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Aromatase and Estrogens: Involvement in Constitutive and Regenerative Neurogenesis in Adult Zebrafish

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INTRODUCTION

Nowadays, with the increase of brain strokes and neurodegenerative diseases, a better understanding of mechanisms triggering constitutive and regenerative neurogenesis is a key challenge for finding treatments and cures. For a long time, estrogens have been known to exert pleiotropic effects on a wide range of tissues and organs. In the central nervous system (CNS), they have neuroprotective properties and modulate neurogenesis, brain repair and synaptic plasticity. In this context, teleost fish exhibit interesting and almost unique features in vertebrates. Among teleosts, the zebrafish (*Danio rerio*) is a useful tool for drug screening and a recognized model organism for studying a wide variety of physiological processes and disorders such as immunity, cancer, neurogenesis and regeneration (Santoriello and Zon, 2012). In this chapter, we aim to emphasize the contribution of fish, and peculiarly zebrafish, in the better understanding of the roles of aromatase and estrogens in

constitutive and reparative neurogenesis. First of all, we will document the unique features exhibited by the brain of teleost fish in terms of aromatase activity, neurogenesis and regenerative capability. We will next focus on neurosteroid synthesis and signaling in the brain of adult zebrafish, and discuss the fact that radial glial cells (RGCs) could be a source of steroids and a target of peripheral and locally produced steroids. Then, we will highlight the role of estrogens in constitutive neurogenesis and their potential involvement in brain repair. Finally, we will compare these data with those of other vertebrates, with a special focus on amphibians.

UNIQUE FEATURES EXHIBITED BY THE BRAIN OF TELEOST FISH

Among vertebrates, teleost fish are unique in many respects and emerge as fascinating models especially in the field of neurosciences (Figure 5.1).

Firstly, pioneer studies in the 1980s documented the presence of high aromatase activity in the brain of adult goldfish and toadfish, reported to be 100 to 1000 times higher than in the corresponding regions in

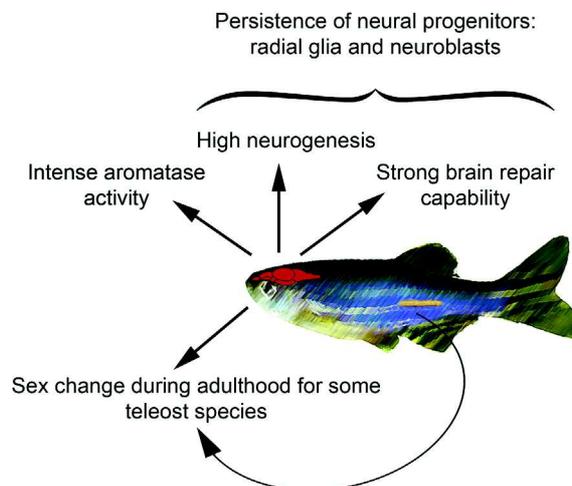FIGURE 5.1 Unique features exhibited by the brain of adult teleost fish. The brain of adult teleost fish displays a strong aromatase activity and a high constitutive and regenerative neurogenesis allowed by the persistence of neural progenitors in the brain (RGCs and further committed precursors). Some teleost species have the capacity to change sex during the lifespan, implying a strong gonadal and neuronal plasticity.

mammals (Callard et al., 1990; Pasmanik and Callard, 1985). This strong aromatase activity was next confirmed in many other species including zebrafish, and was shown to result from the intense expression of a brain-specific aromatase, aromatase B (AroB), encoded by the *cyp19a1b* gene, one of the two *cyp19a1* genes emerging from a genomic duplication event occurring some 325 million years ago in teleost fish (Diotel et al., 2010a; Le Page et al., 2010; Ravi and Venkatesh, 2008). In contrast to other vertebrates in which brain aromatase is mainly expressed in neurons, it is only expressed in radial glial cells (RGCs) in the brain of fish (see section titled "Aromatase B Expression Is Restricted to Radial Glia Acting Like Neural Stem Cells").

Secondly, the brain of teleost fish also displays a widespread and intense neurogenesis during the lifespan (Zupanc, 2001). While there are only two main neurogenic regions in mammals, the dentate gyrus of the hippocampus and the subventricular zone (SVZ) of the lateral ventricle, the brain of adult teleosts exhibits a high density of neurogenic niches throughout the whole brain (Lindsey and Tropepe, 2006; Pellegrini et al., 2007). This strong neurogenic activity is due to the persistence and activity of RGCs during adulthood as well as further committed progenitors (März et al., 2010; Onteniente et al., 1983; Pellegrini et al., 2007). The fact that RGCs are the only ones expressing the estrogen-synthesizing enzyme highlights substantially the potential involvement for aromatase and estrogens in zebrafish neurogenesis (see sections "Aromatase B Expression Is Restricted to Radial Glia Acting Like Neural Stem Cells" and "Involvement of Aromatase and Estrogens in Adult Neurogenesis and Brain Repair in Zebrafish").

