In Vivo and in Vitro Characterization of Novel Neuronal Plasticity Factors Identified following Spinal Cord Injury*

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Following spinal cord injury, there are numerous changes in gene expression that appear to contribute to either neurodegeneration or reparative processes. We utilized high density oligonucleotide microarrays to examine temporal gene profile changes after spinal cord injury in rats with the goal of identifying novel factors involved in neural plasticity. By comparing mRNA changes that were coordinately regulated over time with genes previously implicated in nerve regeneration or plasticity, we found a gene cluster whose members are involved in cell adhesion processes, synaptic plasticity, and/or cytoskeleton remodeling. This group, which included the small GTPase Rab13 and actin-binding protein Coronin 1b, showed significantly increased mRNA expression from 7–28 days after trauma. Overexpression in vitro using PC-12, neuroblastoma, and DRG neurons demonstrated that these genes enhance neurite outgrowth. Moreover, RNAi gene silencing for Coronin 1b or Rab13 in NGF-treated PC-12 cells markedly reduced neurite outgrowth. Coronin 1b and Rab13 proteins were expressed in cultured DRG neurons at the cortical cytoskeleton, and at growth cones along with the pro-plasticity/regeneration protein GAP-43. Finally, Coronin 1b and Rab13 were induced in the injured spinal cord, where they were also co-expressed with GAP-43 in neurons and axons. Modulation of these proteins may provide novel targets for facilitating restorative processes after spinal cord injury.

Traumatic injury to the spinal cord induces delayed biochemical responses that affect both cell loss and subsequent repair. Modulation of reparative processes includes factors involved in either facilitating or inhibiting neurite outgrowth, which are often regulated at the gene and protein levels after injury. Collectively, these factors likely determine in part the degree of anatomical and functional recovery after injury.

Regeneration-associated proteins (RAGs) appear to play a role in plasticity and regeneration following SCI. These include transcription factors (c-Jun), cytoskeletal components (Tao1), microtubule-associated proteins, growth-associated proteins (GAP-43, CAP-23), cell adhesion molecules (N-CAM, L1, TAG1), neurotrophic factors, cytokines, and extracellular matrix components (SNAP25, munc13, and cpg15/neuritin) (1–10). In some cases, these factors share common molecular pathways. GAP-43 and CAP-23 bind downstream to the cofactor PI(4,5)P(2), at plasmalemmal rafts, contributing to the regulation of actin and modulating neurite outgrowth in neuronal-like cell lines (11, 12). In other instances, a common downstream effector, such as neurotrophin-dependent intracellular cAMP, may serve to facilitate axonal regeneration by overcoming inhibition from factors such as myelin-associated glycoprotein (MAG) (13–15).

Microarray technology provides a powerful tool for identifying molecular pathways involved in either endogenous neurotoxicity or regeneration/plasticity after SCI (16–19). It allows concurrent analysis of thousands of genes and the identification of clusters of coordinately regulated transcripts sharing similar functions.

Previously we reported early changes following spinal cord injury in rats, showing induction of specific cell cycle genes in vivo. This paper is available online at http://www.jbc.org

The abbreviations used are: SCI, spinal cord injury; PBS, phosphate-buffered saline; DRG, dorsal root ganglia; RT, reverse transcription; NGF, nerve growth factor; RNAi, RNA interference; eGFP, enhanced green fluorescent protein.
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EXPERIMENTAL PROCEDURES

EXPERIMENTAL SPINAL CORD INJURY AND EXPRESSION PROFILING

Mild contusion spinal cord injury was performed in rats, as previously described (18). Briefly, male Sprague-Dawley rats (275–325 g) were anesthetized with sodium pentobarbital (65 mg/kg, intraperitoneal). Injury was induced using a weight drop method (10 g dropped from 17.5 mm). Animals with this injury level show mild motor impairment on the Basso, Beattie, Bresnahan scale, averaging 12 ± 1, 14 ± 1, and 17 ± 1, respectively at 7, 14, and 28 days after injury. Animals were fully anesthetized with sodium pentobarbital (67 mg/kg) during the operative procedures, and experiments complied fully with the principles set forth in the “Guide for the Care and Use of Laboratory Animals” prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (DHHS Pub. no. (NIH) 85-23, 2885).

A 1-cm section of the spinal cord, centered at T-9, was dissected, and immediately frozen in liquid nitrogen. Spinal cords were collected from 4 injured and 2 sham-operated rats (receiving only laminectomy) for each time point (4 h, 24 h, 7 days, 14 days, and 28 days (3 animals) and two naive controls (rats that did not undergo any surgical procedure) for a total of 31 animals. Seven micrograms of total RNA was used for expression profiling as described previously (18).

