Developmental Cell
Article

# Bmp Signaling at the Tips of Skeletal Muscles Regulates the Number of Fetal Muscle Progenitors and Satellite Cells during Development

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DOI 10.1016/j.devcel.2010.02.008

## SUMMARY

**Muscle progenitors, labeled by the transcription factor Pax7, are responsible for muscle growth during development. The signals that regulate the muscle progenitor number during myogenesis are unknown. We show, through in vivo analysis, that Bmp signaling is involved in regulating fetal skeletal muscle growth. Ectopic activation of Bmp signaling in chick limbs increases the number of fetal muscle progenitors and fibers, while blocking Bmp signaling reduces their numbers, ultimately leading to small muscles. The Bmp effect that we observed during fetal myogenesis is diametrically opposed to that previously observed during embryonic myogenesis and that deduced from in vitro work. We also show that Bmp signaling regulates the number of satellite cells during development. Finally, we demonstrate that Bmp signaling is active in a subpopulation of fetal progenitors and satellite cells at the extremities of muscles. Overall, our results show that Bmp signaling plays differential roles in embryonic and fetal myogenesis.**

## INTRODUCTION

Skeletal muscle development, growth, and regeneration rely on muscle stem cells. An important goal is to understand the source and the nature of the signals regulating these muscle stem cells during myogenesis.

During vertebrate development, the successive phases of primary (embryonic), secondary (fetal), and postnatal (adult) myogenesis leading to the formation of skeletal muscles involve different muscle progenitor populations. Primary myogenesis is the formation of the first multinucleated muscle fibers from embryonic progenitors, which differentiate by fusing with each other. This will establish the scaffold of muscles. This phase is usually considered to take until Embryonic Day (E) 6 in the chick (Stockdale, 1992) and E14.5 in the mouse (Biressi et al., 2007a). Secondary myogenesis depends upon fusion of fetal progenitors, which give rise to secondary fibers. Secondary myogenesis is important for the growth and maturation (fiber type) of muscles during embryonic development. During the perinatal period, there is considerable growth of muscle, mediated by adult muscle progenitors—the satellite cells. During all adult life, satellite cells reside around the muscle fibers in a quiescent state, and are solicited for muscle homeostasis, hypertrophy, and regeneration. Embryonic, fetal, and adult progenitors differ in their in vitro characteristics (Biressi et al., 2007a; Stockdale, 1992). In vivo, they will generate primary, secondary, and adult fibers, respectively, which differ in their morphology, myosin heavy chain isoforms, and muscle genes that they express (Biressi et al., 2007a; Stockdale, 1992). Over time, these muscle progenitors have distinct genetic requirements (Hutcheson et al., 2009; Lepper et al., 2009). However, despite this heterogeneity over time, a pool of resident progenitors is maintained in developing muscles during embryonic development and postnatal growth. The paired-box transcription factors, *Pax3* and *Pax7*, define this progenitor cell population during all the stages of skeletal muscle formation (Hutcheson et al., 2009; Kassar-Duchossoy et al., 2005; Relaix et al., 2005; Schienda et al., 2006). In the absence of both *Pax3* and *Pax7*, muscle development is arrested (Relaix et al., 2005). *Pax3* defines the embryonic myoblast population, and is required for its formation, while *Pax7* labels fetal and adult myoblasts, and is required for their formation (Hutcheson et al., 2009; Kassar-Duchossoy et al., 2005; Relaix et al., 2005). However, *Pax3* and *Pax7* function is dispensable for adult muscle regeneration (Lepper et al., 2009).