Furthermore, in contrast to mammals, teleost fishes exhibit a remarkable ability to regenerate nervous tissue by a process involving extensive neurogenic events (Grandel and Brand, 2013; Kizil et al., 2012). In the last few years, this injury-induced neurogenesis has been intensively studied in zebrafish (Becker and Becker, 2008; Schmidt et al., 2013), and estrogens were suggested to be key players in brain remodeling and regenerative processes (Diotel et al., 2013) (see section "Involvement of Aromatase and Estrogens in Adult Neurogenesis and Brain Repair in Zebrafish").

Last but not least, some teleost fishes show an intriguing capacity to change sex during life (Le Page et al., 2010). This implies that gonad determination and differentiation differ in fish compared to other vertebrates (Guiguen et al., 2010; Nagahama, 2005). It also means that neuronal networks controlling gonadotrophin release, reproductive status and behavior are plastic and not permanently sexualized in contrast to mammals, and that aromatase and estrogens could be key players in such processes (Le Page et al., 2010).

NEUROSTEROID SYNTHESIS AND SIGNALING IN THE BRAIN OF ADULT ZEBRAFISH

Until recently, neurosteroid synthesis was poorly documented in teleost fish (Diotel et al., 2011a,b). Given the high aromatase activity exhibited by the brain of adult zebrafish, the question of local versus peripheral origin of C19 androgens available for aromatization was raised. In 2011, it was shown that the brain of adult male and female zebrafish was able to convert [^{3}H]-pregnenolone into a wide variety of radiolabeled steroids, namely testosterone, progesterone, estrone and estradiol (Diotel et al., 2011a). This study also demonstrates that the main steroidogenic enzymes leading to estrogen synthesis (*cyp11a1* -P450$_{SCC}$-, *3β-hsd*, *cyp17* and *cyp19a1b*) are biologically active and display a similar distribution in numerous brain regions. Interestingly, *cyp11a1*, *3β-hsd* and *cyp17* mRNAs are expressed in some AroB$^+$ RGCs. Apart from aromatase, the neuronal expression of these enzymes is not excluded given their wide distribution. This is further reinforced by the fact that 3βHSD-like immunoreactivity was observed in neurons throughout the adult zebrafish brain (Sakamoto et al., 2001). While these data allow us to consider the brain of adult zebrafish as a true steroidogenic organ (Figure 5.2), the relative and respective abundance of peripheral versus locally produced steroids that impregnate the brain are still unknown. It also raises the question of the targets of steroids in the brain. We will consequently discuss the current knowledge of the expression of nuclear and membrane associated steroid receptors for androgens, progestins and estrogens in the brain of adult zebrafish.

In zebrafish, androgen receptor (AR) was documented to actively bind testosterone (T), 5α-dihydro-T, 11-keto-T, and androstenedione (Jorgensen et al., 2007). In the adult brain, *ar* transcripts were notably detected in the parenchyma but also along the ventricular layers of the preoptic area, hypothalamus and of the periglomerular gray zone of the optic tectum (Gorelick et al., 2008). This distribution strongly argues in favor of AR expression in both neurons and RGCs (Figure 5.2), implying that androgens may possibly modulate the activity of RGCs.

