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Fatty acid binding proteins in brain development and disease

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ABSTRACT Long chain polyunsaturated fatty acids (PUFAs) are critical structural components of the brain and essential for normal brain development. The cellular transportation and physiological actions of PUFAs are mediated by fatty acid binding proteins (FABPs) which are encoded by the intracellular lipid-binding protein gene family. Three of the ten mammalian FABPs identified to date (FABP3, FABP5, FABP7) are expressed in the brain. These three FABPs, along with their fatty acid ligands, have distinct and dynamic spatio-temporal expression profiles that correlate with specific developmental stages and processes in the brain. Functional studies have revealed a variety of roles for FABPs in brain development including the generation of neuronal and/or glial cells, differentiation, neuronal cell migration and axis patterning. A number of transcription factors have been shown to be involved in the developmental regulation of FABP gene expression in the brain. Furthermore, FABPs appear to be major downstream effectors of signaling pathways such as Reelin-Dab1/Notch which mediate neuron-glia crosstalk during brain development. As PUFAs and FABPs play critical roles in brain development, considerable effort has been placed in elucidating their function in the pathogenesis and progression of brain cancers and neuropsychiatric disorders.

KEY WORDS: *fatty acid-binding protein, fatty acid, radial glia, brain development, glioblastoma*

Introduction

Long chain polyunsaturated fatty acids (PUFAs) are enriched in developing brain and are essential for normal development of the central nervous system (reviewed in Neuringer *et al.* 1988; Wainwright 2002). PUFAs play important roles in the development of visual, cognitive, attentional, and learning functions (Neuringer *et al.* 1988; Carlson and Neuringer 1999; Wainwright 2002; McCann and Ames 2005; Carlson 2009). PUFA deficiency in the brain is implicated in various neuropsychiatric/neurodegenerative disorders, such as schizophrenia, depression, attention deficit hyperactivity disorder, Parkinson's disease and Alzheimer's disease (Freeman 2000; Richardson and Puri 2000; Salvati *et al.* 2006).

There are two major types of PUFAs: omega-3 (ω-3 or n-3) and omega-6 (ω-6 or n-6). Docosahexaenoic acid (DHA) and arachidonic acid (AA) represent the most abundant ω-3 and ω-6 PUFAs in brain, respectively. Important roles for PUFAs during brain development include: (i) integration into the phospholipids of cell membranes resulting from continuous membrane remodelling, which in turn modulates signal transduction, neurotransmission, cell motility, and the composition and formation of lipid rafts (Stillwell *et al.* 2005; Grossfield *et al.* 2006); (ii) regulation of the expression of genes implicated in genesis, proliferation, differentiation and migration of neural cells through binding and activation of nuclear receptors such as peroxisome proliferator activating receptors (PPARs) and/or retinoid X receptors (RXRs) (Kitajka *et al.* 2004; Schroeder *et al.* 2008); and (iii) serving as precursors for eicosanoids which are key messengers involved in inter- and intracellular signaling cascades in brain (Purasiri *et al.* 1997; Jump 2002; Venkatraman and Meksawan 2002).

As PUFAs are hydrophobic molecules, their trafficking in the

Abbreviations used in this paper: AA, arachidonic acid; ANS, 1-anilinonaphthalene-8-sulfonate; DAB1, Disabled 1; DHA, docosahexaenoic acid; DS, Down syndrome; Kd, dissociation constant; ED, embryonic day; EPA, eicosapentaenoic acid; FABP, fatty acid-binding protein; LOA, linoleic acid; MUFA, monounsaturated fatty acid; ITC, isothermal titration calorimetry; NF1, nuclear factor I; P, postnatal day; PA, palmitic acid; PPAR, peroxisome proliferator-activating receptor; PUFA, polyunsaturated fatty acid; RXR, retinoid X receptor; SA, stearic acid.

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aqueous cytoplasm is facilitated by intracellular protein vehicles, called fatty acid-binding proteins (FABPs). FABPs belong to the intracellular lipid binding protein (iLBP) family which binds fatty acids, retinoids and other hydrophobic compounds and mediates their physiological functions (reviewed in Storch and Corsico 2008). FABPs exhibit tissue-specific expression patterns and distinct ligand preferences, although they all share a conserved 3-dimensional structure consisting of two orthogonal β-sheets and an α-helical cap (Hanhoff *et al.* 2002; Haunerland and Spener 2004).

Ten FABPs have been identified in mammals to date, with three phylogenetically-related FABPs, FABP3, FABP5 and FABP7, described in the developing and/or adult brain (Fig. 1) (Schoentgen *et al.* 1989; Godbout 1993; Bennett *et al.* 1994; Feng *et al.* 1994; Kurtz *et al.* 1994; Owada *et al.* 1996; Liu *et al.* 1997; Denovan-Wright *et al.* 2000; Liu *et al.* 2004; Liu *et al.* 2007). Each of these three FABPs shows distinct preference for specific fatty acids. The main purpose of this review is to summarize current knowledge regarding the cellular/subcellular distribution, transcriptional regulation and function of these three FABPs in the normal and diseased brain of humans, as well as in the developing brain of model organisms.

Developmental expression patterns of FABPs in the brain

The spatio-temporal expression patterns of FABP3, FABP5 and FABP7 in the developing rodent brain have been thoroughly investigated, with similar results obtained in mouse and rat (Kuhar *et al.* 1993; Feng *et al.* 1994; Kurtz *et al.* 1994; Owada *et al.* 1996). FABP7 levels increase as a function of embryonic mouse brain development, reaching a peak at embryonic day (ED)14, and undergoing significant decreases from post-natal day (P)1 to P14

