Post-transcriptional regulation: **The dawn of PTB**

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The 'polypyrimidine-tract-binding protein' (PTB) participates in the control of alternative processing and translation of various RNAs, and may operate as a multifunctional regulator of tissue-specific gene expression.

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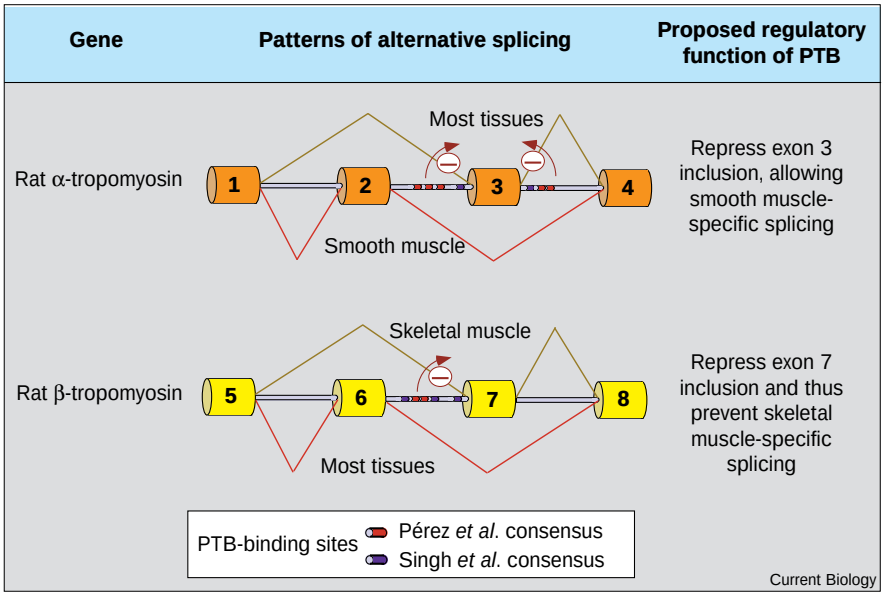
One of a molecular biologist's dreams is to explain complex processes like cell differentiation and development by simple molecular operations, such as the expression of a few master regulatory genes. One instance in which the dream has come true involves the *Drosophila* RNA-binding protein Sex-lethal, which brings about changes in pre-mRNA splicing and mRNA translation of target genes that orchestrate all aspects of female sexual determination. Recent reports suggest that the poly-pyrimidine-tract-binding protein (PTB) also has multiple functions in post-transcriptional regulation. We shall review this evidence and discuss the prospects for PTB as a multifunctional regulator of cell differentiation in mammals.

PTB was first purified as a polypeptide that binds to the polypyrimidine tract that typically precedes the 3′ splice sites of higher eukaryotic mRNA introns [1]. Mutations in the pyrimidine-rich tract were found to have similar effects on PTB binding and on the assembly of splicing complexes, so it was proposed that PTB promotes early steps of the RNA splicing reaction. This view was challenged when PTB was identified as hnRNP I [2], a member of a family of proteins that bind to nascent RNAs and play multiple roles in the nuclear life of transcripts, but that seem to dissociate from the pre-mRNA when splicing complexes assemble [3].

A first hint about the function of PTB came from studies on the alternative splicing of tropomyosin pre-mRNAs. In this process, the same pre-mRNA can follow alternative maturation pathways to generate mRNAs with different coding capacities. One of the alternative splicing events affecting both α- and β-tropomyosin pre-mRNAs is a tissue-specific choice between two exons that are mutually exclusive (Figure 1). Exon 2 of α-tropomyosin is used only in smooth muscle, and exon 7 of β-tropomyosin is used only in skeletal muscle, thus generating different protein isoforms which contribute to the distinct mechanical properties of each type of muscle cell. Several *cis*-acting sequences were found to be important for correct tissue-specific alternative splicing of these pre-mRNAs [4–7], and PTB was found to bind at least some of them [5,6]. In one of these studies [5], it was proposed that PTB recognizes specific pyrimidine-rich sequences, and that PTB binding to its cognate sites results in repression of nearby splice sites.

Figure 1

Proposed activities of PTB in the alternative splicing of α- and β-tropomyosin pre-mRNAs. The relevant exons and introns and splicing pathways, as well as PTB-binding sites predicted by two different studies [8,9] are schematically represented. The arrows point to the splicing events proposed to be regulated by PTB.