Concerning progestagen signaling, a unique progesterone receptor (Pgr) was characterized in zebrafish. It actively binds progesterone (P), 17-hydroxy-P, dihydro-P, and 4-pregnen-17,20β-diol-3-one (Chen et al., 2010; Hanna et al., 2010). In zebrafish brain, Pgr expression was described at the mRNA and protein levels in both neurons and RGCs. These latest display a stronger Pgr expression than neuronal cells (Figure 5.2), suggesting that RGCs could be preferential targets for progesterone (Diotel et al., 2011c). This is of particular interest given the accumulating studies in mammals showing a role of progesterone in
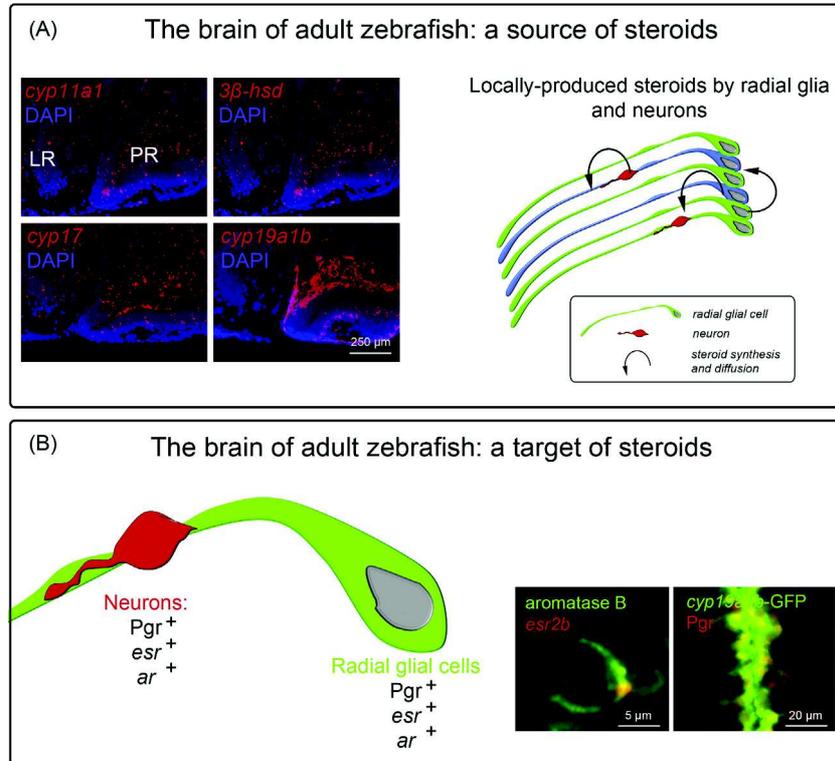
FIGURE 5.2 The brain of fish is a source and a target of steroids. A: *In situ hybridization* for *cyp11a1* (P450$_{scc}$), *cyp17*, *3β-hsd* and *cyp19a1b* (red) shows a similar distribution around the lateral (LR) and posterior recess (PR) of the hypothalamus. Cell nuclei are counter-stained with DAPI (blue). The scheme illustrates neurosteroid synthesis by neurons and RGCs (arrows). B: Accumulating data show that neurons express classical nuclear receptor for progesterone (Pgr), estrogens (*esr1*, *esr2a* and *esr2b*) and androgens (*ar*). RGCs also express Pgr and probably *ar*, *esr2a* and *esr2b*. A weak *esr2b* mRNA expression is notably detected in AroB[+] RGCs and a stronger Pgr expression in *cyp19a1b*-GFP expressing RGCs such as shown in the hypothalamus for both pictures.

neurogenesis (Barha et al., 2011; Zhang et al., 2010). Interestingly, a recent transcriptomic analysis also showed that *pgr* is an estrogen target gene in zebrafish (Hao et al., 2013). It explains that 17β-estradiol upregulates Pgr expression in the brain of larvae and adult zebrafish, by increasing notably the number of Pgr-positive RGCs and Pgr relative expression (Diotel et al., 2011c). Consistently, inhibition of estrogen synthesis leads to a significant decrease of *pgr* expression in the adult brain. Such data reinforce the idea that estrogens could regulate progesterone-mediated effects on RGCs by indirectly modulating Pgr expression.

New data about membrane progestin receptors (mPRα, β and γ) expression recently emerged in zebrafish (Hanna and Zhu, 2009). However, their expression and roles are poorly documented, notably in the CNS in which only pituitary expression was described (Hanna and Zhu, 2009).

Finally, the three zebrafish nuclear estrogen receptor transcripts *esr1* (ERα), *esr2a* (ERβ2) and *esr2b* (ERβ1) were shown to be widely expressed in the brain of adult zebrafish (Diotel et al., 2011b; Menuet et al., 2002; Pellegrini et al., 2005). *In situ* hybridization (ISH) for *esr1*, *esr2a* and *esr2b* show their expression in the subpallium, the anterior and posterior part of the preoptic area and also in the whole hypothalamus. They are detected in neurons and along the ventricular layer, suggesting their possible expression in RGCs. Preliminary data notably reinforce this point showing *esr2b* expression in AroB$^+$ RGCs (Figure 5.2). It would be consistent with *in vivo* and *in vitro* results showing that aromatase expression is regulated by ERs through an estrogen responsive element (ERE) located at -348 bp of the *cyp19a1b* promoter (Menuet et al., 2005; Mouriec et al., 2009). In addition to the classical signaling through nuclear receptors, estrogens could also exert their effect by binding a membrane estrogen receptor called Gper or GPR30, corresponding to a G-protein-coupled receptor. In 2009, a wide *gper* expression was described in parenchymal, periventricular and ventricular cells in the brain of adult zebrafish (Liu et al., 2009), suggesting *gper* expression in RGCs. In addition, taking advantage of the *cyp19a1b*-GFP transgenic zebrafish line, it was shown that acute brain slice exposure to 17β-estradiol (10^{-7}M) results in a rapid modulation of RGCs and parenchymal cell activities, as revealed by calcium imaging (Pellegrini et al., 2013). So, estrogens could locally exert a wide range of effects modulating RGC activities and brain plasticity.

