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Differential thyroid hormone sensitivity of fast cycling progenitors in the neurogenic niches of tadpoles and juvenile frogs

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ABSTRACT

Adult neurogenesis occurs in neural stem cell (NSC) niches where slow cycling stem cells give rise to faster cycling progenitors. In the adult mouse NSC niche thyroid hormone, T_3, and its receptor TRα act as a neurogenic switch promoting progenitor cell cycle completion and neuronal differentiation. Little is known about whether and how T_3 controls proliferation of differentially cycling cells during xenopus neurogenesis. To address this question, we first used Sox3 as a marker of stem cell and progenitor populations and then applied pulse-chase EdU/IdU incorporation experiments to identify Sox3-expressing slow cycling (NSC) and fast cycling progenitor cells. We focused on the lateral ventricle of *Xenopus laevis* and two distinct stages of development: late embryonic development (pre-metamorphic) and juvenile frogs (post-metamorphic). These stages were selected for their relatively stable thyroid hormone availability, either side of the major dynamic phase represented by metamorphosis. TRα expression was found in both pre and post-metamorphic neurogenic regions. However, exogenous T_3 treatment only increased proliferation of the fast cycling Sox3+ cell population in post-metamorphic juveniles, having no detectable effect on proliferation in pre-metamorphic tadpoles. We hypothesised that the resistance of proliferative cells to exogenous T_3 in pre-metamorphic tadpoles could be related to T_3 inactivation by the inactivating Deiodinase 3 enzyme. Expression of *dio3* was widespread in the tadpole neurogenic niche, but not in the juvenile neurogenic niche. Use of a T_3-reporter transgenic line showed that in juveniles, T_3 had a direct transcriptional effect on rapid cycling progenitors. Thus, the fast cycling progenitor cells in the neurogenic niche of tadpoles and juvenile frogs respond differentially to T_3 as a function of developmental stage.

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1. Introduction

In non-mammalian vertebrates, such as teleosts and amphibians, neurogenesis continues throughout life in multiple neurogenic sites. This situation differs from that of mammals where adult neurogenesis is limited to two neurogenic niches (for review see Urbán and Guillemot, 2014): the hippocampal dentate gyrus and the subventricular zone (SVZ) around the lateral ventricles (Alvarez-Buylla and Garcıa-Verdugo, 2002; Alvarez-Buylla and Lois, 1995; Göritz and Frisén, 2012; Kaplan and Bell, 1984). Despite their different anatomical structures the two niches share similar cell types, with multipotent slow-cycling neural stem (NSC) cells giving rise, by asymmetrical division, to fast cycling amplifying progenitors (see Ref. Götz and Huttner, 2005 for review). This progenitor population amplifies by symmetrical division prior to differentiation into one of the three neural cell types (neuron, astrocyte and oligodendrocyte) (Davis and Temple, 1994; Doetsch, 2003; Taupin and Gage, 2002). NSCs are generally characterized by a slower cell cycling speed than progenitors (Morshead et al., 1994).

In the adult mammalian neurogenic niches, NSCs display both an astrocytic morphology and express the astrocyte marker, GFAP (Doetsch et al., 1999; Imura et al., 2003). These adult mammalian NSCs originate from the radial glia that drive embryonic and perinatal neurogenesis (Alvarez-Buylla et al., 2001; Merkle et al., 2004). In contrast, in teleost and amphibians, radial glial cells persist in the adult neurogenic niches, acting as adult NSCs (D'Amico et al., 2011; Ito et al., 2010; Lam et al., 2009).

During xenopus development, neurogenesis in the pre-

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metamorphic stages occurs throughout the ventricular areas whereas, in the adult, the highest levels of neurogenesis are found around the lateral ventricles (D'Amico et al., 2011; Wullimann et al., 2005). The cycling properties of the cells in the xenopus neurogenic niche of the lateral ventricle have not been fully characterized. Most studies have focused on the radial glia that share rapid cycling progenitor characteristics in both tadpole and adult (D'Amico et al., 2011; Gervasi et al., 2000). However, the observation that asymmetrical division can also occur in the niche, generating a radial glia and a neuronal cell, strongly suggests the existence of a NSC population within the more rapidly proliferating pool (Bestman et al., 2012). The continued neurogenesis of the non-mammalian brain can be associated with the ongoing brain growth in adults (reviewed by Lindsey and Tropepe, 2006). This higher neurogenic activity of adult non-mammalian vertebrate brains can be exploited to investigate the regulatory mechanisms governing NSC and progenitor proliferation.

One well-documented factor governing many aspects of neurogenesis in all vertebrates is thyroid hormone (TH). THs activate proliferation in the neurogenic niches during early development in mouse (Hadj-Sahraoui et al., 2000) and in the neurogenic niches of the adult mouse brain (Lemkine et al., 2005; Kapoor et al., 2012; López-Juárez et al., 2012). Notably in the adult mouse SVZ niche, T_3 and its TRα receptor stimulate proliferation and differentiation of the amplifying progenitor pool (López-Juárez et al., 2012).

The majority of studies addressing T_3 action during xenopus neurogenesis focus on the metamorphic period when a T_3 peak orchestrates brain remodeling through differential apoptosis, proliferation and differentiation (Baffoni, 1957; Denver, 1998; Denver et al., 2009, 1997; Marsh-Armstrong et al., 2004, 1999; Schlosser et al., 2002; Schreiber et al., 2001). Endogenous and exogenous T_3 have been shown to stimulate proliferation during metamorphosis (Denver et al., 2009). These authors used BrdU incorporation to follow proliferation. However, the protocol used could not distinguish between NSC and progenitor populations, as these cell populations in the xenopus neurogenic niche had yet to be characterized.

