<Further Development 19_05>

Maturation of Muscle

Myoblasts and myofibers

The cells producing the myogenic regulatory factors are the myoblasts—committed muscle cell precursors—but unlike most cells of the body, muscle cells do not function as “individuals.” Rather, several myoblasts align together and fuse their cell membranes to form a myofiber, a single large cell with several nuclei that is characteristic of muscle tissue (Konigsberg 1963; Mintz and Baker 1967; Richardson et al. 2008). Myofibers in the adult can be the result of thousands of fusion events involving mononucleated cells. Studies on mouse embryos show that by the time a mouse is born, it has the adult number of myofibers and that these multinucleated myofibers grow during the first week after birth by the fusion of singly nucleated myoblasts (Ontell et al. 1988; Abmayr and Pavlath 2012). After the first week, muscle cells can continue to grow by the fusion of muscle stem cells (satellite cells, discussed below) into existing myofibers and by an increase in contractile proteins within the myofibers.

Myoblast fusion. The first step in fusion requires the myoblasts to exit the cell cycle, which involves expression of cyclin D3 (Gurung and Pamaik 2012). Next, the myoblasts secrete fibronectin and other proteins onto their extracellular matrices and bind to it through α5β1 integrin, a major receptor for these extracellular matrix components (Menko and Boettiger 1987; Boettiger et al. 1995; Sunadome et al. 2011). If this adhesion is experimentally blocked, no further muscle development ensues; thus, it appears that the signal from the integrin-fibronectin attachment is critical for instructing myoblasts to differentiate into muscle cells (Figure 1).