AROMATASE B EXPRESSION IS RESTRICTED TO RADIAL GLIA ACTING LIKE NEURAL STEM CELLS

Studies carried out over the last 20 years have managed to identify the nature of AroB$^+$ cells in the brains of various fish species. Using antibodies raised against human aromatase, pioneer investigations identified few positive neuronal cells in the most anterior part of the goldfish brain (Gelinas and Callard, 1997). However, the low number of labeled cells and their restricted distribution contrasted with the massive aromatase activity measured throughout the goldfish brain. The development of homologous molecular tools such as labeled riboprobes and specific antibodies subsequently opened the door to the investigation of the cerebral localization of aromatase-expressing cells

in fish. It finally led to convincing documentation of the distribution of numerous AroB$^+$ cells along the ventricular layer in the forebrain and hindbrain of the midshipman first (Forlano et al., 2001), and next of many other teleost fishes such as rainbow trout (Menuet et al., 2003), zebrafish (Goto-Kazeto et al., 2004; Menuet et al., 2005; Pellegrini et al., 2005), pejer-rey (Strobl-Mazzulla et al., 2005), killifish (Greytak et al., 2005), tilapia (Chang et al., 2005), and also medaka (Okubo et al., 2011). Recently, it was shown in the Japanese eel, a basal teleost fish displaying a single *cyp19a1* gene, that aromatase distribution was similar to that highlighted in teleost species possessing duplicated *cyp19a1* genes (Jeng et al., 2012). In zebra-fish, AroB protein and mRNA overlap along the ventricles of the hind-brain, midbrain, diencephalon, telencephalon and olfactory bulbs (Menuet et al., 2005; Pellegrini et al., 2005).

Whereas neurons are the primary source of aromatase in the brain of mammals and birds (Balthazart and Ball, 1998; Peterson et al., 2005), AroB expression is restricted to RGCs and never expressed in neurons in the adult brain of teleost species studied to date (Diotel et al., 2010a). The radial glia nature of AroB-expressing cells was easily determined considering RGCs specificities including (1) a soma close to the ventri-cles and (2) a peculiar cell morphology characterized by a small cell body with an ovoid nucleus, a short process directed towards the ven-tricle and a long distal extension crossing the cerebral parenchyma and contacting the basal surface of the brain with end feet (Bentivoglio and Mazzarello, 1999; Rakic, 1978). In addition, convincing double-labeling experiments with AroB antibodies and canonical RGC markers such as GFAP, BLBP, GS and S100β definitively confirmed the radial glial iden-tity of AroB-expressing cells in the brain of fish (Forlano et al., 2001; März et al., 2010; Menuet et al., 2003; Pellegrini et al., 2007; Tong et al., 2009). It was also reinforced by the fact that AroB expression never colo-calized with Hu and acetylated tubulin neuronal markers (Forlano et al., 2001; Pellegrini et al., 2007). Curiously, in zebrafish, a strong *cyp19a1b* expression is also detected far away from the ventricular layer in radial processes, corresponding probably to *cyp19a1b* mRNA export in RGC processes far from the cell bodies (Diotel et al., 2011c; Menuet et al., 2005; Pellegrini et al., 2005).

In mammals and birds, RGCs were first described as a guide for newborn neurons migrating to their final destination (Rakic, 1990). However, their roles have been enlarged and their neural progenitor properties are now clearly established (Götz and Huttner, 2005). In mammals, at the end of brain development, most RGCs differentiate into astrocytes, but some are maintained in the neurogenic niches dur-ing adulthood (Brunne et al., 2010; Liu et al., 2006). However, in teleost fishes, RGCs persist during the lifespan and keep neurogenic capacities as we will describe (Onteniente et al., 1983; Pellegrini et al., 2007;

Zupanc and Clint, 2003). Among these RGCs, a huge majority express AroB (Forlano et al., 2001; Menuet et al., 2003; Nagarajan et al., 2011; Pellegrini et al., 2007). The specific ventricular position of AroB$^+$ RGCs highlighted in the previously mentioned works reminds one of the ventricular proliferation that has been documented in different fish species by using incorporation of bromodeoxyuridine (BrdU) or proliferating cell (PCNA$^+$) stainings (Adolf et al., 2006; Ekström et al., 2001; Grandel et al., 2006; Pellegrini et al., 2007; Strobl-Mazzulla et al., 2010; Zupanc and Horschke, 1995). In zebrafish, many ventricular neurogenic niches have been described to generate newborn cells that migrate away from the ventricle and differentiate in mature neurons (Adolf et al., 2006; Chapouton et al., 2007; Grandel et al., 2006). Our group demonstrated for the first time in zebrafish that a large subset of dividing cells corresponds to AroB$^+$ RGCs from the rostral to caudal part of the brain (Pellegrini et al., 2007). The newborn cells slowly migrate to their final destination by moving on the cytoplasmic extensions of AroB$^+$ RGCs and differentiate into mature neurons as evidenced by Hu and acetylated-tubulin stainings (Pellegrini et al., 2007). It was recently shown that in the paraventricular organ of the hypothalamus, a specific area of nonmammalian species, AroB$^+$ RGCs locally give birth to serotoninergic cerebrospinal fluid-contacting neurons (Pérez et al., 2013).

