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Previews

The Long, the Short, and the Micro: A PolyA Tale of Pax3 in Satellite Cells

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The use of alternative polyadenylation sites is emerging as an important regulator of gene expression. In this issue of *Cell Stem Cell*, Boutet et al. (2012) report that alternative 3′UTRs of the Pax3 transcript restrict its expression to axial satellite cells through miR-mediated targeting of one of the isoforms.

The transcription factors Pax3 and Pax7 are key determinants of muscle progenitor fate in adult skeletal muscle (Seale et al., 2000) and during embryonic development (Relaix et al., 2006). In adult skeletal muscles, Pax7 expression is retained by all satellite cells in both their quiescent and their activated states (Seale et al., 2000). In contrast, while Pax3 protein is present in satellite cells in the diaphragm, satellite cells in most other muscle groups, including the hind limb, do not express detectable levels of Pax3 (Boutet et al., 2007; Kuang et al., 2006; Relaix et al., 2006). Indeed, gene expression analysis has indicated that Pax3 mRNA is not detectable in primary myoblasts isolated from hind limb muscles, and in quiescent satellite cells its level is extremely low compared with that of Pax7 (Fukada et al., 2007). The mechanisms responsible for this difference were unknown until now.

In this issue of *Cell Stem Cell*, Boutet et al. (2012) report that hind limb Pax3 expression is regulated by miR-206 (Figure 1A). Interestingly, the authors also demonstrate that satellite cell proliferation is primarily modulated through Pax3. Although miR-206 is expressed at the same level in hind limb and diaphragm muscles, satellite cells of the diaphragm express far higher levels of Pax3 compared with those of hind limb satellite cells. Strikingly, Boutet et al. showed that use of alternative polyA sites results in the formation of multiple Pax3 mRNA isoforms with differing lengths of 3′UTRs in different muscle groups. In the diaphragm, satellite cells express the short isoform of Pax3 mRNA where the 3′UTR lacks the miR-206 seed sequence (Figure 1B).

Initially, the authors investigated the reduced levels of Pax3 expression in hind limb muscle compared with those in diaphragm. Because hind limb satellite cells retain detectable levels of Pax3 mRNA while lacking Pax3 protein (Boutet et al., 2007), Boutet et al. hypothesized that Pax3 may be posttranscriptionally regulated by miRNAs. miRNAs usually bind the 3′UTR by base pairing with homologous sequence and inhibiting the subsequent translation of the targeted mRNA as well as stimulating its degradation. Pax3 3′UTR contains seed sequence motifs for at least three miRNAs: miR-1, miR-206 (Chen et al., 2010), and miR-27b (Crist et al., 2009). Of particular interest, miR-1 and miR-206 belong to the same cluster, are muscle specific, and have previously been shown to play a role in muscle regeneration (Chen et al., 2010).

The authors found miR-206 to be expressed in quiescent satellite cells and to steeply increase during their activation in vivo. Analysis of sorted myoblasts from regenerating hind limb muscles (3.5 days after injury) revealed high levels of miR-206 and almost undetectable levels of Pax3 mRNA. The long isoform of the Pax3 mRNA 3′UTR contains two miR-206 seed sequences and is also recognized by miR-27b. Conventional qPCR analysis and luciferase assays confirmed that these seed sequences are indeed targeted by miR-206. Additionally, treatment of cells with miR-206 generated a pronounced downregulation in Pax3 mRNA level. The opposite trend was found when cells were treated with anti-miR-206, or upon expression of a Pax3 construct containing a 3′UTR with mutated seed sequences. The effect of miR-1 on Pax3 expression was found to be negligible compared with miR-206.

To address the biological significance of miR-206, the authors used the ex vivo myofiber culture approach. It should be noted that satellite cells on myofiber cultures express high level of Pax7, which has also been shown to be a direct target of miR-206 (Chen et al., 2010). The authors report that transfection of myofibers with anti-miR-206 results in an increased number of activated satellite cells expressing the surface marker Syndecan-4. Of interest, this effect was nullified by cotransfection with siRNA against Pax3. This evidence suggests that miR-206 regulates satellite cell proliferation by targeting Pax3. However, it does not explain how Pax7 escapes this regulation.

To determine whether miR-206-dependent regulation of muscle stem cell function was uniquely due to its regulation of Pax3, knockdown experiments were performed. Surprisingly, siRNA knockdown of Pax3, but not Pax7, reduced proliferation. Although the requirement for Pax3 and Pax7 has been suggested to be limited to a critical postnatal juvenile period (Lepper et al., 2009), knockdown of Pax7 in primary myoblasts from 2-month-old mice results in cell cycle arrest and downregulation of Myf5 (McKinnell et al., 2008). Thus, Boutet et al.'s findings argue that Pax3 is also required for satellite cell activation in adult muscle. However, expression of Pax3 in the diaphragm of Pax7-deficient mice does not rescue the satellite cell phenotype (Kuang et al., 2006), suggesting that Pax7 plays an additional unique role in regulating the function of satellite cells.

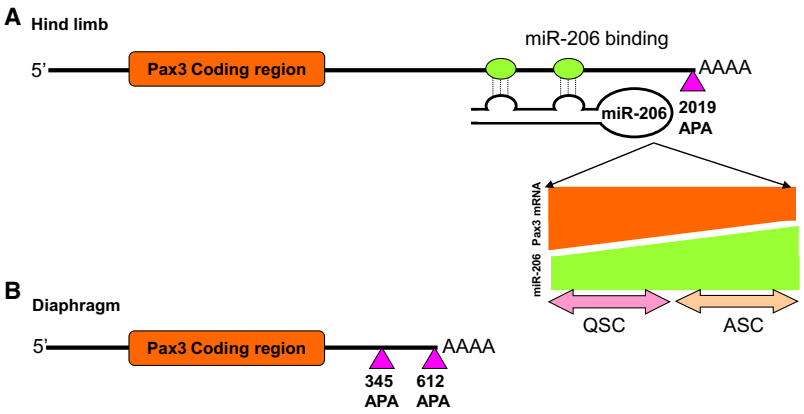

Figure 1. Differential Regulation of Pax3 Expression in Hind Limb and Diaphragm Muscle
(A) In hind limb, alternative polyadenylation (APA) preferentially occurs at the 2019 position, resulting in a 3′UTR containing the miR-206 target sequence and severely decreased expression of Pax3 mRNA in activated satellite cells (ASC) compared with that in quiescent satellite cells (QSC).
(B) In the diaphragm, APA occurs predominantly at positions 345 or 612. Consequently a short Pax3 isoform lacking the miR-206 binding site is produced that escapes the regulation.

The finding that Pax3 mRNA undergoes alternative polyadenylation in satellite cells in a manner dependent on anatomical location is particularly intriguing. Alternative polyadenylation (APA) is the addition of polyA tail to alternative 3′ ends of a given mRNA. APA has been shown to modulate mRNA stability, protein translation, and miRNA targeting. Ultimately APA is involved in a variety of different cellular processes, such as organ development, cell differentiation, or tissue-specific gene expression.

It is clear that use of alternative polyA sites is yet another level of gene regulation because more than 50% of the human genes are regulated by APA (Tian et al., 2005). This work is, to our knowledge, the first one in the muscle field to pinpoint a specific example of gene regulation by the use of APA with a physiological role in stem cell function. Thus, this study has set the stage to further explore the role of APA in modulating stem cell behavior. It is likely that there exist out there even more alternative tales to be told.

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