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Significance of F3/Contactin gene expression in cerebral cortex and nigrostriatal development

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ABSTRACT

F3/Contactin is a neuronal surface glycoprotein, which plays a general role in neural development and, in particular, in neuronal and oligodendrocyte differentiation. In a previous study using the F3/EGFP transgenic mice, which express an EGFP reporter under control of the regulatory region from the mouse F3/Contactin gene, the activation of the F3/Contactin promoter was found to correlate with granule and Purkinje neuron differentiation in developing cerebellar cortex. Here we report that in developing cerebral cortex and basal ganglia the F3/Contactin gene is mostly activated during early commitment of neuronal precursors, thus indicating a region-specific profile of its developmental activation. We also report that, in the same structures of F3/EGFP mice, a downregulation of the endogenous F3/Contactin gene occurs, which correlates with upregulation of the dopaminergic phenotype and with locomotor pattern abnormalities. Therefore, F3/EGFP transgenic mice exhibit morphological and functional phenotypes recapitulating those arising from imbalance of the striatal dopaminergic pathway. As for the underlying mechanisms, we postulate that in F3/EGFP mice F3/Contactin downregulation results from the ability of transgene promoter sequences to interfere with the activation of the endogenous gene, thus realizing an F3/Contactin knockdown model, while dopaminergic upregulation is consistent with a general F3/Contactin inhibitory effect on the neuronal phenotype.

Introduction

Besides that in axonal growth and pathfinding control, axonal adhesive glycoproteins may play relevant roles in further aspects of neural development, including precursor proliferation and migration (Bizzoca et al., 2003; Lu et al., 2008; Maness and Schachner, 2007; Traka et al., 2003), synaptic contact formation and myelination (Laursen et al., 2009; Zhang et al., 2008). The involvement of such molecules in a wide set of developmental events implies that the underlying genes are tightly and coordinately regulated at the cellular levels within distinct developmental steps, which may be relevant for their functional cooperation (Bizzoca et al., 2003; Buttiglione et al., 1998; Charles et al., 2002; Hortsch et al., 2009; Pavlou et al., 2002; Pesheva et al., 2006).

Among the axonally-expressed glycoproteins, F3/Contactin deserves a special mention. This molecule plays relevant roles in distinct developmental events, ranging from precursor proliferation to commitment along the neuronal lineage (Bizzoca et al., 2003, 2009, 2012), but also in differentiation of both the peripheral (Durbec et al., 1992; Gennarini et al., 1989, 1991) and central (Bizzoca et al., 2003, 2012; Buttiglione et al., 1998) neurons. The molecule is also expressed by oligodendrocytes (Koch et al., 1997) of which it modulates differentiation (Hu et al., 2003), while axonal F3/Contactin sustains heterophilic interactions with oligodendrocytes in the earlier stages of myelination (Peles and Salzer, 2000). For these last functions a relevant role is played by F3/Contactin cis-association with the Contactin associated protein CASPR1/Paranodin, a Neurexin family component, and by the trans-interaction of the arising complex with the oligodendroglial immunoglobulin-related glycoprotein Neurofascin 155 (Bonnon et al., 2003; Charles et al., 2002). These interactions are important for the F3/Contactin implication in myelination, as confirmed by the changes in myelinated nerves, which occur in both F3/Contactin (Boyle et al., 2001) and CASPR1 (Bhat et al., 2001) null mutant mice.

Functional interactions among these molecules obviously require coordinated activation of the underlying genes and in previous studies (Bizzoca et al., 2003) we have been addressing the topic of the

Abbreviations: WT, wild-type; PB, phosphate buffer; PFA, paraformaldehyde; AEC, 3-amino-9-ethylcarbazole; E, embryonic day; EGFP, enhanced green fluorescent protein; ON, overnight; PBS, phosphate-buffered saline; BSA, bovine serum albumin; FCS, foetal calf serum; TH, tyrosine hydroxylase; SEM, standard error of mean; P, postnatal day; DA, dopamine; DT, distance travelled; CP, cortical plate; IZ, intermediate zone; SVZ, subventricular zone; VZ, ventricular zone.

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developmental significance of the axonal glycoprotein expression profile by generating transgenic mice in which F3/Contactin was prematurely expressed under control of the promoter region from the gene encoding the Transient Axonal Glycoprotein TAG1 (Furley et al., 1990). These mice (TAG/F3 mice) exhibited developmentally-regulated cerebellar (Bizzoca et al., 2003) and cortical (Bizzoca et al., 2012) phenotypes at both the morphological (Bizzoca et al., 2003, 2012) and functional (Coluccia et al., 2004) levels, which reverted at the same time as F3/Contactin misexpression, thus supporting the hypothesis that the expression profile of this glycoprotein is itself endowed with developmental significance (Bizzoca et al., 2009).

To further address the relationship between F3/Contactin gene expression and neural developmental events we also carefully explored its activation profile by generating transgenic mice bearing a promoter–reporter construct, in which a large part of the F3/Contactin gene 5′ flanking region, including all the identified regulatory sequences, was used to drive an enhanced green fluorescent protein (EGFP) reporter (F3/EGFP mice; De Benedictis et al., 2006). In developing cerebellar cortex, the profile of this transgene largely recapitulated the endogenous gene, indicating that F3/Contactin expression is mostly controlled at the transcriptional level. Together with the above-mentioned data about the cerebellar and cortical phenotypes, which arise from F3/Contactin developmental misexpression this supported the hypothesis that regulated expression of the F3/Contactin gene is an integrant component of its ontogenetic function.