INVOLVEMENT OF AROMATASE AND ESTROGENS IN ADULT NEUROGENESIS AND BRAIN REPAIR IN ZEBRAFISH

The synthesis of estrogens by RGCs (Pellegrini et al., 2007) together with the well-known effects of estradiol in mammalian brain plasticity (Brock et al., 2010; Ormerod et al., 2004) raised the question of a functional relation between these hormones and cell fate in fish neurogenesis. Taking advantage of the zebrafish model, it was shown that Gper knock-down using morpholino results in an impaired neurogenesis during development, showing the importance of estrogen signaling in brain morphogenesis (Shi et al., 2013). In adult zebrafish, the potential role of estradiol in neurogenesis was recently investigated by manipulating the endogenous concentrations of the hormone (Diotel et al., 2013). When aromatase activity is blocked with ATD (10^{-6} M, 15 days), the number of proliferating cells (PCNA$^+$) increases throughout the forebrain, particularly in the olfactory bulbs/telencephalon junction, telencephalon, preoptic area, thalamus and mediobasal hypothalamus, even if this increase does not reach statistical significance. To further clarify the potential role of estradiol in proliferation, ERs were also blocked with ICI$_{182,780}$ (10^{-7} M, 54 h), resulting in a significant increase

in proliferation at the olfactory bulbs/telencephalon junction and in the mediobasal part of the hypothalamus. Consistently, adult zebrafish treated with 17β-estradiol (10^{-7} M, 100 h) exhibit a significant decrease in the number of PCNA^{+} cells in the same brain regions (Diotel et al., 2013). Furthermore, 17β-estradiol was shown to negatively impact cell migration at the olfactory bulbs/telencephalon junction and in the mediobasal hypothalamus, as newborn cells accumulate close to the ventricle and decreases in the parenchyma. However, no clear data were obtained for cell survival and differentiation. Taken together, these data strongly suggest that 17β-estradiol negatively regulates adult neurogenesis in zebrafish in these experimental conditions (Figure 5.3), in contrast to what is generally described in mammals. New data obtained in zebrafish also highlighted a sexual dimorphism in brain cell proliferation in the dorsal telencephalon, the periventricular nucleus of the posterior tuberculum and the ventral pretectal area, mature females exhibiting a stronger number of cycling cells than males (Ampatzis et al., 2012). In contrast, in the dorsal part of the periventricular hypothalamus, the situation is the opposite, with a higher number of dividing cells in males compared to females (Ampatzis et al., 2012). These differences argue in
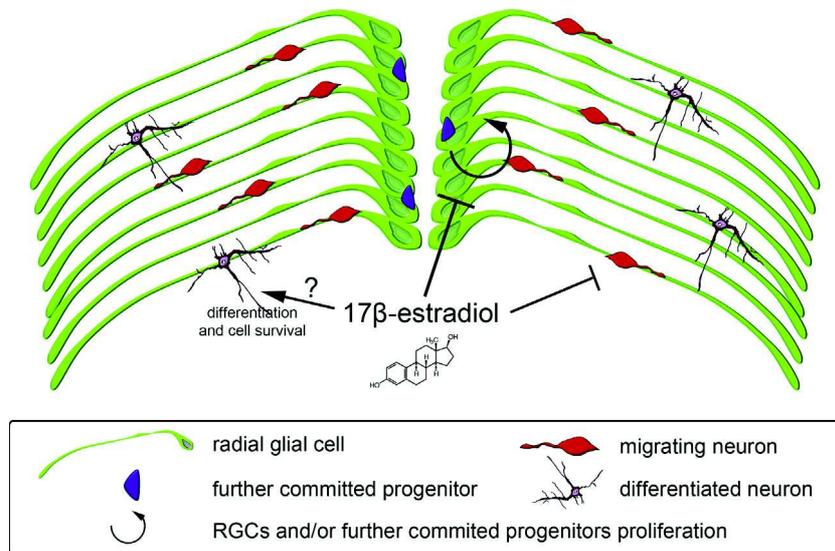
FIGURE 5.3 17β-estradiol modulates adult neurogenesis in zebrafish. Adult zebrafish exposure to 17β-estradiol (10^{-7} M for 100 h) leads to a significant decrease of brain cell proliferation in the ventricular region of the olfactory bulb/telencephalon junction and in the mediobasal hypothalamus. It also impairs neuronal migration after 14 and 28 days of treatment in the same regions. The effects on differentiation and cell survival are not so clear and would require further investigation.

favor of a key contribution of sexual steroids in neurogenic mechanisms. However, for AroB expression, no sexual dimorphism could be detected until now in the adult zebrafish brain, and further investigations would be required for the other stereoidogenic enzymes. In medaka, another fish model used in neurosciences, AroB expression is higher in females, especially in the optic tectum (Okubo et al., 2011). In females, the tectum express a higher level of genes involved in cell proliferation and a lower level of anti-apoptotic genes compared to males (Takeuchi and Okubo, 2013). Such data could imply that a differential level of estradiol resulting from AroB expression dimorphism could affect the cell life cycle in medaka.