The signals regulating the pool of muscle progenitors during embryonic, fetal, and perinatal myogenesis have not been clearly identified. Classical signaling pathways, such as the Notch and Wnt pathways, were thought to be involved in this process. Notch signaling is involved in the maintenance of embryonic and fetal muscle progenitors and in the generation of satellite cells during mouse embryogenesis (Schuster-Gossler et al., 2007; Vasyutina et al., 2007). Components of the canonical and noncanonical Wnt pathways have been implicated in the proliferation of embryonic muscle progenitors in chick somites (Galli et al., 2004), in the maintenance of fetal muscle progenitors in mouse limbs (Hutcheson et al., 2009), and in the expansion of satellite cells (Le Grand et al., 2009; Otto et al., 2008; Perez-Ruiz et al., 2008). However, modification of Wnt signaling has provided conflicting results concerning muscle differentiation, with various manipulations of Wnt signaling reported to both inhibit and promote myogenic differentiation in vitro (Gavard et al., 2004; Goichberg et al., 2001; Kim et al., 2008; Perez-Ruiz et al., 2008) and in vivo (Anakwe et al., 2003; Hutcheson et al., 2009).

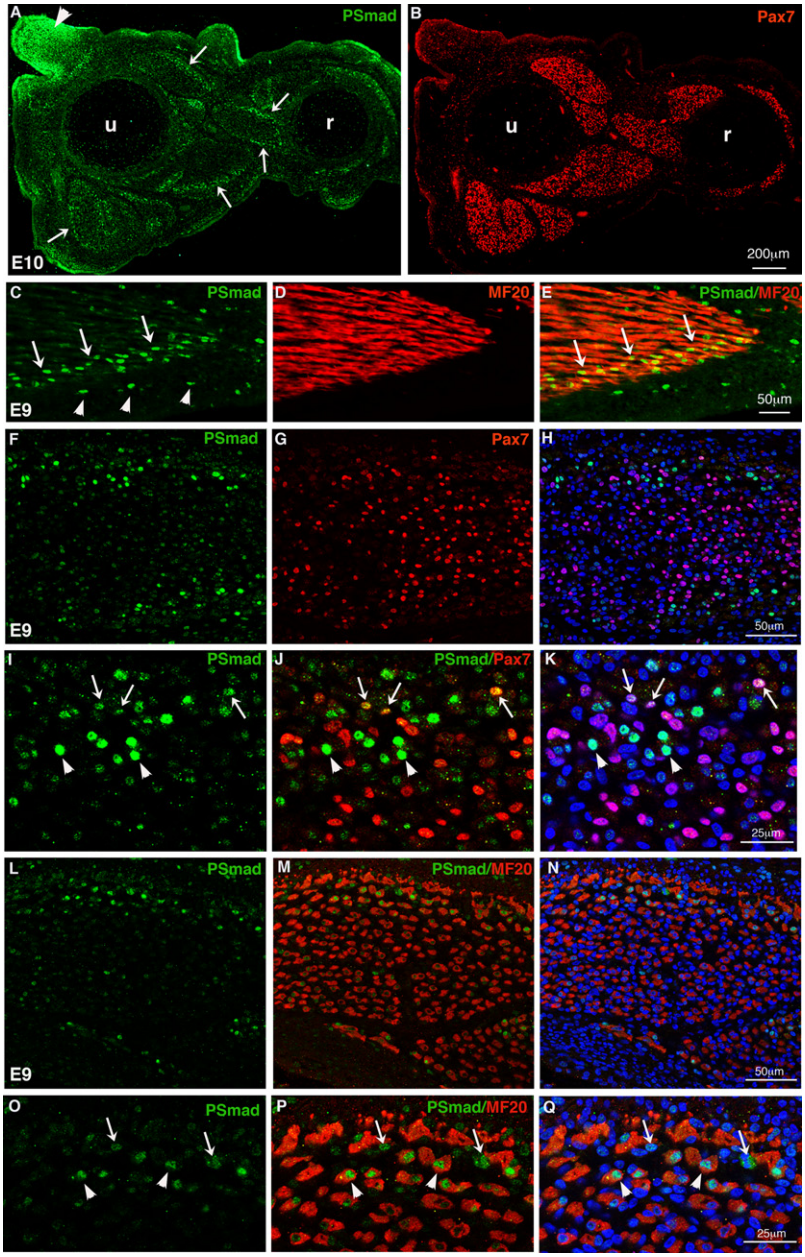


**Figure 1. Bmp Signaling Is Active at the Tendon/Muscle Interface**

(A and B) Transverse E10 limb sections were simultaneously incubated with the PSmad antibody showing active Bmp signaling (green) and the Pax7 antibody (red) revealing the muscles. Arrows point to PSmad expression at the muscle borders, and the arrowhead shows active Bmp signaling in a feather bud.

(C–E) Longitudinal E9 limb sections were simultaneously incubated with the PSmad antibody showing active Bmp signaling (green) and the MF20 antibody (red) revealing the muscles. Arrows point to the PSmad$^+$ cells in muscles, and arrowheads indicate to the PSmad$^+$ cells in tendon regions.

(F–Q) Forelimbs of E9 chick embryos were cut transversely and incubated with either PSmad and Pax7 antibodies (F–K) or PSmad and MF20 antibodies (L–Q).

(I–K) are higher magnifications of (F–H). Arrows point to the Pax7$^+$ cells displaying active Bmp signaling, while arrowheads indicate PSmad$^+$ cells not expressing Pax7.

(L–N) Lower magnifications of (O)–(Q).

(O–Q) Arrowheads indicate the PSmad expression in MF20$^+$ fibers at the borders of muscles, while arrows indicate PSmad$^+$ cells outside MF20$^+$ fibers.

