

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

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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.

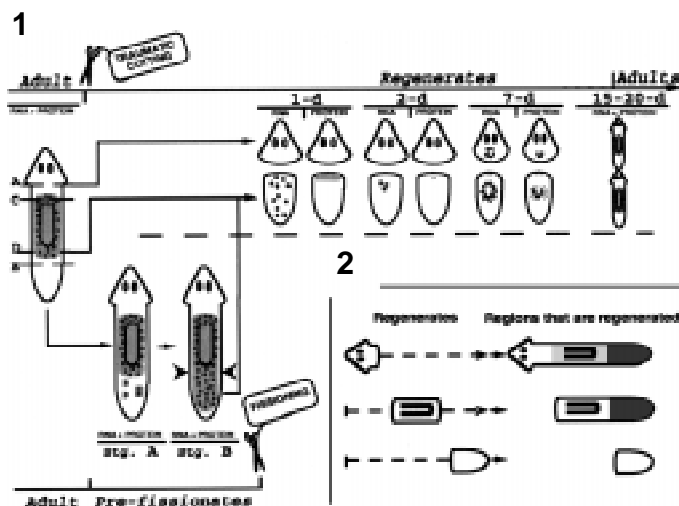


Fig. 1. Summarising scheme of *tcen49* mRNA expression (dark grey) and TCEN49 protein localisation (light grey) at different levels of body cut and during natural occurring fission. Wide arrowheads, area of fissioning.

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,

