The homeobox gene Gsh2 is required for retinoid production in the embryonic mouse telencephalon

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Summary

We have examined the role of the homeobox gene Gsh2 in retinoid production and signaling within the ventral telencephalon of mouse embryos. Gsh2 mutants exhibit altered ventral telencephalic development, including a smaller striatum with fewer DARPP-32 neurons than wild types. We show that the expression of the retinoic acid (RA) synthesis enzyme, retinaldehyde dehydrogenase 3 (Raldh3, also known as Aldh1a3), is reduced in the lateral ganglionic eminence (LGE) of Gsh2 mutants. Moreover, using a retinoid reporter cell assay, we found that retinoid production in the Gsh2 mutants is markedly reduced. The striatal defects in Gsh2 mutants are thought to result from ectopic expression of Pax6 in the LGE. Previously, we had shown that removal of Pax6 from the Gsh2 mutant background improves the molecular identity of the LGE in these double mutants; however, Raldh3 expression is not improved. The Pax6;Gsh2 double mutants possess a larger striatum than the Gsh2 mutants, but the disproportionate reduction in DARPP-32 neurons is not improved. These findings suggest that reduced retinoid production in the Gsh2 mutant contributes to the striatal differentiation defects. As RA promotes the expression of DARPP-32 in differentiating LGE cells in vitro, we examined whether exogenous RA can improve striatal neuron differentiation in the Gsh2 mutants. Indeed, RA supplementation of Gsh2 mutants, during the period of striatal neurogenesis, results in a significant increase in DARPP-32 expression. Thus, in addition to the previously described role for Gsh2 to maintain correct molecular identity in the LGE, our results demonstrate a novel requirement of this gene for retinoid production within the ventral telencephalon.

Key words: DARPP-32 (Ppp1r1b), Pax6, Retinoic acid, Raldh3 (Aldh1a3), Striatum

Introduction

The homeobox gene Gsh2 is required for the normal development of the ventral telencephalon and, in particular, for the formation of the striatum (Corbin et al., 2000; Toresson et al., 2000; Toresson and Campbell, 2001; Yun et al., 2001; Yun et al., 2003). In these mutants, the precursor cells in the lateral ganglionic eminence (LGE) are mispatterned, with ectopic expression of pallial (i.e. dorsal telencephalon) genes such as Pax6, and a loss of ventral genes such as Mash1 (Ascl1 – Mouse Genome Informatics) and Dlx genes. These mutants display severe defects in the development of the striatum, including a greater than 50% reduction in size and a disproportionately reduced number of DARPP-32-expressing striatal neurons (Toresson and Campbell, 2001). DARPP-32 (Ppp1r1b – Mouse Genome Informatics) is a phosphoprotein that marks striatal projection neurons but not striatal interneurons (Anderson and Reiner, 1991). Indeed, generation of striatal interneurons does not appear to be significantly altered in Gsh2 mutants (Toresson and Campbell, 2001). This is probably due to the fact that striatal interneurons derive predominantly from the medial ganglionic eminence (MGE), whereas the projection neurons are generated in the LGE (Olsson et al., 1997; Olsson et al., 1998; Marin et al., 2000; Wichterle et al., 2001). Removal of Pax6 from the Gsh2 mutant background improves the molecular identity of mutant LGE cells, as well as the size of the mutant striatum (Toresson et al., 2000). Thus normal striatal development requires Gsh2 to maintain the correct molecular identity of LGE precursor cells, which is accomplished, largely, by repressing Pax6 expression in the LGE.