Expression profiling was performed using the Affymetrix rat U34 A, B, and C arrays. Each Affymetrix GeneChip was used for one hybridization with RNA isolated from one spinal cord sample (cRNA) synthesis. Expression profiling analysis was performed using each time point (4 h, 24 h, 7 days, 14 days, and 28 days (3 animals) and during the post-PCR temperature ramp. Fold changes were calculated following the manufacturer’s instructions (Invitrogen).

Microarray (Genechip) Quality Control and Normalizations

We employed stringent quality control methods as previously published and detailed on our website: (pepr.cmccresearch.org/browse.do). Expression profiling utilized for analysis fulfilled all quality control measures as detailed in previous reports (18, 21). We used two normalization processes: one for chip-chip comparisons (scaling factors), and one for temporal normalization (normalization to the average of the naive signal intensities for each gene). The scaling factors determinations were done using default Affymetrix algorithms (MAS 5) with a target intensity of chip sector fluorescence to 800. Both pre-amplification (s1) and post-amplification with streptavidin/phycoerythrin (s2) scans were done, and the scans compared by scatter plots and correlation coefficients. Those probe sets showing evidence of saturation of the PMT in s2 were flagged.

Data Scraping and Statistical Analysis

We have recently shown that use of Affymetrix MAS 5.0 signal intensity values, together with a “present call” noise filter achieves an excellent balance relative other probe set analysis methods (dchip, RMA) (39). Data analyses were limited to probe sets that showed one or more “present” (P “calls”) in the 79 gene chip profiles in our complete dataset. Experiment normalization was performed by normalizing gene chips from injured and sham controls to the mean of the two chips from naive animals considered as the baseline gene expression level. Normalized data were then compared for differential gene expression analysis across time points between sham and injured groups. Genes that showed a Welch analysis of variance t test p value <0.05 between sham and injured groups for at least one time point were retained for further analysis. Initial data analysis also included a fold change filter of >1.5 (50% difference) increase or decrease relative to sham operated animals (Affymetrix MAS 5.0). While a p value of <0.05 alone would give many false positive probe sets, the combination of post-amplification, fold change thresholds, and p values thresholds, eliminates most false positives that are obtained with only p < 0.05.

Real Time RT-Multiplex-PCR

We studied selected transcripts by real time PCR using cDNAs extracted from cords of rats subject to spinal cord trauma and sham controls in parallel experiments, at the time point 14 days, to validate our microarrays findings. Fluorophore-labeled LUX primers (forward) and their unlabeled counterparts (reverse) were provided by Invitrogen. LUX primers were designed matching the probe sets sequences for Ninjurin, Rap1b, Rab13, and Corolin 1b and all primers were designed using the software called LUX Designer (Invitrogen, www.invitrogen.com/lux). Each primer was chosen matching the target probe set sequence for the gene of interest. More in detail, primer sequences were:

Ninjurin (forward: cacctTCCGTTTACAGGCGAACG5G5G), reverse: (TTTCCATGGTTTGTGCAGCAC), Rab13 (forward: caacagcAGAACAGGGTGGACAC, reverse: TACGTTGCGAGGCTCTGCT), Rap1b (forward: cacagcTCAGGGTGTGCTGCG, reverse: TTTCCACAATCCATAC), and Corolin 1b (forward: caacTGGACAGCTGTCAGG5G), reverse: GCACATTTGGGCCTGTGGT). We performed multiplex PCR using each experimental gene with the housekeeping gene GAPDH. For each sample, as previously detailed, 50–µl PCR contained 2 µl of cDNA, 10 µl of each gene-specific primer (two pairs for multiplex PCR) and 1× Platinum Quantitative PCR SuperMix-UDG (Invitrogen). Reactions containing fluorogenic LUX primers included 1× SuperMix and were incubated at 25 °C for 2 min, 95 °C for 2 min, and then cycled (45×) using 95 °C for 15 s, 55 °C for 30 s, and 72°C for 30 s, and reactions were incubated at 40 °C for 1 min and then ramped to 95 °C over a period of 19 min followed by incubation at 25 °C for 2 min (ramp for melting curve analysis). Reactions were conducted in a 96-well fluorospectrophotometric thermal cycler (ABI PRISM 7700 Sequence detector system, Applied Biosystems). Fluorescence was monitored during every PCR cycle at the annealing or extension step and during the post-PCR temperature ramp. Fold changes were calculated following the manufacturer’s instructions (Invitrogen).

