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Gene switching at *Xenopus laevis* metamorphosis

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ABSTRACT

During the climax of amphibian metamorphosis many tadpole organs remodel. The different remodeling strategies are controlled by thyroid hormone (TH). The liver, skin, and tail fibroblasts shut off tadpole genes and activate frog genes in the same cell without DNA replication. We refer to this as "gene switching". In contrast, the exocrine pancreas and the intestinal epithelium dedifferentiate to a progenitor state and then redifferentiate to the adult cell type. Tadpole and adult globin are not present in the same cell. Switching from red cells containing tadpole-specific globin to those with frog globin in the liver occurs at a progenitor cell stage of development and is preceded by DNA replication. Red cell switching is the only one of these remodeling strategies that resembles a stem cell mechanism.

Introduction

Thyroid hormone (TH) controls developmental programs during amphibian metamorphosis (Dodd and Dodd, 1976). The climax of metamorphosis for *Xenopus laevis* takes place in about 8 days from the time the front legs emerge (NF59) to the end of tail resorption (NF66) (Brown and Cai, 2007). Most but not all of the metamorphic changes that occur during climax can be described as death and remodeling programs (Tata, 1998, Su et al., 1999, Brown and Cai 2007). Among the organs that remodel at climax are skin (Suzuki et al., 2009), liver (Atkinson et al., 1998), intestine (Ishizuya-Oka and Shi, 2005), pancreas (Mukhi et al. 2008), the immune system (Rollins-Smith, 1998), brain (Kollros, 1981), eye (Hoskins 1986), trunk muscle (Nishikawa and Hayashi, 1994; Shimizu-Nishikawa et al., 2002), fibroblasts (Berry et al., 1998a) and hematopoiesis (Weber, 1996). Molecular and biochemical aspects of remodeling of some of these organs have been studied and reviewed recently (Furlow and Neff, 2006; Buchholz et al., 2006; Tata, 2006; Brown and Cai, 2007).

In this paper our goal has been to identify the tadpole cells that give rise to differentiated frog cells in several organs. The organs or cell types reported on in this paper include, parenchymal and red cells in the liver, epithelial cells of the skin, and fibroblasts in the tail notochord. We propose that there are at least three strategies of remodeling at the climax of metamorphosis. In the dedifferentiation model TH induces differentiated cells of the tadpole exocrine pancreas (Mukhi et al., 2008) and the intestinal epithelium (Schreiber and Brown, 2005) to dedifferentiate to progenitor cells. The subsequent redifferentiation is not controlled by TH. The gene switching model is the subject of most of this paper. TH induces gene switching in differentiated tadpole skin, liver and fibroblast cells demonstrated by the fact that tadpole and frog specific mRNAs coexist for a brief time in the same cells. A third model, "cell-switching," is exemplified by globin switching. TH induces DNA replication of progenitor hematopoietic cells followed by expression of adult globin.

Results

Switching from a growth program to a death program in tail fibroblasts

The tadpole notochord is a hollow collagen rod that extends nearly to the end of the tail. It is lined and surrounded by fibroblasts. At metamorphic climax these fibroblasts switch from a growth program characterized by the synthesis of collagens and extracellular matrix to a hydrolysis program that involves the expression of a variety of proteolytic enzymes that digest the notochord (Berry et al., 1998a; Das et al., 2006) and collapse the tail (Elinson et al., 1999) (Fig. 1). One of the TH-induced MMPs that is absent during premetamorphic growth is collagenase-3 (MMP-13) (Wang and Brown, 1993). As demonstrated in Fig. 1, premetamorphic growing tadpoles express collagen and not collagenase-3 in fibroblasts. After 4 days of exogenous T3 exposure the fibroblasts change their program from synthesizing collagen to collagenase-3. There is a brief time when both sets of mRNAs are present in the same cell (Figs. 1B and E) ruling out a role for DNA replication in the switch. Adjacent sections are adequate to confirm this since every fibroblast that lines the inside of the notochord expresses both mRNAs.

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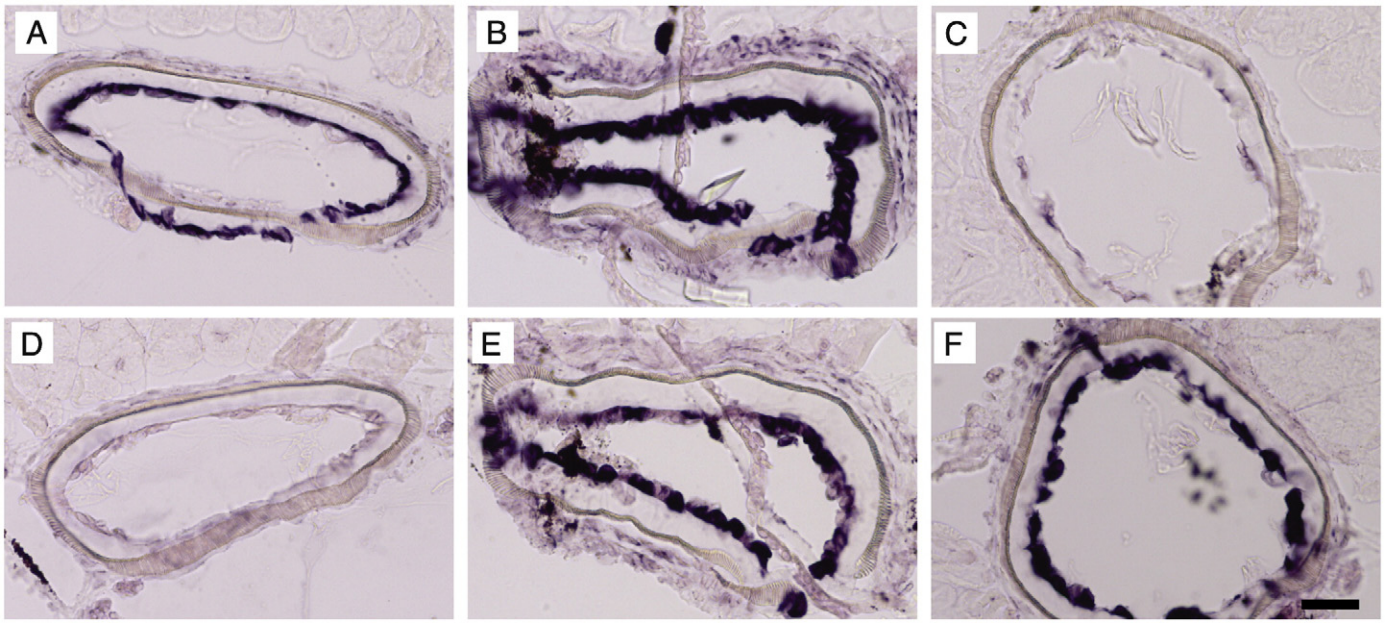

