Int. J. Dev. Biol. 45 (S1): S125-S126 (2001)

Short Report

TNEX59, a planarian regional nuclear factor differentially expressed during regeneration

JUANA FERNÁNDEZ-RODRÍGUEZ*, ALBERT CARDONA, VÍCTOR HERNÁNDEZ, RAFAEL ROMERO and DAVID BUENO

Departament de Genètica, Facultat de Biologia, Universitat de Barcelona, Catalonia, Spain

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.

Introduction

A central issue in developmental biology is to understand how regional differences in body pattern are specified during development and maintained in the adult. Cellular and molecular studies in different developmental systems have identified an increasing number of genes, including nuclear factors, that control body patterning. In freshwater planarians, several genes potentially involved in these processes have been identified, as for example some *Hox* genes (Saló *et al.,* 2001) and genes involved in anteroposterior (A/P) regionalisation and pattern maintenance, such as *tcen49* (Bueno *et al.,* 1996, 2001a, 2001b). This gene codes for a small-secreted protein localised in the central body region, including the pharynx, defining three different molecular regions (anterior, including the brain and eyes; central, including the pharynx; and posterior). It has been suggested that these molecular body regions correspond to a deeper morphological, physiological and functional compartmentalisation (Bueno *et al.,* 2001b).

Freshwater planarians are known for their great power of regeneration and their ability to grow or degrow depending on environmental conditions (for a general review on planarian morphology, regeneration, growth and degrowth, see Baguñà *et al.,* 1990, 1994). The great ability to regenerate exhibited by freshwater planarians makes the mechanisms used for the establishment, maintenance and respecification of A/P polarity during regeneration especially intriguing.

Recently, we have identified a novel nuclear factor, named TNEX59, that is detected mostly in mesenchymal cells in a distribution gradient, with the faintest signal located in the central body region. (Fernández-Rodríguez *et al.,* 2001). However, a few cells located within the epidermis are stained with the mAb-recognising TNEX59. The stained nuclei can be classified in three categories according to the intensity of staining; mild dark, dark and deep dark. When the distribution of these nuclei is analysed along the A/P body axis, we see that the gradient is due to the differential distribution of mild dark and deep dark stained nuclei: deep dark nuclei are present mostly in the anterior (head) and posterior (tail) regions, and mild dark nuclei are present mostly in the central (trunk) region. In this paper we report on the differential dynamics of localisation during head, trunk and tail regeneration. This dynamics suggests that TNEX59 is involved in the formation and/or maintenance of A/P planarian body pattern during regeneration.

Material and Methods

The freshwater planarians used in this work belong to an asexual strain of the species *Girardia tigrina* (Platyhelminthes, Turbellaria, Tricladida). The handling of planarians and immunohistochemistry procedures have been described elsewhere (Bueno *et al.,* 1996). Regenerating organisms were kept at 17±1ºC, and analysed at 1, 3, 5 and 7 days of 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,

***Address correspondence to:** Juana Fernández-Rodríguez. Departament de Genètica, Facultat de Biologia, Universitat de Barcelona, Av. Diagonal 645, 08028 Barcelona, Catalonia, Spain. e-mail: jfernand@porthos.bio.ub.es

