Upregulation of the zebrafish Nogo-A homologue, Rtn4b, in retinal ganglion cells is functionally involved in axon regeneration

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Abstract

Background: In contrast to mammals, zebrafish successfully regenerate retinal ganglion cell (RGC) axons after optic nerve section (ONS). This difference is explained on the one hand by neurite growth inhibitors in mammals (including Nogo-A), as opposed to growth-promoting glial cells in the fish visual pathway, and on the other hand by the neuron-intrinsic properties allowing the upregulation of growth-associated proteins in fish RGCs but not in mammals.

Results: Here, we report that Rtn4b, the zebrafish homologue of mammalian Nogo-A/RTN4-A, is upregulated in axotomized zebrafish RGCs and is primarily associated with the endoplasmic reticulum (ER). Rtn4b functions as a neuron-intrinsic determinant for axon regeneration, as was shown by downregulating Rtn4b through retrogradely transported morpholinos (MOs), applied to the optic nerve at the time of ONS. MO1 and MO2 reduced the number of axons from retina explants in a concentration-dependent manner. With MO1, the reduction was 55% (70 μM MO1) and 74% (140 μM MO1), respectively, with MO2: 59% (70 μM MO2) and 73% (140 μM MO2), respectively (compared to the control MO-treated side). Moreover, regenerating axons 7d after ONS and MO1 or MO2 application were labeled by Alexa488, applied distal to the first lesion. The number of Alexa488 labeled RGCs, containing the Rtn4b MO1 or MO2, was reduced by 54% and 62%, respectively, over control MO.

Conclusions: Thus, Rtn4b is an important neuron-intrinsic component and required for the success of axon regeneration in the zebrafish visual system. The spontaneous lesion-induced upregulation of Rtn4b in fish correlates with an increase in ER, soma size, biosynthetic activity, and thus growth and predicts that mammalian neurons require the same upregulation in order to successfully regenerate RGC axons.

Keywords: Optic nerve lesion, Axon regeneration, Rtn4b upregulation, ER, Morpholino downregulation, Retinal ganglion cells, Neuron-intrinsic determinants, Nogo-A/RTN4A

Background

The visual pathway in teleost fish is well-known for the capacity of the retinal ganglion cells (RGCs) to regenerate axons following optic nerve section (ONS) and to restore function [1]. In contrast, CNS axons in mammals do not spontaneously regenerate because of inhibitory properties of the glial cells in the environment of lesioned axons [2] and the poor neuron-intrinsic properties [3,4]. In fish, the glial cell environment is apparently growth-permissive [5,6]. Moreover, fish RGCs possess the unique ability to spontaneously activate the cellular machinery required for axon regrowth including upregulation of growth-associated proteins [7,8] and rise in the neuron’s biosynthetic activity.

One of the strongest oligodendrocyte and myelin-associated inhibitors in mammals is Nogo-A [2]. More than 95% of Nogo-A is localized at the endoplasmic reticulum (ER) [9,10], and only small amounts emerge at the surface [2]. Nogo-A is also expressed in neurons, particularly in those with far-projecting axons. Neuronal Nogo-A was reported to negatively regulate neuronal plasticity [11,12], but a positive influence on neurite extension has also been observed. Nogo-A promotes sprouting of axons in the lesioned mouse optic nerve...
and regeneration in RGCs with elevated Nogo-A expression levels [13,14].

In zebrafish embryos, the Nogo-A homologue Rtn4b [15] was discovered in many differentiating brain regions [16] including the developing RGCs, and its downregulation caused severe defects in the retinotectal projection.

Here, we asked whether Rtn4b would be upregulated in adult fish RGCs after ONS and promote axon regeneration. This can be assessed by the in vivo application of specific morpholinos (MOs) to the eye-side stump of the lesioned optic nerve as done with reggie-1 and −2, which massively impaired axon regeneration [17]. Our results indeed show that zebrafish RGCs require Rtn4b as a neuron-intrinsic determinant of axon regeneration.

