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Review article

Selecting optimal combinations of transcription factors to promote axon regeneration: Why mechanisms matter

Ishwariya Venkatesh, Murray G. Blackmore*

Department of Biomedical Sciences, Marquette University, 53201, United States

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ABSTRACT

Recovery from injuries to the central nervous system, including spinal cord injury, is constrained in part by the intrinsically low ability of many CNS neurons to mount an effective regenerative growth response. To improve outcomes, it is essential to understand and ultimately reverse these neuron-intrinsic constraints. Genetic manipulation of key transcription factors (TFs), which act to orchestrate production of multiple regeneration-associated genes, has emerged as a promising strategy. It is likely that no single TF will be sufficient to fully restore neuron-intrinsic growth potential, and that multiple, functionally interacting factors will be needed. An extensive literature, mostly from non-neural cell types, has identified potential mechanisms by which TFs can functionally synergize. Here we examine four potential mechanisms of TF/TF interaction; physical interaction, transcriptional cross-regulation, signaling-based cross regulation, and co-occupancy of regulatory DNA. For each mechanism, we consider how existing knowledge can be used to guide the discovery and effective use of TF combinations in the context of regenerative neuroscience. This mechanistic insight into TF interactions is needed to accelerate the design of effective TF-based interventions to relieve neuron-intrinsic constraints to regeneration and to foster recovery from CNS injury.

Contents

* Corresponding author at: Department of Biomedical Sciences, Marquette University, Milwaukee, WI, 53201, USA.
E-mail address: murray.blackmore@marquette.edu (M.G. Blackmore).

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1. Introduction

Coaxing robust, long distance regeneration from injured neurons remains a major unmet challenge in the treatment of spinal cord injury. Although extrinsic barriers to axon regeneration contribute, cell-intrinsic mechanisms within injured CNS neurons also limit axon growth [10,17]. Axon extension requires a profound change in cellular state within injured neurons. Prior to axotomy, neurons are tasked with maintaining intracellular communication and structural homeostasis in far-flung processes; after axotomy, regeneration demands the production, transport, and regulated assembly of enormous amounts of cytoskeletal and membranous material. The sheer number of genes that must be up- or down-regulated to reinitiate axon extension presents a major challenge to targeting the neuron-intrinsic growth state [10,47]. One possible solution is that underlying transcription factors (TFs) might be manipulated in injured neurons, perhaps acting as simple levers to alter the expression of large numbers of downstream regeneration-associated genes (RAGs). A growing number of TFs have been functionally linked to axon growth in a variety of cell types (Table 1). Indeed, manipulation of TFs including KLFs, SOX11, and STAT3 has enhanced regenerative axon growth after spinal injury [11,37,85]. On the other hand, the number and regenerative speed of treated axons likely remains well below the threshold for functional recovery.

One explanation for this limited response may be that no single TF is sufficient to drive a full regenerative program. Instead, groups of functionally interacting factors are likely needed, similar to the situation in induced pluripotency [74]. Indeed, recent work in the optic system makes it plain that combinatorial gene manipulations are most effective in producing axon regeneration [6,49,73]. Although plausible in principle, this combinatorial perspective brings with it the challenge of identifying optimal sets of TFs [77,79]. With well over one thousand TFs in the genome and at least a dozen already linked to regenerative axon growth *in vivo* (Table 1), the number of possible combinations is daunting.

Here we argue that optimal selection of pro-regenerative TF combinations requires careful consideration of the underlying mechanisms of interaction. Fundamentally, the specifics of the various molecular interactions between TFs have profound implications for the discovery and eventual use of TF combinations to improve regenerative axon growth. To illustrate this, we briefly consider four general mechanisms by which TFs can functionally interact. For the sake of clarity, we frame the discussion around two-way interactions between factors, with the understanding that this basic framework must eventually be scaled to accommodate multi-factor networks. For each mechanism we 1) examine instances in which the mechanism has been demonstrated in TFs linked to axon growth 2) examine how the mechanism informs improved discovery of TF/TF interactions and 3) consider the implications of the mechanism for optimal co-manipulations. This consideration of the details of TF/TF interactions is critical to accelerate the discovery of optimal TF mixtures and improve the efficacy of combinatorial manipulations.