Immunoblotting

Cords used for immunoblotting included samples from three injured and two sham spinal cords at the time points 7 and 14 days after injury. For E14 DRGs, 600–600 DRG were isolated for each time point, and E14 DRGs from six well plates for each time point were cultured in Neurobasal medium onto glass coverslips (circular, 13 mm in diameter). Immunofluorescence of cultured cells was performed with 

E14 dorsal root ganglia cells

Immunocytochemistry

Spiral Corde—12-µm frozen sections from three injured and two sham spinal cord sections were cut at 0.4 and 0.5 cm below or above the injury site epicenter, as previously reported (18). Sections were incubated under the same coverslip and processed for immunocytochemistry. They were first dried at room temperature, fixed in 4% paraformaldehyde, rinsed in PBS and incubated with 10% normal serum and 0.2% Triton X in PBS (goat or rabbit depending on the secondary antibodies used) for 60 min to mask nonspecific adsorption sites. Sections were then incubated overnight at 4 °C with one or more of the following primary antibodies: rabbit polyclonal anti-coronin 1b (1:100, custom antibody from Bethyl Laboratories), and rabbit polyclonal anti-Rab13 (1:1000, custom antibody from Bethyl Laboratories).

Omission of the primary antibodies or their replacement by preimmune sera was used for control experiments. Immunocomplexes were visualized with ECL chemiluminescence (Amersham Biosciences).

Dorsal Root Ganglia (DRG) Neurons—E14 dorsal root ganglia cells cultured in Neurobasal medium onto glass coverslips (circular, 13 mm in diameter). Immunofluorescence of cultured cells was performed with 

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RESULTS

Identification of Putative Pro-plasticity Gene Cluster Induced between 7 and 28 Days following SCI—Approximately 24,000 probe sets were examined using Affymetrix high density oligonucleotides arrays (U34 A, B, and C chips) at 4 h, 24 h, 7 days, 14 days, or 28 days after SCI. We applied a present call noise filter (1 in 79 arrays), fold change thresholds (>1.5), and a p value of less than 0.05 as our initial data set for preliminary analysis and pathway identification. This analysis led to further consideration of about 6000 transcripts, of which 5% would be expected to be false positives. Files from all 79 arrays are available from our website, including *.dat, *.cel, and *.txt files (pepr.cnmrcresarch.org/browse.do). This website includes an on-line time series query tool of this data set (pepr.cnmrcresarch.org/jsf/searchForm.jsp). The spreadsheets showing all genes profiling preliminary analysis criteria are also available on the website. A more complete analysis of the global gene changes has also been previously reported by us (18).

Among the significantly altered transcripts, we searched for transcripts previously implicated in neuronal-axonal repair and plasticity after injury. We identified a transcript corresponding to Ninjurin (nerve injury-induced protein), a cell adhesion molecule that is up-regulated after axotomy in neurons and in Schwann cells surrounding the distal nerve segment, and promotes neurite extension of dorsal root ganglion neurons in vitro (20). We then used Ninjurin as an "anchor gene" to fish out a cluster of temporally correlated genes that could potentially have a similar function that is involved in neuronal plasticity after SCI. We used the gene array analysis software GeneSpring (as previously detailed, Refs. 18 and 21) that allows identification of gene profiles with similar temporal regulation, and identified a cluster of temporally correlated genes (correlation coefficient, $R^2 = 0.98$) overexpressed between 7 and 28 days after injury, that included 20 transcripts. Functional classification showed that this group of genes was variably associated with immune response, cell adhesion, axon migration, polarity, growth, guidance, dendrite elaboration, plasticity, and synapse formation (Table I). To further narrow the field to identify a more specific group of temporally correlated transcripts, we performed a more stringent correlation analysis using both Standard and Pearson correlation ($R^2 = 0.99$), which identified five genes other than Ninjurin: Coronin 1b, Rab13, Synaptogyrin, and Synaptotagmin (Fig. 1). Synaptic vesicles associated proteins Synaptotagmin and Synaptogyrin have been localized at the synaptic terminals in several neuronal types and in PC-12 cells, and play a role in synaptic plasticity, neurotransmitter release and neurite outgrowth (for review, see Refs. 22–24). Rap1 is a small GTPase involved in several signal transduction pathways, and is induced by NGF in PC12 cells and is necessary for neurite outgrowth (25, 26). Coronin 1b is an actin-binding protein involved in cytoskeleton remodeling (for review, see Ref. 27), Rab13 is another small GTPase, belonging to the rab family involved in vesicle transport (for review, see Ref. 28). Analogs of Coronin 1b (cijpin C) and a Rab family member (Rab8) have also been localized at cellular protrusions, and neurite tips in PC12 and SH-SY5 cells (29, 30). Therefore, based upon their temporal co-regulation with the other members of the cluster, and upon their known or putative biological function, Coronin 1b, Rab13 appeared to be factors involved in neurite outgrowth and differentiation.