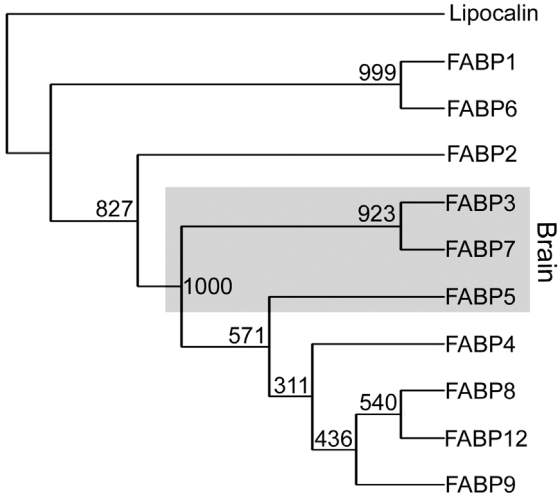

Fig. 1. Phylogenetic tree of human fatty acid binding proteins. *The bootstrap neighbor-joining phylogenetic tree was constructed using* CLUSTALX. *The human lipoclalin 1 protein sequence (LCN1, GenBank accession number NP_002288) was used as an outgroup. The bootstrap values (based on number per 1000 replicates) are indicated on each node. The three phylogenetically-related FABPs with brain functions are highlighted. Amino acid sequences of human FABPs were retrieved from the NCBI website (http://www.ncbi.nlm.nih.gov/).*

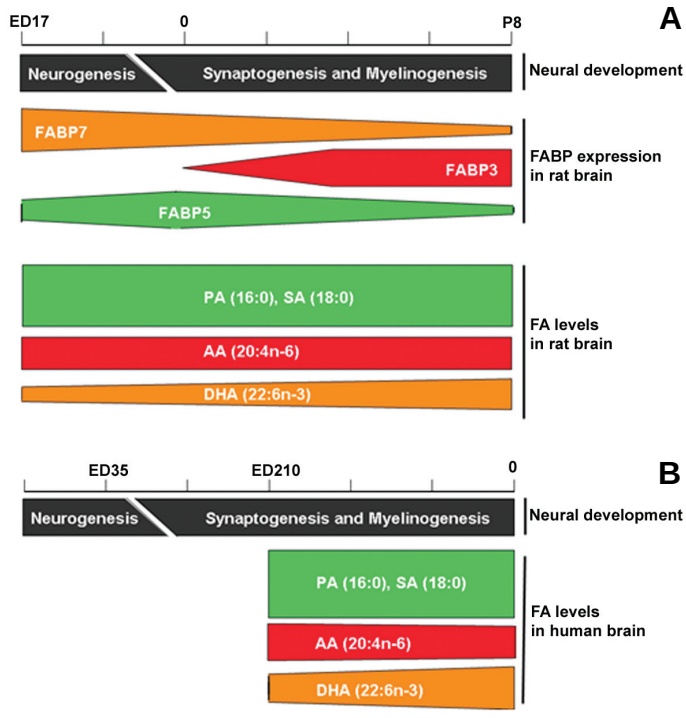

Fig. 2. Temporal correlation of dynamic changes in FABP expression and fatty acid levels in developing rat (A) and human (B) brain. *Temporal expression patterns of* Fabp3, Fabp5 *and* Fabp7 *in rat brain during fetal and postnatal development are based on Northern blot data (Owada* et al. *1996). Dynamic changes in the levels of DHA (22:6n-3), AA (22:4n-6) and PA (18:0) in the developing rat brain and human brain are based on Green and Yavin (1996) and Martinez* et al. *(1992), respectively.*

(Kuhar *et al.* 1993; Feng *et al.* 1994; Owada *et al.* 1996). *Fabp7* mRNA is present at low levels and in restricted regions of the adult olfactory bulb, hippocampus and cerebellum (Kuhar *et al.* 1993; Feng *et al.* 1994; Owada *et al.* 1996; Owada and Kondo 2003). In contrast, *Fabp3* mRNA is barely detectable in brain during embryonic development and the levels gradually increase after birth until adulthood (Owada *et al.* 1996). *Fabp5* mRNA is detected in mid-term embryonic rat brain, reaches its peak at birth, and gradually decreases from P1 to P21 (Owada *et al.* 1996). Like *Fabp7*, *Fabp5* is only weakly expressed in adult rat brain (Owada *et al.* 1996). The temporal pattern of *Fabp7* RNA expression parallels neurogenesis, whereas that of *Fabp3* correlates with synaptogenesis and myelinogenesis (Fig. 2A). The spatial distribution of FABPs in the developing rodent brain has been previously described by Owada *et al.* (1996) and is summarized in Table 1.

Several conclusions can be drawn from the spatial and temporal expression patterns of FABP3, FABP5 and FABP7. First, dynamic changes in expression are observed for all three *Fabp* genes during brain development, with the patterns of expression correlating with specific developmental processes such as establishment of the radial glial fiber system (*Fabp7*), neuronal cell differentiation and migration (*Fabp7*, *Fabp5*), and neurite formation and synapse maturation (*Fabp3*) (Sellner *et al.* 1995; Pu *et al.* 1999; Liu *et al.* 2000; Owada and Kondo 2003; Owada *et al.* 2006). Second, localization of different *Fabps* in the developing brain is either redundant or complementary. For instance, at P7,

TABLE 1

SPATIAL DISTRIBUTION OF FABPs IN DEVELOPING RODENT BRAIN

Gene	Main occurrence	Cell type	Subcellular distribution	References
Fabp7	Cerebral cortex, olfactory bulb, hippocampus, thalamus, hypothalamus, corpus callosum, cerebellum	Radial glia cells, immature astrocytes	Cytoplasm, nucleus	Kurtz et al., 1994; Feng and Heintz, 1994; Owada, 1996
Fabp5	Cerebral cortex, olfactory bulb, hippocampus, thalamus, hypothalamus, corpus callosum, cerebellum, caudate putamen, retina, lens.	Neuron, glia cells	Cell body/soma, processes, nucleus	Liu et al., 2000; Owada et al., 1996
Fabp3	Olfactory bulb, hippocampus, thalamus, hypothalamus, cerebellum, caudate putamen	Neuron	Cytoplasm, nucleus	Sellner et al., 1995; Owada et al., 1996

both *Fabp7* and *Fabp5* mRNA are present in the Bergmann glia of the cerebellum, while *Fabp3* and *Fabp5* mRNA co-localize in the neurons of the olfactory bulb. However, for the most part *Fabp*s show complementary distribution patterns, with transcripts of different *Fabp*s expressed in specific brain regions, cell layers, cell types and developmental stages (Owada and Kondo 2003; Owada *et al.* 2006). Third, transcripts and proteins of all three *Fabp*s localize to both the cytoplasm and nucleus of either glia or neurons (Liu *et al.* 2000; Owada and Kondo 2003). The relative intensity of *Fabp* mRNA and protein in cytoplasm and nucleus changes during development (Liu *et al.* 2000). The presence of FABPs in the nucleus suggests a role in the modulation of gene expression, presumably by controlling the availability of the fatty acid ligands required for nuclear receptor PPAR and/or RXR activity (Kitajka *et al.* 2004; Schroeder *et al.* 2008).