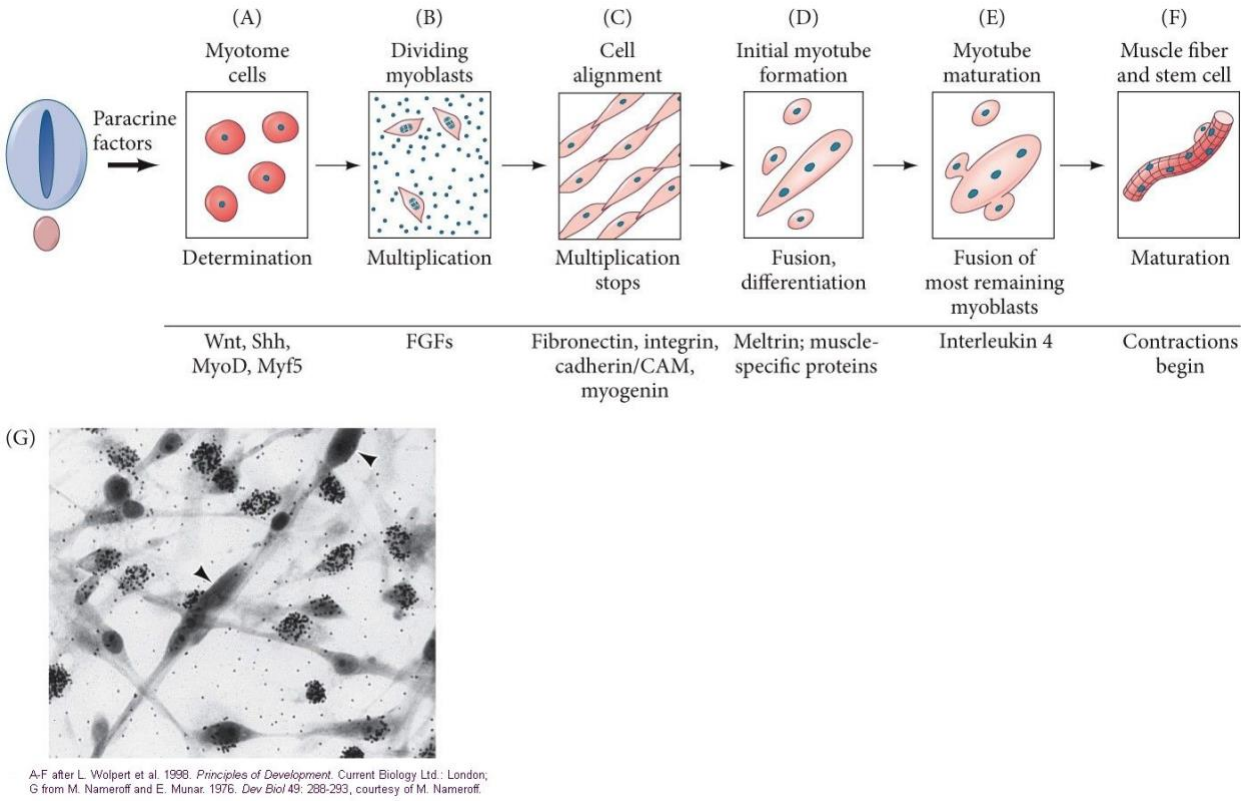

Figure 1 Conversion of myoblasts into muscles in culture. (A) Determination of myotome cells by paracrine factors. (B) Committed myoblasts divide in the presence of growth factors (primarily FGFs) but show no obvious muscle-specific proteins. (C–E) When the growth factors are used up, the myoblasts cease dividing, align, and fuse into myotubes. (F) The myotubes become organized into muscle fibers that spontaneously contract. (G) Autoradiograph showing DNA synthesis in myoblasts and the exit of fusing cells from the cell cycle. Phospholipase C can "freeze" myoblasts after they have aligned with other myoblasts but before their membranes fuse. Cultured myoblasts were treated with phospholipase C and then exposed to radioactive thymidine. Unattached myoblasts continued to divide and thus incorporated the radioactive thymidine into their DNA. Aligned (but not yet fused) cells (arrowheads) did not incorporate the label. (A–F after Wolpert 1998; G from Nameroff and Munar 1976, courtesy of M. Nameroff.)

The third step is the alignment of the myoblasts into chains. This step is mediated by cell membrane glycoproteins, including several cadherins (Knudsen 1985; Knudsen et al. 1990). Recognition and alignment between cells take place only if the cells are myoblasts. Fusion can occur even between chick and rat myoblasts in culture (Yaffe and Feldman 1965); the identity of the species is not critical. The internal cytoplasm is rearranged in preparation for the fusion, with actin regulating the regions of contact between the cells (Duan and Gallagher 2009).

The fourth step is the cell fusion event itself. As in most membrane fusions, calcium ions are critical, and fusion can be activated by calcium transporters, such as A23187, that carry Ca^{2+} across cell membranes (Shainberg et al. 1969; David et al. 1981). Fusion appears to be mediated by a set of metalloproteinases called meltrins. Meltrins were discovered during a search for myoblast proteins that would be homologous to fertilin, a protein implicated in sperm-egg membrane fusion. Yagami-Hiromasa and colleagues (1995) found that one of these proteins, meltrin-α, is expressed in myoblasts at about the same time that fusion begins, and that antisense RNA to the meltrin-α message inhibited fusion when added to myoblasts. As the myoblasts become capable of fusing, another myogenic regulatory factor—myogenin—becomes active. Myogenin binds to the regulatory region of several muscle-specific genes and activates their expression. Thus, whereas MyoD and Myf5 are active in the lineage specification of muscle cells, myogenin appears to mediate muscle cell differentiation (Bergstrom and Tapscott 2001).

Cell fusion ends with the re-sealing (“healing”) of the newly apposed membranes. This step is accomplished by proteins such as myoferlin and dysferlin, which stabilize the membrane phospholipids (Doherty et al. 2005). These proteins are similar to those that re-seal the membranes at axon nerve synapses after membrane vesicle fusion releases neurotransmitters.

Myofiber growth. After the original fusion of myoblasts into a myofiber, the myofiber secretes the paracrine factor interleukin 4 (IL4). Although IL4 was originally believed to work exclusively in the adult immune system, Horsely and colleagues (2003) found that IL4 secreted by new myofibers recruits other myoblasts to fuse with the myotube, thereby forming the mature myofiber (see Figure 1).

The number of muscle fibers in the embryo and the growth of these fibers after birth appear to be negatively regulated by myostatin, a member of the TGF-β family (McPherron et al. 1997; Lee 2004). Myostatin is made by developing and adult skeletal muscle and probably works in an autocrine fashion. Loss-of-function *myostatin* mutations allow both hyperplasia (more fibers) and hypertrophy (larger fibers) of the muscle (see Figure 1 in Further Development Online 3.17 <Make link to Further Development Online 3.17>). These changes give rise to Herculean phenotypes in dogs, cattle, mice, and humans (Figure 2).