Results

Rtn4b expression in zebrafish RGCs and upregulation after optic nerve lesion

The affinity purified antiserum against zebrafish Rtn4b [16] labeled all retinal layers but was brighter over RGC somata compared to other retinal neurons (Figure 1A). The RGC axon layer which was intensely labeled by the anti-MBP antibody (AB) (fish RGC axons are myelinated in their intraretinal path) was only weakly stained by the Rtn4b AB (Figure 1A, B, C). Ten days after ONS, RGC somata had significantly increased expression of Rtn4b indicating that ONS leads to Rtn4b upregulation in neurons (Figure 1B). In the normal optic nerve, Rtn4b labeling was weak (Figure 1D) whereas anti-MBP AB strongly labeled the myelin (Figure 1F, M) in the normal nerve and after ONS. The staining with Rtn4a AB was similar to MBP, but the AB labeled in addition the boundaries of axon fascicles and further subdivisions of the fascicles (Figure 1E). Rtn4a therefore appears to reside in astrocytic structures as suggested earlier [18] and myelin. In the nerve 10 days after ONS, Rtn4b labeling was associated with glial cell processes around fascicles and more strikingly with regenerating RGC axons which were identified by anti-neurolin AB [19] (Figure 1G, H, I, P). Accordingly, axons and growth cones in culture were also labeled (Figure 2E). Rtn4a AB also stains RGC growth cones in vitro [18] but in sections through the nerve strongly stained the fascicle boundaries and subdivisions rather than neuron-positive regenerating axons (Figure 1J, K, L, Q). In the nerve 10 days after ONS, myelin detected by MBP AB was intense and the myelin-positive regenerating axons were located amidst the myelin staining (Figure 1M, N, O, R). Together, this staining shows that regenerating RGC axons in the nerve and in vitro are Rtn4b-positive and cross through MBP-labeled myelin. Rtn4a is in myelin and astrocytic fascicle boundaries and subdivisions but not to the same extent in neuron-positive axons as Rtn4b. Rtn4b appears less prominent in CNS myelin in the retina and optic nerve but is significantly upregulated in RGCs and RGC axons after ONS.

To clarify whether Rtn4b in fish is, as in mammals, localized at the ER, we exposed zebrafish oligodendrocytes in vitro to Rtn4b AB and the AB against CLIMP63 specific for the ER. Rtn4a AB was used for comparison. Rtn4b as well as Rtn4a labeled a reticulum network that is positive for CLIMP63 AB and reached into the cellular processes (Figure 2A, B, C) like Nogo-A in mammals [9]. Growth cones of RGC axons showed Rtn4b and CLIMP63 staining (Figure 2E, F), suggesting that the ER extends into the tips of elongating axons. We also tested if Rtn4b might be visible at the cell surface as reported for Nogo-A in mammalian oligodendrocytes and neurons [2]. We were not able to detect cell surface staining by exposing live cells to Rtn4b AB, either because the protein does not reach the surface or in amounts that are too small to be detected by the present staining procedure (Figure 2D).

Next, we analyzed changes in axotomized RGCs in retina whole mounts (Figure 3A, B, C, D, E, F, G, H, I). RGC somata at 5 days after ONS increased in area by 87% (the cell body reaction), and the cytoplasm was filled entirely by Rtn4b AB labeling (Figure 3D) associated with cloudy structures, typical for ER staining with anti-protein disulfide isomerase (PDI) in mammalian cells (ABCAM home page). Rtn4b staining intensity was upregulated in intensity by 48% when compared to controls (P < 0.01). Ten days after ONS, Rtn4b protein levels were even more elevated with an increase of 54% in comparison to control (P < 0.01).

Quantitative Western blots with retina lysates, control versus 5 and 10 days after ONS, also showed a significant upregulation of 100 kd Rtn4b by 45% (5 days; P < 0.01) and 58% (10 days; P < 0.0001), respectively (Figure 3I).

Rtn4b is essential for RGC axon regeneration

To determine whether Rtn4b is indeed needed for RGC axon regeneration, we downregulated Rtn4b by placing a MO-soaked piece of gel foam directly at the eye-side optic nerve at the time of ONS [17]. The MOs are retrogradely transported into the RGCs as is demonstrated by using lissamine-labeled MOs. To minimize the danger of potential off-target effects, two different MOs, MO1 and MO2, were used and applied at two different concentrations (either 70 or 140 μM) to the left optic nerve in parallel with control MO application to the right nerve within the same animal. That MO1 and MO2 specifically downregulate Rtn4b in a concentration-dependent manner was demonstrated earlier in zebrafish embryos [16]. Five days after MO1 or MO2 application, the Rtn4b immunostaining intensity in RGCs in whole mounts was markedly reduced (Figure 4C,D), whereas RGCs...
Figure 1 (See legend on next page.)
receiving control MO were intensively labeled (Figure 4A, B). That MO1 and MO2 led indeed to Rtn4b downregulation was confirmed by Western blots of retinae, 5 days after ONS and MO application (Figure 4E) showing an overall significant reduction of Rtn4b by 32% with each MO1 and MO2 (\(P < 0.0001\), MO1; \(P < 0.0001\), MO2).

