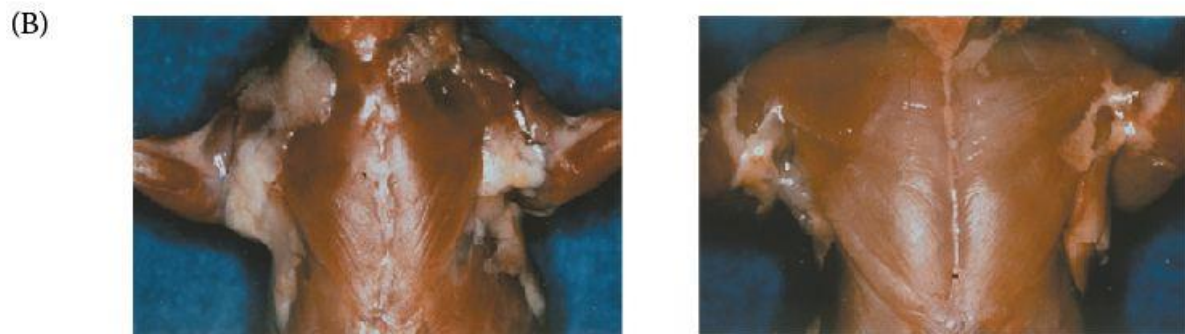
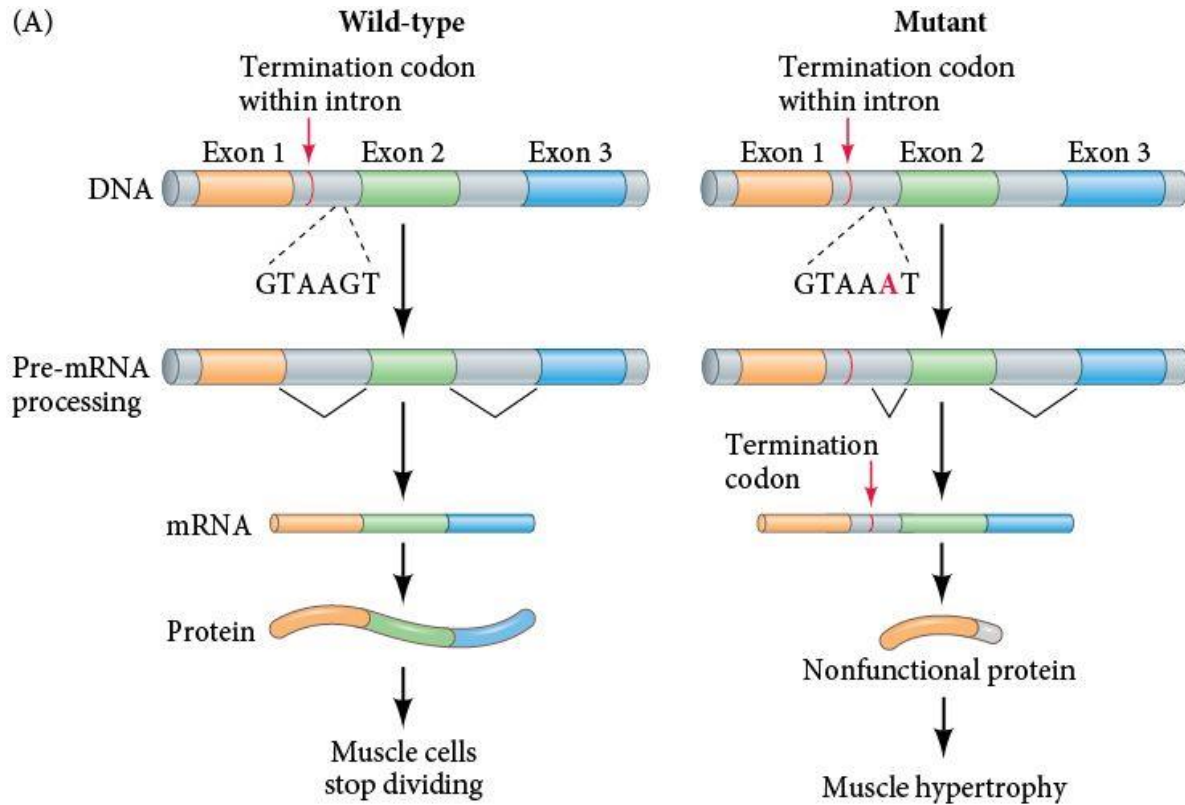


