

Repressing the neuron within

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Summary

A myriad of coordinated signals control cellular differentiation. Reprogramming the cell's proteome drives global changes in cell morphology and function that define cell phenotype. A switch in alternative splicing of many pre-mRNAs encoding neuronal-specific proteins accompanies neuronal differentiation. Three groups recently show that the global splicing repressor, polypyrimidine tract binding protein (PTB), regulates this switch.^(1–3) Although a subset of neuronal genes are turned on in both non-neuronal and neuronal cells, restricted expression of PTB in non-neuronal cells diverts their mRNAs to nonsense-mediated decay and prevents protein expression. When the PTB brake is released, the cell splices like a neuron. *BioEssays* 29:1–4, 2007.

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Introduction

Lesson 8 in the Driver's Education manual includes an optional section for drivers of manual cars: The uphill start. The handbrake is on, you find the "friction point" by balancing accelerator and clutch, more accelerator moves you forward, less you roll back. Energy is consumed but the system is primed and once the brake is released, direction is set by adjusting clutch and accelerator. Similarly, the balance of excitatory/enhancer and inhibitory/repressor signals is used by cells to decide whether to initiate signaling cascades. Inhibitory, braking signals tend to dominate in cells in the resting state. Three recent publications show that the polypyrimidine-tract-binding protein (PTB/PTBP1) is a critical component of a braking mechanism that keeps non-neuronal cells from moving down the neuronal differentiation pathway (Fig. 1).^(1–3) PTB is a splicing repressor of neuron-specific exon selection in a number of pre-mRNAs.^(4,5) PTB contains four RNA recognition motif (RRM)-type domains and binds to

UCUUC and CUCUCU motifs contained within pyrimidine-enriched regions.⁽⁶⁾

Coordinated changes in gene expression drive neuronal differentiation and involve regulation at several stages from transcription, through posttranscriptional RNA processing, to translation. But significant interest surrounds the switch in the pattern of alternative pre-mRNA splicing of a subset of exons that accompanies neuronal differentiation.^(7,8) Pioneering studies of sex determination in *Drosophila melanogaster* provide ample evidence that a coordinated switch in the pattern of alternative pre-mRNA splicing trigger major changes in cell function.⁽⁹⁾ The master controller of female-specific splicing in *Drosophila* is the RNA-binding protein Sex lethal (Sxl). Sxl is only expressed in female flies; it represses male-specific splicing of pre-mRNAs thereby repressing proteins that trigger global cellular signaling cascades required for male development.⁽⁹⁾ Parallels with PTB are notable. Like Sxl, PTB has emerged as a master controller in this case of neuronal-specific alternative splicing^(4,5) and, as we will discuss, again like Sxl, one of its targets is pre-mRNA encoding another splicing factor, in this case nPTB.^(1–3)

Controller, brake and switch

What controls the switch in splicing patterns from non-neuronal to neuronal phenotype? The laboratories of Douglas Black, Tom Maniatis and Christopher Smith show that a sudden decrease in PTB expression is closely associated with the appearance of neuronal-specific alternative pre-mRNA splicing. By knocking down PTB protein *in vivo* using interference RNA methods, they establish that PTB loss is sufficient to trigger neuronal-specific alternative splicing.^(1,2) PTB-binding motifs are contained in a number of pre-mRNAs but, as these authors show, PTB activity is restricted to non-neuronal cells simply because it is not expressed in neurons (Fig. 1, level 1).^(1,2) Interestingly, neurons express a PTB paralog, nPTB (PTBP2/PTBbr), which also acts as a splicing repressor albeit with lower efficacy. In general, nPTB exhibits the converse pattern of expression and is excluded from non-neuronal cells.^(4,10–12) The mutual exclusivity of PTB and nPTB expression, which is illustrated in stunning images from Boutz and colleagues⁽¹⁾ and Makeyev and colleagues,⁽²⁾ has the hallmarks of a negative feedback mechanism where the expression of one PTB inhibits the expression of its paralog⁽¹¹⁾ (Fig. 1). The mechanism of this negative feedback is discussed below.