Retinoic acid (RA) is known to be involved in regulating the differentiation of numerous cell types, including specific neuronal subtypes. In fact, at limb levels, retinoid signaling is required for the formation of the lateral, lateral motor column motoneurons (Sockanathan and Jessell, 1998). Moreover, recent studies have suggested broader requirements for retinoids at spinal cord levels, both in the specification of motoneuron identity (Novitch et al., 2003) and in the development of motor columns at different anteroposterior levels (Sockanathan et al., 2003). Previous studies have demonstrated that retinoids are locally produced in the LGE during the period of striatal neurogenesis (Toresson et al., 1999; Mata de Urquiza et al., 1999). Since then, the rate-limiting enzyme retinaldehyde dehydrogenase 3 (Raldh3) has been identified and shown to be expressed in the LGE (Li et al., 2000). To date, this is the only Raldh enzyme known to be expressed in the LGE. Addition of RA to differentiating LGE cultures increases the number of DARPP-32-expressing cells,
as well as increasing its expression per cell (Toresson et al., 1999). This increase is specific to DARPP-32 expression and is not simply due to an increase in the number of cells differentiating into neurons in the RA-treated cultures. As Gsh2 is required for normal LGE development, it seems likely that retinoid production and/or signaling may be altered in the ventral telencephalon of these mutants. Accordingly, certain aspects of the Gsh2 mutant phenotype (i.e. reduced DARPP-32 neurons) could result from altered retinoid function.

In this study, we have examined the role of Gsh2 in retinoid production and signaling within the ventral telencephalon, and its potential implications for striatal projection neuron differentiation. Our results indicate that Gsh2 is required for normal retinoid production, and that this requirement is distinct from its role in regulating dorsoventral patterning, at least with respect to the repression of Pax6. Furthermore, we have found that exogenous retinoids can improve striatal neuron differentiation in the Gsh2 mutant.

**Materials and methods**

**Animals**

Gsh2 (Szucsik et al., 1997) and Pax6 (Small eye, Sey) (Hill et al., 1991) mouse embryos and adults were genotyped as described previously (Toresson et al., 2000). For staging of embryos, the morning of vaginal plug detection was designated as embryonic day (E) 0.5. Between 3 and 10 embryos of each genotype were used at each stage studied. Embryos were fixed overnight in 4% paraformaldehyde, rinsed extensively in PBS and cryoprotected in 30% sucrose before sectioning at 12 μm on a cryostat.

**Immunohistochemistry**

Primary antibodies were all produced in rabbits and used at the concentrations: Raldh3, 1:5000 (provided by U. Dräger); DARPP-32, 1:1000 (Chemicon); and FoxP1, 1:1000 (provided by E. Morrisey). The secondary antibodies used were biotinylated swine anti-rabbit antibodies (1:200, DAKO). The ABC kit (Vector Laboratories) was used to visualize the reaction product using diaminobenzidine (DAB, Sigma) as the final chromogen.

**In situ hybridization**

In situ hybridization was performed using digoxigenin-labeled cRNA probes as previously described (Toresson et al., 1999). Probes used were: retinoic acid receptor beta (Rarb; EST-clone, GenBank Accession Number BE854385), Retinoic X receptor gamma (Rtxr; EST-clone, GenBank Accession Number AI325376).

**Retinoic acid treatments**

After timed matings of Gsh2 heterozygous parents, pregnant dams were given all-trans RA (5 mg/kg body weight, Sigma) or vehicle control (sunflower oil) by oral gavage. The RA was dissolved in DMSO at 50 mg/ml and diluted in sunflower oil. RA treatments were given twice daily (10-12 hours apart) starting at E11.5 through E17.5. This period covers the majority of striatal neurogenesis (Bayer and Altman, 1995), and is after the sensitive period for the teratogenic effects of RA on embryonic morphology (Nolen, 1986; Simone et al., 1995). Embryos were collected at E18.5, and fixed, genotyped and processed for immunohistochemistry as described earlier. The DARPP-32 neurons were counted in each striatal section from either RA-treated (n=4) or oil-treated (n=4) Gsh2 mutants and a total for each embryo was determined.

**Results**

**Gsh2 mutants exhibit reduced retinoid production in the ventral telencephalon**

Previous studies (Corbin et al., 2000; Toresson et al., 2000; Toresson and Campbell, 2001; Yun et al., 2001; Yun et al., 2003) have shown that Gsh2 is required for normal development of the striatum. The striatum of these mutants is severely reduced in size and contains disproportionately fewer DARPP-32-positive neurons (Corbin et al., 2000; Toresson and Campbell, 2001). Our previous work indicated that RA promotes DARPP-32 expression in differentiating striatal neurons in vitro (Toresson et al., 1999). As the LGE is a localized source of retinoids (Toresson et al., 1999; Mata de Urquiza et al., 1999), we set out to determine whether alterations in retinoid production and/or signaling underlie the striatal differentiation defects observed in the Gsh2 mutant telencephalon.

