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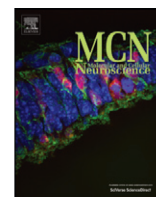

Heterochronic misexpression of *Ascl1* in the *Atoh7* retinal cell lineage blocks cell cycle exit

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ABSTRACT

Retinal neurons and glia arise from a common progenitor pool in a temporal order, with retinal ganglion cells (RGCs) appearing first, and Müller glia last. The transcription factors *Atoh7*/*Math5* and *Ascl1*/*Mash1* represent divergent bHLH clades, and exhibit distinct spatial and temporal retinal expression patterns, with little overlap during early development. Here, we tested the ability of *Ascl1* to change the fate of cells in the *Atoh7* lineage when misexpressed from the *Atoh7* locus, using an *Ascl1*-IRES-DsRed2 knock-in allele. In $Atoh7^{Ascl1KI/+}$ and $Atoh7^{Ascl1KI/Ascl1KI}$ embryos, ectopic *Ascl1* delayed cell cycle exit and differentiation, even in cells coexpressing *Atoh7*. The heterozygous retinas recovered, and eventually produced a normal complement of RGCs, while homozygous substitution of *Ascl1* for *Atoh7* did not promote postnatal retinal fates precociously, nor rescue *Atoh7* mutant phenotypes. However, our analyses revealed two unexpected findings. First, ectopic *Ascl1* disrupted cell cycle progression within the marked *Atoh7* lineage, but also nonautonomously in other retinal cells. Second, the size of the *Atoh7* retinal lineage was unaffected, supporting the idea of a compensatory shift of the non-proliferative cohort to maintain lineage size. Overall, we conclude that *Ascl1* acts dominantly to block cell cycle exit, but is incapable of redirecting the fates of early RPCs.

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Introduction

The murine retina is an attractive model for studying cell fate specification. From a common neuroepithelium, four cell types (retinal ganglion cells (RGCs), cone photoreceptors, amacrine and horizontal interneurons) arise largely prenatally. During late embryonic and postnatal development, the bulk of rod photoreceptors, bipolar interneurons and Müller glia are generated (Cepko et al., 1996; Jeon et al., 1998; Sidman, 1961; Young, 1985). When the mouse optic cup forms at E10.5, retinal progenitor cells (RPCs) are multipotent, but over time exhibit restricted developmental potential.

Retinal birthdating experiments demonstrated that neurons and glia exit mitosis in a regular sequence, with one or more cell types arising simultaneously, as well as from sequential cell divisions. Lineage tracing and clonal analyses have also highlighted the multipotency of individual RPCs. Moreover, when early and late RPCs were co-cultured, neither population was temporally reprogrammed, exemplifying the importance of intrinsic determinants in dictating cell fate. Although extrinsic factors influence the relative proportions of cell types produced, they cannot supersede intrinsic regulation of the order in which cell types appear (reviewed in Agathocleous and Harris, 2009; Cayouette et al., 2006; Livesey and Cepko, 2001; Rapaport, 2006).

Numerous retinal studies have focused on basic helix–loop–helix (bHLH) transcription factors, due to their prominent roles in neuronal specification. Indeed, their distinct spatiotemporal expression patterns and loss-of-function phenotypes have solidified key roles during retinal neurogenesis. For example, *Atoh7*/*Math5* appears at the initiation of retinogenesis, and is critically required for RGC formation, and the suppression of cone photoreceptors (Brown et al., 1998, 2001; Kanekar et al., 1997; Kay et al., 2001; Wang et al., 2001). *Ascl1*/*Mash1* expression becomes apparent two days later than *Atoh7* in the mouse retina, and is required for normal bipolar interneurons and suppression of Müller glia differentiation (Brzezinski et al., 2011; Jasoni and Reh, 1996; Tomita et al., 1996). This suggests that *Atoh7* and *Ascl1* act via inherently different mechanisms, which is further supported by the evolutionary

