

Regeneration of gross molecular body regions in planaria: from molecules to organs

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

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

ABSTRACT For many organisms, the establishment and subsequent maintenance of anteroposterior polarity is governed by gene expression in several molecular regions. We have identified two novel regionally expressed molecules, TCEN49 and TNEX59, that may be involved in such processes. The analysis of *tcen49* mRNA expression and TCEN49 protein localisation in a variety of physiological conditions, the specific arrest of anterior regeneration by exogenous retinoic acid administration, the functional analysis of *tcen49* by RNA interference, and the structural similarities between TCEN49 and TNEX59, all suggest that planarian molecular regions reflect deeper morphological, physiological and functional compartmentalisation.

Introduction

In many organisms, including invertebrates and vertebrates, the establishment of anteroposterior (A/P) polarity is preceded by genetically controlled activity which causes molecular regions to be expressed differently. Once established, these regions are maintained. This is crucial in organisms that exhibit great morphological plasticity, such as freshwater planarians (for a general review on planarian morphology, regeneration, growth and degrowth, see Baguña *et al.*, 1990, 1994). Usually, these morphological and molecular regions exhibit distinctive functional and/or physiological features. The discovery and examination of an increasing number of genes involved in these phenomena help to give an integrated view of the mechanisms that govern the formation and maintenance of a defined body plan. Although some *Hox* genes have already been identified in planarians (Saló *et al.*, 2001), the cellular and molecular mechanisms underlying the patterning of cells for a specific region along the A/P axis remain obscure.

In this paper we report on the role of two distinct but structurally related molecules, TCEN49, a secreted protein present in the central body region, and TNEX59, a nuclear protein present mostly in anterior and posterior regions, both of which may be involved in the formation and/or maintenance of A/P planarian body regions during regeneration and asexual fission processes.

Materials and Methods

The freshwater planarians used belong to an asexual strain of *Girardia tigrina* (Platyhelminthes, Turbellaria, Tricladida). The handling of planarians, whole mount *in situ* hybridisation and immunohistochemistry procedures, retinoic acid (RA) treatments, and RNAi (RNA interference) experiments, have been described elsewhere (Bueno *et al.*, 1996, 1997; Romero and Bueno, 2001; Sánchez-Alvarado and Newmark, 1999). To induce fission, 10-mm long

organisms were isolated in Petri dishes at $17\pm 1^\circ\text{C}$ for day-night fotoperiods and fed once a day.

Results and Discussion

Immunohistochemistry of TCEN49 on intact and regenerating planarians derived from traumatic cutting by using a specific planarian monoclonal antibody has been described elsewhere (Bueno *et al.*, 1996). TCEN49 is a secreted 5-kDa protein present in the central body region of adult planarians.

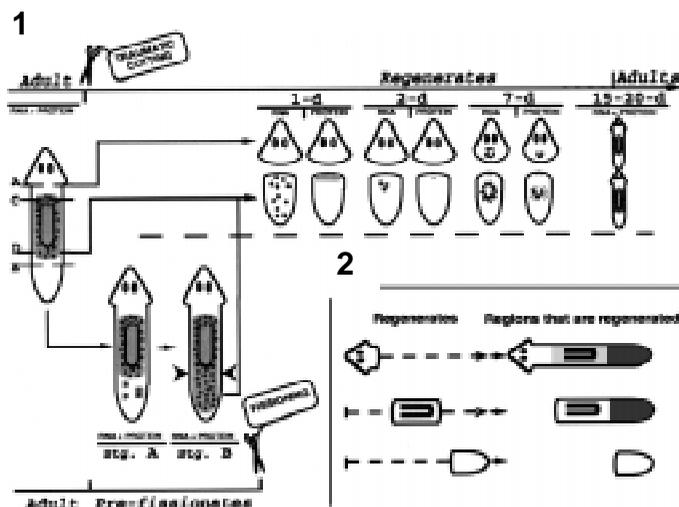


Fig. 1. Summarising scheme of *tcen49* mRNA expression (dark grey) and TCEN49 protein localisation (light grey) at different levels of body cut and during natural occurring fission. Wide arrowheads, area of fissioning.