Retinaldehyde dehydrogenase 3 (Raldh3), the gene encoding a rate-limiting enzyme in retinoid production, has previously been shown to be restricted to the LGE during telencephalic development (Li et al., 2000). Recent studies have confirmed that Raldh3 protein expression closely matches that of the endogenous gene expression (Wagner et al., 2002). At E12.5, Raldh3 is expressed in the subventricular zone of the ventromedial LGE, and in the sulcus between the LGE and MGE (Fig. 1A). This expression domain is considerably truncated in Gsh2 mutants, leaving mostly low-level expression in and around the LGE/MGE sulcus (Fig. 1B). The Gsh2 mutant LGE exhibts a partial recovery at later stages of neurogenesis (Corbin et al., 2000; Toresson et al., 2000; Yun et al., 2001), which has been shown to depend on a compensatory response by the family member Gsh1 (Toresson and Campbell, 2001; Yun et al., 2003). Despite this, only a modest recovery of Raldh3 expression is observed in the Gsh2 mutant LGE at E16.5 (Fig. 1D), as compared to wild type (Fig. 1C). These findings are consistent with Raldh3 gene expression results in Gsh2 mutants (Toresson and Campbell, 2001) (data not shown).

The reduction in Raldh3 expression in the Gsh2 mutant suggests that retinoid production is decreased in the LGE. However, retinoid synthesis can occur through multiple biochemical pathways, including three other RALDH enzymes (Raldh1, Raldh2 and Raldh4) (Lee et al., 1991; Lin et al., 2003; Wang et al., 1996; Zhao et al., 1996). To determine whether the reduced expression of Raldh3 in the Gsh2 mutant LGE correlates with reduced retinoid production, we employed a
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retinoid reporter cell assay, which uses a retinoic acid response element (RARE) to drive lacZ in stably transfected F9 cells (Wagner et al., 1992). This reporter cell assay has been used to detect production of retinoids in explants of various embryonic CNS regions (e.g. Wagner et al., 1992; Luo et al., 2004). LGE explants were dissected from E12.5 and E16.5 Gsh2<sup>+/+</sup>, Gsh2<sup>+/−</sup> and Gsh2<sup>−/−</sup> embryos. As controls, MGE (E12.5) and cortex (E16.5) were dissected from the same embryos. Co-culture of LGE explants from the Gsh2<sup>+/+</sup> and Gsh2<sup>+/−</sup> embryos with the F9 reporter cells resulted in many X-gal-positive cells (Fig. 2A, B). However, the reporter cells co-cultured with E12.5 Gsh2<sup>−/−</sup> LGE explants displayed very little X-gal staining (Fig. 2C), indicating that these explants produced significantly less RA than the wild types or heterozygotes (Fig. 2D). There were no significant differences in the co-cultures with MGE explants from all three genotypes, which contained low levels of retinoids, similar to the mutant LGEs. At E16.5, Gsh2<sup>−/−</sup> LGE explants still induce a significantly lower level of lacZ gene expression than the Gsh2<sup>+/+</sup> and Gsh2<sup>+/−</sup> LGE explants (Fig. 2E), indicating a continued reduction in retinoid production. It should be noted, however, that there was more variability in the response to the Gsh2<sup>−/−</sup> LGEs at E16.5. While some of the mutant explants activated the lacZ gene to levels near the range of the wild types and heterozygotes, others were severely deficient. As was the case with the MGE explants, cortical explants only contain low levels of retinoids, which do not differ between genotype. Overall, the RA reporter cell data are consistent with the findings of Raldh3 expression in Gsh2 mutants, and demonstrate that retinoid production in these mutants is markedly reduced. Moreover, these findings indicate that Raldh3 expression itself is a good indicator of retinoid production in the embryonic mouse ventral telencephalon.

