Molecular and Cellular Neuroscience xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne

Regulation of gene expression in mammalian nervous system through alternative pre-mRNA splicing coupled with RNA quality control mechanisms

Karen Yap, Eugene V. Makeyev *

School of Biological Sciences, Nanyang Technological University, Singapore 637551, Singapore

ARTICLE INFO

Article history:
Received 26 October 2012
Accepted 17 January 2013
Available online xxxx

Keywords:
Post-transcriptional regulation of gene expression
Alternative pre-mRNA splicing
RNA quality control
Nonsense-mediated decay
Nuclear retention and elimination of incompletely spliced transcripts
Nervous system development and function

ABSTRACT

Eukaryotic gene expression is orchestrated on a genome-wide scale through several post-transcriptional mechanisms. Of these, alternative pre-mRNA splicing expands the proteome diversity and modulates mRNA stability through downstream RNA quality control (QC) pathways including nonsense-mediated decay (NMD) of mRNAs containing premature termination codons and nuclear retention and elimination (NRE) of intron-containing transcripts. Although originally identified as mechanisms for eliminating aberrant transcripts, a growing body of evidence suggests that NMD and NRE coupled with deliberate changes in pre-mRNA splicing patterns are also used in a number of biological contexts for deterministic control of gene expression. Here we review recent studies elucidating molecular mechanisms and biological significance of these gene regulation strategies with a specific focus on their roles in nervous system development and physiology. This article is part of a Special Issue entitled 'RNA and splicing regulation in neurodegeneration'.

Contents

Introduction

The discovery that different metazoan organisms depend on largely similar repertoires of protein-encoding genes has suggested that evolutionary processes within this clade may rely on elaboration of transcriptional and posttranscriptional gene regulation mechanisms (Keren et al., 2010; Lenhard et al., 2012; Levine and Tjian, 2003; Licatalosi and Darnell, 2010; Moore and Proudfoot, 2009; Nilsen and Graveley, 2010). One such mechanism is based on alternative splicing of multiexon pre-mRNA transcripts into two or more distinct mRNA products (Black, 2003; Calarco et al., 2011; Nilsen and Graveley, 2010; Wang and Burge, 2008).

Several common alternative splicing patterns have been described including cassette exons, mutually exclusive exons, alternative 5′ and 3′ splice sites, alternative 5′ and 3′ exons, and alternative intron retention events (Black, 2003; Wang and Burge, 2008) (Fig. 1). In each case, the choice between the alternatives is regulated through an interplay between constitutive splicing motifs (5′ splice sites, branch points, polypyrimidine tracts and 3′ splice sites) and components of the core splicing machinery, as well as optional cis-regulatory elements (exonic and intronic splicing enhancers and silencers referred to as ESE, ISE, ESS, and ISS, respectively) and a range of trans-factors (normally RNA-binding proteins, or RBPs) interacting with these elements (Black, 2003; Wang and Burge, 2008).

Recent transcriptome-wide analyses suggest that >90% of human genes may give rise to alternatively spliced transcripts (Calarco et al., 2011; Chen and Manley, 2009; Pan et al., 2008; Wang et al., 2008). Approximately 100,000 intermediate- to high-abundance alternatively spliced events have been identified with the largest fraction occurring in

* Corresponding author at: School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, SBS-02n-45, Singapore 637551, Singapore.
E-mail address: makeyev@ntu.edu.sg (E.V. Makeyev).

