A novel regulator for the generation of muscle stem cells

The skeletal muscle is the largest organ in human body and plays a key role in locomotion and systemic energy homeostasis. Muscle dystrophy and atrophy often leads to frailty and fatality, underscoring the importance for maintaining proper muscle mass – a function mediated by a population of muscle resident stem cells called satellite cells. Satellite cells are derived from proliferating embryonic myogenic progenitor cells (MPCs) that mostly differentiate and fuse to form multinuclear muscle cells called myofibers. A small proportion of MPCs withdraws from the cell cycle, adopts a reversible quiescent state and resides on the surface of nascent myofibers to form satellite cells. What regulates the balance between differentiation and generation of satellite cells from MPCs during development is not well understood.

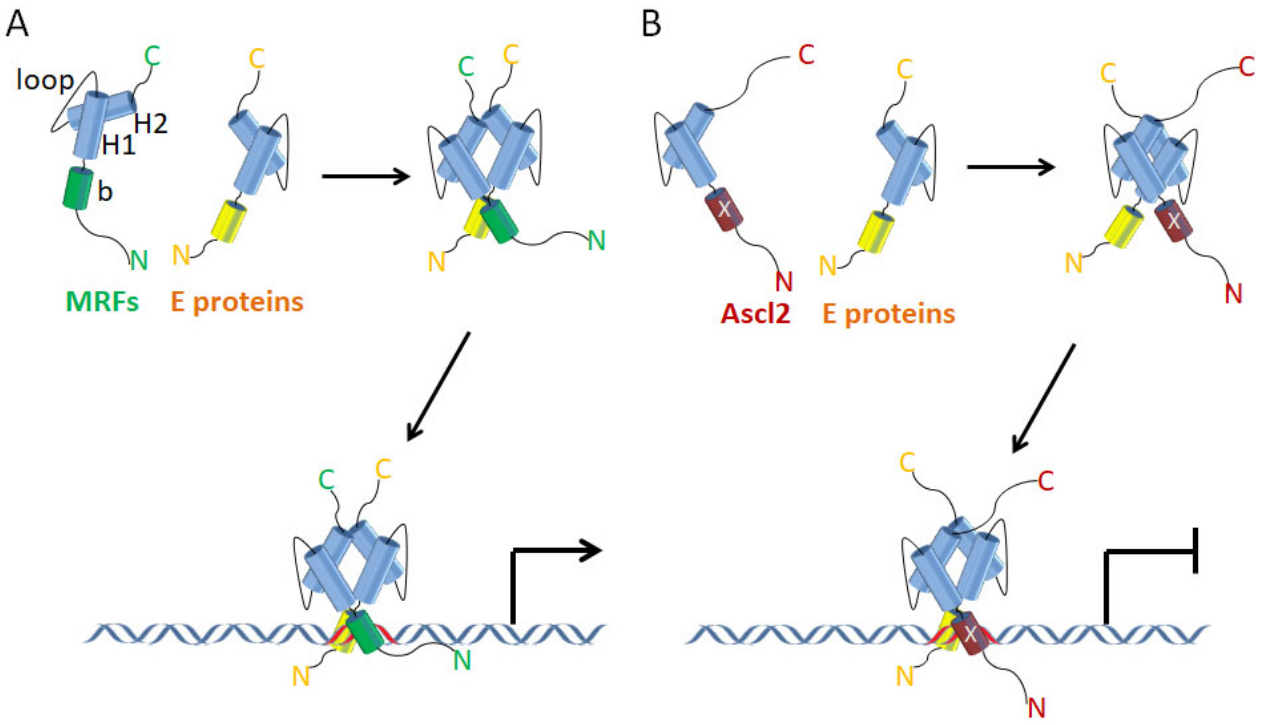

Fig. 1. A graphical model summarizing how Ascl2 modulates myogenesis
(A) Schematic of how MRFs regulate gene expression. MRFs and E proteins form heterodimers. The heterodimers subsequently bind to a hexameric consensus DNA sequence (highlighted by red color), the E-box to activate the transcription of target genes. (B) Schematic of how Ascl2 represses the activity of MRFs. Ascl2 has mutated basic region and thus loses the transcriptional activity. Ascl2 represses MRFs by competitively binding with E proteins and E-boxes, and inhibits the transcription of MRFs-targeted genes. H1, H2, loop and b indicate helix motifs, loop motif and basic region, respectively. Basic regions of MRFs, E proteins and Ascl2 have different colors. The basic region of Ascl2 is labeled by X, indicating the loss of myogenic activation ability.

Several transcriptional factors have been shown to regulate MPCs during development of limb and trunk muscles. In particular, MPCs are derived from somatic progenitor cells expressing paired box transcription factors, Pax3 and Pax7. A subpopulation of these Pax3/7^{+} cells subsequently express myogenic regulatory factors (MRFs), including Myf5, MyoD, Myog and Mrf4. Expression of MRFs commit the Pax3/7^{+} cells to MPCs (also called myoblasts) and drive their differentiation into muscle cells. At the end of the fetal stage, a portion of Pax7^{+}MRF^{+} myoblasts revert to Pax7^{+}MRF^{-} cells to become satellite cells. Hence, the process of generating satellite cells involves timely repression of MRFs. What represses the activity and expression of MRFs, however, has been elusive.

In this study, we identify the achaete-scute homologue 2 (Ascl2) as a novel repressor of MRFs during the generation of satellite cells. All MRFs contain a basic Helix-loop-Helix (bHLH) domain that has two functions. First, the bHLH of MRFs typically form heterodimers with ubiquitous bHLH proteins, also known as E proteins. Second, the heterodimeric MRF/E-proteins bind to a hexameric consensus DNA sequence, the E-box (CANNTG), to activate the transcription of target genes (Fig. 1A). Ascl2 also contains a bHLH domain, but its basic region lacks key amino acids that are responsible for transcriptional activation. In turn, when Ascl2 dimerizes with an E-protein, the heterodimer would still be able to bind to E-box, but cannot activate transcription of muscle genes (Fig. 1B). This way, Ascl2 inhibits differentiation of MPCs and promotes their entry to a quiescent state, thus facilitating the generation of satellite cells.

In the context of skeletal muscle development, it is crucial that Ascl2 expression is temporally and spatially regulated so that the majority of MPCs can undergo normal differentiation into muscles before being made into satellite cells. In this regard, Ascl2 is transiently detected in a subpopulation of MPCs that are destined to become satellite cells prior to birth. Knockout of Ascl2 increases the activity and expression of MRFs and impedes the generation of satellite cells. Conversely, overexpression of *Ascl2* inhibits the activity and expression of MRFs, and promotes generation of quiescent satellite cells. Importantly, forced expression of MyoD or Myog is sufficient to negate the effect of ectopic Ascl2. These data demonstrate that Ascl2 inhibits myogenesis through repressing the transcriptional activities of MRFs, and identify Ascl2 as a novel member of the repressive bHLH factor that are key for the generation of satellite cells.

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