The first avian FABP7 (also known as R-FABP) was identified by Godbout (1993) by screening a cDNA library prepared from embryonic chick retina at ED3.5. Northern blot analysis revealed elevated levels of *FABP7* mRNA in the ED3.5 retina, with a 50 to 100-fold decrease in transcript levels observed from ED3.5 to ED19. In contrast, *FABP7* mRNA levels increased 30 to 40-fold in the differentiating brain from ED3.5 to ED19 (Godbout 1993). Godbout *et al*. (1995) later analyzed the cellular and subcellular distribution of *FABP7* mRNA and protein in the developing chick retina. At early stages of development, from ED3 to ED7, FABP7 was found throughout the retina, with accumulation in the neurites of ganglion cells. By ED11, FABP7 was mainly found in the inner nuclear layer, inner plexiform layer, optic nerve fiber layer and non-pigmented ciliary epithelium (Godbout *et al.* 1995). In contrast to mammalian brain FABP7 which is primarily found in radial glial cells (Kurtz *et al.* 1994; Feng and Heintz 1995), chicken FABP7 localizes to retinal neurons (Sellner 1993). The spatio-temporal expression pattern of FABP7 and other FABPs in the developing avian brain has yet to be described.

In zebrafish, five *fabp* genes have been identified in developing and/or adult brain. These include duplicate *fabp7* genes (*fabp7a* and *fabp7b*), duplicate *fabp11* genes (*fabp11a* and *fabp11b*) and a single *fabp3* gene (Liu *et al.* 2003a; Liu *et al.* 2003b; Liu *et al.* 2004; Liu *et al.* 2007; Karanth *et al.* 2008). As *fabp7b* and *fabp11b* transcripts are expressed at negligible levels in the developing brain, we compared the spatio-temporal pattern of *fabp7a*, *fabp11a* and *fabp3* in the developing zebrafish brain (Fig. 3). Of all *fabps* expressed in zebrafish brain, *fabp7a* transcripts were the most abundant and showed the widest distribution in the developing forebrain, midbrain, hindbrain, spinal cord as well as the retina. Similar to mammalian *FABP7*, zebrafish *fabp7a* is also the earliest gene expressed during brain development, although *fabp7a* mRNA is not spatially restricted when it is first detected at the early segmentation stage (Liu *et al.* 2004). The levels of zebrafish *fabp3* transcripts decrease as the brain develops, with barely detectable levels observed 48 hours post-fertilization (hpf). In contrast, *fabp7a* and *fabp11a* transcripts are still abundant at this stage. Thus, the relative temporal expression patterns of the fish *fabp3 and fabp7* in the developing brain are different from their mamma-

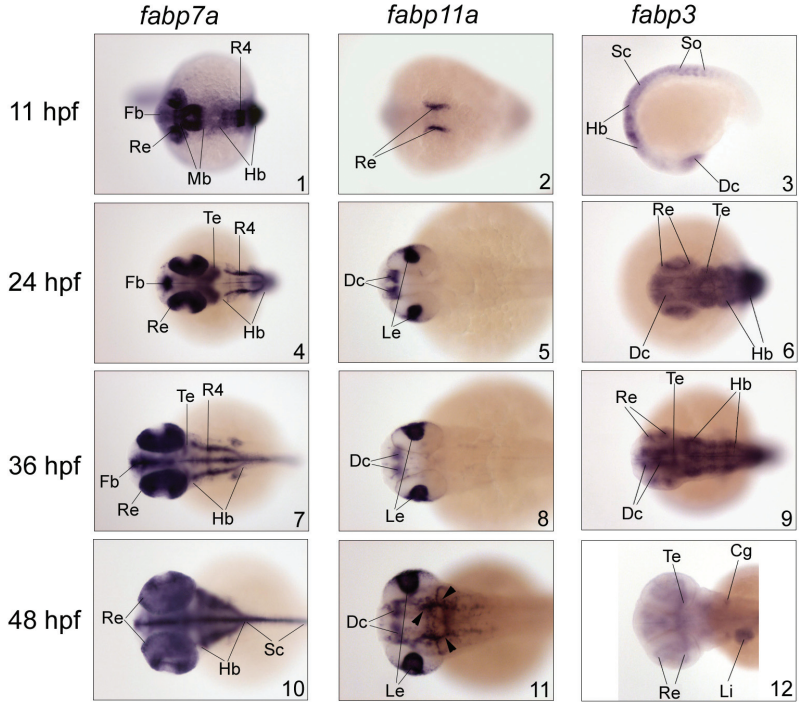

Fig. 3. Comparative expression patterns of FABPs in the developing brain of zebrafish (*Danio rerio*). fabp7a, fabp11a *and* fabp3 *transcripts in zebrafish embryos at 11, 24, 36 and 48 hours post-fertilization (hpf) were detected by whole mount* in situ *hybridization using specific DIG-dUTP-labeled antisense RNA probes. Images are dorsal views of the zebrafish embryos except for III-3, which is a lateral view. The head is on the left. Abbreviations: Fb, forebrain; Te, tectum; Re, retina; Hb, hindbrain; R4, rhombomere 4; Dc, diencephalon; Le, lens; Sc, spinal cord; So, somites; Li, liver; Cg, Cranial ganglion. Arrowheads indicate brain vasculature. The data shown in this figure were compiled from our previous publications (Liu* et al. *2004; Liu* et al. *2007) with permission from the editor.*

lian counterparts (Owada and Kondo 2003; Liu *et al.* 2004). The zebrafish *fabp11a* (also named *fabp4*) shows restricted expression in the developing diencephalon, lens and brain vasculature (Agulleiro *et al.* 2007; Liu *et al.* 2007). As *fabp11a* is the only FABP gene other than *fabp7* and *fabp3* that is expressed in the developing brain, this suggests a possible orthologous relationship with the mammalian *Fabp5*. Of note, both the zebrafish *fabp11a* and the mammalian *Fabp5* are also expressed in lens (Wen *et al.* 1995; Liu *et al.* 2007).