1044-7431/$ – see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.mcn.2013.01.003

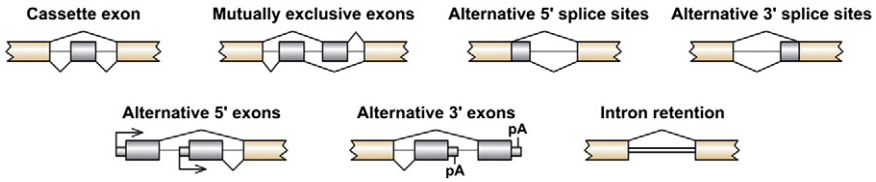

Fig. 1. Possible alternative splicing topologies.
Modified from Black (2003).

the nervous system (NS) (Calarco et al., 2011; Li et al., 2007; Pan et al., 2008). Since the human genome is estimated to contain ~20,000–25,000 protein-coding genes (International Human Genome Sequencing Consortium, 2004), this potentially translates to a >4-fold gain in the effective coding capacity. In several exceptional cases, individual genes are known to give rise to hundreds and sometimes thousands of distinct splice forms (Hattori et al., 2009; Nilsen and Graveley, 2010; Park and Graveley, 2007).

Biogenesis of alternative mRNA isoforms may additionally modulate mRNA stability, translational efficiency and intracellular localization (Andreassi and Riccio, 2009; Barrett et al., 2012; Lareau et al., 2007a; Lutz and Moreira, 2011). Notably, 20–35% of alternatively spliced mRNAs are predicted to contain premature termination codons (PTCs) in the mRNA open reading frame (ORF) (Baek and Green, 2005; Green et al., 2003; Lewis et al., 2003). This is expected to destabilize these transcripts through nonsense-mediated decay (NMD), an evolutionarily conserved cytoplasmic mRNA quality control (QC) mechanism (Chang et al., 2007; Isken and Maquat, 2007; Lareau et al., 2007a). Although most of the PTC-containing transcripts likely appear due to random splicing errors, at least 10–20% of these may correspond to bona fide gene regulation events (Pan et al., 2006). Moreover, a subset of unproductively spliced transcripts retaining intronic sequences may be intercepted by a distinct RNA QC pathway that we refer to as nuclear retention and elimination (NRE) of intron-containing transcripts (Yap et al., 2012). In addition to its well-known role in quality control, NRE can be integrated into gene regulation networks (Yap et al., 2012).

Compared to other human organs, brain is known to express the largest number of distinct alternatively spliced transcripts, which both diversifies the proteome and increases the gene regulation complexity (Calarco et al., 2011; Li et al., 2007; Norris and Calarco, 2012; Pan et al., 2008; Wang et al., 2008) (and see below). Here we summarize recent studies examining the functional interface between alternative splicing and RNA QC in the mammalian NS. Other biological functions of alternative splicing and RNA QC as well as the underlying molecular mechanisms have been discussed earlier in several excellent reviews (Bicknell et al., 2012; Black, 2003; Calarco et al., 2011; Chang et al., 2007; Doma and Parker, 2007; Huang and Wilkinson, 2012; Hwang and Maquat, 2011; Isken and Maquat, 2007; Kervestin and Jacobson, 2012; Li et al., 2007; Nicholson et al., 2010; Schoenberg and Maquat, 2012; Wang and Burge, 2008).

Alternative splicing coupled with nonsense-mediated decay

Nonsense-mediated decay has been originally described as a cytoplasmic mRNA QC mechanism targeting aberrant transcripts that emerge as a result of nonsense mutations and RNA-processing errors (Chang et al., 2007; Isken and Maquat, 2007; Kervestin and Jacobson, 2012). Several NMD components identified by genetic screens in budding yeast *Saccharomyces cerevisiae* and nematode *Caenorhabditis elegans* are evolutionarily conserved. These include the RNA-helicase UPF1, its associated proteins UPF2 and UPF3, protein kinase Smg1 activating UPF1 by phosphorylation, as well as Smg5, Smg6 and Smg7 proteins that interact with phosphorylated UPF1 and destabilize PTC-containing mRNAs (Chang et al., 2007; Isken and Maquat, 2007; Kervestin and Jacobson, 2012; Neu-Yilik and Kulozik, 2008; Nicholson and Muhlemann, 2010; Nicholson et al., 2010; Schoenberg and Maquat, 2012). Yet two additional proteins called Smg8 and Smg9 control Smg1 activity (Schoenberg and Maquat, 2012; Yamashita et al., 2009). NMD strictly depends on mRNA translation and requires nuclear cap-binding protein complex CBP80/20, translation termination factors eRF1 and eRF3, cytoplasmic poly(A)-binding protein PABPC1 and a subset of cytoplasmic mRNA degradation enzymes (Chang et al., 2007; Isken and Maquat, 2007; Kervestin and Jacobson, 2012; Nicholson et al., 2010; Schoenberg and Maquat, 2012).

