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swap70 Promotes neural precursor cell cycle exit and oligodendrocyte formation

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ABSTRACT

Multipotent neural precursors produce oligodendrocyte lineage cells, which then migrate throughout the central nervous system and extend multiple, long membrane processes to wrap and myelinate axons. These dynamic cellular behaviors imply dynamic regulation of the cytoskeleton. In a previous microarray screen for new oligodendrocyte genes we identified *swap70*, which encodes a protein with domains that predict numerous signaling activities. Because mouse Swap70 can promote cell motility by functioning as a guanine nucleotide exchange factor for Rac1, we hypothesized that zebrafish Swap70 promotes oligodendrocyte progenitor cell (OPC) motility and axon wrapping. To test this we investigated Swap70 localization in OPCs and differentiating oligodendrocytes and we performed a series of gain and loss of function experiments. Our tests of gene function did not provide evidence that Swap70 regulates oligodendrocyte lineage cell behavior. Instead, we found that *swap70* deficient larvae had excess neural precursors and a deficit of OPCs. Cells associated with neural proliferative zones express *swap70*. Therefore, our data reveal a potential new role for Swap70 in regulating transition of dividing neural precursors to specified OPCs.

Introduction

During vertebrate development, dividing neuroepithelial precursors produce first neurons and then glial cells. Diversity of neuronal and glial cell type is achieved, in part, by subdivision of the developing CNS into spatial domains of precursors that give rise to subsets of neurons and glia. In particular, pMN precursors of the spinal cord generate motor neurons and then oligodendrocyte progenitor cells (OPCs), which continue to divide as they migrate to become dispersed throughout the neural tube(Rowitch and Kriegstein, 2010). As OPCs migrate, they continuously extend and retract multiple long membrane processes, which might serve as a surveillance mechanism that influences the number of OPCs and their distribution (Kirby et al., 2006). Near the end of embryogenesis and continuing into the postnatal stage, a subset of OPCs stop dividing, wrap multiple neighboring axons with membrane and form myelin. Therefore, successful myelination requires integration of mechanisms that promote specification of OPCs from dividing, multipotent neural precursors, regulate OPC division to ensure formation of sufficient myelinating cells, guide OPCs to their target axons and mediate recognition and wrapping of axons by differentiating oligodendrocytes.

To search for factors that might be important for oligodendrocyte development, we performed a microarray screen that uncovered several genes expressed by oligodendrocyte lineage cells (Takada and Appel, 2010). Because of the highly dynamic nature of OPC membrane processes during migration and axon wrapping, we were particularly interested in genes that might control the cytoskeleton. One gene, *swap70*, seemed to be a good candidate because it encodes a protein that can bind the lipid second messenger phosphatidylinositol-3,4,5-triphosphate (PtdIns(3,4,5)P3) and can function as a GEF for Rac1 GTPase (Shinohara et al., 2002), which influences actin cytoskeletal rearrangements and motility (Bosco et al., 2009). To test the possibility that *swap70* gene function influences OPC behavior, we examined localization of fusion proteins and performed gain and loss-of-function experiments in zebrafish. Although we found evidence that Swap70 occupies both nuclear and cytoplasmic compartments, our data do not support the hypothesis that Swap70 regulates OPC motility or membrane extension and axon wrapping. Instead, we found that larvae lacking *swap70* function had a deficit of OPCs and myelinating oligodendrocytes. These larvae upregulated expression of *sox2*, a marker of neural precursor cells, and maintained elevated numbers of spinal cord cells in the cell cycle. Therefore, *swap70* function may be necessary for the transition of dividing precursors to specified neural cells.

Results

Neural precursors and oligodendrocyte lineage cells express swap70

To characterize *swap70* expression, we performed in situ RNA hybridization on zebrafish embryos collected at different stages of

Abbreviations: CNS, central nervous system; OPCs, oligodendrocyte progenitor cells; GEF, guanine nucleotide exchange factor; PIP_3, phosphatidylinositol-3,4,5-triphosphate.

