Gene Expression Patterns 11 (2011) 239–243

Contents lists available at ScienceDirect

Gene Expression Patterns

journal homepage: www.elsevier.com/locate/gep

Amphioxus Tbx6/16 and Tbx20 embryonic expression patterns reveal ancestral functions in chordates

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ARTICLE INFO

Article history:
Received 11 October 2010
Received in revised form 7 December 2010
Accepted 18 December 2010
Available online 24 December 2010

Keywords:
Amphioxus
T-box
AmphiTbx6/16
AmphiTbx20
TBX6
TBX16
TBX24
MGA
VegT
Ventral mesoderm
Heart
Pre-oral pit
Posterior mesoderm

ABSTRACT

T-box transcription factors are found in all metazoans and play diverse roles during embryogenesis. In the cephalochordate amphioxus, nine T-box genes were previously identified. In this work we undertook the analysis of the embryonic expression pattern of *Tbx6/16* and *Tbx20*, the last two T-box genes for which no such data are available. We found that *Tbx6/16* is expressed in the unsegmented paraxial mesoderm, in a subpopulation of neurons, and in the tail epidermis. Comparison with the expression patterns of the different vertebrate orthologues indicates a conserved role of those genes in posterior mesoderm formation in chordates. *Tbx20* expression is detected in the ventral mesoderm of amphioxus embryos, in cells that are proposed to be precursors of the amphioxus myocardium, in some neurons of the neural tube, and in the pre-oral pit which is thought to be the homologue of the vertebrate adenohypophysis. In vertebrates, *Tbx20* is also one of the first genes expressed in the embryonic heart field, suggesting that the function of this gene in heart development has been conserved during chordate evolution.

T-box genes (*Tbx*) code for transcription factors found in all metazoans in which they are implicated in diverse developmental processes. They are characterized by a highly conserved DNA binding domain (T-domain) which recognizes a consensus palindrome site (T-site) of 20 bp. Eighteen T-box genes, classically divided into five families, are known in vertebrates, whereas much less genes are found in non-vertebrate species (Minguillon and Logan, 2003; Naiche et al., 2005). In the urochordate *Ciona intestinalis*, a member of the sister group of vertebrates, eight T-box genes, that are orthologues of seven vertebrate paralogy groups, are known (Takatori et al., 2004). In the cephalochordate amphioxus, a representative of the most basally divergent group among chordates, nine T-box genes were previously described, namely *AmphiTbx1/10*, *AmphiTbx2/3*, *AmphiTbx4/5*, *AmphiTbx6/16*, *AmphiTbx15/18/22*, *AmphiTbx20*, *AmphiBra1*, *AmphiBra2* and *AmphiTbr1/Eomes/Tbx21* (Beaster-Jones et al., 2006; Holland et al., 1995; Horton and Gibson-Brown, 2002; Horton et al., 2008; Mahadevan et al., 2004; Minguillon et al., 2009; Ruvinsky et al., 2000). Amphioxus is an amenable animal model to study the invertebrate-chordate to vertebrate transition, due to its morphological, developmental and genomic characteristics that are little derived compared to the chordate ancestor (Schubert et al., 2006). Studying the expression pattern of T-box genes in amphioxus can therefore shed light on the functional evolution of this gene family in the chordate lineage. The embryonic expression pattern of all T-box genes from amphioxus have been described, except for *AmphiTbx6/16* and *AmphiTbx20*. Moreover, only a partial short sequence of *AmphiTbx20* is present in the databases, rendering impossible a formal phylogenetic reconstruction. In this study we undertook the cloning of *Tbx6/16* and *Tbx20* from the European amphioxus *Branchiostoma lanceolatum*, we assessed their orthology relationships with vertebrate T-box genes, and we determined their expression pattern during embryonic development.

1. Results

1.1. Cloning and phylogenetic study

Partial cDNA of *B. lanceolatum Tbx6/16* and *Tbx20*, of 474 and 439 bp, respectively, were cloned from embryonic total mRNA. We used the predicted sequences of these two genes present in

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doi:10.1016/j.gep.2010.12.006

the genome of *B. floridae* for phylogenetic study. The tree constructed using Maximum Likelihood inference shows that *Tbx6/16* is the orthologue of vertebrate *Tbx6*, *Tbx16*, *Tbx24* and *VegT*, and that *Tbx20* is the orthologue of vertebrate *Tbx20*, although the branch support for *Tbx6/16* is low (Fig. 1).

1.2. *Tbx6/16* expression pattern

We analyzed the expression of *Tbx6/16* in *B. lanceolatum* by whole mount *in situ* hybridization from the four cells stage to the larval stage. No expression could be detected using this technique before the gastrula stage. In gastrulae, *Tbx6/16* is expressed in the ventral posterior mesendoderm and ectoderm as well as in the dorsal posterior mesendoderm (Fig. 2A and B). The same expression pattern is observed in later gastrulae, the dorsal expression being more expanded in the paraxial domain (Fig. 2C and D). Then, at the mid and mid-late neurula stage, expression keeps on being restricted to the posterior part of the embryo, in the future tailbud, and in the ventral posterior epidermis (Fig. 2E–H). This posterior expression is still observed in late neurulae before the mouth opens and labelling is first observed at this stage in some lateral neurons posterior to the fisrt pigment spot (Fig. 2I–I'', J). In the larva, *Tbx6/16* is expressed in the tailbud, in the epidermis of the tailfin and the neural expression posterior to the first pigment spot is expanded compared to earlier stage (Fig. 2K–M).

