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SHORT NOTES

Gradients and regeneration: the case of TNEX59 in the planarian *Girardia tigrina* (Platyhelminthes, Tricladida)

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During development, multicellular organisms determine and then differentiate the distinct regions that constitute the body's architecture. These regions are established and controlled by a number of molecules, including nuclear factors, that drive the organism from the egg to its final shape. We studied the molecules involved in the regionalisation of the freshwater planarian body (Platyhelminthes, Turbellaria, Tricladida). These organisms are known for their great power of regeneration and their ability to grow or degrow depending on environmental conditions (1). Using a monoclonal antibody (MAb) from a planarian-specific MAbs library (2), we identified TNEX59, a molecule detected in a regional fashion. Here we present the preliminary results on its localition in intact and regenerating organisms.

The specimens used in this study belong to an asexual race of *Girardia tigrina*. The MAbs used were obtained following standard procedures (3) with some modifications (4). Immunohistochemistry on sagittal paraffin sections was performed as described in (4), and Adobe Photoshop was run to determine pixel intensity of nuclear staining.

TNEX59 localisation in intact organisms

TNEX59 is a nuclear protein detected mostly in mesenchymal cells in a distribution gradient, with a higher proportion of nuclei with the faintest signal located in the central body region (Fig. 1A). However, a few cells located within the epidermis are stained with the MAb-recognising TNEX59.

To explore the gradient of TNEX59, the stained nuclei of sagittal sections were analysed. Five areas along the antero-posterior (A/P) axis were selected (Fig. 1A), and staining of their nuclei was quantified relative to the darker nuclei of each area.

Stained nuclei were classified in three categories, according to the intensity of staining: Type I nuclei (mild dark), Type II nuclei (dark) and Type III nuclei (deep dark). The plot of the percentage of nuclei for each staining category and for each area analysed shows that (Fig. 1B): (a) the most central areas have more Type I nuclei (mild dark); (b) the most anterior (head) and posterior (tail) areas have more Type III (deep dark) nuclei; and (c) Type II nuclei are equally distributed along the A/P body axis.

It can be deduced from these data that the gradient is due to the distribution of Type I and III nuclei along the A/P body axis. Two hypotheses can account for this gradient: (a) different categories of nuclei correspond to different cell types, and the gradient depends on the distribution of these cell types along the A/P body axis; (b) The gradient is independent of the cell types and depends merely on the respective position of mesodermal cells along the A/P body axis. Immunostaining on pla-

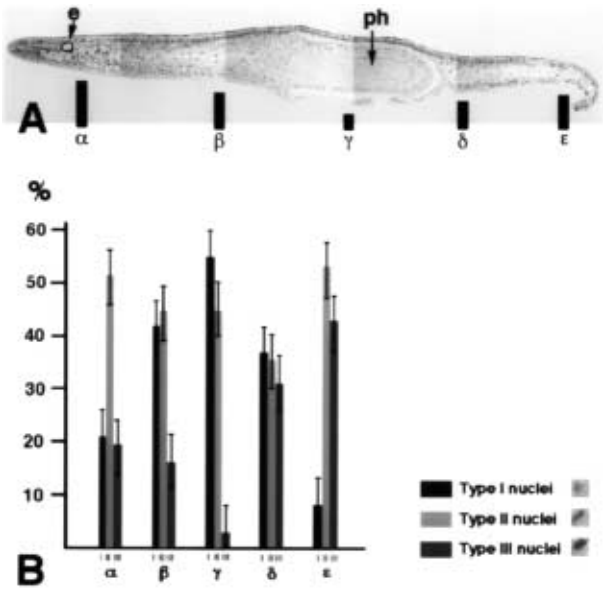

Fig. 1. – A). Intact adult planarian immunostained with TNEX59. Sagittal section. Anterior is to the left, and dorsal to the top. Real size: 8mm. Areas of nuclei quantification are marked. B) Plot of the percentage of nuclei for each staining category and for each area analysed. Several sections of three specimens were examined. Error bars represent SD (SD = 7.5). Abbreviations: e, eye; ph, pharynx.

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narian macerated cells (work in progress) would help to answer this question.