(H, K, N, and Q) The merged pictures of (F) and (G), (I) and (J), (L) and (M), and (O) and (P), respectively, combined with Hoechst labeling. r, radius; u, ulna.

Bmps (bone morphogenetic proteins) constitute a subgroup of the transforming growth factor (TGF)-β super family, the members of which act through heteromeric complex of serine/threonine kinase receptors. Smad1, Smad5, and Smad8 (referred as Smad1/5/8) are the specific intracellular transducers of Bmp ligands. Upon Bmp stimulation, Smad1/5/8 are phosphorylated by Bmp-activated receptors, associate with Smad4, and translocate into the nucleus to regulate gene expression (Massague, 2008; Nohe et al., 2004). Bmps are usually considered potent inhibitors of embryonic, fetal, and adult muscle differentiation (Amthor et al., 1998; Biressi et al., 2007b; Dahlqvist et al., 2003; Frank et al., 2006; Tzahor et al., 2003), although application of low levels of Bmps upregulates *Pax3* expression during primary myogenesis in early chick limbs and somites (Amthor et al., 1998, 1999). The in vivo role of Bmp signaling on fetal muscle growth and satellite cell formation during development has never been addressed.

In the present study, we show that Bmp gain-of-function experiments increased the number of fetal muscle progenitors and satellite cells. The increase in fetal progenitor number was accompanied by an increase in the number of fetal muscle fibers. Conversely, blocking of Bmp signaling led to the opposite phenotype (i.e., a global diminution in the number of fetal progenitors and of satellite cells, ultimately leading to small muscles). We have identified a subpopulation of Pax7$^+$ cells responding to Bmp signaling at the extremities of the muscles close to the tendons, which produced Bmp4. We conclude that active Bmp signaling at the tips of muscles regulates the correct number of fetal progenitors and satellite cells during myogenesis.

## RESULTS

### Active Bmp Signaling Is Increased at the Muscle-Tendon Interface

In order to define the cells in which Bmp signaling was active, we used an antibody that detected phosphorylated Smad1/5/8 (referred as PSmad), reflecting active Bmp signaling pathway (Faure et al., 2002). In chick limbs, this PSmad antibody labeled the ectodermal buds (Figure 1A, arrowhead), consistent with the presence of *Bmp4* transcripts in this tissue (see Figure S1A [arrowhead] available online). In E10 muscles, Bmp activity