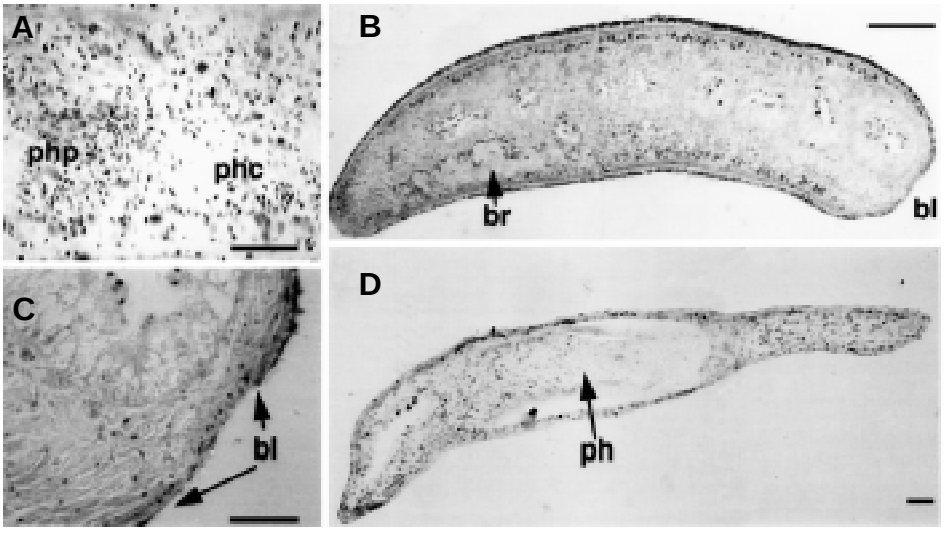

Fig.1. Regenerating planarians immunostained with mAb TNEX-59. *Sagittal sections. Anterior is to the left, and dorsal to the top.* **(A)** *Regenerating tail at 3 days of regeneration. Note pharynx primordium staining.* **(B)** *Regenerating head at 1 day of regeneration. Note the D/V regions.* **(C)** *Regenerating head at 1 day of regeneration. Note the blastema staining.* **(D)** *Regenerating trunk at 7 days of regeneration. Note that the tip of the pharynx (to the right) is faint stained, and the implantation zone is still deep stained. Abbreviations: phc, pharynx cavity; php, pharynx primordium; br, brain; bl, blastema; ph, pharynx. Scale bars: A and C, 0.125 mm; B and D, 0.25 mm.*

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 remainded 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

BAGUÑÀ, J., ROMERO, R., SALÓ, E., COLLET, J., AULADELL, C., RIBAS, M., RIUTORT, M., GARCIA-FERNÀNDEZ, J., BURGAYA, F. and BUENO, D. (1990). Growth, degrowth and regeneration as developmental phenomena in adult freshwater planarians. In *Experimental Embryology in Aquatic Plants and Animals* (H. J. Marthy, ED.), pp. 129-162. Plenum Press, NATO-ASI Series, New York.

BAGUÑÀ, J., SALÓ, E., ROMERO, R., GARCIA-FERNANDEZ, J., BUENO, D., MUÑOZ-MARMOL, A. M., BAYASCAS-RAMIREZ, J. R. and CASALI, A. (1994). Regeneration and pattern formation in planarians: *Cells, molecules and genes. Zool. Sci* 11: 781-795.

BUENO, D., BAGUÑÀ, J. and ROMERO, R. (1996). A central body region defined by a position-specific molecule in the planarian *Dugesia (Girardia) tigrina*: spatial and temporal variations during regeneration. *Dev. Biol.* 178: 446-458.

BUENO, D., BAGUÑÀ, J. and ROMERO, R. (1997). Cell-, tissue- and position-specific monoclonal antibodies against the planarian *Dugesia (Girardia) tigrina. Histochemistry and Cell Biology* 107: 139-149.

BUENO, D., VISPO, M., SANCHO, V. and ROMERO, R. (2001a). Maintenance of A/P body regions in planarians by TCEN49, a putative cystine-knot neurotrophin. *Belg. J. Zool.* 131 (Sppl): 89-95.

BUENO, D., FERNÁNDEZ-RODRÍGUEZ, J., CARDONA, A., HERNÁNDEZ-HERNÁNDEZ, V. AND ROMERO, R. (2001b). Regeneration of gross molecular regions in planaria: from molecules to organs. *Int. J. Dev. Biol.* (in this issue).

FERNÁNDEZ-RODRÍGUEZ, J., REIGADA, S., ROMERO, R. and BUENO, D. (2001). Gradients and regeneration: the case of TNEX59 in planarians. *Belg. J. Zool.* 131 (Sppl): 143-145.

ROMERO, R. and BUENO, D. (2001). Disto-proximal regional determination and intercalary regeneration in planarians, revealed by retinoic acid induced disruption of regeneration. *Int. J. Dev. Biol.* 45: 669-673.

SALÓ, E. (1984).Formació del blastema i reespecificació del patró durant la regeneració de las planàries *S. mediterranea* i *G. tigrina*. Anàlisi morfològic, molecular i bioquímic. Ph.D. Thesis. Universitat de Barcelona.

SALÓ, E., TAULER, J., JIMÉNEZ, E., BAYASCAS, J.R., GONZÁLEZ-LINARES, J., GARCIA-FERNÀNDEZ, J. and BAGUÑÀ, J. (2001) Hox and ParaHox genes in flatworms. Characterization and expression. *Am. Zool.* (in press).