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development. At 2 somite stage, which occurs about 10.5 hours post fertilization (hpf), the eye primordia prominently expressed *swap70*, whereas transcript levels appeared to be at lower levels throughout other regions of the embryo (Fig. 1A). At 20 hpf, *swap70* expression remained high within the developing eyes and was at high level in the pronephric ducts (Fig. 1B). By 50 hpf, cells occupying the ventral spinal cord expressed *swap70* (Figs. 1C,I) similar to the pattern of cells that expressed *sox10* (Figs. 1D,J), which marks newly specified OPCs. At this stage no spinal cord cells expressed *plp1a* (Fig. 1E), a marker of myelinating oligodendrocytes. At 80 hpf, cells in both ventral and dorsal spinal cords expressed *swap70* (Figs. 1F,K), consistent with the distribution of differentiating oligodendrocytes marked by *sox10* (Figs. 1G,L) and *plp1a* (Figs. 1H,M) expression. These data therefore indicate that OPCs initiate *swap70* expression prior to their differentiation as myelinating cells. We also noticed that cells lining the medial septum and central canal of the spinal cord at 50 hpf expressed *swap70* (Fig. 1I). Medial expression was maintained through 80 hpf but restricted to the ventral half of the spinal cord (Fig. 1K). The medial septum is lined initially by dividing neuroepithelial precursors and later radial glia. Similarly, *swap70* expression was evident at proliferative ventricular zones of the forebrain and hindbrain (Figs. 1N,O). These expression data indicate that at least a subset of zebrafish neural precursor cells express *swap70*. Notably, the hippocampus, subventricular zones, olfactory bulb and cerebellum of adult mouse brain appear to express *Swap70* (Lein et al., 2007) and Swap70 was purified from bovine brain lysate (Shinohara et al., 2002) raising the possibility that Swap70 has a role in neural precursor maintenance or specification.

To confirm that oligodendrocyte lineage cells express *swap70* we examined homozygous *sox10*^{tw11} mutant embryos and larvae. *sox10*^{tw11} mutants produce OPCs but the myelinating subpopulation of oligodendrocytes, which express *nkx2.2a*, die soon after initiation of axon wrapping (Takada et al., 2010). *sox10*^{tw11} mutant larvae had a deficit of *swap70*$^{+}$ cells relative to wild type at 60 and 80 hpf (Fig. 2) consistent with the notion that myelinating oligodendrocytes express *swap70.* Notably, remaining OPCs of mutant larvae appeared to express *swap70* at normal levels. Therefore, *swap70* expression in oligodendrocyte lineage cells does not appear to be under control of the Sox10 transcription factor.

Swap70 accumulates at the plasma membrane to promote membrane ruffling (Shinohara et al., 2002) but also can move to the cell nucleus to influence B cell switching (Borggrefe et al., 1998; Borggrefe et al., 1999). To assess Swap70 localization in neural cells, we crossed fish carrying *Tg*(*UAS*:*cherry-swap70*), which encodes Cherry fluorescent protein fused to full-length Swap70, to *Tg*(*hsp70l*:*Gal4vp16*);*Tg*(*olig2*:*EGFP*) fish and induced expression in embryos using heat shock. This produced a mosaic pattern of expression within the spinal cord, which revealed that Cherry-Swap70 protein localized to the cytoplasm and not the nucleus of most cells (Figs. 3A,B). However, some cells usually associated with the proliferative zone of the medial spinal cord, including *olig2*:EGFP^{+} cells, had nuclear localized Cherry-Swap70 protein (Figs. 3A,B). These data raise the possibility that Swap70 can occupy different cellular compartments.

To more thoroughly investigate subcellular localization we next expressed Cherry-Swap70 in OPCs also expressing membrane-tethered GFP. To do so, we co-injected *UAS*:*cherry-swap70* plasmid with *sox10*:*Gal4vp16* plasmid into *Tg*(*nkx2.2a*:*mEGFP*) embryos, which express membrane-tethered EGFP in the myelinating subset of OPCs and oligodendrocytes (Kirby et al., 2006). In migrating OPCs, Cherry-Swap70 protein accumulated at the cell membrane and the tips of extending membrane processes (Fig. 4A). In myelinating oligodendrocytes, Cherry-Swap70 protein was apparent at the axon-ensheathing internode membrane (Fig. 4C). By comparison, when expressed alone Cherry fluorescent protein appeared to accumulate mostly in the soma of oligodendrocytes and, although evident in membrane processes, did not reveal ensheathing internodes as distinctly as the fusion protein (Fig. 4B). To attempt to identify Swap70 domains necessary for localization, we expressed deletion constructs. First, we deleted C-terminal sequence, which includes the Dbl-homology domain (Cherry-Swap70ΔGEF). This truncated fusion protein showed localization similar to that of full-length Cherry-Swap70 (Fig. 4D). By contrast, a fusion protein containing only the Pleckstrin Homology (PH) domain of Swap70 (Cherry-Swap70PH) was localized primarily to the cytoplasm and did not show significant internode accumulation (Fig. 4E). Therefore, Swap70 protein localizes to myelinating membrane processes of oligodendrocytes in a PH domain-dependent manner.