Real Time PCR Confirmed Microarray Results—Real time PCR experiments using cDNAs from a parallel set of animals at 14 days after SCI was performed on several members of the gene cluster to validate results obtained by microarrays. The
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Comparison of expression levels between sham and injured cords for Ninjurin cluster transcripts induced 14 days after SCI

These transcripts represent genes that are temporally co-regulated with Ninjurin gene based upon a standard correlation coefficient \( R^2 \) of 0.98. GenBank™ gene names, fold changes, and \( t \)-test \( p \) values are reported for each transcript.

| Fold change | \( t \)-test \( p \) value (injured 14 d vs sham 14 d) |
|-------------|-----------------------------------------------|
| GenBank™ Gene name |                                         |
| AI232103 | Collagen XVIII | 2.34 | <0.01 |
| AI169104 | Platelet factor 4 (Pf4) | 4.22 | <0.05 |
| AI104907 | Protein degradation (Pame4) | 2.67 | <0.01 |
| AI009460 | Filamin | 1.87 | <0.05 |
| AA925081 | Synaptotagmin | 1.52 | <0.05 |
| AA924748 | Synaptobrevin family (Vamp 5) | 2.07 | <0.05 |
| AA799879 | Synaptogyrin | 2.1 | <0.05 |
| X82396 | Cathepsin B | 1.65 | <0.01 |
| X81654 | CD63 antigen | 1.43 | <0.01 |
| X07365 | Glutathione peroxidase 1 | 1.87 | <0.05 |
| U72660 | Ninjurin | 2.62 | <0.01 |
| U47031 | P2x4 | 2.96 | <0.05 |
| M3678 | RAB13 | 2 | <0.05 |
| M31018 | RT1 class Ib gene | 1.79 | <0.05 |
| M24026 | RT1 class Ib gene | 1.81 | <0.05 |
| L40362 | RT1 class Ib gene | 1.59 | <0.01 |
| D89655 | CD36 antigen | 2.32 | <0.05 |
| AJ048984 | Coronin, actin-binding protein, 1B | 1.25 | <0.05 |
| AF083209 | Actin-related protein complex 1b (Coronin 1b) | 1.96 | <0.01 |
| AA893443 | RAP1B | 1.44 | <0.05 |

Fig. 1. Dendogram showing temporally co-regulated transcripts induced between 7 and 28 days after SCI. Shown is a dendogram (gene tree) obtained using Ninjurin as anchor gene to nucleate co-regulated transcripts based upon a correlation coefficient \( R^2 \) of 0.99 (both Standard and Pearson correlation). Expression levels in sham and injured cords at different time points after SCI are represented with a specific color code. Bar graph on the right shows the color code for gene expression level from low (blue) to high (red). These transcripts are up-regulated between 7 and 28 days after SCI (asterisk indicates a Welch \( t \) test \( p \) value <0.05 between sham and injured at the corresponding time point).

TABLE II

Comparison of mRNA changes by Affymetrix and real time RT-PCR for several transcripts up-regulated 14 days following SCI

| Gene name | Affymetrix fold changes | \( p \) value | Real time PCR fold changes | \( p \) value |
|-----------|-------------------------|-------------|---------------------------|-------------|
| Ninjurin  | 2.62 ± 1.25 | <0.01 | 6.60 ± 0.91 | <0.01 |
| Synaptotagmin | 2.1 ± 0.21 | <0.01 | 2.06 ± 0.86 | <0.01 |
| Rab13 | 2.00 ± 0.48 | 0.023 | 7.76 ± 0.94 | <0.01 |
| Rap1b | 1.44 ± 0.51 | <0.01 | 3.92 ± 0.78 | <0.01 |
| Coronin 1b | 1.8 ± 0.47 | <0.01 | 3.5 ± 0.54 | <0.01 |

