Gene Expression Patterns 10 (2010) 306–313

Contents lists available at ScienceDirect

Gene Expression Patterns

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

An oligochaete homologue of the *Brachyury* gene is expressed transiently in the third quartette of micromeres

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ARTICLE INFO

Article history:
Available online 4 July 2010

Keywords:
Brachyury
Annelid
Tubifex tubifex
Micromeres
Teloblasts
Blastopore
Direct development

ABSTRACT

We have isolated a *Brachyury* homologue (*Ttu-Bra*) from the oligochaete annelid *Tubifex tubifex* which displays a direct mode of development. Developmental RT-PCR analysis showed that *Ttu-Bra* transcripts are present in embryos at stages 9–11, 16 and 17, but undetectable at the remaining embryonic stages. Whole-mount *in situ* hybridization demonstrated that *Ttu-Bra* is expressed transiently in the third quartette of micromeres, which are located at the prospective stomodaeum (at stages 9–11). The second burst of *Ttu-Bra* expression occurs at the posterior end of stage 16 embryo that undergoes body elongation. *Ttu-Bra*-expressing cells, which are organized in a circle at stage 16, become aggregated at the proctodaeum at stage 17. Consistent with the results of the RT-PCR analysis, there is no sign of *Ttu-Bra*-expressing cells in embryos that undergo gastrulation during stages 12–15.

1. Results and discussion

The gene *Brachyury* encodes a transcriptional factor that has a conserved DNA-binding domain called T-domain in N-terminal half of the protein. Although *Brachyury* homologues have been isolated in many metazoan phyla, their developmental roles have been analyzed only in *Drosophila* and vertebrates. In *Drosophila*, *brachyenteron* (*Brachyury* homologue) is involved in the morphogenesis of the caudal part of the gut and its derivatives (e.g. anal pads) and the development of the caudal visceral mesoderm (Kispert et al., 1994; Kusch and Reuter, 1999). In vertebrates, *Brachyury* has a conserved role in mesoderm differentiation (Showell et al., 2004).

The spatial patterns of *Brachyury* expression have been examined in many organisms. Most intriguingly, in all of the animals examined, *Brachyury* is expressed around the blastopore of the developing embryos and larvae. Recent comparative analyses of *Brachyury* expression patterns in basal members of Lophotrochozoa, Ecdysozoa and Deuterostomia have suggested that the original expression of *Brachyury* in Bilaterians was in the blastopore and its derived structures, the stomodaeum/foregut and the proctodaeum/hindgut in the primary larva (Arendt et al., 2001; Technau, 2001; Scholz and Technau, 2003). Such "original" traits of *Brachyury* expression appear to have been preserved in animals that display an indirect mode of development via primary, ciliary larvae (such as trochophora, bipinnaria, pluteus, and tornaria larvae); these animals include the polychaete annelid *Platynereis dumerilii* (Arendt et al., 2001), the molluscs *Patella vulgata* (Lartillot et al., 2002) and *Saccostrea kegaki* (Kin et al., 2009), the hemichordate *Ptychodera flava* (Tagawa et al., 1998), the starfish *Asterina pectinifera* (Shoguchi et al., 1999), and the sea urchins *Lytechinus variegatus* (Gross and McClay, 2001) and *Paracentrotus lividus* (Croce et al., 2001). In the acoel flatworm *Convolutriloba longifissura* which has only one opening to its digestive system, *Brachyury* homologue is expressed not only in association with the mouth but also in small region at the posterior end of the animal (Hejnol and Martindale, 2008). In contrast, in animals that display a direct mode of development without larval stages or develop indirectly by means of larvae other than ciliary larvae (such as tunicates, acraniates, vertebrates, insects and nematodes), there is no stomodeal *Brachyury* expression; *Brachyury* expression is restricted to the small caudal region (Yasuo and Satoh, 1994; Holland et al., 1995; Kispert et al., 1994; Woollard and Hodgkin, 2000). It should be mentioned, however, that there has been reported a directly developing organism (a chaetognath *Paraspadella gotoi*) in which a *Brachyury* homologue is expressed not only in the blastopore region but also in the stomodaeum region (Takada et al., 2002).

During the course of examining the temporal and spatial aspects of embryonic expression of a *Brachyury* homologue (*Ttu-Bra*) in an oligochaete annelid *Tubifex tubifex* which uses a direct mode of development via teloblasts, we found that *Ttu-Bra* is expressed not only in a small ring of cells encircling the caudal region but also in an anterior region corresponding to the prospective stomodaeum; this expression pattern is apparently similar to the expression patterns reported for organisms displaying the indirect mode of development through ciliary larvae.

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doi:10.1016/j.gep.2010.07.001

1.1. Cloning of Tubifex homologue of Brachyury

Using a set of degenerate oligonucleotide primers, we amplified a *Brachyury* homologue from *T. tubifex* cDNA generated from mixed embryonic stages. The amplified fragment was 361 bp long and the deduced amino acid sequence contained a putative T-domain, which was more homologous to T-domain of Brachyury than that of other T-box family members.

To obtain additional sequence for the entire T-domain, not only 3′RACE but also 5′RACE were performed using gene-specific primers. The amplified fragments contained 1419 bp in 3′RACE and 661 bp in 5′RACE. Nucleotide sequences thus obtained were aligned with each other using an AutoAssembler 2.0 software (Applied Biosystems). The results showed that a cDNA of *T. tubifex* homologue of *Brachyury* (*Ttu-Bra*) contained 2100 bp (except for a poly-A tail) and that an open reading frame of 1524 bp encodes a polypeptide of 508 amino acids (Accession No. AB553740).

