Mini-Symposium

Transcriptomic Approaches to Neural Repair

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Understanding why adult CNS neurons fail to regenerate their axons following injury remains a central challenge of neuroscience research. A more complete appreciation of the biological mechanisms shaping the injured nervous system is a crucial prerequisite for the development of robust therapies to promote neural repair. Historically, the identification of regeneration associated signaling pathways has been impeded by the limitations of available genetic and molecular tools. As we progress into an era in which the high-throughput interrogation of gene expression is commonplace and our knowledge base of interactome data is rapidly expanding, we can now begin to assemble a more comprehensive view of the complex biology governing axon regeneration. Here, we highlight current and ongoing work featuring transcriptomic approaches toward the discovery of novel molecular mechanisms that can be manipulated to promote neural repair.

Significance Statement

Transcriptional profiling is a powerful technique with broad applications in the field of neuroscience. Recent advances such as single-cell transcriptomics, CNS cell type-specific and developmental stage-specific expression libraries are rapidly enhancing the power of transcriptomics for neuroscience applications. However, extracting biologically meaningful information from large transcriptomic datasets remains a formidable challenge. This mini-symposium will highlight current work using transcriptomic approaches to identify regulatory networks in the injured nervous system. We will discuss analytical strategies for transcriptomics data, the significance of noncoding RNA networks, and the utility of multiomic data integration. Though the studies featured here specifically focus on neural repair, the approaches highlighted in this mini-symposium will be of broad interest and utility to neuroscientists working in diverse areas of the field.

Introduction

Unlike neurons of the peripheral nervous system (PNS), those of the CNS do not spontaneously regenerate their axons following injury; hence, trauma to the brain or spinal cord frequently results in permanent neurological deficits. The development of effective therapies that can restore lost neurological function is therefore critically dependent on strategies to promote robust axon regeneration. Over the past several decades, a great body of work has chipped away at elucidating the biological mechanisms limiting the regeneration of injured CNS axons. We now recognize that a host of extrinsic factors—chiefly, the presence of myelin-associated inhibitors such as Nogo, OMgp, MAG, and netrins (Filbin, 2003; Giger et al., 2010; Geoffroy and Zheng, 2014); deposition of a reactive glial scar tissue surrounding the lesion site (Silver and Miller, 2004; Yiu and He, 2006; Busch and Silver, 2007; Fitch and Silver, 2008); a paucity of supportive growth factors (Gordon, 2009; Harvey et al., 2012; McCall et al., 2012), as well as persistent inflammation (Donnelly and Popovich, 2008; Benowitz and Popovich, 2011; Raposo et al., 2014)—contribute to an extracellular milieu that is highly inhibitory for axon regeneration. It is also clear that the growth capacity of CNS

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neurons declines sharply after development, and that their ability to assume an intrinsic growth-enabled state following injury is tremendously limited. This is in stark contrast to dorsal root ganglion (DRG) neurons of the PNS, which exhibit rapid and prolonged activation of an injury-induced transcriptional program encompassing hundreds of regeneration associated genes, enabling robust regeneration of injured axons (Seijffers and Benowitz, 2008). The evident failure of CNS neurons to activate such a transcriptional response to injury has led to a major ongoing effort to identify genes and signaling pathways that can be manipulated to promote neuronal intrinsic growth capacity in the injured CNS.

Historically, single-gene manipulation in experimental PNS and CNS injury models has served as the dominant approach in the search for key molecular players of regeneration programs. In this way, several genes and signaling pathways whose manipulation can enhance (or inhibit) axon regeneration have been identified (Seijffers and Benowitz, 2008; Liu et al., 2011; Doron-Mandel et al., 2015). However, the robust regrowth of injured peripheral axons is associated with the activation of broad genetic programs in the soma (Blackmore, 2012). Thus, many questions still remain to be answered. What are the core, upstream molecular regulators of transcriptional programs driving regeneration in the PNS? Beyond gene expression, what other molecular factors—noncoding RNAs, epigenetic modulators, small molecules, and post-translational modifications—might be critical determinants of regeneration? How do the intrinsic responses of different types of neurons to axotomy differ? Importantly, can recapitulating developmental or peripheral growth programs enable regeneration in multiple distinct classes of CNS neurons?