swap70 loss of function results in fewer OPCs

Expression of *swap70* by neural precursors and oligodendrocytes raised the possibility that it has distinct functional roles in specification or maintenance of precursors and in oligodendrocyte differentiation. However, expression of neither full-length nor deleted versions of Swap70 described above had any discernable effect on oligodendrocyte development. Therefore, we sought to further test these possibilities by reducing *swap70* function using an antisense morpholino oligonucleotide (MO) approach. We designed two different translation-blocking MOs (MO$^{swap70\text{-}UTR}$ and MO$^{swap70\text{-}ATG}$) and one splice-blocking MO (MO$^{swap70\text{-}sp}$). MO$^{swap70\text{-}sp}$ was designed to target the boundary between exon 5 and intron 5 with the prediction that it would cause exclusion of exon 5, which encodes a portion of the PH domain, from the processed mRNA (Fig. 5A). We tested this prediction using RT-PCR. Whereas primers that target sequences within exons 4 and 6 produced a band of the predicted size of 500 bp from control embryos, a smaller product of about 375 bp was produced by RT-PCR from embryos injected with MO$^{swap70\text{-}sp}$ (Fig. 5B). Sequence analysis showed that *swap70* mRNA modified by MO$^{swap70\text{-}sp}$ encodes a protein lacking the portion of the PH domain encoded by exon 5 (Fig. 5C). Because the PH domain is necessary for PtdIns(3,4,5)P3 binding and Swap70 function (Shinohara et al., 2002), we expect that MO$^{swap70\text{-}sp}$ produces a loss-of-function effect.

At 3 dpf, larvae injected at single cell stage with up to 4 ng of any of the *swap70* MOs had no discernable morphological defects (Figs. 6A–C). To investigate the effect of *swap70* loss-of-function on oligodendrocyte development, we examined the number and distribution of oligodendrocyte lineage cells marked by EGFP encoded by a *Tg*(*olig2*:*EGFP*) transgene. This revealed that larvae injected with either MO$^{swap70\text{-}ATG}$ or MO$^{swap70\text{-}sp}$ had fewer dorsal spinal cord EGFP^{+} cells then control larvae (Figs. 6A′–C″). We confirmed that injected larvae had statistically fewer oligodendrocyte lineage cells by counting Sox10^{+} cells in the spinal cord (Figs. 6D,E). To further validate our MO approach, we tested the ability of Myc epitope-tagged Swap70 (Myc-swap70) encoded by synthetic mRNA to suppress the oligodendrocyte phenotype when co-injected with MO$^{swap70\text{-}SP}$. This partially restored the number of Sox10^{+} cells at 3

Fig. 1. Developmental expression of *swap70*. (A,B) Lateral views of embryos hybridized with *swap70* antisense RNA probe at 2 somite (11 hpf) and 20 hpf. The eye primordia (arrows) and pronephric ducts (arrowhead) express *swap70*. (C–H) Lateral views of whole embryos focused on the trunk spinal cord, with dorsal up. The bracket marks spinal cord (sc). Expression of *swap70* (C,F) is similar to that of *sox10* (D,G), a marker of OPCs (arrows) at 50 and 80 hpf. By contrast, expression of *plp1a*, which marks differentiating oligodendrocytes, is not evident until 80 hpf (E,H). (I–M) Transverse sections through trunk spinal cord, dorsal up. *swap70* (I,K) is expressed similarly to *sox10* (J,L) in OPCs (arrows) except that cells that line the proliferative ventricular zone (arrowheads) also express *swap70* but not *sox10*. (M) *plp1a* expression marking oligodendrocytes (arrows). (N,O) Transverse sections through forebrain (N) and hindbrain (O). *swap70* expression is evident at ventricular zones of the tectum (te), diencephelon (di) and at the boundary between cerebellum (ce) and hindbrain (hb).