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Expression patterns of genes encoding small GTPases Ras-dva-1 and Ras-dva-2 in the *Xenopus laevis* tadpoles

Maria B. Tereshina, Andrey V. Bayramov, Andrey G. Zaraisky *

Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

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ABSTRACT

Small GTPases of the recently discovered Ras-dva family are specific to the Vertebrate phylum. In *Xenopus laevis*, *Ras-dva-1* is expressed during gastrulation and neurulation in the anterior ectoderm where it regulates the early development of the forebrain and cranial placodes (Tereshina et al., 2006). In the present work, we studied the expression of *Ras-dva-1* at later developmental stages. As a result, the *Ras-dva-1* expression was revealed in the eye retina, epiphysis (pineal gland), hypophysis (pituitary), branchial arches, pharynx, oesophagus, stomach and gall bladder of swimming tadpoles. Additionally, we investigated for the first time the expression pattern of *Ras-dva-2*. This gene encodes a protein belonging to a novel sub-group of Ras-dva GTPases that we identified by phylogenetic analysis within Ras-dva family. In contrast to *Ras-dva-1*, *Ras-dva-2* is not expressed before the swimming tadpole stage. At the swimming tadpole stage, however, *Ras-dva-2* transcripts can be detected in the eye retina and brain. Later in development, the expression of *Ras-dva-2* can also be revealed in the mesonephros and stomach.

Small GTPases are monomeric regulatory GTP-hydrolases that circulate between active, GTP-bound, and inactive, GDP-bound, states. More than 200 proteins have been identified that constitute nine families of small GTPases according to the homology of their primary structure. These families include the following: Ras, Rab, Rho, Ran, RJL, Arf/Sar, Ras-dva, RGK and Gie (Finlin et al., 2000; Takai et al., 2001; Nepomuceno-Silva et al., 2004; Okai et al., 2004; Tereshina et al., 2006). These proteins are involved in the regulation of many cellular processes, including signal transduction (Ras, Rho), cytoskeleton reorganisation (Rho, RGK, Gie), and vesicular and nuclear–cytoplasmic transport (Rab, Arf/Sar and Ran). Recently, we investigated the expression pattern and biological function of one representative of Ras-dva GTPases, the *Xenopus laevis* Ras-dva-1 (former Ras-dva). The expression of *Ras-dva-1* begins at the midgastrula stage and is seen throughout the anterior ectoderm. By neurulation, its expression is confined to the crescent-shape area of the non-neural ectoderm that surrounds the anterior neural plate and gives rise to the cement and hatching glands (Novoselov et al., 2003). We also demonstrated, by using various approaches, that the normal functioning of Ras-dva-1 is critical during this early period of development for the further differentiation of the forebrain and cranial placodes (Tereshina et al., 2006). These data indicate the potential importance of Ras-dva-1 GTPase for the development of other tissues and organs in which the gene is expressed. To begin to address this issue, we investigated the expression pattern of *Ras-dva-1* at later developmental stages. Based on recently accumulated genomic sequencing data, we also re-examined the phylogeny of Ras-dva GTPases in the different classes belonging to the Vertebrate phylum. As a result of this examination, we found two sub-groups of proteins within this family of small GTPases, which we designated as Ras-dva-1 and Ras-dva-2. To compare the expression patterns of genes from these two sub-groups, we cloned the cDNA sequence of *Ras-dva-2* of *X. laevis* and investigated its expression at different developmental stages. From these experiments, we find that, unlike *Ras-dva-1*, *Ras-dva-2* is not expressed before the swimming tadpole stage. At later stages, both genes have non-overlapping expression patterns in many organs that are derived from different tissues. Despite their different origins, each organ shares a common feature,

* Corresponding author. Address: Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Miklukho-Maklaya 16/10, Moscow 117997, Russia. Tel./fax: +7 495 336 36 22.
E-mail address: azaraisky@yahoo.com (A.G. Zaraisky).

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which is that each organ has a pronounced secretory activity. This finding indicates that one of the intracellular functions of Ras-dva GTPases may be associated with the regulation of cellular secretion.

1. Results and discussion

1.1. Ras-dva-1 expression at late developmental stages in X. laevis embryos

In previous work, we investigated the expression of *Ras-dva-1* from the beginning of gastrulation to the late tail-bud stage (stage 30). During this time, it is expressed in the transient secretory embryonic organs derived from the non-neural anterior ectoderm, in the cement and hatching glands. To understand whether such *Ras-dva-1* expression pattern is determined by its tissue (ectoderm) or some functional (possibly secretion) specificity, we examined the expression pattern of *Ras-dva-1* at later developmental stages (stage 30–46) when many more tissue types have acquired their final identity.