The advent of transcriptomics has equipped us with the ability to address such questions with greater sensitivity and resolution than previously possible (Belgard and Geschwind, 2014). We can now take transcriptional "snapshots" of specific neuronal and glial subtypes, during development or in adulthood, within the axon or the cell body, from minutes after axotomy to the far chronic stage of injury. Moreover, next-generation RNA sequencing (RNA-Seq) can give us multidimensional insight into the transcriptional state of a tissue or cell population, yielding information about alternative splicing and novel isoforms (Roberts et al., 2011; Voineagu et al., 2011), diverse classes of noncoding RNA (Shao et al., 2010; Belgard et al., 2011), and post-transcriptional RNA editing (Danecek et al., 2012). Although high-throughput omics are sometimes criticized as "fishing expeditions," discovery-based transcriptional profiling is proving to be a powerful engine for the generation of novel testable hypotheses (Belgard and Geschwind, 2014). Indeed, we are now beginning to appreciate the potential of high-throughput screens to enrich and accelerate neuroscience research. Here, we provide an overview of current work that is united by a common theme: the use of transcriptomics in the search for biological targets to promote neural repair.

Mechanisms of peripheral axon regeneration

Coding and noncoding RNA networks in regenerating PNS neurons (J. Lerch)

Strategies to identify gene networks involved in the axon regeneration process have used microarray and/or subtractive hybridization analysis extensively to compare expression in regenerating (PNS or early embryonic and postnatal neurons) and non-regenerating (adult CNS neurons) populations (Kubo et al., 2002; Smith et al., 2011; Buchser et al., 2012; Siddiq et al., 2015). These approaches have identified numerous transcription factors as master regulators of axon regrowth, including, for example, Stat3 (Smith et al., 2011); Klf4, Klf6, and Klf7 (Stam et al., 2007; Moore et al., 2009; Blackmore et al., 2010); and Atf3 (Stam et al., 2007).

We now appreciate that gene expression regulatory networks extend beyond single or multiple differentially expressed transcription factors to include a variety of regulatory RNAs [e.g., microRNAs (miRNAs); Lee et al., 1993]. Approximately 92–94% of human genes undergo alternative splicing that is frequently tissue specific or disease dependent (Wang et al., 2008), and remarkably little is understood about the functions of these specific transcripts or how they are regulated. RNA-Seq technology greatly improves upon and expands our ability to understand gene expression network regulation, because, unlike microarrays, it can identify isoform-specific gene regulators (Mortazavi et al., 2008; Langmead et al., 2009; Trapnell et al., 2009; Yuan and Licatalosi, 2014). RNA-Seq allows us to define the precise transcription start site and 3′ untranslated region (UTR) for each differentially expressed isoform of a gene. This information can be used to determine isoform-specific *cis*-regulatory elements, such as transcription factor binding sites in the promoter region and miRNA binding sites in the 3′UTR.

We took such an approach to characterize the regulators of isoform diversity in purified regeneration-enabled sensory neurons and non-regenerating central neurons (Lerch et al., 2012). We detected >8000 differentially expressed isoforms between these two populations, and observed higher overall isoform expression and diversity in DRG neurons. Notably, among these were multiple isoforms of Atf3, Klf7, Pten, and Stat3, implicating potential roles for cell-specific isoforms in modulating the activity of these known transcriptional regulators of axon regeneration. We next sought to identify potential interaction networks regulating isoform diversity in central and peripheral neurons. To accomplish this, we used bioinformatics analysis to identify transcription factor binding sites and miRNA response elements that were enriched in the regulatory regions of genes with cell-specific isoform expression. We employed hierarchical clustering analysis to identify clusters of transcription factor binding sites and miRNA response elements enriched in regenerating DRG neurons, then used these clusters to generate an interaction network between transcription factors and miRNAs. A major node in this network was serum response factor, which is known to be critical for embryonic DRG target innervation, cytoskeletal dynamics, and CNS neurite outgrowth (Knöll et al., 2006; Wickramasinghe et al., 2008), as well as axon regeneration in facial nerve (Stern et al., 2013). Together, these experimental validations support our *in silico* approach to predicting key upstream regulators of sensory axon regeneration. We can now go beyond *in silico* prediction of regulatory RNA expression and can profile the expression of miRNAs as well as long noncoding RNAs in RNA-Seq datasets. Future studies that combine RNA-Seq with regulatory RNA expression profiling will significantly extend our understanding of the RNA interaction networks that regulate gene expression to promote or inhibit axon growth.