Fatty acid ligand preference for FABPs in developing brain

Commonly used approaches for measuring binding of FABPs to their fatty acid ligands include: Isothermal Titration Calorimetry (ITC), ANS Fluorometry and Lipidex 1000. Despite method-dependent and species-dependent variation in absolute dissociation constant (K_d) values, FABP3, FABP5 and FABP7 each demonstrate preferences for specific classes of fatty acids (Table 2). For example, ITC analysis of human FABP3 in the presence of ω-3 PUFA (DHA, EPA), ω-6 PUFA (LOA) and monounsaturated fatty acid (MUFA) [oleic acid (OA)] yields K_d values in the range of 3-4 μM, 1 μM and 0.8 μM, respectively (Balendiran *et al.* 2000). In support of a preference for ω-6 PUFA, *Fabp3* gene-ablated mice show impairment of AA incorporation into the brain (Murphy *et al.* 2005). These *in vivo* data suggest a requirement for FABP3 binding to ω-6 PUFA that is not compensated by expression of other FABPs in the brain (FABP5 and FABP7).

Rat and human FABP5 both show a preference for the more saturated fatty acids, generating K_d values of 0.168 μM (ANS Fluorometry) and 0.290 μM (Lipidex) for stearic acid (SA) compared to K_d values ≥0.4 μM for ω-3 and ω-6 PUFAs (Liu *et al.* 2008; Hohoff *et al.* 1999). In comparison to FABP3 and FABP5, human FABP7 has the highest affinity for ω-3 PUFA, with a K_d of 0.053 μM when bound to DHA. This is in contrast to K_d values of 0.207 μM for FABP7-AA and 7.1 μM for FABP7-stearic acid (SA) (Balendiran *et al.* 2000). Although not numerically consistent with the K_d of 0.010 μM reported for murine FABP7-DHA using Lipidex (Xu *et al.* 1996), both ITC and Lipidex demonstrate that FABP7 preferentially binds to ω-3 PUFA.

Green *et al*. (1996) and Martinez *et al*. (1992) have examined the fatty acid composition of the developing rat and human brain, respectively. In rat, the levels of saturated fatty acids (16:0; 18:0) and AA (ω-6 PUFA) remain stable throughout neural development. In contrast, the levels of DHA in rat brain are relatively low during neurogenesis (~50% of AA levels and 25% of PA and SA levels) and increase during synaptogenesis (to ~50% of PA and SA levels and close to 100% of AA levels by P8) (Fig. 2A). A similar trend is observed in the developing human brain, with stable levels of saturated fatty acids from 30 weeks gestation to birth. A slight decrease in AA levels is observed during this period of time, whereas synaptogenesis and myelinogenesis is accompanied by an ~20% increase in DHA levels (Fig. 2B). As there is no data on the fatty acid composition of the human brain during neurogenesis, one can only postulate that, similar to what has been observed in rat brain, the transition from neurogenesis to synaptogenesis is accompanied by a significant increase in DHA in the developing human brain.

TABLE 2

LIGAND PREFERENCE OF FABPs EXPRESSED IN DEVELOPING BRAIN

FABP	Fatty Acid Classification	Fatty Acid	Kd (nM)	Method	Reference
Hs-FABP3	ω-3 PUFA	DHA (22:6)	4100 ± 6	ITC	(Balendiran *et al*., 2000)
		EPA (20:5)	3300 ± 10	ITC	(Balendiran *et al*., 2000)
	ω-6 PUFA	LOA (18:2)	970 ± 8	ITC	(Balendiran *et al*., 2000)
		AA (20:4)	370 ± 20	Lipidex	(Veerkamp *et al*., 1999)
	MUFA	OA (18:1)	820 ± 10	ITC	(Balendiran *et al*., 2000)
		OA (18:1)	440 ± 50	Lipidex	(Veerkamp *et al*., 1999)
	Saturated FA	PA (16:0)	960 ± 50	Lipidex	(Veerkamp *et al*., 1999)
Rn-FABP5	ω-3 PUFA	DHA (22:6)	422.2 ± 58.1	ANS Fluorometry	(Liu *et al*., 2008)
		EPA (20:5)	598 ± 100.5	ANS Fluorometry	(Liu *et al*., 2008)
	ω-6 PUFA	AA (20:4)	390.9 ± 54.2	ANS Fluorometry	(Liu *et al*., 2008)
		LOA (18:2)	512.0 ± 34.5	ANS Fluorometry	(Liu *et al*., 2008)
	MUFA	OA (18:1)	154.6 ± 35.3	ANS Fluorometry	(Liu *et al*., 2008)
	Saturated FA	SA (18:0)	168.1 ± 38.1	ANS Fluorometry	(Liu *et al*., 2008)
Hs-FABP5	ω-6 PUFA	AA (20:4)	1730 ± 250	Lipidex	(Hohoff *et al*., 1999)
	MUFA	OA (18:1)	1600 ± 200	Lipidex	(Hohoff *et al*., 1999)
	Saturated FA	SA (18:0)	290 ± 60	Lipidex	(Hohoff *et al*., 1999)
Hs-FABP7	ω-3 PUFA	DHA (22:6)	53.4 ± 4.1	ITC	(Balendiran *et al*., 2000)
		EPA (20:5)	48.1 ± 21	ITC	(Balendiran *et al*., 2000)
	ω-6 PUFA	AA (20:4)	207 ± 19	ITC	(Balendiran *et al*., 2000)
		LOA (18:2)	115 ± 19	ITC	(Balendiran *et al*., 2000)
	MUFA	OA (18:1)	46.7 ± 1.4	ITC	(Balendiran *et al*., 2000)
	Saturated FA	SA (18:0)	7100	Lipidex	(Balendiran *et al*., 2000)
		PA (16:0)	13500	Lipidex	(Balendiran *et al*., 2000)
		LA (12:0)	443 ± 55	ITC	(Balendiran *et al*., 2000)
Mm-FABP7	ω-3 PUFA	DHA (22:6)	10 ± 2	Lipidex	(Xu *et al*., 1996)
	ω-6 PUFA	AA (20:4)	250 ± 34	Lipidex	(Xu *et al*., 1996)
	MUFA	OA (18:1)	440 ± 27	Lipidex	(Xu *et al*., 1996)