We set out to fill this knowledge gap and better define the cycling properties of the cell populations in the lateral ventricle neurogenic niche. Given, the strong effects of thyroid hormones on proliferation (Denver et al., 2009; Lemkine et al., 2005) and neuronal commitment (López-Juárez et al., 2012) we needed to focus on developmental stages where thyroid hormone homeostasis was relatively stable. The most obvious parallel to adult mammalian neurogenesis is the post-metamorphic frog. We examined cell cycling dynamics in the post-metamorphic juvenile, and compared results to a larval stage, the pre-metamorphic tadpole at NF stage 48, as thyroid homeostasis is relatively stable at both stages. Our experiments focused on the ventricular zone (VZ) and the SVZ around the lateral ventricle. In the adult xenopus, this area corresponds to the well-characterized SVZ in adult mammals and displays the highest level of continued neurogenesis (D'Amico et al., 2011; Wullimann et al., 2005).

One of major problems facing any study addressing the identity of populations in a stem cell niche is the question of markers. Transitions from NSC to progenitors to neuroblast and differentiated states are gradual, such that most markers overlap between one cell type and another. As a consequence, there is no specific marker limited to each population (reviewed by Mamber et al., 2013). This continuum in marker expressions was recently confirmed at the transcriptional level in mice SVZ (Llorens-Bobadilla et al., 2015). This characteristic also underlines the importance of pulse-chase DNA labeling to accurately discriminate slow cycling and fast cycling cells in the niche (Mamber et al., 2013). Nevertheless, some markers, such as the Sox B1 family are widely expressed in vertebrate NSCs and progenitors (Archer et al., 2011; Rogers et al., 2013; Wang et al., 2006; Wegner and Stolt, 2005).

Our primary aim was to identify NSCs and progenitors population in the neurogenic niche of xenopus brain. For this purpose we combined differential labeling of slow and fast cycling cells with markers of NSCs versus differentiated cells. Sox3 was chosen as a multipotency marker, as it is first expressed during neural specification in xenopus (Rogers et al., 2009), then in neural precursors and neural progenitors (Archer et al., 2011; Bylund et al., 2003) and its expression persists in neuronal progenitors throughout life (Rogers et al., 2013; Wang et al., 2006). Our second aim was to address the question of the contribution of thyroid signaling to controlling neurogenic niche activity in pre- and post-metamorphic developmental stages. We examined TRα expression distribution by immunocytochemistry and used *in situ hybridization* to follow the expression of the inactivating deiodinase, *dio3*, expression. The choice to examine *dio3* expression was determined by the differential responses to exogenous T_3 treatment observed in post-metamorphic juveniles that was absent in pre-metamorphic tadpoles.

With the new markers and knowledge on cell cycling characteristics gained from this work the question of how this niche and the different populations respond during the complex and dynamic process of metamorphosis can be addressed in future studies.

2. Material and Methods

2.1. Reagents

T_3 (sodium salt, >98% purity) was purchased from Sigma–Aldrich (ref T6397, Saint Quentin Fallavier, France). A stock solution was prepared by dissolving T_3 in NaOH 1 M to obtain a 3.3 10^{-2} M solution. This solution was diluted (3.3×) in pure water to obtain 10^{-2} M T_3 aliquots which were stored at −20 °C. For each experiment an aliquot was extemporaneously diluted to obtain a 10^{-5} M T_3 working solution. T_3 was then added to tank water to achieve 5.10^{-9} M (5 nM) T_3. This dilution had no effect on pH of aquarium water that remained stable at pH 8.1.

EdU (5-ethynyl-2′-deoxyuridine, >90% purity) was purchased from Invitrogen (Carlsbad, CA, USA) and dissolved (10 mg/ml) in PBS (pH 7.4). Aliquots were stored at −20 °C. EdU (1 mg/ml for injection and 200 μM in aquaria) was diluted extemporaneously in PBS from a 10 mg/ml aliquot.

IdU (5-Iodo-2′-deoxyuridine, >99% purity) was purchased from Sigma–Aldrich (Saint Quentin Fallavier, France). For treatment in aquaria, a 2 mM IdU solution was prepared the day before the experiment and dissolved overnight at 4 °C. The 200 μM IdU dilution in tadpole water was prepared extemporaneously from the 2 mM solution stock. For injection, a 10 mg/ml solution in PBS was prepared the day before the experiment and dissolved overnight at 4 °C. The IdU solution at 1 mg/ml was prepared extemporaneously by dilution of the 10 mg/ml solution in PBS.

2.2. Animals

Xenopus laevis tadpoles and juveniles were obtained by breeding adult frogs injected with human chorionic gonadotropin (Chorulon) (400 U/female and 200 U/male) or from the CRB (Xenope Biology Resources Center, *Centre de Ressource Biologie Xénope*), France (University of Rennes1; http://xenopus.univ-rennes1.fr/). Animals were reared under a 12 h light/12 h dark cycle at 22–23 °C. Animals were staged according to the Nieuwkoop and Faber (NF) table (1956). Juveniles were used when they had developed beyond NF66 and weighed between 1 and 2 g. All animal studies were carried out in accordance with the European Union regulations

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