In Vitro Overexpression of Selected Gene Cluster Members in PC-12, SH-SY5 Neuroblastoma, and DRG Cells Promotes Neurite Outgrowth—In order to evaluate whether gene cluster members Ninjurin, Rap1b, Synaptotagmin, Coronin 1b, and Rab13 were involved in neuronal plasticity, we tested them using in vitro assays. We transfected plasmid cDNAs for each gene and overexpressed them in the neuronal-like cell lines PC-12, and SH-SY5 neuroblastoma cells, as well as in rat primary DRGs, evaluating neurite outgrowth by measuring the longest neurite per cell, and the number of neurite branches. Percentage of cells with neurites lengths at least two times the cell bodies were evaluated in PC-12 and SH-SY5 neuroblastoma cells. Neurite length as percentage of length in control cells was measured in DRG-transfected cells. Transfected genes promoted neurite outgrowth in the two cell lines (Fig. 2, panels A and B), as well as in DRGs (Fig. 2, panel C). These data confirmed and extended previous findings for Ninjurin, Rap1, Synaptotagmin, and Synaptogyrin, which suggested a possible role for these factors in neurite extension and neuronal differentiation; they also suggested a novel function for Coronin 1b, and Rab13 in neurite outgrowth. The greatest effect on neurite outgrowth was achieved with Ninjurin, Rab13, and Coronin 1b. Ninjurin increased the number of cells with extended neurites by 2.3-fold in PC-12 cells and by 5.2-fold in SH-SY5 neuroblastoma cells. Rab13 overexpression resulted in 2.7- and 9-fold increase, respectively, in PC-12 and SH cells. Coronin 1b induced a 2.4-fold increase in PC-12 and 5.4 in SH cells. Experiments in primary neuronal cells, such as DRGs, also confirmed the significant effect of these genes on neurite...
outgrowth. Ninjurin increased neurite length by 52%, Rab13 by 54%, and Coronin 1b by 63% (Fig. 2). The number of neurite branches was not affected by transfections.

Expression of Rab13 and Coronin 1b in Cultured DRG Neurons—Further in vitro and in vivo experiments were conducted for Coronin 1b and Rab13, whose roles in the nervous system are still largely unknown. To verify if Coronin 1b and Rab13 protein expression were regulated during neurite outgrowth in cultured DRG neurons, we performed immunoblotting experiments in E14 DRG cells from days 1–7. Coronin 1b and Rab13 expression were strongly induced between 1 and 3 days (Fig. 3). Immunofluorescence experiments at days 1, 3, 5, 7, 9, and 14 in culture showed expression for Coronin 1b and Rab13 in the cytoplasm, and in particular at the cortical cytoskeleton as shown by co-localization with F-actin (data not shown); they were also found at the neurite terminals and growth cone, where staining pattern suggested co-localization with the plasticity/regeneration marker GAP-43 (Fig. 4). Consistent with immunoblot experiments, an increased immunofluorescence signal was detected for both proteins between days 1 and 3. No difference in the intensity of the staining was observed beyond day 3 (data not shown).

RNA Interference Experiments Show That Coronin 1b and Rab13 Repression Inhibit Neurite Extension in PC-12 Cells after NGF—To examine whether members of this gene cluster were involved in neurite outgrowth during differentiation, we performed gene silencing experiments using RNAi for Coronin 1b and Rab13 in PC-12 cells treated with NGF. Coronin 1b and Rab13 RNAi gene silencing, which resulted in strong reduction of protein expression (Fig. 5, panels A and C) significantly reduced the extent of neurite outgrowth in PC-12 cells after NGF treatment by 53 and 44%, respectively (Fig. 5, panels B and D).
Coronin 1b, and Rab13 Protein Expression Is Induced after SCI Preferentially in Neurons and Axons Undergoing Plasticity—

The combination of the gene expression changes in vitro and the findings from the experiments in vivo suggested that these genes and proteins may modulate neural plasticity in the spinal cord after injury. In accordance with gene array experiments, we examined changes in protein expression by immunoblotting for Coronin 1b and Rab13 in injured spinal cord at 7 and 14 days post-trauma and compared them to corresponding sham samples. Both proteins were similarly increased after injury at these time points (Fig. 6). We then studied expression of these proteins at the cellular level by immunofluorescence in sham and injured spinal cords 14 days after injury. The proteins were co-examined with cell specific markers for neurons (anti-NeuN, and anti-β-III tubulin antibody), astrocytes (anti-GFAP), oligodendrocytes (anti-O2), and microglia (anti-CD11). Increased expression for Coronin 1b and Rab13 were found after injury (Fig. 7, A and B), primarily in the cell membrane and cytoplasm, and showed a preferential localization in neurons. Only weak expression was detected in astrocytes or in microglial cells (data not shown).