No expression of *Ras-dva-1* was detected by whole-mount in situ hybridisation on stages earlier than stage 33, except for in the cement and hatching glands. Just after this stage, *Ras-dva-1*-positive cells were also detected in the pineal gland (epiphysis), and this expression pattern was seen up to the tadpole stage 46 (the last stage to be analysed in the present study) (Fig. 1A, B and C). The morphological specification of the pineal gland begins

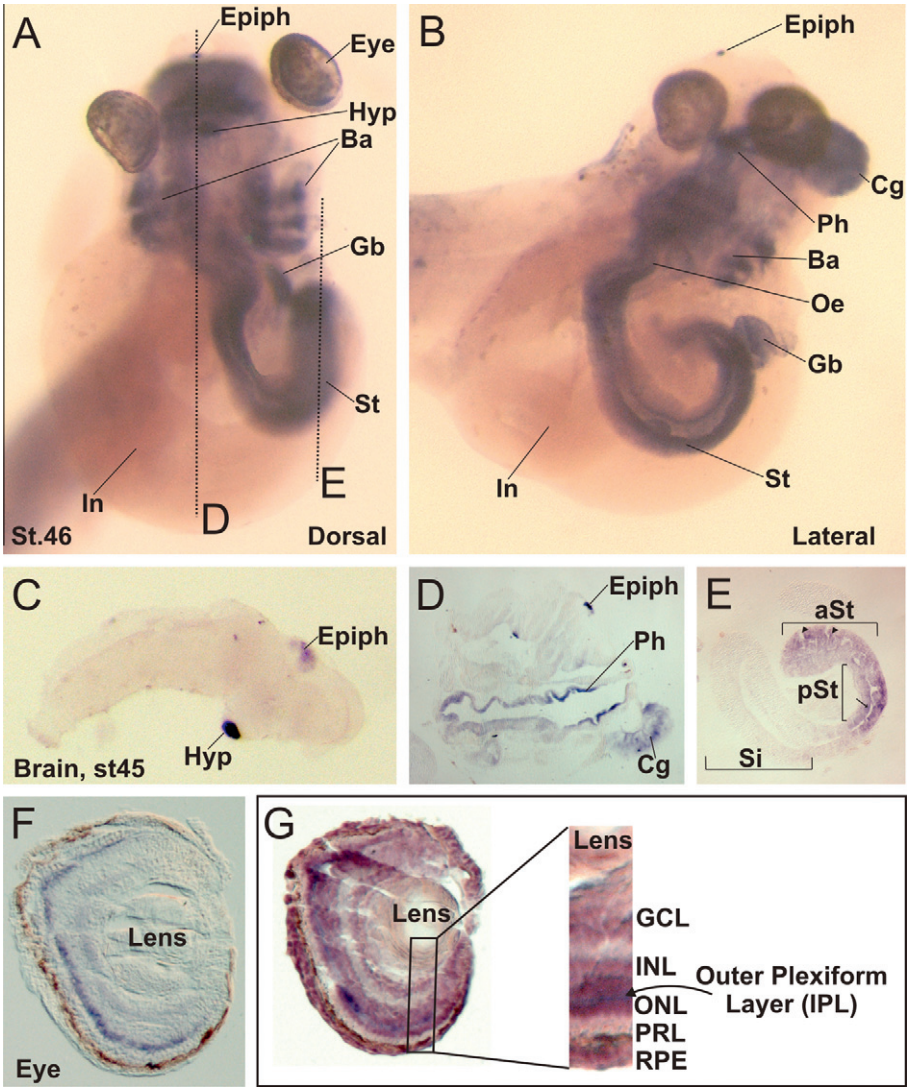

Fig. 1. Expression pattern of *Ras-dva-1* in *Xenopus laevis* tadpoles. (A and B) In situ hybridisation of the whole tadpole at stage 46. The tadpole is shown from the dorsal (A) and right (B) sides. *Ras-dva-1* positive cells are localised in the epiphysis, hypophysis, eye, branchial arches (gills), cement gland, pharynx, oesophagus, stomach and gall bladder. The dotted lines indicate the positions of the sagittal sections shown in D and E. (C) The in situ hybridisation on the whole brain extirpated from a tadpole at stage 45 demonstrates that *Ras-dva-1* is specifically expressed in the epiphysis and hypophysis. Dorsal side up, anterior to the left (see scheme of the brain units on Fig. 4E). (D) *Ras-dva-1* expression in the cement gland, pharynx and epiphysis as it is seen on the medial sagittal section (see position of the section in A). (E) Para-sagittal section of the tadpole (see position of the section in A) shows that *Ras-dva-1* is expressed only in the anterior and posterior regions of the stomach and not in the small intestine. In the anterior stomach region, the strongest *Ras-dva-1* expression can be detected in the areas of acinar glands (arrowheads), which are typical for that part of the gut where they secret different proteolytic enzymes and mucous. Additionally, in the posterior stomach region *Ras-dva-1* expression is stronger in the mucous epithelium (arrow), which lines the stomach cavity and secretes different compounds of the defensive mucous layer. (F) The transverse section of the eye shows that *Ras-dva-1* expression is localised in the retina. (G) After staining by haematoxylin, the *Ras-dva-1* expressing region can be identified as the Outer Plexiform Layer (OPL) of the retina. Ba – branchial arches (gills), Cg – cement gland, Epiph – epiphysis, Gb – gall bladder, GCL – ganglion cell layer, Hyp – hypophysis, In – intestine, INL – inner nuclear layer, Oe – oesophagus, ONL – outer nuclear layer, Ph – pharynx, PRL – photoreceptor layer, RPE – retinal pigment epithelium, Si – small intestine, St – stomach (aSt – anterior stomach region, pSt – posterior stomach region).