**Fig. 1.** Altered expression of Raldh3 detected by immunohistochemistry (IHC) in Gsh2 mutants. (A) At E12.5, Raldh3 is normally expressed in the SVZ of the ventromedial LGE. (B) In Gsh2 mutants, the Raldh3 staining is reduced and its normal expression domain in the LGE is truncated (arrow). However, the expression of Raldh3 in the nasal epithelium (NE) and retina (R) remains at wild-type levels. (C) At E16.5, Raldh3 is expressed in the SVZ of the LGE and septum, and (D) remains severely reduced in the Gsh2 mutant LGE. NE, nasal epithelium; R, retina; Stm, striatum.

Reduced retinoid production in Gsh2 mutants is independent of ectopic Pax6

Previous studies have shown that Gsh2 is required for the correct patterning of LGE precursors, and, in its absence, pallial (i.e. dorsal) genes such as Pax6 are ectopically expressed at high levels in LGE precursors (Corbin et al., 2000; Toresson et al., 2000; Yun et al., 2001). Moreover, there is a concomitant loss of subpallial (i.e. ventral) regulators, such as Mash1 and Dlx genes, in the Gsh2 mutant LGE. This altered patterning leads to the reduced striatal size observed in the Gsh2 mutants, because the removal of Pax6 gene function in the Gsh2 mutant background (i.e. Pax6;Gsh2 double mutants) improves the molecular identity of the LGE and results in the formation of a larger striatum (Toresson et al., 2000). It is possible, therefore, that the decrease in Raldh3 expression and the reduced retinoid production in the Gsh2 mutants may be due to the ectopic expression of Pax6 in the mutant LGE. To examine whether this is the case, we analyzed Pax6 single and Pax6;Gsh2 double mutants at E12.5.

**Fig. 2.** Gsh2 mutants exhibit reduced retinoid production in the ventral telencephalon. (A-C) Representative pictures of X-gal-positive F9 cells, stably transfected with RARE-lacZ, that were co-cultured for 24 hours with E12.5 LGE explants from wild-type embryos (A), Gsh2 heterozygotes (het, B) or Gsh2 homozygous mutants (KO, C). (D, E) Bar graphs showing the reduced numbers of X-gal-positive cells from LGE (black bars) or MGE (white bars) explants at E12.5 (D), and LGE (black bars) or cortex (white bars) at E16.5 (E). ∗P<0.05, ∗∗P<0.01, one-way ANOVA using a Fisher test as a post-hoc. E12.5, n=4 WT, 12 +/- and 6 –/−; E16.5, n=3 WT, 15 +/- and 5 –/−.
Raldh3 appears to be expressed in a wild-type pattern in the Pax6 mutant telencephalon (Fig. 3A). However, unlike the case for Mash1 and Dlx genes (Toresson et al., 2000), Raldh3 expression is not improved, even marginally, in the Pax6;Gsh2 mutants (Fig. 3B). Its expression remains reduced, very similar to that observed in the Gsh2 mutant (Fig. 1B). These data strongly suggest that the decreased retinoid production in the Gsh2 mutant is independent of ectopic Pax6 in LGE progenitors.

