISHIN, N.V. (2003). Structural classification of zinc fingers: SURVEY AND SUMMARY. *Nuc. Acid. Res.* 31: 532-550.

LAITY, J.H., LEE, B.M. and WRIGHT, P.E. (2001). Zinc finger proteins: new insights into structural and functional diversity. *Curr. Opin. Struct. Biol.* 11: 39-46.

MAGUIRE, R.J., ISAACS, H.V. and ELIZABETH POWNALL, M. (2012). Early transcriptional targets of MyoD link myogenesis and somitogenesis. *Dev. Biol.* 371: 256-268.

NIETO, M., SARGENT, M., WILKINSON, D. and COOKE, J. (1994). Control of cell behavior during vertebrate development by Slug, a zinc finger gene. *Science* 264: 835-839.

PETERKIN, T., GIBSON, A. and PATIENT, R. (2003). GATA-6 maintains BMP-4 and Nkx2 expression during cardiomyocyte precursor maturation. *EMBO J* 22.

REITER, J.F., ALEXANDER, J., RODAWAY, A., YELON, D., PATIENT, R., HOLDER, N. and STAINIER, D.Y.R. (1999). Gata5 is required for the development of the heart and endoderm in zebrafish. *Genes Dev.* 13: 2983-2995.

SANDERS, E. and PARKER., E. (2001). Ablation of axial structures activates apoptotic pathways in somite cells of the chick embryo. *Anat Embryol (Berl)* 204: 389-98.

SATER, A.K. and JACOBSON, A.G. (1989). The specification of heart mesoderm occurs during gastrulation in *Xenopus laevis*. *Development* 105: 821-830.

ZHAO, R., WATT, A.J., LI, J., LUEBKE-WHEELER, J., MORRISEY, E.E. and DUNCAN, S.A. (2005). GATA6 Is Essential for Embryonic Development of the Liver but Dispensable for Early Heart Formation. *Molec. Cell Biol.* 25: 2622-2631.

Further Related Reading, published previously in the *Int. J. Dev. Biol.*

***Sox17*-dependent gene expression and early heart and gut development in *Sox17*-deficient mouse embryos**
Sabine Pfister, Vanessa J. Jones, Melinda Power, Germaine L. Truisi, Poh-Lynn Khoo, Kirsten A. Steiner, Masami Kanai-Azuma, Yoshiakira Kanai, Patrick P. L. Tam and David A. F. Loebel
Int. J. Dev. Biol. (2011) 55: 45-58
http://dx.doi.org/10.1387/ijdb.103158sp

Building the vertebrate heart - an evolutionary approach to cardiac development
José M. Pérez-Pomares, Juan M. González-Rosa and Ramón Muñoz-Chápuli
Int. J. Dev. Biol. (2009) 53: 1427-1443
http://dx.doi.org/10.1387/ijdb.072409jp

Myoskeletin, a factor related to Myocardin, is expressed in somites and required for hypaxial muscle formation in *Xenopus*
Hui Zhao, Martha L. Rebbert and Igor B. Dawid
Int. J. Dev. Biol. (2007) 51: 315-320
http://dx.doi.org/10.1387/ijdb.062260hz

Msx1 and Msx2 have shared essential functions in neural crest but may be dispensable in epidermis and axis formation in *Xenopus*
Deepak Khadka, Ting Luo and Thomas D. Sargent
Int. J. Dev. Biol. (2006) 50: 499-502
http://dx.doi.org/10.1387/ijdb.052115dk

Early stages of neural crest ontogeny: formation and regulation of cell delamination
Chaya Kalcheim and Tal Burstyn-Cohen
Int. J. Dev. Biol. (2005) 49: 105-116
http://dx.doi.org/10.1387/ijdb.041949ck

5 yr ISI Impact Factor (2011) = 2.959