In contrast to the compromised capacities of mammals to repair nervous structures after injury, nonmammalian vertebrates, and particularly teleost fish, possess a great power to regenerate damaged tissue by stimulating reparative neurogenesis mechanisms leading to almost complete recovery of the lost function (Schmidt et al., 2013; Zupanc, 2009). The zebrafish has recently emerged as a suitable model to address the question of cellular and molecular processes induced during the regeneration (Grandel and Brand, 2013; Kishimoto et al., 2012; März et al., 2011). As adult zebrafish regenerate neural tissue after injuries so efficiently, a model of lesions of the telencephalon has been developed in order to investigate the regenerative mechanisms and highlight the potential role of estradiol in this response (Diotel et al., 2013). As described in recent papers dedicated to reparative processes in zebrafish, a strong increase in proliferation is observed in the telencephalon after the lesion compared to the control side (Kishimoto et al., 2012; Kroehne et al., 2011; März et al., 2011). The surge in the number of proliferating cells noticeable from 24 hours in the injured parenchyma corresponds to oligodendrocytes and microglia recruitment at the injury site (März et al., 2011). At later times (from day 2 to 7), the number of dividing cells decreased in the parenchyma while more and more cycling cells appeared in the ventricular zones of the telencephalon. At these stages, the identity of the increasing number of ventricular proliferative cells in the injured telencephalon has been investigated using different markers such as AroB/BLBP/GFAP/S100β (RGC markers), Sox2 (neural progenitor marker) and PSA-NCAM (further committed progenitors marker). It allows characterizing the ventricular dividing cells as BLBP/GFAP/S100β$^+$ RGCs and as further committed progenitors, but not as AroB$^+$ RGCs (Baumgart et al., 2012; Diotel et al., 2013; März et al., 2011). Interestingly, at the same stages, the *cyp19a1b* gene and AroB expressions decrease in the injured telencephalon compared to the control hemisphere. This AroB downregulation correlates with the surge in proliferative activity and is in agreement with the inhibitory effect of estradiol on proliferation in homeostatic conditions. So, in order to evaluate

the involvement of estradiol in the cellular events evidenced after mechanical lesion in the telencephalon, zebrafish have been treated with 17β-estradiol (10^{-7} M, for 48 h and 7 days) or with ICI. The results do not point to a significant impact on injury-induced proliferation in such experimental conditions (Diotel et al., 2013). However, the apparent absence of estradiol effect on proliferation after injury does not necessarily mean that survival or differentiation of newborn neurons generated after the lesion are not affected. Very interestingly, some AroB$^+$ cells were observed in brain parenchyma 72h after the telencephalon injury. The identity of these *de novo* AroB expressing cells is currently unknown and could correspond to neurons or oligodendrocytes (Diotel et al., 2013). Even if this point would require further investigation, it is quite relevant given that AroB is only detected in RGCs and never observed in the parenchymal cells in normal conditions.

COMPARISON WITH MAMMALIAN AND NONMAMMALIAN MODELS

In contrast to teleosts, mammals and birds exhibit only restricted to moderate constitutive and regenerative neurogenic capabilities, as well as a relatively weak aromatase activity during adulthood. In general, estrogens have been described for increasing neurogenesis by improving neural progenitor cell proliferation, differentiation and cell survival (Bowers et al., 2010; Brinton, 2009; Martinez-Cerdeño et al., 2006; Ormerod et al., 2004; Wang et al., 2003; Wang et al., 2008). Such data could appear in contrast to what was shown in adult zebrafish. However, recent works in mice show that estradiol negatively regulates neural progenitor proliferation in the SVZ (Brock et al., 2010; Veyrac and Bakker, 2011), and estrogens were shown to exert opposite effects on neuroplasticity and cognition according to their nature and concentration (Barha and Galea, 2010). Furthermore, under physiological conditions, aromatase is mainly expressed by neurons in mammals and birds. However, after chemical or mechanical lesions, aromatase is *de novo* expressed in reactive astrocytes in rats and birds (Azcoitia et al., 2003; Garcia-Segura et al., 1999; Peterson et al., 2001) but also in RGCs facing the lesion in birds (Peterson et al., 2004). This is in striking contrast to teleosts and notably zebrafish in which brain aromatase is only expressed in RGCs under physiological conditions, while a decreased expression in RGCs and a *de novo* expression is observed in parenchymal cells after mechanical injury of the telencephalon (Diotel et al., 2013). So far, it appears that similarities and discrepancies occur between models (Figure 5.4), and push researchers to better take into