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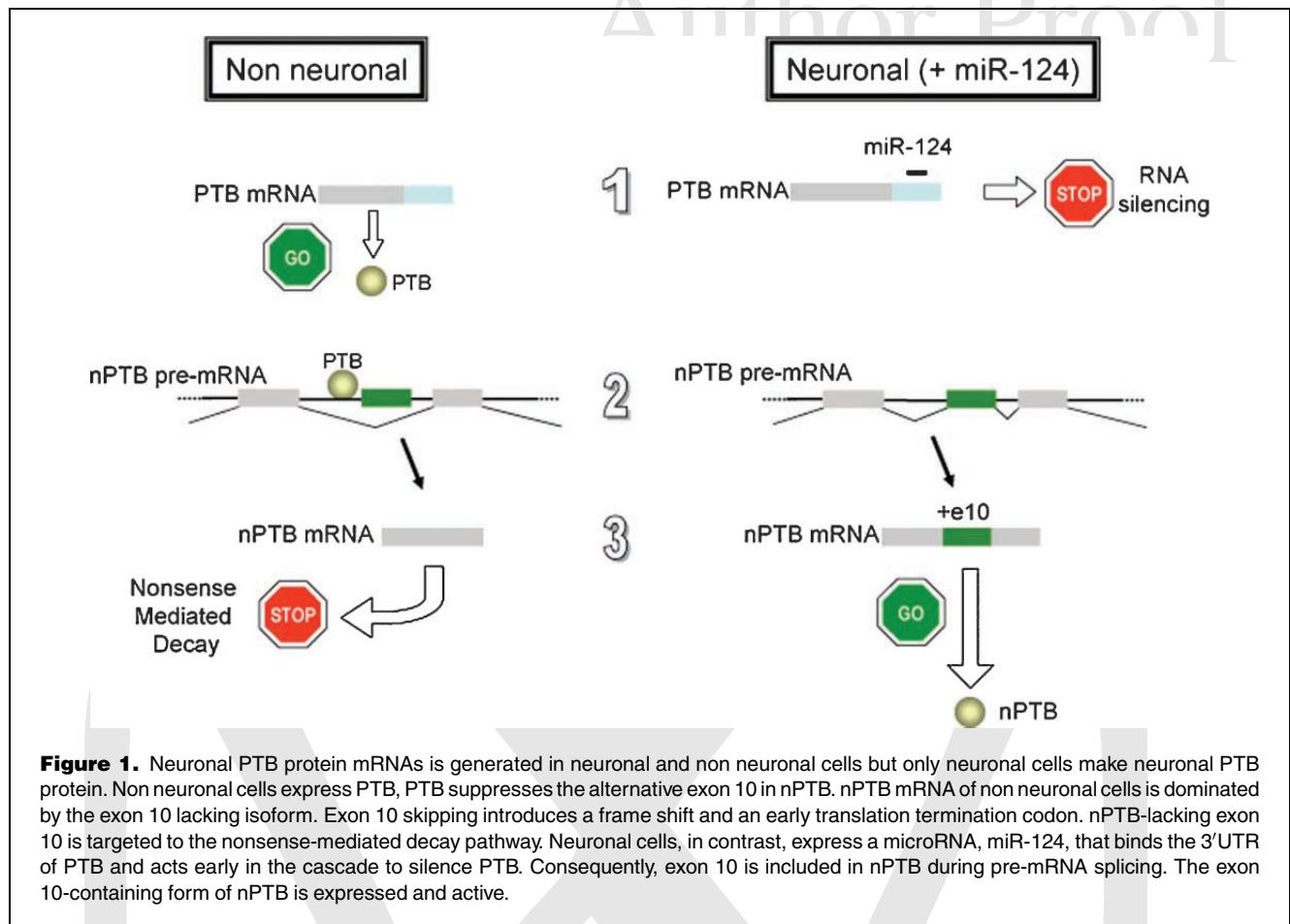
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Functionally, nPTB partly substitutes for the loss of PTB in neurons by maintaining repression of a subset of PTB-targeted exons. In the P19 cell line, which differentiates into neurons in response to retinoic acid, PTB and nPTB regulate a distinct but overlapping set of exons.^(1,2) In HeLa cells, Christopher Smith and colleagues show the overlap in PTB and nPTB function is almost complete. They used quantitative proteomics to compare protein composition of HeLa cell extracts following siRNA knockdown of either PTB or nPTB alone, and in combination.⁽³⁾ Given its reputation as a master repressor of alternatively spliced exons, the group anticipated PTB loss would alter the HeLa cell proteome significantly. But, as they so often do, the data proved more interesting. Levels of nPTB increased substantially following PTB knockdown, which is consistent with the theory that PTB inhibits the expression of nPTB. However, unexpectedly, an increase in nPTB protein levels was the only significant change in the proteome. Simultaneous knockdown of both PTB and nPTB was required to effect substantial and widespread changes in protein expression; strong evidence of their overlapping activity as repressors of alternatively expressed exons.