Splicing Enhancers and Recognition Factors

The mechanisms of differential pre-mRNA processing involve both *cis*-acting sequences on the pre-mRNA and *trans*-acting protein factors that bind to these regions (Black 2003). The *cis*-acting sequences on pre-mRNA are usually close to their potential 5' or 3' splice sites. These sequences are called splicing enhancers because they promote the assembly of spliceosomes at RNA cleavage sites. (Conversely, these same sequences can be “splicing silencers” if they act to exclude exons from an mRNA sequence.) These sequences are recognized by *trans*-acting proteins, most of which can recruit SPLICEOSOMES to that area. Some *trans*-acting proteins, however, such as the polypyrimidine tract-binding proteins (PTPs), repress spliceosome formation where they bind. Indeed, different PTPs can control the splicing of batteries of pre-mRNAs. For example, PTPb prevents the adult neuron-specific splicing of the neural pre-mRNAs controlling cell fate, cell proliferation, and actin cytoskeleton, thereby keeping the neuronal precursors in a proliferating, immature state (Licatalosi et al. 2012).

As might be expected, some splicing enhancers appear to be specific for certain tissues. Muscle-specific splicing enhancers have been found around those exons characterizing muscle cell messages. They are recognized by certain proteins that are found in the muscle cells early in their development (Ryan and Cooper 1996; Charlet-B et al. 2002). Their presence is able to compete with the PTP that would otherwise prevent the inclusion of the muscle-specific exon into the mature message. In this way, an entire battery of muscle-specific isoforms can be generated. The context dependency of splicing is too complex to delineate by merely comparing sequences, however. Computational studies—in which the computer is asked to identify (1) the combination of sequence elements, (2) the proximity of these sequences to the splice junctions, and (3) the differences of splicing outcomes in different cell types—are providing our first look at a “splicing code” that may allow us to predict which exons will persist in one cell and not in others (Barash et al. 2010).

Mutations in the splicing sites can lead to alternative developmental phenotypes. Most splice site mutations lead to nonfunctional proteins and serious diseases. For instance, a single base change at the 5' end of intron 2 in the human β -globin gene prevents splicing from occurring and generates a nonfunctional mRNA (Baird et al. 1981). That causes the absence of any β -globin from this gene and thus a severe (and often life-threatening) type of anemia. Similarly, a mutation in the *DYSTROPHIN* gene at a particular splice site causes the skipping of that exon and a severe form of muscular dystrophy (Sironi et al. 2001). In at least one such case of aberrant splicing, the splice site mutation was not dangerous and actually gave the patient greater strength. In this case, Schuelke and colleagues (2004) described a family in which individuals in four generations had a splice site mutation in the *MYOSTATIN* gene. Among the family members were professional athletes and a 4-year-old toddler who was able to hold two 3-kg dumbbells with his arms fully extended. The product of the normal *MYOSTATIN* gene is a factor that tells muscle precursor cells to stop dividing; that is, it is a negative regulator. In mammals (including humans and mice) with the mutation, the factor is nonfunctional, and the muscle precursors are not told to differentiate until they have undergone many more rounds of cell division; the result is larger muscles (Figure 1).



A after Schuelke et al. 2004; B from McPherron et al. 1997

Figure 1 Muscle hypertrophy through misspliced RNA. This mutation results in a deficiency of the negative growth regulator myostatin in the muscle cells. (A) Molecular analysis of the mutation. There is no mutation in the coding sequence of the gene, but in the first intron, a mutation from a G to an A creates a new (and widely used) splicing site, which causes aberrant pre-mRNA splicing and the inclusion of an early protein synthesis termination codon into the mRNA. Thus, proteins made from that message are short and nonfunctional. (B) Pectoral musculature of a “mighty mouse” with the mutation (right) compared with the muscles of a wild-type mouse (left). (A after Schuelke et al. 2004; B from McPherron et al. 1997.)

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