We previously demonstrated that the size of the striatal complex, as marked by the LIM homeobox protein Islet1, is improved in a Pax6;Gsh2 double mutant, as compared with a Gsh2 mutant, at least at E16.5 (Toresson et al., 2000). We show here that this is also the case at E18.5, using an antibody against Foxp1, another marker of the developing striatum (Ferland et al., 2003; Takahashi et al., 2003) (Fig. 4A-D). To further investigate the improved Pax6;Gsh2 double mutant striatum, we analyzed the expression of the striatal projection neuron marker DARPP-32 (Fig. 4E) (Ouimet et al., 1984; Anderson and Reiner, 1991). In addition to the reduction in striatal volume observed in the Gsh2 mutant, the number of DARPP-32-expressing cells appears to be disproportionately reduced (Fig. 4F) (Corbin et al., 2000; Toresson and Campbell, 2001). DARPP-32 expression is not improved in the Pax6;Gsh2 double mutant striatum (Fig. 4H), despite its considerably larger volume compared with the Gsh2 mutant (Fig. 4B,D). As was the case with Raldh3 expression, DARPP-32 expression in Pax6;Gsh2 double mutant striatum appears to be more similar to the Gsh2 mutant striatum than the Pax6 mutant striatum (Fig. 4F-H). Thus the removal of Pax6 from the Gsh2 mutant background improves striatal size but does not improve striatal neuron differentiation. The former effect is likely to be due to the improvement in molecular identity of progenitor cells in the double mutant LGE, whereas the lack of improvement in DARPP-32 expression could be the result of reduced retinoid production and/or signaling in both the Gsh2 and Pax6;Gsh2 mutants. These findings suggest, therefore, that there are at least two independent requirements for Gsh2 in striatal development: first, to repress Pax6 expression in the LGE and thereby maintain the correct molecular identity of striatal progenitors; and second, for normal Raldh3 expression and ultimately retinoid production in the LGE. This latter
requirement is likely to be involved in the correct
differentiation of striatal projection neurons.

**Exogenous retinoids improve striatal neuron
differentiation in the Gsh2 mutant**

As mentioned above, decreased retinoid production in Gsh2 mutants may be responsible for the disproportionate reduction in DARPP-32 neurons. However, it is possible that RA signaling is also affected in these mutants and, even if retinoids were available at normal levels, the mutant striatal cells would not be capable of responding. The RA receptor heterodimer that is expressed in the developing and mature striatum consists of Rarb (Fig. 5A) and Rxrg (Fig. 5C) (Ruberte et al., 1993; Dolle et al., 1994; Krezel et al., 1998; Toresson et al., 1999). At E16.5, Gsh2 mutants maintain expression of both Rarb (Fig. 5B) and Rxrg (Fig. 5D); however, the domain of expression is reduced in accordance with the reduction in size of the mutant striatum. These findings suggest that the Gsh2 mutant striatum has the ability to respond to retinoids. Thus, if the signaling machinery is functional, the altered striatal differentiation phenotype in the Gsh2 mutant may be a result of a retinoid deficiency in the LGE, which is likely to be due to the decreased Raldh3 expression. As described earlier, DARPP-32 expression can be enhanced in striatal neuron cultures in vitro with exogenous retinoids (Toresson et al., 1999). Therefore, we attempted to improve striatal differentiation in the Gsh2 mutant by maternal RA administration. RA (5 mg/kg) was given twice daily from E11.5 to E17.5, which covers the major period of striatal neurogenesis (Bayer and Altman, 1995), and is after the sensitive period for teratogenic effects on brain morphology (Nolen, 1986; Simeone et al., 1995). At E18.5, RA-treated Gsh2 mutant embryos (Fig. 6B,D) showed an increase in DARPP-32 expression compared with vehicle (oil)-treated Gsh2 mutant embryos (Fig. 6A,C). The effect was both on the level of expression per cell and on the number of detectable DARPP-32-positive neurons, as was previously described after RA treatment of LGE cells in vitro (Toresson et al., 1999). RA treatment resulted in a 75% increase (P<0.01, using a Student’s unpaired t-test) of DARPP-32 neurons in the Gsh2 mutant striatum, as compared with oil-treated mutants (RA, 876.8±96.8 DARPP-32 cells, n=4; oil, 502.1±33.4 DARPP-32 cells, n=4). The size of the Gsh2 mutant striatum, as marked by Foxp1 expression, remained reduced after RA treatment, similar to that in the oil-treated mutant striatum (Fig. 6E-H). Moreover, the effect was specific to DARPP-32 expression, as the expression of the calcium-binding protein calbindin was not changed in the Gsh2 mutant striatum regardless of RA or oil treatment (data not shown). These findings demonstrate that reduced retinoid production in Gsh2 mutants impairs normal striatal neuron differentiation, at least with respect to DARPP-32 expression, which can be improved by the addition of exogenous RA.