Transcriptional programs activated by cAMP-induced CREB-sufficient/activator protein 1-dependent growth programs (D. Willis)

Studying the transcriptional response of regeneration-competent neurons to axon injury has implicated several individual regeneration associated genes in axon regeneration; hence, many studies have investigated the specific contributions of such genes to the regeneration process. However, the response to injury likely involves

regulated expression of functionally coordinated gene groups, emphasizing the importance of the transcriptional regulation of gene programs. cAMP-mediated gene transcription is an important contributor to the injury-induced regeneration associated gene response seen in peripheral DRG neurons (Smith and Skene, 1997; Gao et al., 2004; Hannila and Filbin, 2008), but the upregulation of cAMP alone may not harness the robustness of the injury response. In studies directly comparing axon growth following injury with other stimuli that increase cAMP (Udina et al., 2008; Blesch et al., 2012), the peripheral conditioning lesion shows superior efficacy for axonal regeneration.

Our work has focused on characterizing the transcriptional determinants of cAMP actions. Our initial focus was CREB, as it is a well characterized transcriptional mediator of cAMP signaling (Lonze and Ginty, 2002). Using a constitutively active version of CREB (CREB-CA), we found that either driving CREB activity in DRG neurons or the application of dibutyryl-cAMP (db-cAMP) alone enhanced axon growth to a similar degree. Surprisingly, axon growth was significantly increased when CREB-CA-expressing neurons were treated with db-cAMP, which was paralleled by an increase in the transcription of candidate genes. This suggested that levels of transcription stimulated by CREB or cAMP alone were insufficient for the maximum observed axon growth. This put forth the notion that CREB may serve as an important hub for regeneration, but that physiological activation of CREB after injury is insufficient to recruit the network needed to promote robust regeneration (Ma et al., 2014). Using arginase 1 as a model regeneration associated gene, we identified a binding site for the transcription factor activator protein 1 (AP-1) in the proximal promoter. Blocking AP-1 activity with a dominant-negative Fos inhibited both CREB-CA and cAMP-mediated axon growth. Constitutively active Fos only modestly increased axon growth, suggesting that the AP-1-controlled genes cooperate with other CREB targets to stimulate regeneration (Ma et al., 2014).

The strong induction of regeneration associated gene transcription by cAMP plus CREB-CA may induce supraphysiological expression of injury-induced genes. Driving AP-1 activity plus cAMP alone did not recapitulate the effects of CREB-CA plus cAMP, further reinforcing the notion that the successful axon regeneration requires a concerted and broad transcriptional response. Indeed, the strong activation of CREB recruited other previously identified hub proteins, such as ATF3, suggesting that these responses may contain functional modules that mediate specific aspects of axon growth (Ma et al., 2014). In ongoing work in our laboratory, the analysis of this CREB-activated, AP-1-dependent gene module by nonbiased weighted gene coexpression network analysis (WGCNA) of RNA-Seq data shows that >1600 genes belong to this group. Many of these genes are traditional regeneration associated genes with experimentally validated roles in the axon regeneration process, though others may be nonessential for regeneration. Understanding the differences between these two populations may be critical for identifying the "optimal" approach for driving regeneration.