Although there is some overlap in the temporal expression pattern and fatty acid binding affinities of FABP3, FABP5 and FABP7, it is clear from *in vitro* binding studies that these FABPs have specific fatty acid ligand preferences (i.e. ω-6 PUFA for FABP3, saturated fatty acids for FABP5 and ω-3 PUFA for FABP7). The temporal expression patterns of FABP3, FABP5 and FABP7 during brain development, combined with the temporal availability of saturated fatty acids, ω-6 PUFA and ω-3 PUFA, suggest that different FABPs and their fatty acid ligands play specialized roles during neurogenesis and synaptogenesis.

Emerging functions for FABPs in brain development

FABP7 and neuronal cell migration

During brain development, neuronal precursor cells proliferate in the ventricular zone of the developing neocortex. Neurons then travel from their origin or "birth place" using radial glial fibers that serve as a scaffold for migrating neuronal cells. Each subsequent wave of migrating cells travel past their predecessors, forming layers in an inside-out manner, i.e. the youngest neurons are the closest to the cortical surface (Rakic 1972; Nadarajah and Parnavelas 2002). It has been estimated that as much as 90% of neuronal migration in the cortex is mediated by radial glial cells (Sidman and Rakic 1973; Hatten 1999).

The mammalian FABP7 is specifically expressed in radial glial cells during brain development (Feng *et al.* 1994; Kurtz *et al.* 1994; Hartfuss *et al.* 2001). Feng *et al*. (1994) used an *in vitro* neuron-glia co-culture system to investigate whether FABP7 plays a role in the establishment of a glial fiber system and neuronal migration. When purified anti-FABP7 antibody was added to the neuron-glia culture, both glial cell processes extension and neuronal migration were blocked, while cell proliferation and adhesion were not affected (Feng *et al.* 1994). Subsequent investigations further demonstrated that expression of FABP7 in radial glial cells is induced by the presence of migrating neurons (Feng and Heintz 1995) and that this induction is mediated by the Notch signalling pathway (Anthony *et al.* 2005).

Pax6 and GGF/neuregulin also mediate migrating neuron-radial glia interactions. Both these molecules control FABP7 expression in the radial glial cells of the developing rat brain (Anton *et al.* 1997; Arai *et al.* 2005). These studies suggest that FABP7 is a critical molecule in radial glial cells, on one hand functioning as a sensor for neuronal signals, and on the other hand modulating radial glia differentiation and neuronal migration.

FABP7-expressing cells as neural progenitors

As mentioned above, FABP7 is present at very early developmental stages in both mammalian and fish brains. Mammalian FABP7 is specifically expressed in the neuroepithelial cells and radial glial cells of the ventricular and subventricular zones, the hot spots of neurogenesis (Feng *et al.* 1994; Kurtz *et al.* 1994; Owada *et al.* 2006). In addition to their role in neuronal cell guidance, radial glial cells have been shown to act as neural stem/progenitor cells during brain development (Malatesta *et al.* 2000; Miyata *et al.* 2001; Noctor *et al.* 2001; Malatesta *et al.* 2003; Anthony *et al.* 2004).

Anthony *et al*. (2004) used a 1.6 kb *FABP7* promoter-driven Cre/Rosa26 Cre reporter double transgenic mice for fate mapping analysis. They found that all FABP7-expressing radial glial cells, regardless of location within the brain, go through a neuron generating stage. These authors concluded that FABP7-expressing radial glial cells give rise to most of the neurons in the brain. Furthermore, analysis of *Fabp7-/-* mice revealed dramatic decreases in the number of astrocytes, neural stem cells and early progenitor cells in the developing brain (Watanabe *et al.* 2007). *Fabp7-/-* mice also showed decreased numbers of late progenitor cells and attenuated neurogenesis in the developing hippocampus compared to wild-type mice (Watanabe *et al.* 2007). Knockdown of FABP7 in cultured rat neuroepithelial cells results in decreased proliferation, loss of processes and induction of premature neurogenesis, suggesting a role for FABP7 in the maintenance of proliferation (Arai *et al.* 2005). FABP7 may therefore play a key role in neurogenesis, by maintaining the pool of neural stem/progenitor cells (Watanabe *et al.* 2007).

Role of FABP3 and FABP5 in neuronal differentiation

During brain development, specific biochemical, physiological and morphological properties must be acquired by neuroprogenitor cells to allow differentiation along the neuronal cell lineage. The coordinated regulation of gene expression is fundamental to the control of neuronal cell differentiation. FAPB5 is specifically found in the differentiating neurons of the developing rat cerebral cortex and retina. FABP5 mRNA and protein levels are markedly higher in the prenatal and early postnatal neurons compared to adult neurons, suggesting a function for this FABP in neuronal differentiation (Liu *et al.* 1997; Liu *et al.* 2000). Other evidence in support of a role for FABP5 in neuronal cell differentiation is the observation that FABP5 is induced in injured and regenerating neurons (De Leon *et al.* 1996; Owada *et al.* 1997). FABP5 is also upregulated with NGF-induced neurite growth in rat pheochromocytoma cells (PC12), a model system for neuronal differentiation (Allen *et al.* 2000). Depletion of FABP5 in PC12 using an antisense *Fabp5* construct significantly reduces NGF-mediated neurite growth, suggesting a role for FABP5 in axonal development and regeneration (Allen *et al.* 2000). Of note, no brain phenotype has been reported thus far in *Fabp5* knockout mice, suggesting that other FABPs may compensate for loss of FABP5 function (Owada *et al.* 2002).