**Ex vivo outgrowth assay**

Next, we analyzed whether Rtn4b MOs impair axon regeneration. In the so-called outgrowth assay, the retinas were divided into mini-explants at 5 days after ONS and MO application (Figure 4E) showing an overall significant reduction of Rtn4b by 32% with each MO1 and MO2 (\(P < 0.0001\), MO1; \(P < 0.0001\), MO2).

**In vivo regeneration assay**

In a second assay, the optic nerve of fish after ONS and MO treatment was re-sectioned at 7 days, 2 to 3 mm distal from the first lesion, and Alexa488-dextran was applied to retrogradely label RGCs with regenerating axons [17]. Two days later, the dextran-labeled RGCs were counted in left and right retina whole mounts (left side: Rtn4b MO1 and Rtn4b MO2, respectively; right side: control MO) in seven independent experiments (Figure 6A, B, C, D, E, F). The number of dextran-labeled RGCs was on average reduced by 54% over controls with MO1 (\(P < 0.001\)) and by 62% with MO2 (\(P < 0.001\)) (Figure 6G). Thus, downregulation of Rtn4b with two unrelated MOs blocks RGC axon regeneration.
Discussion

This study showed that zebrafish upregulate Rtn4b after ONS and need Rtn4b for axon regeneration. This was demonstrated by employing the MO-mediated downregulation of Rtn4b in vivo, with two different MOs and at different concentrations. Moreover, impaired axon regeneration was observed in two independent experimental approaches: the ex vivo outgrowth and the in vivo regeneration assay. Therefore, we conclude that Rtn4b belongs to the group of growth-associated proteins that axotomized fish RGCs upregulate in order to regenerate axons and that are indicative of the growth-supportive neuron-intrinsic properties of these neurons.

The present technique involving downregulation of specific growth-associated proteins in vivo by MO application to lesioned zebrafish CNS fiber tracts has been successfully applied in the past [20,21,17,22]. The danger of potential MO side effects was minimized by employing two different MOs against Rtn4b and different MO concentrations, in parallel with control MO. That MO1 and MO2 specifically target Rtn4b is further supported by Western blots in retinae and embryos. In zebrafish embryos, the appropriate rescues involving co-injection of MO-resistant RNAs partially restored the defects caused by Rtn4b downregulation [16]. Such rescue experiments are not feasible in the present experimental setting since lesioned axons do not take up or retrogradely transport RNAs or vectors, nor can RGCs be transfected by injecting the agents into the vitreous. Still, present and earlier controls together with results from several studies using...
the in vivo MO application to downregulate specific proteins speak for the reliability of our results showing that Rtn4b is essential for axon regeneration.

In a previous study, MO-mediated downregulation of reggie-1 and -2 impaired RGC axon regrowth by up to 70%. Reggies are intracellular membrane-associated proteins involved in Rab11-dependent cargo recycling and trafficking [4,23]. Their downregulation affects the machinery that regulates the delivery of membrane and proteins to the elongating growth cone [24]. This function is essential for neurite elongation and explains why the reduction in axon regeneration was massive with reggie MOs. The present results suggest that Rtn4b is equally important even though the molecular mechanism is unclear (see below).