1.3. *Tbx20* expression pattern

Amphioxus *Tbx20* expression is first detected in mid-late neurula in the ventral mesoderm (Fig. 3A and B). As already mentioned by others (Panopoulou et al., 1998), the morphological ventral midline in amphioxus is displaced to the right side of the embryo; this is why the *Tbx20* expression on a ventral view does not match the topographical midline (Fig. 3B). At a later stage, before the mouth opens, expression is still observed in the ventral mesoderm from the pharyngeal region to the most posterior part of the embryo (Fig. 3C, E and F). Again, the expression in the pharynx is displaced to the right (Fig. 3E and F). Moreover at this stage expression is also detected in some few ventral neurons, the number and position of which vary among specimens (Fig. 3C and D). In the larva, the same overall expression pattern is observed with additional labelling in the pre-oral pit (Fig. 3H–H''', J).

2. Discussion

2.1. Amphioxus *Tbx6/16* expression pattern reflects those of its vertebrate and urochordate orthologues

The phylogenetic analysis (Fig. 1) shows that amphioxus *Tbx6/16* is the orthologue of vertebrate *Tbx6*, *Tbx16*, *Tbx24* and *VegT* genes. The *Mga* (MAX gene associated) T-box domain also belongs to this group although *Mga*, unlike other T-box genes, includes a bHLHZip domain coding region (Lardelli, 2003). The T-box region of *Mga* has been proposed to be the result of a reverse transcription event (Lardelli, 2003). In the urochordate *C. intestinalis* three orthologues of the *Tbx6/16* group have been described, namely *Ci-tbx6a*, *Ci-tbx6b* and *Ci-tbx6c* (Takatori et al., 2004). All three are expressed at early developmental stages in the B-line muscle and mesenchyme cells. Then, during gastrulation, they are expressed in muscle cells. Later on, *Ci-tbx6a* expression becomes restricted to the tip of the tail and to posterior epidermal cells, whereas expression is no longer visible in the tadpole stage for *Ci-tbx6b* and *Ci-tbx6c*. In vertebrates, all the members of the *Tbx6/16* group are expressed in the paraxial unsegmented

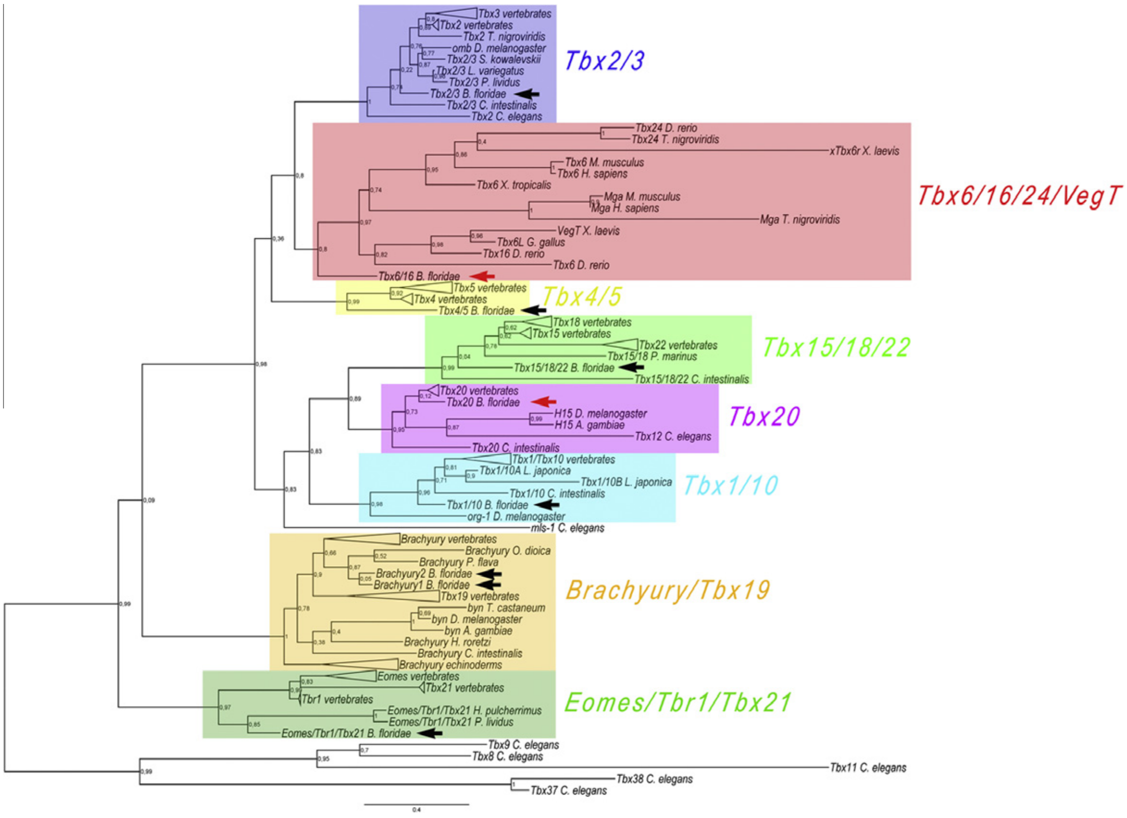

Fig. 1. Phylogeny of the *Tbx* gene family. For clarity, vertebrate orthology groups were collapsed. Each *Tbx* group is highlighted with a specific colour. *B. floridae Tbx* are indicated by a black arrow. *B. floridae Tbx6/16* and *Tbx20* are indicated by a red arrow. Scale bar represents 0.4 amino acid substitution per site.