TNEX59 localisation and dynamics in regenerating organisms

The dynamics of TNEX59 localisation during regeneration were analysed in regenerating tails kept at 17±1ºC (organisms cut at postpharyngeal level expected to regenerate new central and anterior regions). In brief:

1st day of regeneration

TNEX 59 is located in the nuclei of blastema cells early in regeneration, as soon as the blastema can be identified, and the pattern of expression is re-established, although transiently (Figs 2A,B). Surprisingly, most nuclei of epithelial cells express TNEX59, in contrast with intact adult organisms.

3rd day of regeneration

TNEX59 expression fades 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 (5) attenuates the gradient.

5th day of regeneration

The nuclei of pharynx primordium cells are strongly stained with the MAb recognising TNEX59 (Fig. 2C), which reduces the gradient.

7th day of regeneration

TNEX59 expression in the nuclei of pharyngeal cells fades. The A-P gradient is definitively re-established.

From these complex dynamics of expression, several characteristics should be highlighted:

(1) The A/P gradient is transiently re-established as early as day 1 of regeneration. This suggests that this factor is an early activator of the re-establishment of the planarian body pattern and supports the hypothesis that the gradient is independent of the cell type and depends on the position of mesenchymal cells along the A/P body axis.

(2) At 1-2 days of regeneration, the nuclei of epithelial cells transiently express TNEX59, perhaps reflecting a general territorial reorganisation, as shown by use of other molecular markers in the same species (TCEN49 [6]; GtPOU-1 [7]).

(3) The strong TNEX59 staining of the nuclei of pharynx primordium cells at 3-5 days of regeneration parallels the strong staining of the apical regenerative blastema, suggesting a similar origin for both structures. In a broad sense, the pharynx primordium can be considered an inner blastema, as both structures are formed by accumulation of undifferentiated but probably committed neoblasts. When the new pharynx starts its maturation, TNEX59 expression fades. The strong TNEX59 staining in the nuclei of both blastema and pharyngeal primordium cells may also suggest that TNEX59 is a regulative factor overexpressed in differentiating cells.

In summary, TNEX59 is a nuclear factor that may be involved in the A/P patterning of mesenchymal cells.

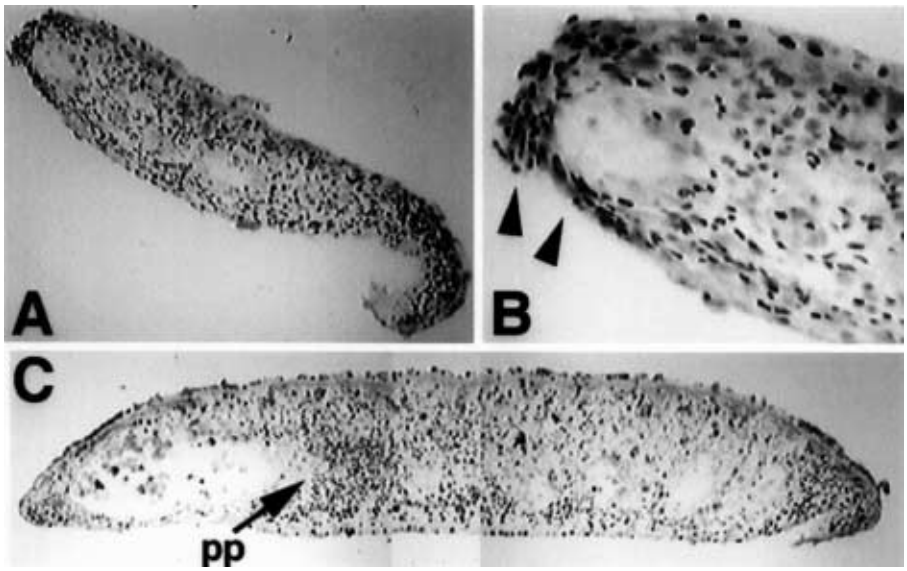

Fig. 2. – Regenerating planarians cut at postpharyngeal level immunostained with TNEX59. Sagittal sections. Anterior is to the left, and dorsal to the top. A) 1st day of regeneration. B) Magnification of the anterior part of A). Note that darker nuclei affect both the blastema and the postblastema (arrowheads). Also note that most nuclei from the epidermis are reactive to TNEX59. C) 5th day of regeneration. Note that nuclei from pharynx blastema cells are strongly stained (arrow). Abbreviations: pp, pharynx primordium.

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