To get an impression of the significance of a given protein for axon growth, its forced upregulation in mammalian RGCs can be informative. For instance, adeno-associated virus (AAV)-mediated upregulation of reggie-1 in rat RGCs increased the number and length of regenerating axons in the optic nerve [25]. In a similar experiment, AAV-mediated upregulation of Nogo-A enhanced sprouting of lesioned axons in vitro after MO1 and MO2 application to the optic nerve, in comparison to axon number from control (Co) MO-treated fish (100%). Bars indicate standard deviation. The differences between groups are statistically significantly different. Quantification was done on three replicates from three different experiments, and for statistical analysis, Student’s T-test was used. *P < 0.05, **P < 0.01.
localized at the ER as shown by the present immunostainings. The increase in Rtn4b staining in the retina and RGCs is consistent with an expansion of the ER in response to ONS during the upregulation of protein synthesis (cell body response, [8,7]). It is possible that Rtn4b enhances growth through its ER structuring ability [9], or it plays a direct role in the production of specific proteins and the upregulation of growth-associated molecules in regeneration-competent neurons. Rtn4b and CLIMP63 staining reaches into the axons and RGC growth cones which is consistent with the notion that they contain ER and synthesize molecules relevant for growth and guidance [27]. RTN4a as an ER shaping protein is reportedly involved in the redistribution of PDI in superoxide dismutase (SOD)1 dependent amyotrophic lateral sclerosis [28] emphasizing the importance of ER-associated functions of RTN4. Moreover, the ER structure in axons depends on microtubules and GTPases like atlastin-1. Atlastin-1 loss of function inhibits axon elongation [29-31], probably due to impairment of ER structure and distribution. Therefore, it is conceivable that zebrafish Rtn4b subserves similar important functions for the integrity of the ER and growth, including reforming growth cones and axons.

The other functionally relevant location of Nogo-A/RTN4-A in mammals is the cell surface. Growing axons typically are inhibited by Nogo-A exposed on the surface of oligodendrocytes and CNS myelin [2]. Surface-exposed Nogo-A is also known as an inhibitor of neuronal plasticity and regulator of structural integrity of neuronal connections. Whether a fraction of zebrafish Rtn4b is exposed on the cell surface is relevant for experiments testing its function as potential growth inhibitor associated with glial cells and CNS myelin. In mammals, surface-exposed Nogo-A acts as a ligand for two receptor complexes [2,32] connecting to signaling cascades that inhibit axon growth. Whether zebrafish Rtn4b exerts inhibition on growing axons with Nogo receptors needs to be analyzed. However, even though Rtn4b is expressed in fish oligodendrocytes (ER), the present staining with Rtn4b AB in MBP-rich regions of the normal and regenerating optic nerve is relatively weak, particularly when compared to Rtn4a AB. Rtn4a consists mainly of the reticulon homology domain (RHD) and a short N-terminal sequence but

Figure 6 Rtn4b MO-induced reduction in axon regeneration in the in vivo regeneration assays. (A-F) After application of Alexa488 to the regenerating axons (distal from the original lesion and MO application site), the retrogradely labeled RGCs are counted in retina whole mounts. Many more Alexa488-labeled RGCs are recognized 9 days after ONS and control (Co) MO application (A) than on the contralateral retina (D) belonging to the nerve that received Rtn4b MO1 (or MO2). (B, E) The RGCs contain lissamine associated with the MOs. (C,F) Merge of (A,B) and (D,E). Scale bar, 50 μm. (G) The histogram demonstrates the decline in the number of Alexa-labeled RGCs after MO1 and MO2 application to the optic nerve, in comparison to axon number from control (Co) MO-treated fish (100%). Bars indicate standard deviation. Three different experiments with n, 10 retinal squares (300 × 300 μm) for each experimental group were statistically evaluated using Student’s T-test. The differences between groups are statistically significantly different, ***P < 0.001.
the long N-terminal region of Rtn4b with homology to the mammalian Nogo-A-specific region is absent from Rtn4a [15]. Unlike mammalian Nogo-66, zebrafish Nogo-66 within the RHD of Rtn4a is not inhibitory to axon growth but rather seems to promote growth [18]. Rtn4b is significantly upregulated in axotomized RGCs and regenerating RGC axons. Strikingly, the regenerating Neurolin-positive axons were amidst optic nerve myelin [33] consistent with the notion that fish CNS myelin is not or by far less inhibitory than mammalian CNS myelin [5,6].

Conclusions
Notwithstanding the inhibitory activity of surface-exposed mammalian Nogo-A, our study has demonstrated that neuron-intrinsic Rtn4b/zebrafish Nogo is upregulated after optic nerve lesion in RGCs and contributes to axon regeneration as an important ER-associated factor. Robust upregulation of RTN4-A/Nogo-A and growth of the ER in mammalian RGCs concomitant with a decrease of RTN-4/Nogo-A in optic nerve myelin might increase the RGC's competence for regeneration.