In mammalian cells, PTC-containing transcripts are normally recognized based on the position of the translation termination codon relative to the last (3′-proximal) exon–exon junction (Chang et al., 2007; Isken and Maquat, 2007; Kervestin and Jacobson, 2012). Termination codons located >50 nucleotides (nt) upstream of the last exon–exon junction are typically recognized as premature, whereas stop codons located <50 nt upstream or downstream are, as a rule, considered normal. This rule is implemented through the deposition of so-called exon junction complexes (EJCs) ~20–25 nucleotides upstream of most exon–exon junctions (Le Hir et al., 2000; Sauliere et al., 2012; Singh et al., 2012). EJC is composed of 4 core subunits, eIF4AIII, MAGOH, MNL51/BTZ and Y14, and a number of associated factors including UPF3, UPF2 and UPF1 (Chang et al., 2007; Isken and Maquat, 2007; Kervestin and Jacobson, 2012). EJCs survive mRNA export from the nucleus to the cytoplasm. However, they are dislodged by translating ribosomes during the "pioneer" round of translation unless associated with exon–exon junctions positioned >50 nt downstream of the termination codon. mRNAs retaining one or several EJCs following the pioneer round of translation are normally subjected to NMD (Chang et al., 2007; Isken and Maquat, 2007; Kervestin and Jacobson, 2012). Interestingly, requirements for specific NMD factors may differ for different targets and some mammalian mRNAs containing long 3′UTRs may undergo NMD in an EJC-independent manner (Chang et al., 2007; Huang and Wilkinson, 2012; Isken and Maquat, 2007; Nicholson et al., 2010).

Besides its role in eliminating aberrant mRNAs, NMD is known to contribute to gene regulation programs through an alternative splicing-dependent mechanism referred to as AS-NMD or sometimes RUST (from "regulation by unproductive splicing and translation") (Lareau et al., 2007a; McGlincy and Smith, 2008; Neu-Yilik and Kulozik, 2008). Different estimates predict that AS-NMD may control the abundance of ~2% to 35% of alternatively spliced transcripts with a considerable fraction of regulated splicing events showing interspecies conservation (Baek and Green, 2005; de Lima Morais and Harrison, 2010; Green et al., 2003; Lewis et al., 2003; Mudge et al., 2011; Pan et al., 2006; Zhang et al., 2009). Notably, a number of genes known to be under AS-NMD control encode RBPs and other proteins involved in cellular RNA metabolism and in many of these cases, the corresponding RBPs auto-regulate the AS-NMD process through a negative feedback loop (Cuccurese et al., 2005; Lareau et al., 2007a, 2007b; McGlincy and Smith, 2008; Ni et al., 2007; Saltzman et al., 2008, 2011).

RBPs that normally function as splicing repressors (including a large fraction of hnRNP proteins) tend to stimulate NMD of their own mRNAs by inhibiting "ORF-maintaining" cassette exons [(McGlincy and Smith, 2008) and see below] (Fig. 2A). Since the lengths of these exons are not divisible by 3, their skipping is expected to shift the ORF register and result in the appearance of a downstream PTC (Magen and Ast, 2005). On the other hand, splicing activators (e.g., SR and hnRNP proteins interacting with splicing enhancer sequences) usually stimulate

Please cite this article as: Yap, K., Makeyev, E.V., Regulation of gene expression in mammalian nervous system through alternative pre-mRNA splicing coupled with RNA..., Mol. Cell. Neurosci. (2013), http://dx.doi.org/10.1016/j.mcn.2013.01.003