If this model were correct, the identification of PTB-binding sites in pre-mRNAs would also serve to identify target genes and their mode of regulation by PTB. As usual in these days of *in vitro* genetics, iterative selection from random pools of RNA was used to investigate the precise binding specificity of PTB [8,9]. Related, but distinct, consensus sequences were obtained, depending on the detailed conditions of the selection procedure, the simplest high-affinity binding sites being represented by the sequence UCUU(C) — and possibly also UUCU/C — in a pyrimidine-rich context [9,10]. Sequences matching the various consensus sequences occur in the introns adjacent to regulated exons of a variety of genes, and others are likely to be found in the near future. Below we discuss the functional evidence that PTB regulates these genes.

In the case of tropomyosins (Figure 1), mutation of the PTB-binding sites disrupts regulation *in vivo* [9], and placing these sequences close to splice sites confers PTB-mediated splice-site repression *in vitro* [8,11]. Taken together, these data indicate that repression of nearby exons induced by PTB binding results in inclusion of a smooth-muscle-specific exon in α-tropomyosin mRNAs and skipping of a skeletal-muscle-specific exon in β-tropomyosin mRNAs, thus allowing the coordinated expression of protein isoforms in a particular cell type.

Recent reports implicate PTB in coordinating another set of tissue-specific splicing decisions: the inhibition of neuron-specific processing patterns in non-neural cells. The pre-mRNAs of *c-src* and the $GABA_A$ receptor γ2 subunit gene contain exons which are included in the mature mRNA only in neurons, and sequences resembling PTB consensus sites are present within previously described *cis*-acting regulatory signals located in the flanking introns [10,12] (Figure 2). Although these exons are quite small, their presence significantly affects the amino-acid sequence of key domains of the encoded proteins, indicating that regulation of exon inclusion has important functional consequences.

Evidence for a regulatory role of PTB came from *in vitro* studies using nuclear extracts from HeLa cells, in which the use of the neuron-specific splice sites is repressed. Addition of short RNAs corresponding to PTB-binding sites to these extracts allowed neuron-specific splicing, presumably by titrating PTB and thus relieving its repressive activity. Two additional observations are consistent with this interpretation. First, the extent to which splicing was activated correlated with the affinity of the interaction between different RNAs and PTB. Second, addition of purified PTB restored repression [10,12].

Taken together, these results support a role for PTB in preventing neuron-specific splicing events. It should be noted, however, that more definitive depletion/reconstitution experiments have not been possible with the reagents available, and therefore it cannot be formally excluded that the effects are mediated by binding factors other than PTB. It is also unclear why addition of purified PTB from HeLa cells does not repress splicing of *c-src* pre-mRNA in nuclear extracts derived from a neuronal cell line [12]. In this regard, it is important to consider that PTB acts in concert with other signals and factors that affect regulation, both positively and negatively, most likely reflecting the need for versatile fine tuning of splice-site usage in different cells at distinct times during development.

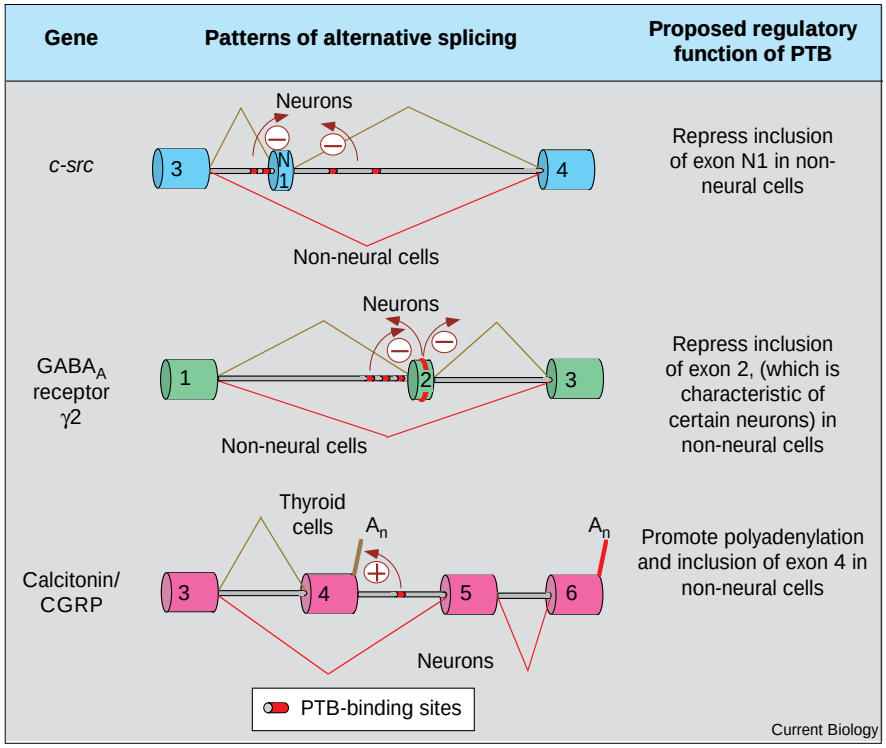
How is splicing inhibited by the presence of PTB? Some PTB-binding sites are within the polypyrimidine tract recognized by the splicing factor U2AF [5,6,8,9,12]. Other sites overlap with the binding site for U2 snRNP [10] or with enhancer regulatory sequences [10,12]. A simple model for regulation is that PTB sterically interferes with the binding of splicing factors that recognize these or nearby sequences, and indeed PTB has been shown to compete with the binding of U2AF to some polypyrimidine tracts [8,11]. The more distant location of other PTB sites from splice signals [7,10,12], together with the observation that PTB bound to a 3′ splice-site region can inhibit the use of the 5′ splice site across the exon [5,10,12], suggests that more complex mechanisms of regulation by PTB are also likely to operate.