Methods
Animals, ONS, and morpholino application
Zebrafish were maintained at 28°C in the animal facility (TFA) of the University of Konstanz. For in vivo knockdown of the Rtn4b protein, the optic nerves of zebrafish, male and female, aged 4 to 8 months, were severed under 3-aminobenzoic ethylester anesthesia (MS222, 250 mg/l; Sigma-Aldrich, St. Louis, MO, USA) in compliance with animal welfare legislation. Procedures were performed three times with n, 80 explants per experimental group.

Quantitative in vivo regeneration assay
To assess the regeneration capacities of RGCs under rtn4b knockdown in vivo, the optic nerve of MO-treated zebrafish was re-sectioned 7 days later, 2 to 3 mm distal to the first lesion, and Alexa488-dextran (Invitrogen, Carlsbad, CA, USA) was applied on the second lesion in order to retrogradely label RGCs that had regenerated their axons. At this time, the previously spared fascicles were also severed and served as control for the successful retrograde transport of the dye. After 48 h, the number of dextran-labeled RGCs was counted in retina whole mounts of control and Rtn4b knockdown retinae [17]. The experiment was performed four times with each Rtn4b and CoMO. Per experiment, n = 10 images were evaluated for each group. Results were statistically evaluated using Student’s T-test.

Immunostaining of retina whole mounts and cryosections
For retina whole mount stainings, retinas were prepared as described above, fixed in 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) at RT for 30 min, permeabilized by incubation in 1% Triton in PBS for 1 min at RT and exposed to immunoaffinity purified polyclonal K1121 against Rtn4b [16] 1:500 and anti-MBP 1:100 (kindly provided by William S. Talbot, Stanford University, USA), diluted in 1% BSA/PBS, overnight at 4°C. Nuclei were stained with 4’,6-diamidino-2-phenylindole (DAPI) (100 ng/ml) and cells with Alexa488-coupled Phalloidin (Invitrogen, Carlsbad, CA, USA), applied together with the secondary AB Cy3-coupled donkey anti-rabbit or Alexa488-coupled goat anti-rabbit (Jackson ImmunoResearch, West Groove, PA, USA) for 2 h at RT. For cryosections, the eye and optic nerve were isolated, transferred directly into TissueTek (Sakura, Alphen aan den Rijn, The Netherlands) at ~20°C and cut on a cryostat. The 10-μm-thick sections were transferred to pLys-coated slides and allowed to dry and either stored at ~20°C or subjected directly to immunostainings with Rtn4b AB, anti-MBP, IK964 against Rtn4a, or anti-neurolin (N518) against growing axons [19]. After washes in PBS, sections were coveredslipped with Mowiol (Calbiochem, San Diego, CA, USA). Images were acquired at a confocal laser-scanning microscope (LSM700 META; Carl Zeiss Inc., Oberkochen, Germany).
Germany) with an Apochromat 63×/1.4 oil immersion lens. For quantitative analysis of RGC size, Rtn4b-labeled cells were encircled in retinae at 5 days after ONS and normal control retinae at the LSM, evaluated by ImageJ. Fluorescence mean intensities of Rtn4b staining in control and ONS retinae were scored in three separate experiments for 12 images per group using ImageJ. Zebrafish oligodendrocytes were obtained (as described [5]) from the regenerating optic nerve/tract by explanting pieces of tissue between two cover slips in the same medium as retin-a explants. Cells emigrate from the nerve/tract explants, and some divide over 10 to 14 days in vitro. Oligodendrocytes and RGC axons were immunolabeled with Rtn4a AB, Rtn4b AB, or CLIMP63 (kindly provided by Hess-Farhan, University of Konstanz, Germany) after PFA fixation or exposed to Rtn4b AB live, then fixed and exposed to secondary ABs.

Western blots
For Western blot analysis, isolated retinae were lysed in RIPA buffer. Blots were exposed to Rtn4b AB (diluted 1:1,000) and anti-α-Tubulin AB. The intensity of protein bands was determined by ImageJ, the Rtn4b band normalized to the loading control and statistically evaluated using the Student’s T test. Blots for evaluation of upregulation of Rtn4b after nerve transection were repeated four times for 5 and 10 days ONS; experiments for assessing downregulation by MO were performed five times with two retinae used per lysate.