2. Physical interaction

TFs can directly bind to one another and reciprocally influence activity (Fig. 1). Indeed, some families of TFs, notably bZIP, bHLH, and STATs, are obligate dimers; the ability to bind DNA is conferred by the presence of two subunits [reviewed in [2]]. Obligate dimers form both homo- and heterodimers, commonly with related family members. Importantly, transcriptional activity can be increased or suppressed depending on the specific partnerships formed, creating a system for graded control of transcription. A highly relevant example involves the bZIP AP1 factors, JUN and ATF3. Previous work indicates that JUN homodimers drive moderate activation of target genes and JUN-ATF3 heterodimers drive strong activation, whereas ATF3 homodimers can act to repress transcription [4]. JUN and ATF3 have been individually linked to axon regeneration [65,67], and single overexpression of each has been attempted to enhance regenerative outcomes. Intriguingly, it was recently shown that forced co-expression of both factors is more effective in promoting axon growth in sensory neurons than either alone [13]. These data raise the possibility that the synergistic effects of co-expressed JUN and ATF3 in sensory axon growth might be explained by direct binding, although this possibility has yet to be directly tested.

In addition to obligate dimers, physical interaction between TFs is also common across TF classes, and between TFs that normally function as individual subunits (e.g. zinc finger TFs). In one highly relevant example from optic nerve regeneration, KLF4, which acts to inhibit axon growth in this system, physically associates with and inhibits pro-regenerative STAT3 (Qin et al.). In addition, a wide range of physical interactions between RAG TFs, shown mostly in non-neural cell types, are summarized in Fig. 2 (references provided as hyperlinks in Supplementary Table S1). Notably, p53 (TP53) can bind seven of the twelve RAG TFs (STAT3, KLF6, MYC, ATF3, CREB, HIF1A, SMAD1), and STAT3 binds five (KLF4, ATF3, SMAD1, p53, HIF1A). In summary, although data in neurons remain sparse, evidence from non-neural cell types strongly supports the possibility that TFs implicated in regeneration may influence one another's activity in part through direct physical association.

2.1. Implications for discovery

Physical binding between TFs is perhaps the most straightforward type of interaction to identify. Datasets and network tools that include physical interactions, although built largely from non-neural cell types [14,24,31,44,60], are readily available and are already being used to help prioritize TFs of interest in the context of regeneration research [13,77,79]. A driving assumption of this approach is that TFs with large numbers of known interactions act as hub proteins and are thus high priority targets for functional intervention. Although certainly valid, an important caveat to this assumption is that the number of known physical interactions for each TF is highly influenced by the interest that TF has previously received, mostly in non-neural cell types. For example, a Pubmed search for p53 identifies >80,000 manuscripts, STAT3 identifies >15000, and a search for KLF6 yields less than 400. Thus it is perhaps unsurprising that the number of known interactions with other RAG TFs is higher for p53 and STAT3. When extrapolating available network data to prioritize TFs for regenerative axon growth, care must to taken to avoid a self-reinforcing interest in well-studied factors, at the expense of TFs that may be less well studied but functionally important.

Unbiased methods are available to discover physical interactions between TFs. For example, novel TF binding partners can be identified by proteomic analysis involving immunoprecipitation with mass spectrometry [28]. In a complementary approach, the spatial distribution of TF binding in the genome can be used to predict possible physical interactions [82]. First, chromatin immunoprecipitation and high throughput sequencing (ChIP-Seq) can be used to determine genome-wide locations of binding by TFs of interest. Then, bioinformatic tools are used to scan adjacent sequences for recognition motifs of potential partner TFs, with particular attention paid to promoter and enhancer regions for genes of interest, in this case, regeneration-associated genes. If two TFs bind one another and then additionally bind DNA, this can be detected in TF binding sites in very close proximity. Software packages are now available for this approach [50]. In this way, starting from a TF that is known to promote regeneration, it would be possible to iden-