«Constitutive» aromatase expression	RGC	neuron	neuron	neuron
Aromatase expression following brain injury	- decrease in RGCs - *de novo* expression in parenchymal cells	?	*de novo* expression in RGC-like	*de novo* expression in reactive astrocytes
Adult neurogenesis	intense and widespread	intense and widespread	moderate	restricted
Brain repair capability	very high	very high	moderate	restricted

FIGURE 5.4 Similarities and discrepancies concerning brain aromatase expression and adult neurogenesis in homeostatic or injured conditions in teleost fish, amphibians, birds and rodents during adulthood.

consideration sex, estrogenic concentration and duration of treatment, brain structures analyzed and developmental stages studied for their experiments.

Among vertebrates, amphibians share some common features with teleost fish. They notably emerged as a leading model for studying constitutive and regenerative neurogenesis. Firstly, the brain of adult frog exhibits a widespread proliferation such as shown recently in *Xenopus laevis* (D'Amico et al., 2011), and gives rise to newborn neurons and oligodendrocytes (D'Amico et al., 2011; Simmons et al., 2008). Secondly, RGCs are also maintained during adulthood in many frog brain subdivisions (D'Amico et al., 2011, 2013). Finally, amphibians are also highly regenerative animals and have been used in various CNS regeneration studies (Tanaka and Ferretti, 2009). As well, the brain of adult amphibian produces neurosteroids, which might have important developmental or physiological roles in the CNS and in neurogenesis (Do Rego et al., 2009; Vaudry et al., 2011). The estrogen-synthesis enzyme P450 aromatase, encoded by one single *cyp19a1* gene, has been shown to exhibit a strong biological activity in the preoptic area and the hypothalamus of different adult frog species (Callard et al., 1978; Mensah-Nyagan et al., 1999), and aromatase-expressing cells were abundantly detected in these neuroendocrine regions in adult lizard (Cohen and Wade, 2011).

Interestingly, similar to what was described in fish, estrogens probably exert a positive autoregulatory feedback loop on aromatase expression given (1) the presence of a half ERE on aromatase promoter in frog and (2) that exposure to estrogens or ethinylestradiol increases *cyp19a1* expression in the brain (Akatsuka et al., 2005). Recently, ISH experiments precisely described the distribution of *cyp19a1* transcripts in the brain of *Xenopus laevis* during development and postmetamorphosis

(Coumailleau and Kah, 2014). From larval stage 42 until postmetamorphic stages, a very strong *cyp19a1* expression was detected in the preoptic area and the caudal hypothalamus (Coumailleau and Kah, 2014). While recently published data using mammalian aromatase antibodies further describes aromatase protein distribution in the frog brain (Burrone et al., 2012), its exact neuroanatomical distribution remains to be investigated using specific antibodies. However, in striking contrast to fish, under normal conditions, *cyp19a1* transcripts were exclusively detected in neurons, and not in RGCs or progenitor cells (Coumailleau and Kah, 2014). In *Xenopus Laevis* and *Xenopus tropicalis*, two (ERα, ERβ) or three (ERα1, ERα2, ERβ) isoforms of estrogen receptor have been identified respectively (Iwabuchi et al., 2008; Takase and Iguchi, 2007). In *Xenopus laevis* and in the urodele *Taricha granulosa*, a widespread distribution of estrogen-concentrating nerve cells was notably observed in the hypothalamic and limbic structures (Davis and Moore, 1996; Morrell et al., 1975). In addition, in the male roughskin newt (*Taricha granulosa*), the preoptic area and hypothalamus were found to contain estrogen receptors positive cells (Davis and Moore, 1996). Clearly, cyp19a1/aromatase and estrogen receptor expression studies revealed an overlapping distribution in specific brain areas, notably in the preoptic area and in the caudal hypothalamus. The apparent neuroanatomical distribution of aromatase and estrogen receptors also reveals an intriguing degree of consistency among vertebrates and suggests that estrogen signaling is involved in controlling similar brain functions and behaviors through evolution. It will be interesting to understand the roles of aromatase and estrogens in constitutive and regenerative neurogenesis in amphibians.

HYPOTHESIS AND PERSPECTIVES FOR ESTROGEN INVOLVEMENT IN NEUROGENESIS REGARDING NEW ADVANCES IN ZEBRAFISH AND NEW LITERATURE IN MAMMALS

In the past few years, constitutive and regenerative neurogenesis has been extensively studied in adult zebrafish, allowing the scientific community to reach a better understanding of neurogenic events. Here, we decided to highlight future directions that would be interesting to investigate for a better understanding of the roles of estrogens in neurogenic processes.