The T-domain of *Ttu-Bra* aligned well with Brachyury proteins of other animals (such as molluscs, polychaete annelids, insects and vertebrates) with more than 69% identity (Fig. 1A), suggesting

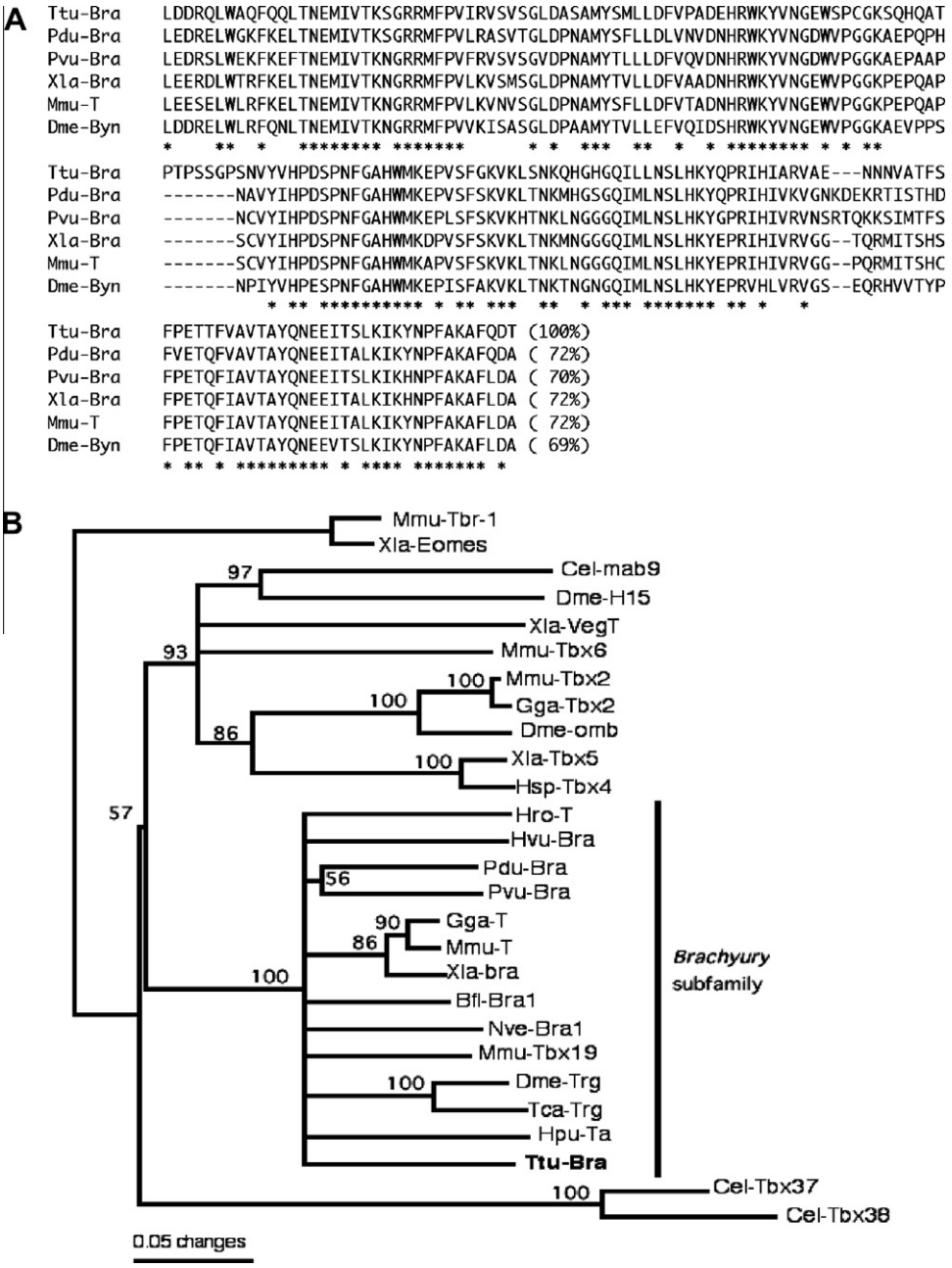

Fig. 1. Characterization of *Ttu-Bra*, a *Brachyury* homologue from *Tubifex tubifex*. (A) Alignment of the T-domain of Ttu-Bra with known Brachyury class proteins. The numbers of amino acid residues in the T-domain presented are 222 for Ttu-Bra, 218 for Pdu-Bra and Pvu-Bra, and 216 for Xla-Bra, Mmu-T and Dme-Byn. Asterisks represent amino acid identity. Numbers in parentheses indicate the percentage amino acid identity with the T-domain of Ttu-Bra. (B) Molecular phylogenetic relationship of Ttu-Bra to other T-box proteins. The phylogenetic tree was generated by the neighbor joining method using PAUP* 4.0b10. Mmu-Tbr-1 and Xla-Eomes were used as outgroups. Numbers are bootstrap values (as percentages of 1000 replications). Lengths of branches are drawn to the scale indicated. Species abbreviations: Bfl, *Branchiostoma floridae* (amphioxus); Cel, *Caenorhabditis elegans* (nematode); Dme *Drosophila melanogaster* (fruit fly); Gga, *Gallus gallus* (chick); Hpu, *Hemicentrotus pulcherrimus* (sea urchin); Hro, *Halocynthia rotetzi* (tunicate); Hsa, *Homo sapiens* (human); Hvu, *Hydra vulgaris* (hydra); Mmu *Mus musculus* (mouse); Nve, *Nematostella vectensis* (cnidaria); Pdu, *Platynereis dumerilii* (annelid); Pvu, *Patella vulgata* (mollusc); Tca, *Tribolium castaneum* (beetle); Ttu, *Tubifex tubifex* (annelid); Xla, *Xenopus laevis* (frog).