To study the possible involvement of these proteins in neuronal and axonal plasticity, we examined the degree of Coronin 1b, and Rab13 co-expression and co-localization with the well established pro-plasticity/regeneration protein GAP-43. Using confocal microscopy, immunofluorescence experiments showed that the proteins were highly expressed in GAP-43-positive neurons and axons after injury, including expression in dorsal root ganglia, and each likely co-localized with GAP-43 (Fig. 8).

**DISCUSSION**

Using a hypothesis-driven approach, combined with specific bioinformatics tools to analyze coordinated gene expression profiles, we have identified a cluster of genes induced between...
gyrin, and Rap1b, have already been partially characterized in injury (20, 35).

Reduced by peripheral nerve injury, and promotes axonal and adhesion molecule Ninjurin has also been reported to be in-

way, leading to axonal elongation in PC-12 cells (26). The cell plays a role in the activation of the NGF-dependent ERK path-

NGF, and can promote neurite outgrowth (34). Rap1 itself is a small GTPase belonging to Ras superfamily of proteins, whose mem-

member, which regulates intracellular vesicle trafficking to and from the plasma membrane, and mediates exocytosis within the trans-Golgi network (TGN) (28). Interestingly, Rab8, an analog to Rab13, also belonging to TGN network, has been found on cellular protrusions at the cortical cytoskeleton, and is co-localized with actin filaments (30). Coronin 1b is an actin-binding protein, belonging to the Coronin family, and may bind also to tubulin (27). It is important in cytoskeleton remodeling, lamellipodia extensions and mitosis; moreover,
Coronin 1b is localized to the cortical cytoskeleton, where it is co-localized with F-actin (36, 37). It is known that cytoskeletal organization and remodeling are essential cellular modifications during sprouting and axonal elongation (for review, see Ref. 38). Importantly, cilpin C, a human Coronin-like homolog protein, which is highly expressed in brain, has been identified in stress fibers and neurite tips in SH-SY5 neuronal cells during neurite outgrowth (29).

Based upon these functional features of the identified gene cluster, transfection experiments were performed in PC-12, SH neuroblastoma neuronal like cell lines, as well as in DRG neurons, in order to examine whether expression of these genes promote neurite elongation or sprouting. While expression of each of these genes enhanced neurite outgrowth, Ninjurin, Rab13, and Coronin 1b were most effective. Moreover, Coronin 1b, and Rab13 RNAi experiments in PC-12 cells showed that inhibition of Coronin 1b, and Rab13 protein expression reduced neurite outgrowth after NGF treatment. While Rab13 gene knockout has never been reported before, previous work in Dictyostelium discoideum, showed that mutant cells lacking Coronin grow and migrate more slowly than wild-type cells, probably by affecting cytokinesis (31). Also, in vertebrate cells Coronin expression at lamellipodia was disrupted by overexpression of truncated mutants, inhibiting cell spreading and locomotion (32).

In cultured DRG neurons, Coronin 1b, and Rab13 were also co-expressed with GAP-43 at neurite terminals and growth cones, and their expression increased during the first days in culture along with the increase in neurite outgrowth. Parallel studies in vivo demonstrated that Coronin 1b, and Rab13 protein expression were increased at both 7 and 14 days after rat SCI. They were primarily expressed in neurons, although low level of expression was also present in astrocytes. Moreover, they were co-expressed and likely co-localized with the pre-regeneration marker GAP-43 in neurons and axonal membranes throughout the spinal cord, as well as in DRGs. The possible functional relationship between cytoskeleton and membrane-bound proteins Coronin 1b, Rab13 with GAP-43 is also supported by the fact that GAP-43 itself accumulates in the pseudopods of spreading cells and interacts with cortical actin-containing filaments and the cell membrane at the growth cones (11).

Taken together, these data support a role for Coronin 1b and Rab13 in neuronal and axonal plasticity. For most effective regeneration to occur after injury, multiple molecular pathways may need to operate together in a coordinated fashion. These include the ability of the damaged axons to extend the growth cone, to make cell contacts with the extracellular matrix (guidance), to form connections with nearby cells, and to achieve functional synapses. The genes and proteins in the gene cluster here described play a known or putative role in several of the above mechanisms; together their coordinated action may induce more effective plasticity and regeneration than that resulting from expression of a single protein.

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