Fig. 1. Reprogramming of fibroblasts in the tail notochord. TH induces a change in gene expression in the tail that causes regression of the tail. *In situ* hybridization of adjacent sections with (A–C) collagen and (D–F) collagenase-3. (A, D) NF55 control; (B, E) 10 nM T3 for 2 days; (C, F) 10 nM T3 for 4 days. (scale bar = 40 μm).

Gene switching in notochord fibroblasts can be inhibited in two ways. Transgenics in which the rat collagen promoter drives the dominant negative form of the thyroid receptor alpha (TRDN) express the transgene widely in tadpoles and interferes with many TH-inducible changes (Schreiber et al., 2001) including the fibroblasts of the tail notochord making them resistant to downregulation of collagen (Fig. 2A) and upregulation of collagenase-3 (Fig. 2B). Overexpression of prolactin makes tail fibroblasts resistant to TH (Huang and Brown, 2000). Tadpoles transgenic for prolactin cannot resorb their tail fins or digest their notochords. TH does not induce downregulation of collagen (Fig. 2C) or upregulation of collagenase (Fig. 2D) in transgenic tadpoles expressing prolactin.

Switching from a tadpole to a frog skin

The tadpole skin is induced to change by TH at the climax of metamorphosis. This change occurs throughout the body including the growing limbs, the sites of the sections described here. The tadpole skin consists of three replicating cell layers (Yoshizato, 2007) that synthesize a number of tadpole specific genes (Furlow et al., 1997; Suzuki et al., 2009) including a tadpole-specific keratin named DG 118 (Miyatani et al., 1986) (Fig. 3A). These cells do not express adult-specific keratin genes (Fig. 3D). In contrast frog skin is a typical germinative epithelium in which only the basal cells replicate. The skin has switched from expressing tadpole-keratin

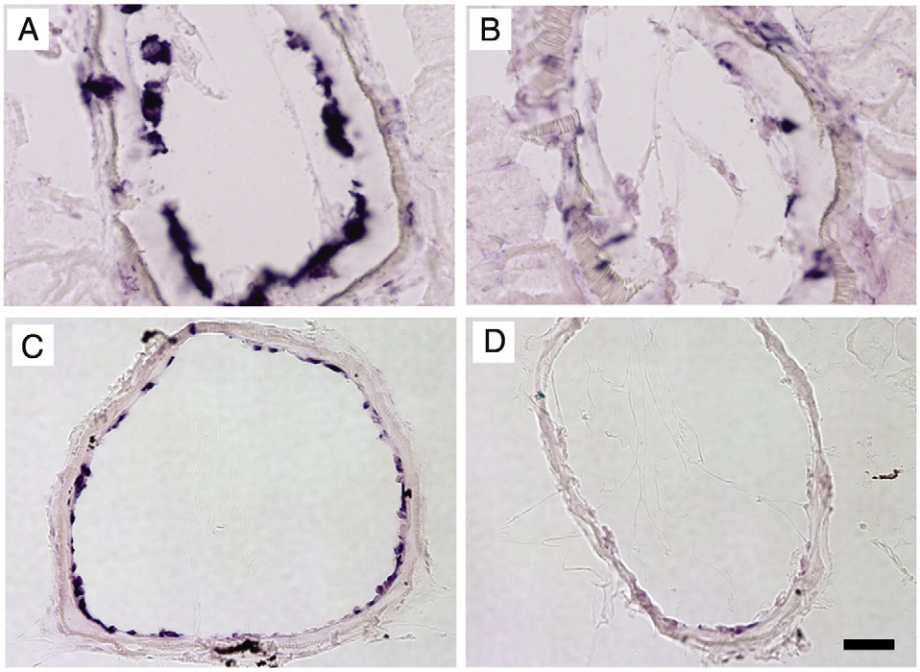

Fig. 2. The inhibition of fibroblast reprogramming. *In situ* hybridization with (A, C) collagen; (B, D) collagenase-3. (A, B) NF55 Col-TRDN transgenic tadpoles treated with 10 nM T3 for 4 days. (C, D) NF55 transgenic for ovine prolactin treated for 4 days with 10 nM T3. (scale bar = 40 μm).