The biological significance of this degree of functional redundancy between PTB and nPTB is not completely understood but the overall lower efficacy of nPTB compared to PTB in exon repression is likely to be important. Perhaps gradual easing of repression prevents stalling and permits a smoother transition in proteome and cell function? More generally, these and other studies point to a hierarchy of splicing repressors with PTB positioned at the top. Loss of PTB is needed for neuronal differentiation, but its removal is accompanied by a concomitant increase in nPTB.^(1–3)

Repressing the repressor of the repressor

Although PTB acts on a number of pre-mRNAs to regulate expression, nPTB is a critical target. All three papers highlighted here reveal how PTB keeps nPTB levels in check.^(1–3,13)

PTB directly suppresses expression of nPTB in non-neuronal cells by a mechanism that involves exon repression and mRNA silencing via nonsense-mediated decay (NMD) (Fig. 1).^(1–3) By repressing exon 10 inclusion in nPTB, PTB shifts the nPTB mRNA reading frame and introduces a premature translation termination codon. This frame shift diverts nPTB mRNA to the NMD pathway (Fig. 1, levels 2, 3).

At first glance, controlling nPTB levels by making and then immediately degrading mRNA seems remarkably wasteful. Why not keep nPTB transcription turned off until it is needed? One simple reason is that a primed system, sitting at its friction point, can react much more quickly when the brake is released than a system that has to be started from cold. As noted by Douglas Black and colleagues,^(1–3) studies addressing the time course of protein regulation and tracing the ratio of PTB:nPTB expression during early neurogenesis should be informative in defining temporal changes in the level of these splicing factors.

Why is PTB protein excluded from neurons and what signal triggers its loss during neuronal differentiation? PTB is a predicted target of the neuronal-specific microRNA miR-124.⁽¹⁾ In an elegant series of experiments, Makeyev and colleagues show that miR-124 indeed directly targets the 3'UTR of PTB, silences PTB expression, releases the PTB brake on neuronal-specific splicing and promotes neuronal differentiation (Fig. 1, level 1).⁽²⁾ These authors show that miR-124 is necessary to trigger a decrease in PTB and consequently sufficient to affect a switch in the pattern of alternative pre-mRNA splicing from non-neuronal to neuronal. Overexpression of miR-124 could not induce neuronal differentiation but it dramatically augmented retinoic-induced differentiation of P19 cells.⁽²⁾ A remarkably similar regulatory mechanism controls muscle cell differentiation. The muscle-restricted micro RNA, miR-133, is turned on during muscle maturation and targets nPTB mRNA and probably PTB mRNA. Silencing of nPTB and downregulation of PTB are processes tightly linked to the expression of mature muscle-specific exons in target mRNAs.⁽¹³⁾ Interestingly, a decrease in the expression of both nPTB and PTB seems to be required to support muscle-specific exon splicing.⁽¹³⁾ Now the quest is on for the regulators of these microRNAs.

Splicing decisions depend on integrating multiple inputs

While the repressor activities of PTB are the focus of this discussion, PTB is one of only a handful of splicing factors that have been studied in extensive detail. PTB represses exon inclusion during pre-mRNA splicing by targeting the interactions involved in exon and intron definition.⁽¹⁴⁾ Splicing activators such as Nova and members of the serine/arginine-rich (SR) protein family are also relatively well studied. These proteins generally bind to enhancer elements and contain arginine-serine (RS) RNA-binding domains that may also promote interaction with other splicing proteins.⁽³⁾ Exon selection *in vivo* therefore depends on the concerted actions of multiple enhancer and repressor elements. This probably in part explains the very different behaviors exhibited by different exons in response to knockdown of PTB, nPTB and their combination.^(1,3) While many alternatively spliced exons are

repressed by PTB and nPTB, different subsets are targeted by both, by one or other, and yet others appear to be enhanced by PTB.⁽¹⁾ Consistent with this, early studies demonstrated splicing enhancer activity of PTB-like proteins stimulate splicing when bound at the intronic positions.^(15,16) Various models have been developed to explain how different combinations of cis-elements and their splicing factors are integrated and “read” by the spliceosome in the final splicing choice. Exons and intron sequences identified as PTB and nPTB targets from these *in vivo* studies should prove invaluable in defining biologically relevant cis-elements that direct PTB-dependent alternative splicing.

Conclusion

Dynamic control of splicing choices is an exciting and rapidly changing field. The mechanisms that underlie neuronal-specific splicing of pre-mRNAs during neuronal differentiation are also likely to be relevant to studies of the mature nervous system. Parallels exist between cellular changes that occur during neuronal differentiation and those that underlie more local synaptic changes in the mature nervous system that are thought to mediate learning and memory. MicroRNAs are already implicated in controlling the expression of proteins that regulate synaptic efficacy.^(17,18) Likewise, many pre-mRNAs that encode proteins important in synaptic plasticity are regulated by alternative splicing.^(7,8) It seems only a matter of time before microRNAs, alternative splicing and synaptic plasticity collide.

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What the papers say

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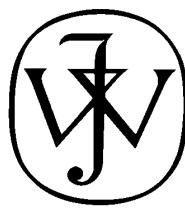
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