FABP3 is expressed in mouse brain at relatively late developmental stages compared to *Fabp7* and *Fabp5*, with barely detectable levels until ED17-19 (Sellner *et al.* 1995; Owada *et al.* 1996). Based on its spatio-temporal expression pattern in mouse brain, Sellner *et al*. (1995) proposed that FABP3 is involved in neurite formation and synapse maturation. As *Fabp3*-null mouse brains show a reduction in both the incorporation of AA and the proportion of total ω-6 fatty acids in the major phospolipid classes, it was concluded that FABP3 may be involved in the uptake and metabolism of ω-6 fatty acids in the developing brain (Murphy *et al.* 2005).

Role of FABPs in axis patterning of the developing brain

As the brain develops from the neural plate, different regions of the brain need to establish spatial identities to generate the precise cytoarchitecture of the mature brain. Patterning along both the anterior-posterior (AP) axis and dorsal-ventral (DV) axis starts after the generation of the neural tube, which involves precisely-timed expression of developmentally regulated genes (Lumsden and Krumlauf 1996). Several organizing centers in the

early developing vertebrate brain are involved in local patterning of axis formation, including the midbrain-hindbrain boundary (reviewed by Rhinn and Brand 2001; Wurst and Bally-Cuif 2001), the forebrain organizer (Shimamura and Rubenstein 1997; Houart *et al.* 1998) and the hindbrain-spinal cord border (Maden 2002). However, the signaling mechanisms from organizing centers involved in neuronal and glial cell differentiation are not well understood. Isolating genes with restricted patterns of expression in organizing centers combined with mutant screening using model organisms would be a powerful approach toward understanding the signaling pathways governing axis patterning in vertebrate brain.

As FABP7 is expressed in the early developing brain and has cell signaling properties (Feng *et al.* 1994), it is logical to postulate that FABP7 may play a role in neural axis patterning. For instance, the vertebrate hindbrain consists of several subdivided segments, called rhombomeres, each of which has distinct cellular and molecular characteristics (reviewed by Lumsden and Krumlauf 1996). Rhombomere 4 (r4) is the first one to form in the developing vertebrate hindbrain and acts as a signaling center in hindbrain patterning (Graham and Lumsden 1993; Graham and Lumsden 1996; Maves *et al.* 2002). In zebrafish, *fabp7a* transcripts specifically localize to a restricted region of r4 during the middle and late segmentation phase, suggesting an important role for the *fabp7a* product in patterning of the hindbrain (Liu *et al.* 2004). Recent data indicate that Pbx homeodomain proteins may play a role in the patterning of zebrafish retina and optic tectum, and that one of the major downstream effectors of these transcription factors is FABP7 (French *et al.* 2007). Indeed, zebrafish *fabp7a* mRNA is abundantly expressed in the developing retina and tectum from early embryonic stages (Liu *et al.* 2004) and its levels are markedly reduced in the *pbx2/4*-null embryos compared with the wild-type (French *et al.* 2007).

Regulation of FABP expression in developing brain

Nuclear factor I (NF1)

The nuclear factor I (NFI) gene family, including *NFIA*, *NFIB*, *NFIC* and *NFIX*, plays important roles in development (reviewed by Gronostajski 2000). NFIs are expressed in both glial cells and neurons. Examination of the neural development abnormalities in *Nfia-/-* mice provide direct evidence for the importance of this transcription factor in regulating gene expression in the developing brain (das Neves *et al.* 1999; Shu *et al.* 2003; Wong *et al.* 2007). cDNA microarray analysis of genes expressed in the brains of *Nfia-/-* and *Nfia+/+* mice identify *Fabp7* as significantly up-regulated (2.59-fold) as a consequence of NFIA disruption (Wong *et al.* 2007). Three NFI-binding sites in the proximal promoter region of human *FABP7* have been mapped (Bisgrove *et al.* 2000; Brun *et al.* 2009). FABP7 expression is modulated by differential phosphorylation of NFI in human malignant glioma cells (Bisgrove *et al.* 2000). Whether transcription of *FABP7* in the developing brain is regulated by the same mechanism is not known.

The Notch signaling pathway

Notch proteins are single-pass transmembrane receptors which include four family members, NOTCH1, NOTCH2, NOTCH3 and NOTCH4. Notch proteins play a role in a variety of developmental processes by controlling cell fate decisions (reviewed by Lewis 1998; Gaiano and Fishell 2002; Rodriguez-Rivera *et al.* 2009). Both neural stem cells and intermediate neural progenitors respond to Notch receptor activation, but through differential action of the downstream effector C-promoter binding factor 1 (CBF1) (Mizutani *et al.* 2007). A single binding site for CBF1 (5'-GTTCCCAGGC-3', conserved nucleotides underlined) has been mapped within the proximal promoter region of mouse *Fabp7* and is required for *Fabp7* expression in radial glial cells (Anthony *et al.* 2005). Furthermore, FABP7 protein levels are significantly down-