Identification of a core transcriptional regeneration program using a comprehensive systems biology approach (V. Chandran)

As illustrated above, comparing gene expression profiles across multiple regenerating and non-regenerating neuronal populations is a powerful strategy for the identification of growth-associated signaling networks. Our understanding of the complex regulation of such networks can be greatly enhanced by bioinformatics tools that enable the identification of upstream transcriptional regulators, correlation of RNA-Seq and protein expression data, and the prediction of small molecules that can activate the expression of genetic programs. Our group recently took such an approach to identify core transcriptional programs driving the regeneration of injured peripheral axons (Chandran et al., unpublished observations). By applying WGCNA to transcriptomics data derived from multiple, independent peripheral nerve injury experiments, we defined 14 gene coexpression modules that were highly preserved across sciatic nerve lesion datasets. Of these, two modules of upregulated genes and three modules of downregulated genes were strongly associated with regeneration, and gene ontology analysis of these modules revealed strong enrichment for terms related to regeneration and axon outgrowth. Upon examining the top interconnected hub genes across these modules, we found that coexpression was reliably preserved across PNS but not CNS injury datasets, supporting the idea that CNS neurons fail to activate transcriptional regeneration programs.

To identify potential upstream regulators of these modules, we performed transcription factor binding site analysis and found that the top two modules of "up" genes were enriched for binding sites of 15 transcription factors associated with neuronal injury and axonal regeneration; remarkably, eight of these (Atf3, Egr1, Fos, Jun, Myc, Rela, Smad1, and Stat3) were themselves present in the top up module. Moreover, several of these transcription factors exhibit experimentally validated physical interactions. Together, our findings indicate that the activation of a core transcriptional network, involving interactions between multiple transcription factors, coordinates gene expression to promote axon regeneration after peripheral nerve injury. Hence, recapitulating peripheral growth programs in non-regenerating neuronal populations is likely to be achieved by activating this network as a whole, rather than manipulating single genes.

We compared our regeneration associated gene network to drug-related expression profiles available from the Connectivity Map (www.broadinstitute.org/cmap; Lamb et al., 2006) to identify small molecules that can mimic the gene expression program induced by sciatic nerve injury. Among the drugs with top matching expression patterns was a sodium and calcium channel blocker used clinically as a mucolytic. Treating DRG neurons with this candidate drug *in vitro* induced the expression of regeneration associated genes, including Atf3, Fos, Jun, Smad1, and Sp1, and significantly enhanced neurite outgrowth. We predicted that if treatment with this small molecule could activate transcriptional regeneration programs in CNS neurons, it might thereby promote regeneration of injured CNS axons. Indeed, we observed a significant enhancement of optic nerve axon regeneration (1.5-fold increase between 200 and 500 μm, $p < 0.04$) at 2 weeks following treatment with this candidate drug after optic nerve crush; furthermore, drug treatment further increased axon regeneration in *Pten*$^{-/-}$ mice at 2 weeks following crush (2.9-fold increase at 2500 μm, $p = 0.02$). Together, these findings validate our comprehensive systems biology approach taken to identify such transcriptional networks and potential small-molecule regulators, and provide strong support for the hypothesis that the activation of core regeneration-associated transcriptional programs in injured CNS neurons can promote axon regeneration.

Data analysis approaches to identify genetic pathways dysregulated upon PNS injury (A. Antunes-Martins)

High-throughput transcriptomics technologies are able to query virtually the entire genome (Hammer et al., 2010; Perkins et al., 2014), providing comprehensive information about the overall transcriptional profile of the tissue of interest. Extracting useful information from such rich datasets relies on integrative approaches that identify

commonalities across the set of genes of interest; use gene ontology, regulatory, and protein interaction information to capture the biological processes compromised in the system of interest; and can find regulators amenable to functional intervention. Injury to the peripheral axons of sensory neurons results in marked transcriptional dysregulation in the cell body. This includes genes involved in the injury response and axon outgrowth as well as genes that shape the pathogenesis of neuropathic pain (Hammer et al., 2010). Understanding the relationship of altered gene expression to these distinct functional outcomes is an important challenge requiring thoughtful integration of data.