Fig. 2. Arrestment of regeneration in RA treated planarians. White, anterior region (head); light gray, central region; dark gray, posterior region (tail).

Expression of *tcen49* in intact adult organisms

tcen49 mRNA was detected exclusively in cells localised within the parenchyma of the central body region except within the pharynx. The area of immunochemical localisation of the TCEN49 protein in adult intact organisms coincides with the area where *tcen49*-expressing cells are distributed (Bueno *et al.*, 1996; Fig. 1).

Expression of *tcen49* mRNA and localisation of TCEN49 protein in fissionates derived from natural occurring fission

During asexual reproduction, *G. tigrina* fissions at a level approximately equivalent to level D for traumatic cutting. In adult organisms,

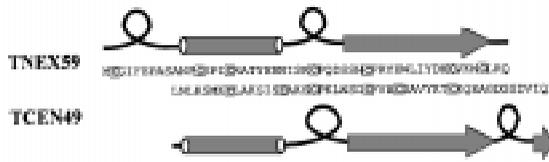


Fig. 3. Comparison of the amino acid sequences and predicted 2ary structures of TCEN49 and putative TNEX59. Cylinder, α -helix; arrow, β -sheet.

the expression of *tcen49* mRNA and the localisation of TCEN49 protein are modified at stages prior to fission, well before any morphological evidence of this process. When fission was underway (Stages A and B; Fig. 1), we detected *tcen49*/TCEN49 all along the central and posterior regions. After Stage B, organisms undergo fission. This suggests that TCEN49 may be necessary for, or related to, the process of fission. After fission, both fissionates regenerated a new complete organism. Taking all the results on *tcen49*/TCEN49 localisation together, we can clearly distinguish three different molecular regions in planarians: (1) the anterior region (including eyes and brain) where *tcen49*/TCEN49 are never detected; (2) the central region (including the pharynx), where *tcen49*/TCEN49 are always detected; and (3) the posterior region, where *tcen49*/TCEN49 are detected depending on the physiological conditions of the organism (stages prior to fission, and the initial stages of tail regeneration).

Effects of exogenous retinoic acid administration on regenerating planarians

As exogenous retinoic acid affects development and regeneration in other systems, we treated regenerating planarians with this morphogen. Exogenous retinoic acid administration on regenerating planarians disrupted anterior but not posterior regeneration (Romero and Bueno, 2001). Regenerating trunks, which have to regenerate new anterior (head) and posterior (tail) regions, regenerated a new complete posterior region, but the regeneration of the anterior region was completely arrested; regenerating heads regenerated new complete central and posterior regions; but regenerating tails did not regenerate either the anterior or the central regions (Fig. 2). Whether or not RA is acting through a morphogenetic or specific toxic effect, this differential disruption of gross molecular regions regeneration suggests that at least some processes of anterior regeneration differ from those found during posterior regeneration, and that these molecular regions are physiologically and functionally distinct. We suggest that these molecular regions correspond to a deeper level of functional and physiological compartmentalisation that could be related to the distinct organs and/or processes present in each region (brain and eyes in the anterior region, pharynx in the central region, and the fission capability in the border between central and posterior regions).

RNA interference of *tcen49* function during regeneration

RNAi experiments were performed on regenerating tails by injecting *tcen49* double-stranded RNA (dsRNA) just after amputation, and repeating the injection every 3 days. In the absence of TCEN49, the regeneration proceeded normally until the 9th day of regeneration. That is, a complete new pharynx was formed. However, at 9-12 days of regeneration, when the pharynx usually recovers functionality and TCEN49 starts to be secreted, the organisms lysated. It is important to note that just before lysis, cells and tissues of the "central" and "posterior" regions fused and degenerated, and the pharynx was expelled. The anterior region was unaffected. This suggests that in the absence of TCEN49, the "central" region becomes "posterior", as it is able to express *tcen49*, but does not. This may explain why these two regions fuse (both are "posterior"), which in turn causes their cellular and tissular degeneration. In this situation, the pharynx is expelled, as it is unable to find its proper region. We conclude that TCEN49 is not necessary for either the first stages of regeneration or the formation of a new pharynx, but is indeed necessary for the maintenance of functional body regions in planarians and the organs contained within them.

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