A third example of neural-specific processing patterns modulated by PTB involves the gene that encodes both the hormone calcitonin and the neuropeptide CGRP (Figure 2). In thyroid C cells and — if ectopically expressed — most other tissues, calcitonin mRNA is generated by inclusion of exon 4 and cleavage/polyadenylation of the transcript at the end of this exon. In neurons, however, exon 4 is skipped — and the associated polyadenylation site ignored — so that the alternative CGRP mRNA is made. A complex enhancer sequence present in the downstream intron is important for exon 4 inclusion [13]. The enhancer sequence is composed of a pyrimidine-rich stretch followed by a 5′ splice site that binds splicing factors.

Consensus sequences for PTB were found within the pyrimidine-rich stretch of the calcitonin/CGRP pre-mRNA, and competition experiments, similar to those described above for *c-src* and the γ2 $GABA_A$ receptor gene, indicated that PTB stimulates cleavage/polyadenylation of the transcript at the end of exon 4, which in turn forces the selection of the non-neural splicing pattern [13]. It is unclear at present whether PTB has a direct role in promoting 3′ end formation or whether this effect is indirectly caused by inhibiting the assembly of splicing factors close to the sites of cleavage/polyadenylation. In any case, repressive effects on splicing or activating effects on polyadenylation have the same consequence: to promote non-neural patterns of gene expression.

Figure 2

Proposed activities of PTB in the alternative splicing of *c-src*, γ2 $GABA_A$ receptor and calcitonin/CGRP pre-mRNAs. Schemes and symbols are as in Figure 1.



An additional ability of PTB was unexpectedly revealed by studies of picornaviruses. The genomic RNA of these viruses is translated from internal ribosome entry sites (IRES) by mechanisms distinct from those governing the cap-dependent initiation of translation of most eukaryotic mRNAs. *In vitro* translation studies suggested that the efficient use of IRES requires cellular components in addition to canonical translation initiation factors. In a search for these factors, PTB was found associated with many picornavirus IRES [14], and depletion/reconstitution experiments demonstrated that PTB stimulates translation initiated from at least two of them [15,16]. The mechanisms used by PTB to enhance IRES-dependent translation are presently unknown. Although a role for PTB in the translation of cellular mRNAs remains to be demonstrated, the presence of IRES and PTB-binding sites in the 5′ untranslated region of a variety of cellular mRNAs suggests that the observations made in viruses may be revealing a more widespread — albeit specific — mechanism of translational control.

The results that we have discussed hold the promise of exciting developments in PTB research in the near future and pose two general questions. The first general question is whether the multiple activities of PTB have a common mechanistic basis. Does PTB, for example, induce conformational changes in RNA that occlude or reveal structural features of the transcript which need to be recognized by the machineries of RNA processing and translation? The second general question is how does PTB regulate posttranscriptional processing in a tissue-specific and physiologically meaningful fashion?

For PTB to be a physiologically important regulator, there must be a way, or ways, of varying its activity in different cell types. One simple way of achieving this would involve cell-type-specific variation in the concentration of PTB, relative to other splicing, polyadenylation or translation factors. Indeed, tissue-specific and cell-line-specific differences in PTB levels have been reported [10,12,17]. A general correlation between PTB abundance — or PTB activity, possibly modulated by post-translational modifications — and the choice of processing patterns has not yet been made. An alternative possibility involves the presence of tissue-specific PTB variants. In support of this idea, alternative RNA splicing generates at least four different PTB isoforms [2,17,18], and a brain-specific protein that reacts with antibodies against PTB has recently been identified [10]. A third way that PTB activity might be regulated is by differential association with other molecules. Proteins such as the PTB-associated splicing factor (PSF), an essential splicing factor that interacts with PTB [19], might associate with PTB only in particular circumstances [12], and significantly change the effect of PTB interaction with a cognate site. These mechanisms are likely to be the focus of future work that will show whether the dawn of PTB as a regulator of gene expression turns into a sunny day.

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