Work in our laboratory has focused on using transcriptional profiling to identify biological pathways, dysregulated upon nerve injury, that contribute to the development of injury-associated neuropathic pain; hence, we have examined different analytical approaches to identify relevant biological networks in transcriptomics data generated from DRG neurons following sciatic nerve or sham injury. We investigated the time course of gene expression after sciatic nerve crush in the absence of neuregulin-1 (Nrg1) signaling, using a tamoxifen-inducible mouse model (Fricker et al., 2013). Such a study required a complex design accounting for multiple time points postinjury and multiple genotypes. Although pairwise comparisons of genotype effects at each time point were informative, a clear picture of the role of Nrg1 in regeneration was better observed when genes were clustered according to overall expression patterns (Theocharidis et al., 2009). This approach allowed the identification of clusters of genes that, despite following a parallel pattern of activation in both genotypes, were dysregulated to a different extent in Nrg1 mutants, as well as clusters of genes where levels of expression remained unchanged after injury. Additional advantages of clustering approaches stem from the ability of the approach to account for variability within experimental groups, thus avoiding missing out on relevant genes that would not reach statistical significance due to high variability within a given experimental group.

Bioinformatics analysis of defined subsets of genes can provide general information about normal physiological or pathological mechanisms in experimental systems, highlighting the biological mechanisms involved. Interrogation of gene ontology, using a variety of web-based resources and commercial packages, allows the experimenter to identify statistically overrepresented annotations. Gene ontology information can be general (e.g., enrichment in "inflammation-related genes" 7 d after sciatic nerve transection; Perkins et al., 2014) or more detailed in relation to pathological mechanisms. For example, we observed an overrepresentation of myelination-related functions among the clusters of genes expressed at lower levels in *Nrg1*-mutant mice 10 d after nerve crush, an observation that is consistent with observations of slower remyelination in the mutants (Fricker et al., 2013). More importantly, expression data can also be used to infer regulatory networks. In a very elegant study, Chang et al. (2013) developed InteGraNet, a computational pipeline that allows the combination of transcriptomics data with transcription factor and miRNA–target interaction data (verified and predicted). This pipeline was tested in a model of spinal cord gene expression following peripheral nerve injury to characterize the network of miRNA and transcription factor interactions governing various coexpression clusters of injury-response genes. The authors identified a network of regulatory interactions around the early growth response-2 (*Egr2*) gene. Such integrative approaches also allow the prediction of relevant canonical pathways, the activation status of signaling pathways based on the differential expression of known downstream target genes, or whether any these pathways are amenable to intervention by chemical compounds. Other insights into signaling can be provided by combining transcriptomics data with phosphoproteomics data (Michaelevski et al., 2010). Protein interaction data can also provide useful insights into the involvement of the candidate gene or genes of interest in particular, and can be integrated with gene expression data. Resources such as PainNetworks (www.painnetworks.org) allow the visualization of proteins encoded by the genes of interest alongside known interactors and thus infer putative involvement of the proteins of interest in the same mechanisms as their interaction partners.

An important consideration in the interpretation of transcriptional profiling data at the whole-tissue level stems from heterogeneity in the overall cellular composition of the tissue. Analysis of whole tissue samples can make it difficult to determine whether differential expression results from the activation or repression of transcriptional programs in the cells of interest, or from the presence of infiltrating cells that may dilute the contribution of resident cells to the overall transcriptome. Recent progress in the field has equipped us with powerful methods such as magnetic cell sorting (Thakur et al., 2014), fluorescence-activated cell sorting (Chiu et al., 2014), laser-capture microdissection (Solga et al., 2015), RiboTag (Sanz et al., 2009), and translating ribosome affinity purification technology (Heiman et al., 2008), which allow the investigation of transcriptional changes that operate in a cell type-specific manner. As newer methods such as single-cell transcriptomics (Tang et al., 2011) become more commonplace, it will become easier to characterize how specific cell types in the nervous system differentially respond to perturbations such as injury.

Mechanisms of regeneration in the CNS

CNS axon regeneration via activin signaling (M. Costigan)