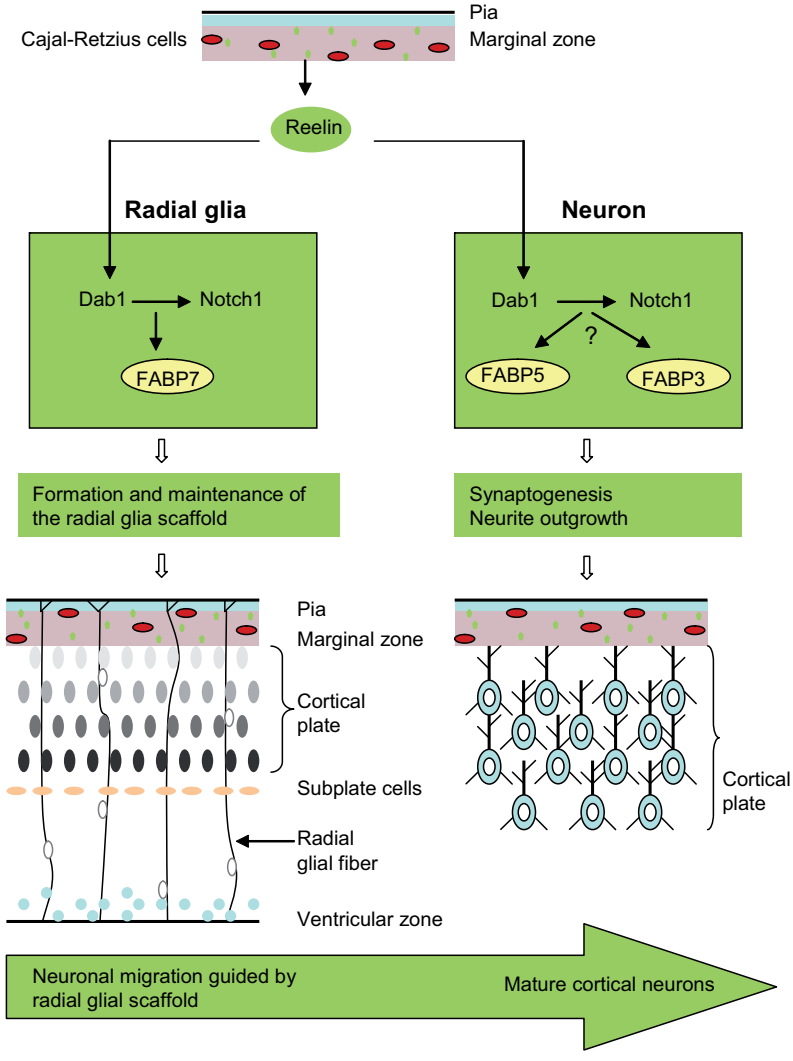

Fig. 4. Schematic illustration of a proposed Reelin-Dab1/Notch/FABP signaling pathway involved in neural development. *FABP7 expressed in radial glial cells is a major target of Reelin-Dab1/Notch signaling, which coordinates interaction between migrating neurons and radial glia during cortical neural development. We propose a model whereby FABP5 and FABP3 could function as downstream effectors of Reelin-Dab1/Notch signaling in neuronal cells.*

regulated in the developing forebrain of mice lacking *Notch1* and *Notch3*, indicating an essential role for Notch signaling in modulating *Fabp7* expression in developing mouse brain (Anthony *et al.* 2005).

The Reelin-Dab1 pathway

Reelin is a large secreted glycoprotein which controls cell-cell interactions critical for cell positioning and neuronal migration during brain development (D'Arcangelo *et al.* 1995). Disabled-1 (DAB1) serves as the intracellular adaptor for Reelin and has also been shown to be involved in neuronal cell positioning in the developing brain (Howell *et al.* 1997; Howell *et al.* 1999). DAB1 is tyrosine phosphorylated when Reelin binds to the lipoprotein receptors Very Low Density Lipoprotein Receptor (VLDLR) and Apolipoprotein E Receptor 2 (APOER2) on the cell membrane (Huang *et al.* 2005). In mutant mice deficient for Reelin, DAB1, or Reelin receptors (VLDLR and APOER2), radial glial morphology is altered and the radial glial scaffold is impaired in the developing brain (Forster *et al.* 2002; Hartfuss *et al.* 2003; Weiss *et al.* 2003). Reelin signalling affects FABP7 expression in radial glial cells both *in vitro* and *in vivo* (Hartfuss *et al.* 2003; Keilani and Sugaya 2008) and DAB1 is required for Reelin-mediated FABP7 expression (Hartfuss *et al.* 2003).

A recent study of the role of Reelin in FABP7 induction in human neural progenitor cells revealed cross-talk between the Reelin and Notch signaling pathways (Keilani and Sugaya 2008). Reelin treatment of these cells increased the levels of both the NOTCH1 intracellular domain (NICD) and FABP7, and DAB1 co-immunoprecipitated with NICD, indicating physical interaction between these molecules (Keilani and Sugaya 2008). Interaction between Reelin-Dab1, Notch and FABP7 (BLBP) has also been demonstrated using the radial glial cells of Reeler mice (Sibbe *et al.* 2009). The inter-relationship between these major signaling molecules may be of significance in elucidating the links between the neuronal-radial glial signaling networks which regulate glial cell development, neuronal generation and migration.

Reelin is primarily secreted by neurons termed Cajal–Retzius cells, which establish early neuronal circuitry in the developing brain (Aguilo *et al.* 1999). Secreted Reelin promotes the differentiation of progenitor cells into radial glia and affects the orientation of radial glia fibers, which serve as guides for migrating neurons (Fig. 4). Interaction between Reelin and Notch signaling pathways has also been demonstrated in the migrating and differentiating neurons of the developing cerebral cortex (Hashimoto-Torii *et al.* 2008). Reelin may therefore serve as a general signaling messenger to integrate the events associated with radial glia fiber formation and neuronal cell migration (Fig. 4). Thus, Reelin-Dab1/Notch/FABP7 may represent a major and essential signaling pathway mediating neuronal-glial interaction during the early development of the brain.

As FABP3 and FABP5 are expressed in the neurons of developing brain and their expression patterns correlate with neuronal cell differentiation and/or migration (Sellner *et al.* 1995; Liu *et al.* 2000), it will be interesting to know whether the neuronal Reelin-Dab1/Notch signaling pathway also functions through a neuronal FABP (FABP3 or FABP5) downstream effector, as it does for FABP7 in radial glial cells (Anthony *et al.* 2005; Keilani and Sugaya 2008; Sibbe *et al.* 2009).