**Discussion**

The results of this study demonstrate a novel requirement for Gsh2 in retinoid production within the ventral telencephalon of the mouse embryo. Our results indicate that the observed reduction in retinoids within the Gsh2 mutant LGE is responsible for the decreased number of DARPP-32-positive striatal neurons in the Gsh2 mutant (Corbin et al., 2000; Toresson and Campbell, 2001). Moreover, the present findings demonstrate that this function of Gsh2 is distinct from its previously described role in dorsoventral patterning, through the repression of Pax6 gene expression in the LGE (Corbin et al., 2000; Toresson et al., 2000; Yun et al., 2001).

Although our results show that Gsh2 is required for the normal expression of Raldh3 in the LGE, and thus normal levels of retinoids within this structure, the mechanism by which Gsh2 is required for this retinoid synthesizing enzyme to be correctly expressed remains unclear. It seems unlikely that this is direct because Raldh3 expression in the LGE is much more restricted than that of Gsh2. Gsh2 is expressed in the ventricular zone of the entire LGE, with its highest levels observed in the dorsal half (Toresson et al., 2000; Yun et al., 2001), whereas Raldh3 is only found in the subventricular zone of the rostral LGE, where it is largely confined to the ventral portion (Fig. 1) (Li et al., 2000) (data not shown). Thus it seems more likely that Gsh2 plays a permissive role in the expression of this enzyme, allowing for other factors to regulate its precise expression domain within the LGE. It is possible that Raldh3 expression in the LGE is induced by retinoid producing mesenchymal cells that have previously been shown to signal to the ventrolateral telencephalon prior to (and concurrent with) the appearance of the LGE (LaMantia et al., 1993). This process may require Gsh2 expression in the receptive cells in order to establish and/or maintain Raldh3 expression within the LGE.
An important aspect of the Gsh2 mutant phenotype in the telencephalon is the compensation by its family member Gsh1 (Toresson and Campbell, 2001; Yun et al., 2003). As a result of this compensation, the molecular identity (i.e. Mash1 and Dlx gene expression) of the Gsh2 mutant LGE improves at late stages of embryogenesis (e.g. E16.5). The present results show that Raldh3 expression in the Gsh2 mutant LGE does not significantly improve, indicating that Gsh1 cannot fully compensate for Gsh2 in the regulation of this gene. However, this does not exclude a role for Gsh1 in regulating Raldh3 expression because this gene appears to be completely absent in Gsh1/2 double mutants (Toresson and Campbell, 2001). In any case, our results suggest that in the Gsh2 mutant, Gsh1 is not as efficient in regulating Raldh3 expression as it is in restoring Dlx and Mash1 gene expression at later embryonic stages (Corbin et al., 2000; Toresson et al., 2000; Yun et al., 2001). This reduced efficacy may simply be due to timing, as the Gsh1 compensation does not occur until around E14 (Toresson and Campbell, 2001; Yun et al., 2001; Yun et al., 2003). It may be that for normal Raldh3 expression in the LGE, Gsh gene expression is required at early stages (i.e. between E10.5 and E14). This timeframe corresponds well with the period during which RA-producing mesenchymal cells are signaling to the ventrolateral telencephalon (i.e. the presumptive LGE) (LaMantia et al., 1993).

The previously established role for Gsh2 in the developing telencephalon was to establish the correct dorsoventral identity of telencephalic progenitor cells (Corbin et al., 2000; Toresson et al., 2000; Yun et al., 2001). The Gsh2 mutant LGE cells ectopically express dorsal telencephalic genes (i.e. Pax6) and lose their expression of ventral regulators (e.g. Dlx and Mash1 genes). Removal of Pax6 from the Gsh2 mutant background (i.e. Pax6;Gsh2 double homozygous mutants) improves the molecular identity of LGE cells (i.e. Dlx and Mash1 gene expression) and results in a larger striatum, as compared with Gsh2 mutants (Toresson et al., 2000) (data presented here). However, the present results show that Pax6;Gsh2 double mutants do not improve all aspects of the Gsh2 mutant phenotype. In particular, Raldh3 expression remains reduced in the Pax6;Gsh2 double mutant LGE, showing no improvement over that observed in the Gsh2 mutant LGE. Therefore, the role that Gsh2 plays in regulating retinoid production within the LGE is novel and independent of its role in repressing Pax6 gene expression (and thus maintaining the correct dorsoventral identity in LGE cells). Furthermore, the number of DARPP-32 neurons in the Pax6;Gsh2 double mutant striatum continues to be severely reduced, similar to that seen in the Gsh2 mutant striatum, suggesting that the observed retinoid deficiency underlies this differentiation defect.