Axonal regeneration is almost completely absent in the injured adult mammalian CNS. This loss in growth competence likely evolved to protect CNS synaptic structure, allowing us to retain our thoughts at the cost of overall plasticity (Sun and He, 2010). In the mammalian PNS and, crucially, in the CNS of some lower organisms, neurons, once damaged, can functionally regenerate (Huebner and Strittmatter, 2009; Zhang et al., 2012). Peripheral nerves regenerate by a complex set of changes including Wallerian degeneration, a transdifferentiation of the distal region of the nerve into a growth permissive state to allow subsequent axon regrowth (Stoll and Müller, 1999). At the same time, DRG neurons become primed internally into a progrowth state, a change occurring at the transcriptional level that has been well studied (Tedeschi, 2012; Scheib and Höke, 2013). Some sensory neurons project a peripheral axon into outlying tissue and a central process to the brainstem via the spinal cord. Interestingly, if these cells are preinjured peripherally they will mount a much more vigorous growth response to a second injury of the central axonal branch in the CNS (Neumann and Woolf, 1999; Kadoya et al., 2009). Although the sequence of events of a "conditioning" peripheral lesion followed by a central injury in the same neuron is unusual and does not reflect real life, it offers us the very important clue that to grow well in the CNS, neurons must be in a progrowth mode, and results using genetically modified animals support this concept (Sun et al., 2011).

We used this phenomenon as the basis of a genetic screen of inbred mice to look for alterations in injury-induced axonal growth in the CNS. We found that DRG neurons from one strain, CAST/Ei mice, were able to extend injured axons on CNS myelin to a far greater extent than all other strains tested (Omura et al., 2015).

CAST/Ei mice also demonstrate much more robust axonal growth *in vivo* in the injured spinal cord and optic nerve, and enhanced cortical neuron plasticity following stroke. Detailed analysis of this strain relative to control C57BL/6 mice showed that the level of preconditioned growth in the periphery was equivalent between strains, but what distinguished CAST/Ei mice from the others was strong preconditioned growth in the CNS, enabled by an entirely new genetic program specific to CAST/Ei mice (Omura et al., 2015).

To identify the biological mechanism enabling such robust CNS axon regeneration in CAST/Ei mice, we performed two independent genome-wide transcriptional profiling screens of naive and preconditioned DRGs across several mouse strains. This led to the identification of the activin pathway as the main signaling cascade responsible for this growth, and subsequent functional assays both *in vitro* and *in vivo* have confirmed a role for the activin pathway in robust CNS axonal regeneration (Omura et al., 2015). Interestingly, independent genomic screens in some lower species, where neural and tissue regeneration can occur, have previously pointed to activin signaling as the definitive mechanism in this regrowth (Zheng et al., 2003; Jaźwińska et al., 2007; Gilbert et al., 2013). We have also demonstrated that growing neurons use the Smad2/3 signaling system downstream of activin to initiate the strong central growth program. These data suggest that axonal regeneration is possible in the mammalian CNS, but that it requires an intrinsic growth-like state coupled with active nuclear Smad2/3 signaling.

Other studies have used genetic manipulations to promote intrinsic growth capacity in CNS neurons; however, this has frequently been achieved by using the deletion of multiple tumor suppressor genes, which, although very effective, may lead to unacceptable side effects such as tumor growth (Sun et al., 2011). By studying a phenomenon that occurs naturally in mice, as opposed to artificial genetic constructs, it may be possible to one day find ways to selectively and focally increase growth in the injured CNS to enable the functional regrowth of injured tracts and at the same time retain overall synaptic structure.

Biological networks in injured and regenerating corticospinal neurons (J. Dulin)

The repair of injured CNS motor systems remains a great translational goal. The corticospinal tract is the most functionally important pathway for voluntary motor control in humans; however, the intrinsic regenerative capacity of corticospinal axons is limited (Raineteau and Schwab, 2001; Oudega and Perez, 2012). To date, efforts to promote regeneration of the lesioned corticospinal tract into spinal cord lesion sites have achieved very limited success (Hollis et al., 2009; Liu et al., 2010; Sun et al., 2011; Blackmore et al., 2012; Floriddia et al., 2012; Ghosh et al., 2012; Lang et al., 2013; Lewandowski and Steward, 2014; Danilov and Steward, 2015; Wang et al., 2015). Recent work in our laboratory has demonstrated the ability of injured corticospinal tract axons to regenerate robustly into transplanted neural progenitor cell grafts and to form functional synaptic connections with grafted neurons (Kadoya et al., unpublished observations). This unprecedented extent of regeneration illustrates that injured adult corticospinal neurons can indeed activate an intrinsic growth state in the absence of experimental genetic manipulation, upon provision of a permissive cellular substrate. Hence, we now aim to characterize the intrinsic growth programs activated in the regenerating corticospinal tract and to identify signaling pathways that can potentially be manipulated to achieve greater growth over longer distances.

