ABSTRACT We have identified a novel planarian molecule, named TNEX59, that is regionally expressed. This molecule is localised mainly in the nuclei of mesenchymal cells in intact adult planarians in a distribution gradient, with the faintest signal located in the central body region, which includes the pharynx. The dynamics of localisation during regeneration depend on the regions to be regenerated. Our results suggest that TNEX59 is involved in the formation and/or maintenance of A/P planarian body pattern during regeneration.

Results and Discussion

TNEX59 was first identified by using a planarian specific monoclonal antibody (mAb) from a specific mAbs library (Bueno et al., 1997).

Dynamics of TNEX59 localisation in regenerating tails

Tail regenerates were cut at postpharyngeal level and were expected to regenerate new central and anterior regions. Just after amputation, TNEX59 was distributed as in an intact adult tail; that is, the deepest signal of the gradient. At 1 day of regeneration, TNEX59 was located in the nuclei of blastema cells, as soon as the blastema could be identified. The distribution gradient of expression was re-established, although transiently. Surprisingly, most nuclei of epithelial cells expressed TNEX59, in contrast with intact adult organisms. At 3 days of regeneration, TNEX59 expression faded in most nuclei of epithelial cells, as in intact adult organisms. The accumulation of highly stained morphologically undifferentiated cell nuclei in the central area that will generate the pharynx primordium attenuated the gradient (Fig. 1A). At 5 days of regeneration, the nuclei of pharynx primordium cells were strongly stained with the mAb recognising TNEX59, which still reduced the gradient. At 7 days of regeneration, TNEX59 expression in the nuclei of pharyngeal cells faded. The A-P gradient was definitively re-established. Subsequently, the adult proportions were restored through an epimorphic-morphallactic process.

Dynamics of TNEX59 localisation in regenerating heads

Head regenerates were cut at prepharyngeal level and were expected to regenerate new central and posterior regions. Just after amputation, TNEX59 was distributed as in an intact adult head; that is, the deepest signal of the gradient. At 1 day of regeneration,
TNEX59 was located in the nuclei of blastema cells, as soon as the blastema could be identified, and as described by regenerating tails. The A/P gradient, however, was not re-established; nuclei from the ventral mesenchyma cells of the regenerate were deep stained, especially those that surround the cephalic ganglia and the ventral nerve chords, whereas nuclei from dorsal mesenchyma cells were faintly stained or not stained at all. This forms two transient dorsoventral (D/V) regions: a stained ventral region, and a non-stained dorsal region (Fig. 1 B,C). Moreover, at this stage, nuclei of some epithelial cells also expressed TNEX59. The pattern of mesenchymal staining remained until day 7 of regeneration, when the pharynx primordium started to be formed. Cells that form the pharynx primordium showed a faint TNEX59 staining. From day 7 on, nuclei from dorsal mesenchyma cells started to be deep stained; the transient D/V regions disappeared, and the A/P gradient was re-established. Subsequently, the adult proportions were restored through an epimorphic-morphallactic process.

**Dynamics of TNEX59 localisation in regenerating trunks**

Trunk regenerates were cut at prepharyngeal and postpharyngeal level and were expected to regenerate new anterior and posterior regions. Just after amputation, TNEX59 was distributed as in an intact adult trunk; that is, the faintest signal of the gradient. At 1 day of regeneration, TNEX59 was located in the nuclei of blastema cells as soon as the blastema could be identified, and as described by regenerating heads and tails. The A/P gradient, however, was not re-established, as nuclei of the pharyngeal cells were deep stained. Moreover, at this stage, nuclei of some epithelial cells also expressed TNEX59. The pattern of blastema and pharynx deep staining remained until day 7 of regeneration, when the A/P gradient was re-established following a posterior-anterior direction, from the tip of the pharynx (which first recovered the faintest staining) to the implantation zone (which remained deep stained a bit longer) (Fig. 1D). Subsequently, the adult proportions were restored through an epimorphic-morphallactic process.

**The dynamics of TNEX59 localisation and planarian pattern formation**

The dynamics of TNEX59 localisation during regeneration depend on the regions to be regenerated. The reported differences suggest that the processes of determination and/or differentiation of the planarian body regions and/or the organs and structures contained within them differ from anterior to posterior regeneration. This is in agreement with recent reports using retinoic acid to selectively arrest anterior but not posterior regeneration (Romero and Bueno, 2001). Moreover, it is worth noting that the first changes of TNEX59 localisation occur as early as 1 day of regeneration. It has been reported that territorial determination during regeneration occurs within 24-36 hours of regeneration (Saló, 1984). This suggests that TNEX59 is involved in the formation and/or maintenance of A/P planarian body pattern during regeneration.

**References**