The fact that Gsh2 mutants maintain expression of the RA receptor heterodimer RARβ and RXRγ suggests that the mutant striatum has the ability to respond to retinoids. It has previously been shown that RA promotes the expression of DARPP-32 in wild-type LGE cultures (Toresson et al., 1999). Accordingly, exogenous retinoids also increase the number of DARPP-32-positive striatal neurons in vivo, improving striatal differentiation in the Gsh2 mutants. These results show that the retinoid signaling machinery is functional in Gsh2 mutants but that it is not actively used because of the reduction in retinoids. As expected, exogenous RA had no effect on the size of the Gsh2 mutant striatum, which remains severely truncated, typical of the Gsh2 mutant striatum. In our experimental paradigm, we provided RA to the Gsh2 mutant striatum during the period of striatal neurogenesis. It is possible, however, that retinoids may play an earlier role (i.e. prior to E11) in LGE/striatal development (e.g. in regulating the size of the striatal progenitor pool), which would not be affected by our treatments. Nevertheless, the present findings strongly support the notion that the decreased retinoid production in the Gsh2 mutant LGE is responsible for the disproportionate reduction of DARPP-32-positive striatal neurons seen in these mutants.

Fig. 6. Exogenous retinoids improve striatal neuron differentiation in the Gsh2 mutant. At E18.5, DARPP-32 expression (detected by IHC) is increased at all levels of the striatum in the RA-treated Gsh2 mutants (B,D) compared with vehicle (oil)-treated Gsh2 mutants (A,C). RA treatment of the Gsh2 mutants does not alter striatal size, as marked by Foxp1 staining (F,H). Indeed, the size of the RA-treated striatum appears to be very similar to that of the control oil treatments (E,G). GP, globus pallidus.
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Taken together, our results indicate that Gsh2 is required both for the repression of Pax6 in LGE cells (so that the correct number of striatal progenitors are maintained throughout striatogenesis) and for the production of retinoids within the LGE (which are needed for the correct differentiation of at least a portion of the striatal projection neurons).

The LGE of Gsh1/2 double mutants does not express detectable levels of Raldh3 (Toresson and Campbell, 2001). Moreover, these double mutant embryos completely lack DARPP-32-expressing neurons in their striatum. This suggests that a complete retinoid deficiency may exist in the Gsh1/2 double mutants. Unlike the case in Gsh2 mutants, the Gsh1/2 double mutants do not show a recovery of Dlx and Mash1 gene expression within the LGE (Toresson and Campbell, 2001; Yun et al., 2003). Thus the specification of LGE precursor cells in Gsh1/2 double mutants remains abnormal. For this reason, it seems unlikely that exogenous retinoids could improve striatal projection neuron differentiation in these double mutants.

Our findings add further support to our previous suggestion that retinoids regulate striatal neuron differentiation (Toresson et al., 1999). Moreover, our results show that Gsh2 is required for the correct expression of the retinoid synthesizing enzyme Raldh3, and thus for normal retinoid production in the ventral telencephalon. However, it remains unclear what aspect(s) of striatal neuron differentiation retinoids promote. Although DARPP-32 is a marker of all striatal projection neurons in the mature striatum (Ouimet et al., 1984; Anderson and Reiner, 1991), at birth striatal neurons expressing this protein are largely confined to the patch compartment (Foster et al., 1987). Because the Gsh2 mutants and the Pax6;Gsh2 double mutants die at birth, analysis of the mature striatum has, thus far, not been possible. Future studies will be focused on identifying the specific role of retinoids during striatal projection neuron differentiation in both wild types and Gsh2 mutants.

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