To accomplish these goals, we have embarked upon a "top-down" multiomics strategy. We first performed RNA-Seq of corticospinal motor neurons at time points corresponding to the induction and peak growth phases of corticospinal tract regeneration into neural progenitor cell grafts (Poplawski and Tuszynski, unpublished observations). We detected differential expression of thousands of genes within regenerating corticospinal motor neurons, and network analysis showed the activation of biological networks associated with transcriptional regulation and axon growth. Integrated analysis of transcriptomics and phosphoproteomics networks has been successfully used by others to identify key signaling pathways in the peripheral axon retrograde injury response (Michaelevski et al., 2010). To gain insight into signaling events occurring within the regenerating corticospinal axon, we performed phosphoproteomics screens of corticospinal axons isolated from spinal cord tissue at several time points under injured [spinal cord injury (SCI) only] or regenerating (SCI plus neural progenitor cell graft) conditions. Bioinformatics analysis using kinase–substrate interaction networks such as PhosphoNetworks (www.phosphonetworks.org; Hu et al., 2014) predicted key kinase "hubs" that may function as modulators of growth in the regenerating corticospinal axon. We are currently working to integrate our transcriptomics and phosphoproteomics datasets toward the goal of identifying upstream genetic regulators that might modulate growth associated signaling events within the axon.

The robust regeneration of corticospinal tract axons into neural progenitor cell grafts strongly implies that these grafts contain permissive factors that support ingrowth and synaptic connectivity of injured corticospinal axons. To identify potential biological mechanisms underlying the permissiveness of these grafts, we performed proteomics screens of spinal cord-derived (permissive for regeneration) and forebrain-derived (nonpermissive) neural progenitor cell graft tissue. Gene ontology analysis enabled the identification of proteins enriched in permissive grafts that are involved in cell–cell adhesion, axon guidance, and synapse formation. Using protein–protein interaction networks, we then compared graft proteomics data with a list of upregulated guidance and adhesion molecules mined from our transcriptomics data of corticospinal neurons. We have now begun to perform *in vivo* screens of candidate proteins identified using this approach to identify key cell–surface protein interactions involved in corticospinal growth and synapse formation.

Ultimately, the long-distance growth and connectivity exhibited by neural grafts transplanted into sites of SCI (Lu et al., 2012; Lu et al., 2014) suggest great potential for the formation of *de novo* functional relays between regenerating host axons and graft-derived neurons. By developing a better understanding of the intrinsic mechanisms enabling robust regeneration of the injured corticospinal tract into permissive cell grafts, we hope to identify new strategies by which to potentially enhance the growth of injured motor axons and shape connectivity in a way that promotes meaningful functional recovery.

The future of transcriptomics in neuroscience

Resources for the interpretation of transcriptomics data and its assimilation into a global biological framework are rapidly becoming more comprehensive, sophisticated, and user friendly. Researchers can access a treasure trove of useful on-line resources such as ENCODE (www.encodeproject.org), an annotated encyclopedia of functional elements in the human genome; the Brain-

Span transcriptomics atlas (www.brainspan.org); and the CNS cell type-specific transcriptome database Brain RNA-Seq (Zhang et al., 2014), to name just a few. These and many other publicly available curated databases (listed on-line at www.omictools.com/transcriptomics) are invaluable tools for mining specific information from extraordinarily large and complex omics data sets. Additionally, the power and sophistication of bioinformatics analytical methods continue to grow. It is becoming clear that the integration of RNA-Seq data with other modalities (e.g., proteomics, phosphoproteomics, epigenomics, and metabolomics) will yield greater insight into the overall biological state of the cell or tissue of interest than can be gained from assessing RNA expression alone (Ritchie et al., 2015). However, tools for multiomic data integration are still in their infancy, and we have far to go before these types of analyses become routine. At present, the potential for high-throughput omics approaches to enhance our understanding of neurological development, disease, and repair is rapidly on the rise.

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