Abbreviations
AAV: adeno-associated virus; AB: antibody; CNS: central nervous system; Co: Control; DAPI: 4′,6-diamidino-2-phenylindole; ER: endoplasmic reticulum; MO: morpholino; MS222: 3-aminobenzoic ethylester; ONS: optic nerve section; PBS: phosphate-buffered saline; PDI: protein disulfide isomerase; PFA: paraformaldehyde; RGCs: retinal ganglion cells; RHD: reticulon homology domain; RTN: reticulon.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
CW performed the experiments, outgrowth assay, in vivo regeneration assay, immunostaining, analyzed the data, and performed the statistical test. SE performed earlier outgrowth assays, analyzed them, and proved statistical significance. CS designed the research, discussed the background and results with CW and SE, and wrote the paper. All authors read and approved the final manuscript.

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References
1. Gaze RM. The formation of nerve connections. London: Academic; 1970.
2. Schwab ME. Functions of Nogo proteins and their receptors in the nervous system. Nat Rev Neurosci. 2010;11:799–811.
3. Eva R, Andrews MR, Fransen EH, Fawcett JW. Intrinsic mechanisms regulating axon regeneration: an integrin perspective. Int Rev Neurobiol. 2012;106:75–104.
4. Stuermer CA. The reggie/flotillin connection to growth. Trends Cell Biol. 2010;20:6–13.
5. Bastmeyer M, Beckmann M, Schwab ME, Stuermer CA. Growth of regenerating goldfish axons is inhibited by rat oligodendrocytes and CNS myelin but not by goldfish optic nerve tract oligodendrocyte-like cells and fish CNS myelin. J Neurosci. 1991;11:626–40.
6. Wanner M, Lang DM, Bandtlow CE, Schwab ME, Bastmeyer M, Stuermer CA. Reevaluation of the growth-permissive substrate properties of goldfish optic nerve myelin and myelin proteins. J Neurosci. 1995;15:7500–8.
7. Grafstein B, McQuarrie IG. The role of the nerve cell in axonal regeneration. In: ‘Neuronal plasticity’ (CW Cotman ed) pp155-195, Raven, New York. 1978.
8. Skene JH, Jacobson RD, Snipes GJ, McGuire CB, Norden JI, Freeman JA. A protein induced during nerve growth (GAP-43) is a major component of growth-cone membranes. Science. 1986;233:783–6.
9. Voetz GK, Prinz WA, Shibata Y, Rist JM, Rapoport TA. A class of membrane proteins shaping the tubular endoplasmic reticulum. Cell. 2006;124:73–86.
10. Shibata Y, Hu J, Kozlov MM, Rapoport TA. Mechanisms shaping the membranes of cellular organelles. Annu Rev Cell Dev Biol. 2009;25:329–54.
11. Vanek P, Thallmair M, Schwab ME, Kapellhammer JP. Increased lesion-induced sprouting of corticospinal fibers in the myelin-free rat spinal cord. Eur J Neurosci. 1998;10:45–56.
12. Zembran O, Weismann O, Kellner Y, Yu X, Vicente R, Gullu M, et al. Neutralization of Nogo-A enhances synaptic plasticity in the rodent motor cortex and improves motor learning in vivo. J Neurosci. 2013;34:4685–96.
13. Perem V, Joly S, Dalkara D, Schwarz O, Christ F, Schaffer D, et al. Neuronal Nogo-A upregulation does not contribute to ER stress-associated apoptosis but participates in the regenerative response in the axotomized adult retina. Cell Death Differ. 2012;19:1096–118.
14. Vajda F, Jordi N, Dalkara D, Joly S, Christ F, Tews B, et al. Cell type-specific Nogo-A gene ablation promotes axonal regeneration in the injured adult optic nerve. Cell Death Differ. 2014;22:323–35.
15. Shyptysna A, Malaga-Trillo E, Reuter A, Stuermer CA. Origin of Nogo-A by domain shuffling in an early jawed vertebrate. Mol Biol Evol. 2011;28:1363–70.
16. Pinzon-Olaja A, Welte C, Abdesselem H, Malaga-Trillo E, Stuermer CA. Essential roles of zebrafish mRNAGopro paradigues in embryonic development. Neural Dev. 2014;9:8.
17. Munderloh C, Solis GP, Bodrikov V, Jaeger FA, Weichers M, Malaga-Trillo E, et al. Reggies/flottillins regulate retinal axon regeneration in the zebrafish optic nerve and determination of hippocampal and Nr2a neurons. J Neurosci. 2009;29:6607–15.
18. Abdesselem H, Shyptysna A, Solis GP, Bodrikov V, Stuermer CA. No Nogo66- and Nr8-mediated inhibition of regenerating axons in the zebrafish optic nerve. J Neurosci. 2009;29:15489–98.
19. Diekmann H, Stuermer CA. Zebrafish neurolin-a and -b, orthologs of ALCAM, are involved in retinal ganglion cell differentiation and retinal axon pathfinding. J Comp Neurol. 2009;513:38–50.
20. Becker CG, Lieberoth BC, Morellini F, Feldner J, Becker T, Schachner M. L1.1 is involved in spinal cord regeneration in adult zebrafish. J Neurosci. 2004;24:7837–42.
21. Elsaeidi F, Bemben MA, Zhao XF, Goldman D. Jak/Stat signaling stimulates sprouting of corticospinal fibers in the myelin-free rat spinal cord. Eur J Neurosci. 2013;51:168–76.
22. Veldman MB, Bemben MA, Thompson RC, Goldman D. Gene expression analysis of zebrafish retinal ganglion cells during optic nerve regeneration identifies KLFea and KLF7a as important regulators of axon regeneration. Dev Biol. 2007;312:596–612.
23. Solis GP, Hülsbusch N, Radon Y, Katanaev VL, Plattner H, Stuermer CA. Development through reggie/flotillin-dependent N-cadherin trafficking. Mol Biol Cell. 2013;24:2689–702.
24. Bodrikov V, Solis GP, Stuermer CA. Prion protein promotes growth cone development through reggie/flotillin-dependent N-cadherin trafficking. J Neurosci. 2011;31:18013–25.
25. Koch JC, Solis GP, Bodrikov V, Micheli U, HaraLampiavea D, Shyptysna A, et al. Upregulation of reggie-1/flotillin-2 promotes axon regeneration in the rat optic nerve in vivo and neurite growth in vitro. Neurobiol Dis. 2013;51:168–76.
26. Kurowska Z, Brundin P, Schwab ME, Li JY. Intracellular Nogo-A facilitates initiation of neurite formation in mouse midbrain neurons in vitro. Neuroscience. 2014;256:456–66.