PAX6

The transcription factor PAX6 affects cell fate, cell proliferation and patterning during brain development in various species (Haubst *et al.* 2004). In *Pax6*-deficient mice, the morphology, quantity and cell cycle distribution of radial glial cells in the developing cortex are altered (Gotz *et al.* 1998). In the embryonic brains of rats, there is a strong correlation in the distribution patterns of PAX6 and FABP7. *Pax6* mutation in rat embryonic brains results in dramatically reduced FABP7 whereas over-expression of PAX6 induces FABP7 expression, suggesting a role for PAX6 in the regulation of *Fabp7* (Arai *et al.* 2005). Although a putative *cis*-binding site for PAX6 was identified in the mouse *Fabp7* promoter (Feng and Heintz 1995), it is not known whether PAX6 regulates *Fabp7* transcription by directly interacting with this *cis* element or through other downstream effectors. It is noteworthy that FABP7 levels are not significantly affected by depletion of PAX6 in mouse brain (Gotz *et al.* 1998; Arai *et al.* 2005), suggesting that PAX6-mediated regulation of FABP7 expression is species-dependent.

POU-domain proteins

POU-domain proteins are widely expressed in developing brain with distinct temporal and spatial patterns (He *et al.* 1989; Alvarez-Bolado *et al.* 1995). Using an *in vivo* reporter transgenic mouse model, Josephson *et al*. (1998) tested the ability of serial deletions of the proximal 5' upstream region of *Fabp7* (Feng and Heintz 1995) to drive developmentally-regulated *Fabp7* expression in the embryonic CNS. A POU-domain protein PBX-1 and BRN-1 binding element was subsequently identified in the *Fabp7* promoter (Josephson *et al.* 1998). Mutant mice with germ-line deletion of the 9 bp POU-domain binding element lacked *Fabp7* expression in the developing forebrain and midbrain, suggesting an essential role for POU-domain proteins in *Fabp7* regulation (Josephson *et al.* 1998).

In zebrafish, the levels of the *fabp7a* transcripts are dramatically reduced in the embryonic brain and retina when the POU-domain protein is knocked-down by morpholino transfection (French *et al.* 2007), directly implicating this POU-domain protein in the regulation of *fabp7* expression.

FABPs and brain disease

FABP7 is detected in human malignant glioma tumor biopsies and cell lines (Godbout *et al.* 1998). cDNA array analysis of human glioblastoma tumor tissues has revealed a significant correlation between FABP7 and survival, suggesting that FABP7 could be an effective prognostic marker for glioblastoma patients with the worst survival rates (Liang *et al.* 2005; Kaloshi *et al.* 2007). Furthermore, the nuclear distribution of FABP7 in epidermal growth factor receptor (EGFR)-positive glioblastoma tumors has been shown to be associated with a poor prognosis (Liang *et al.* 2006). Expression of FABP7 in FABP-negative human malignant glioma cell lines enhances cell migration (Liang *et al.* 2005; Mita *et al.* 2007), whereas FABP7 depletion by RNA interference suppresses migration (Mita *et al.* 2007). Taken together, both clinical investigations and *in vitro* functional studies support an important role for FABP7 in the invasion and progression of human glioblastoma tumors. Further *in vivo* studies using animal

models and exploration of the mechanism of action of FABP7 in the nucleus will shed light on its role in human glioblastoma.

Mental retardation is a typical phenotypic feature of Down syndrome (DS) patients. As FABPs are involved in development, establishment and maintenance of the central nervous system, it is reasonable to propose that FABPs may be implicated in DS pathogenesis. FABP7 has been found to be overexpressed in DS adult (Cheon *et al.* 2003) and fetal brains (Sanchez-Font *et al.* 2003), whereas FABP3 is significantly decreased in DS adult brains (Cheon *et al.* 2003). Furthermore, FABP7 upregulation in DS brains correlates with *PKNOX1* gene-dosage imbalance. As PKNOX1 is a POU domain protein, it may directly control FABP7 expression by interacting with the Pbx/POU binding element in the *FABP7* promoter (Sanchez-Font *et al.* 2003).

Different lines of investigation directly associate FABP7 with schizophrenia, a psychiatric illness characterized by abnormalities in the perception or expression of reality (Watanabe *et al.* 2007). Firstly, a quantitative trait locus (QTL) screening in a reference mice population demonstrates that *Fabp7* is a promising candidate gene responsible for deficits in prepulse inhibition (PPI), a biological marker for schizophenia. Secondly, *Fabp7*-deficient mice show decreased PPI and impaired hippocampus neurogenesis. Thirdly, human *FABP7* mRNA levels are significantly upregulated in the postmortum brains of schizophrenia patients compared with normal controls. Fourthly, there is a correlation between a single nucleotide polymorphism (SNP) variant within the second exon of human *FABP7* and schizophrenia pathology (Watanabe *et al.* 2007). As PPI impairment has been demonstrated in many other neuropsychiatric disorders (e.g. Alzheimer's disease, autism, bipolar disorders, Tourette syndrome, etc.) in addition to schizophrenia (Braff *et al.* 2001; Hejl *et al.* 2004), the association between *FABP7* and PPI status suggest that this gene is involved in the pathology of a wide range of neuropsychiatric and/or neurodegenerative diseases. Relationships between FABP3 and FABP5, and neurodegenerative diseases have also been reported (Gearhart *et al.* 2002; Steinacker *et al.* 2004).

Summary

FABP3, FABP5 and FABP7 are expressed in specific spatiotemporal patterns in the developing brain, with each showing preferential binding to different categories of long chain fatty acid ligands. Although the precise roles of these three FABPs in brain development awaits more in-depth studies, investigations using animal models have revealed a number of possible functions in neurogenesis, neuronal migration and differentiation, and axis patterning. Data to date indicate that FABP3, FABP5 and FABP7 are multi-functional proteins regulated by complex signaling networks and transcription factors. In particular, FABP7 in radial glial cells may be a major downstream effector of the Reelin-Dab1/Notch signal pathway which plays a key role in the formation and maintenance of the radial glia scaffold and neuronal cell migration in the developing brain. FABP7 may serve as a mediator for the signaling crosstalk required between migrating neurons and radial glial cells. So far, FABP expression, its cellular distribution and genetic variation have been implicated in a number of brain diseases including human glioblastoma and neuropsychiatric disorders. Future investigations will shed additional light on the role of FABPs and their mechanisms of action in the development and health of the brain.

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