Recent data have shown that estrogens upregulate the cxcr4 receptor and its chemokine cxcl12 (Sdf1) in various cell-types in mammals (Boudot et al., 2011; Li et al., 2013). This chemokine/receptor couple is

implicated in constitutive and regenerative neurogenesis, increasing neural stem cell survival (Zhu et al., 2012), regulating neuronal migration and differentiation (Cui et al., 2013; Yang et al., 2013), and acting on RGC morphology and cell fate in mammals (Mithal et al., 2013). Given these data and the recently described *cxcr4a/cxcl12b* expression in RGCs of adult zebrafish (Diotel et al., 2010b), it will be interesting to further investigate the roles of cxcr4a/cxcl12b in neurogenesis and its potential estrogenic regulation in zebrafish.

Recently, inflammation appeared as a positive regulator of neuronal regeneration in the zebrafish CNS. Indeed, acute inflammation initiates brain regeneration by increasing the neurogenic activity of RGCs (Kyritsis et al., 2012), notably as stab-wounded fish treated with the anti-inflammatory dexamethasone display a significant decrease in reactive neurogenesis (Kyritsis et al., 2012). However, the question of how inflammation enhances neurogenesis either by amplifying some signaling pathways or by initiating new and specific regenerative programs remains open. Given the complex pro- and anti-inflammatory effects of estrogens in mammals (Straub, 2007), notably during neuroinflammation and neurodegeneration (Spence and Voskuhl, 2012; Vegeto et al., 2001), it suggests that estrogens could participate in the regulation of inflammatory signals triggering injury-induced neurogenesis in zebrafish.

Another interesting direction to further explore is the link between notch and estrogen signaling. The notch pathway is well known for its involvement in the maintenance of neural progenitors and for regulating embryonic and adult neurogenesis (Imayoshi et al., 2010). In mice, recent evidence indicates that notch and estradiol signaling interact in the hippocampus, estradiol reducing notch signaling and finally favoring neuritogenesis (Arevalo et al., 2011; Wei et al., 2012). In zebrafish, notch signaling was recently described for its roles in constitutive and regenerative neurogenesis (Chapouton et al., 2010; Kishimoto et al., 2012; Rothenaigner et al., 2011). In the adult zebrafish, around 90% of proliferating RGCs express *notch1a*, *notch1b* and *notch3* while the other neural progenitors of the brain express these receptors at different levels and proportions (de Oliveira-Carlos et al., 2013). Taken together these data suggest that estradiol and notch signaling could interact for regulating neurogenic processes in fish.

Finally, there are accumulating data showing key roles for short (19−25 nucleotides) noncoding RNA called the microRNAs (miRNAs) in a wide range of physiological processes. miRNAs act by base-pairing with the 3′ untranslated region of target mRNAs, leading to translation blockade or transcript degradation. Recently, new studies documented their roles in neurogenesis in both mammals and zebrafish (Coolen et al., 2013; Ji et al., 2013; Lopez-Ramirez and Nicoli, 2013). Interestingly, some miRNAs appeared to be regulated by estrogens in

mammals and zebrafish (Cohen and Smith, 2014; Klinge, 2009, 2012). Consequently, it would be interesting to study (1) how estrogen signaling could modulate miRNA expression and, reciprocally, (2) how miRNAs could regulate estrogen signaling in zebrafish during constitutive and reparative neurogenesis. However, miRNA targets and miRNA regulation are still poorly understood.

CONCLUSION

In vertebrates, there is increasing evidence showing that aromatase and estrogens are strongly implicated in constitutive and regenerative neurogenesis during development and adulthood. They notably act on neuroprotection, brain plasticity, spatial and working memory as well as behavior. For all these reasons, aromatase and estrogens are interesting candidates from a therapeutic point of view for fighting brain strokes and neurodegenerative diseases. However, we are still far from understanding their precise effects, given the different properties exhibited among estrogens, their pleiotropic effects, their complex signaling, and also their multifaceted combinatory effects with other steroids. A better understanding of these mechanisms is crucial for the development of new therapeutics, and also given the accumulating data showing how some endocrine disruptor chemicals could disturb estrogen signaling. In this chapter, we highlighted the power of zebrafish for studying the roles of estrogens and aromatase on adult neurogenesis and brain repair mechanisms. Altogether, these data suggest similarities but also major differences between fish and other vertebrates with respect to estrogen production and functions in adult and reparative neurogenesis (Figure 5.4). The rise of the zebrafish model will be reinforced in the next few years given its relevant and conserved physiological properties, and its utility for rapid drug screening, notably for assessing combined effects of estrogenic chemicals (Petersen et al., 2013), and the debate concerning the use of animals in laboratories.

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