27. Merianda TT, Lin AC, Lam JS, Vuppalanchi D, Willis DE, Karin N, et al. A functional equivalent of endoplasmic reticulum and Golgi in axons for secretion of locally synthesized proteins. Mol Cell Neurosci. 2009;40:128–42.

28. Yang YS, Harel NY, Strittmatter SM. Reticulon-4A (Nogo-A) redistributes protein disulfide isomerase to protect mice from SOD1-dependent amyotrophic lateral sclerosis. J Neurosci. 2009;29:13850–9.

29. Rismanchi N, Soderblom C, Stadler J, Zhu PP, Blackstone C. Atlastin GTPases are required for Golgi apparatus and ER morphogenesis. Hum Mol Genet. 2008;17:1591–604.

30. Renvoise B, Blackstone C. Emerging themes of ER organization in the development and maintenance of axons. Curr Opin Neurobiol. 2010;20:531–7.

31. Gonzalez C, Couve A. The axonal endoplasmic reticulum and protein trafficking: cellular bootlegging south of the soma. Semin Cell Dev Biol. 2014;27:23–31.

32. Schmandke A, Schwab ME. Nogo-A: multiple roles in CNS development, maintenance, and disease. Neuroscientist. 2014;20:372–86.

33. Strobel G, Stuermer CA. Growth cones of regenerating retinal axons contact a variety of cellular profiles in the transected goldfish optic nerve. J Comp Neurol. 1994;346:435–48.

34. Bernhardt RR. Cellular and molecular bases of axonal regeneration in the fish central nervous system. Exp Neurol. 1999;157:223–40.

35. Meyer RL, Sakurai K, Schauwecker E. Topography of regenerating optic fibers in goldfish traced with local wheat germ injections into retina: evidence for discontinuous microtopography in the retinotectal projection. J Comp Neurol. 1985;239:27–43.

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