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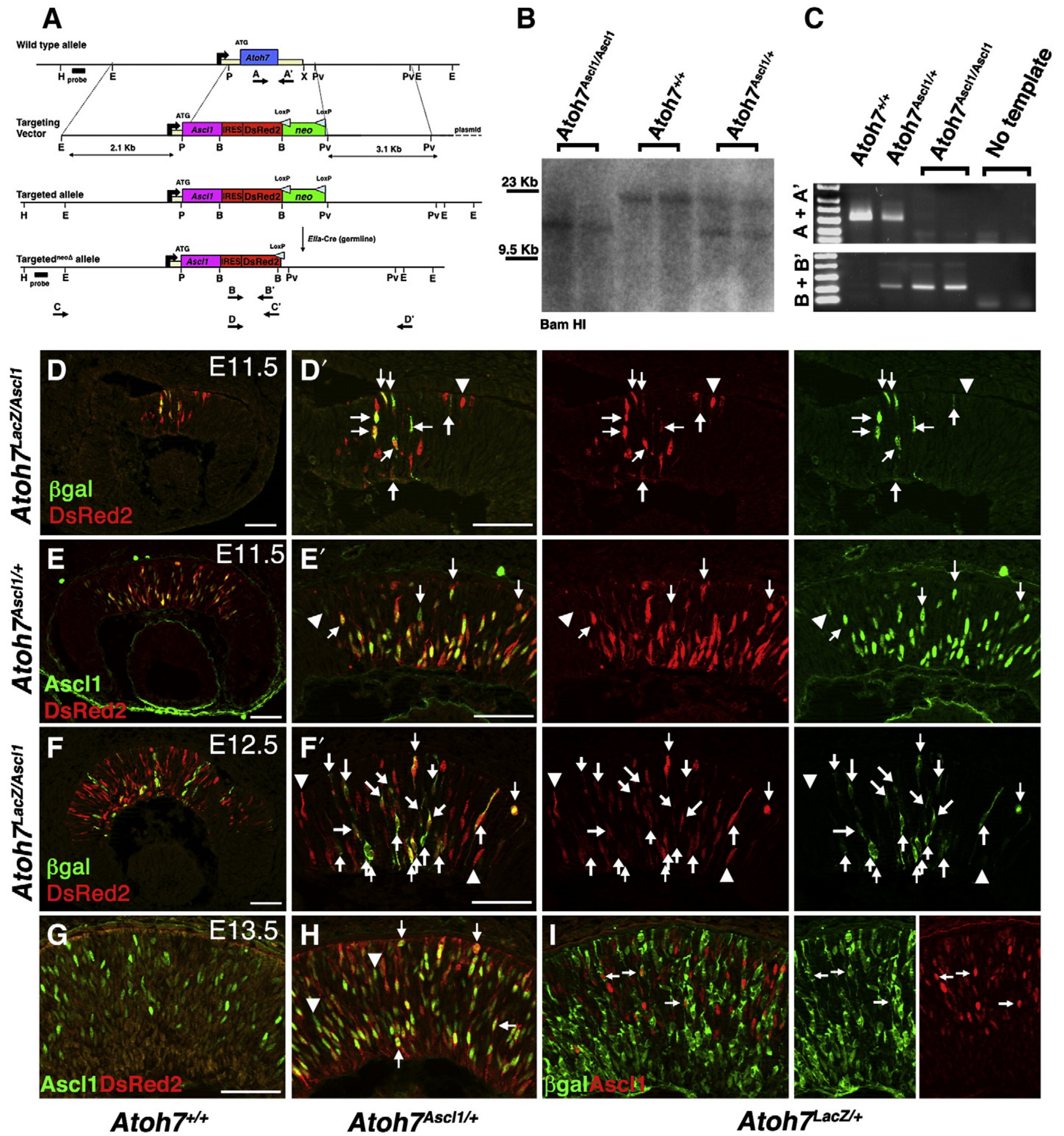

Fig. 1. Homologous recombination of *Ascl1* into the *Atoh7* gene locus. A) Targeting strategy for *Atoh7* coding exon + 3′ UTR replacement with an *Ascl1* coding + 3′UTR-IRES-DsRed2 expression cassette. The positions of single-copy genomic DNA probe, routine genotyping PCR primers (A + A′, B + B′), and long range PCR primers (C + C′, D + D′) are indicated. A reverse orientation PGK-neo-pA cassette was removed by Cre-LoxP mediated recombination, by crossing to the EIIa-Cre driver mouse. B) Southern analysis of Bam HI digested mouse tail genomic DNA yielded predicted 22 Kb wild type and 14 Kb targeted bands. C) Routine PCR genotyping of mouse tail DNA from F_2 crosses. Internal primers amplified allele-specific PCR products (A + A′ = 350 bp wild type band, B + B′ = 232 bp mutant band). D–D′) Direct comparison of DsRed2 and βgal expression in the E11.5 optic cup, showing all βgal+ cells coexpress DsRed2 (arrows), but also some DsRed2+; βgal-negative (red-only) cells (arrowhead) at the leading edges of neurogenesis. E–E′) At E11.5, all DsRed2 cells also coexpress ectopic Ascl1 (arrows). A rare cell presumably expressing endogenous Ascl1 (green-only) can be seen in E′ (arrowhead). F–F′) E12.5 retinal sections also exhibit complete overlap of βgal and DsRed2 (arrows), as well as some cells only expressing DsRed2 (red-only, arrowheads). G) Endogenous Ascl1 protein (green-only) in a population of E13.5 retinal cells. H) Extensive coexpression of ectopic DsRed2 and Ascl1 in *Atoh7*$^{Ascl1KI/+}$retinal lineage (arrows). A smaller population of endogenous Ascl1+ (green-only) cells is also apparent (arrowheads). I) In E13.5 *Atoh7*^{LacZ} cells (green-only), there are very few βgal+Ascl1+ coexpressing cells (arrows). H = Hind III; E = Eco RI, P = Pst I, X = Xba I, Pv = Pvu I, B = Bam HI; Scleral is up in D–I; Mag bars = 20 μm in all panels.

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