In this study, by using the F3/EGFP transgenic mice as a model, we report on the activation profile of the F3/Contactin gene in developing forebrain, with special reference to the cerebral cortex and the basal ganglia. In addition, in these mice, unexpectedly, we demonstrate a functional phenotype, which mostly consists in the occurrence of a circling behaviour, typical of basal nuclei dysfunction (Ishiguro et al., 2007). We infer that activation of the F3/Contactin promoter sequences is itself able to perturb basic neurodevelopmental events, most likely by interfering with the expression of the endogenous gene. To address this possibility we explore here the neuromorphological and functional phenotypes, which occur in F3/EGFP mice versus wild-type littermates, in comparison with the expression profile of the F3/EGFP transgene and of the endogenous F3/Contactin gene.

Results

Expression of the F3/EGFP transgene in the forebrain

In coronal sections from E16 frontal cortex (Fig. 1Aa) faint and diffuse expression of the F3/EGFP transgene was observed within the cortical plate (CP) and the subplate, with lower to undetectable levels in the intermediate (IZ), subventricular (SVZ) and ventricular (VZ) zones. By contrast strong transgene activation occurred on scattered cells (see also arrowhead and arrow in the inset). These cells lacked neuronal (NeuN), glial (Vimentin) and oligodendroglial (O4) markers (not shown), suggesting their nature of uncommitted precursors. This mode of transgene activation at the cellular level was also observed in the striatum, in which cells expressing different levels of the transgene were demonstrated (Fig. 1Ab, arrow and arrowhead in the inset); in the olfactory bulb strong transgene activation was demonstrated in the mitral cells (Fig. 1Ac), again with different levels in different neurons (arrows in the inset).

In newborn mice (P0), stronger transgene expression was observed in the CP, mostly in its deepest region (Fig. 1Ba–c), in particular in layer VI, but not in the VZ (Fig. 1Bb,c). Scattered strong-expressing cells were still observed along the cortical layers while faint, but detectable transgene expression occurred on radially-oriented fibres projecting towards the cortical surface (Fig. 1Bb,c). In the striatum, strong transgene expression on scattered perikarya or fainter activation on cell bodies and processes of cells bearing a neuronal-like morphology could be seen (Fig. 1Bd, see arrow and arrowhead in the inset); in the olfactory bulb transgene expression was still restricted to the mitral cell layer with, again, a variable level of immunostaining at the cellular level (Fig. 1Be, see also the inset).

At P3, transgene expression was further increased in the cortical plate (Fig. 1Ca, compare to Fig. 1Ba), with highest levels in the deep layers, mostly layer VI (Fig. 1Ca–d). In addition, as in the previous stage, transgene expression still occurred on scattered, strong-expressing cells and on radially-oriented fibres spanning the whole cortical plate (Fig. 1Cc–d), the ventricular zone being still essentially devoid of immunostaining (Fig. 1Ca). In the striatum, neuron-like cells expressing low transgene levels (Fig. 1Ce inset, arrow) were intermingled with scattered strong-expressing cells (Fig. 1Ce inset, arrowheads). In the olfactory bulb transgene was still expressed in the mitral cell layer, although with lower intensity (Fig. 1Cf).

At P8 the transgene was partially downregulated within the cortex, in particular in the upper cortical layers (Fig. 2Aa), in which, however, relatively strong activation was observed on the somata of scattered cells (Fig. 2Ab; see also the inset), while fainter expression on lower layers displayed a cytoplasmic profile (Fig. 2Ac and inset therein). Strong cytoplasmic expression was demonstrated in the striatum (Fig. 2Ad and inset therein), while in the olfactory bulb transgene was still expressed in the mitral cell layer, although its levels were significantly reduced compared to the previous stage (Fig. 2Ae).

At P16, the transgene was sharply downregulated throughout the whole cortex (Fig. 2Ba). Scattered cells expressing high transgene levels were still predominant in the more superficial layers (Fig. 2Bb and inset therein), while lower, mostly cytoplasmic immunostaining was found in layers III–IV (Fig. 2Bc and inset) and mostly V–VI (Fig. 2Bd, see also the inset).

Altogether, these data indicated that, while the two modes of transgene expression at the cellular level co-existed during cortical development, cytoplasmic expression occurred mostly on early developing precursors committed to the lower cortical layers, while those committed to the upper layers rather displayed strong membrane-like transgene expression. At this stage transgene was still detected in the striatum, in which expression in scattered cell bodies was predominant (Fig. 2Be, see also the inset).

From P21 to P90 (Fig. 3) fainter F3/EGFP transgene activation throughout the whole cortex indicated promoter downregulation. However, although with lower density, strong-expressing cells with branched extensions still populated layers I–II and V–VI (arrows) intermingled with cells bearing a lower cytoplasmic immunostaining (arrowheads), predominant in layers III–IV (inset in Fig. 3e). Expression in the striatum was low at P21, P40 and P90, with higher levels, however, at the two latter developmental stages (b, d, and f).

Expression profiles of F3/EGFP transgene at the cellular level

From the above it could be inferred that in developing cortex F3/Contactin promoter activation essentially followed two distinct profiles at the cellular level: i) strong expression on scattered cells, mostly typical of the upper cortical layers, suggestive of transgene activation in membrane structures and most likely at the cell surface; ii) a lower profile of transgene activation, rather consistent with cytoplasmic expression, typical of the intermediate and lower cortical layers.

The time course of these two different profiles was studied by a morphometric approach. For this, transgene activation was expressed as the ratio of high or low versus total expression in fields of identical size. As an alternative, the density was measured of high or low expressing cells compared to the one of the overall population of EGFP-labelled cells. In Fig. 4 the relative profile of high or low expression is reported from P0 to P40, both in the cortex (A) and in the striatum (B). In both cases high transgene expression was predominant in the earlier postnatal development, mostly in newborn mice, then decreasing to reach the lower value at P16 (Figs. 4A,Ba). By contrast, the lower transgene expression mode increased progressively during development the highest