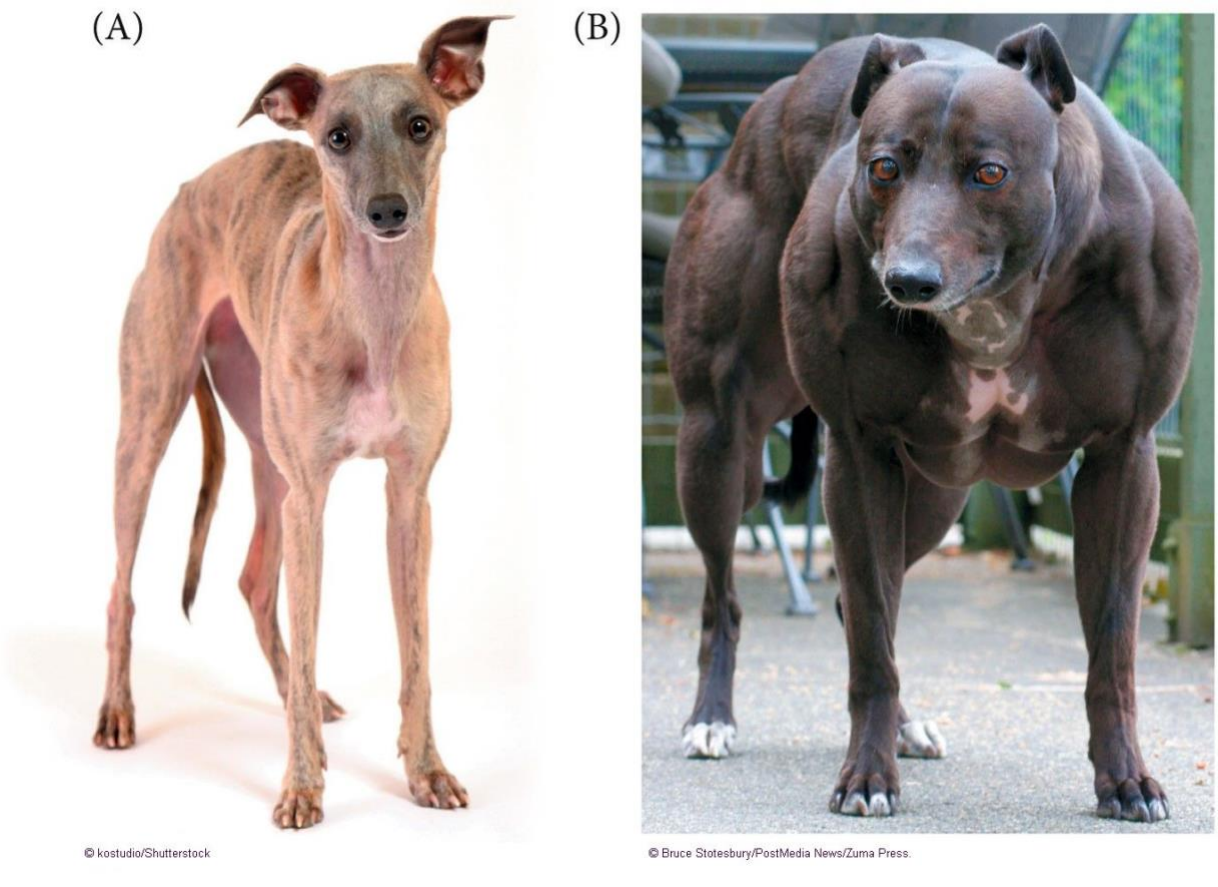

Figure 2 A loss-of-function mutation in the *Myostatin* gene of whippets. (A) Whippets are a typically slender breed, bred for speed and dog racing. (B) Although the homozygous loss-of-function condition is not advantageous, heterozygotes have more muscle power and are significantly overrepresented among the top racers (see Mosher et al. 2007). (A © kustudio/Shutterstock; B © Bruce Stotesbury/PostMedia News/Zuma Press.)

Satellite cells: Unfused muscle progenitor cells

Any dancer, athlete, or sports fan knows that (1) adult muscles grow larger when they are exercised, and (2) muscles are capable of limited regeneration following injury. The growth and regeneration of muscles both arise from satellite cells, populations of stem cells and progenitor cells that reside alongside the adult muscle fibers. Satellite cells respond to injury or exercise by proliferating into myogenic cells that fuse and form new muscle fibers. Lineage tracing using chick-quail chimeras indicates that satellite cells are somite-derived myoblasts that have not fused and that remain potentially available throughout adult life (Armand et al. 1983).

The source of mouse and chick satellite cells appears to be the central part of the dermomyotome (Ben-Yair and Kalcheim 2005; Gros et al. 2005; Kassar-Duchossoy et al. 2005; Relaix et al. 2005). Although the myoblast-forming cells of the dermomyotome form at the lips and express Myf5 and MyoD, the cells that enter the myotome from the central region usually express Pax3 and Pax7 as well as microRNAs miRNA-489 and miRNA-31. The combination of Pax3 and Pax7 appears to inhibit MyoD expression (and thus muscle differentiation) in these cells; Pax7 also protects the satellite cells against apoptosis (Olguin and Olwin 2004; Kassar-Duchossoy et al. 2005; Buckingham et al. 2006). The two microRNAs appear to prevent the translation of factors such as Myf5 that would promote muscle cell differentiation (Cheung et al. 2012; Crist et al. 2012).

Satellite cells are not a homogeneous population; rather, they contain both stem cells and progenitor cells. The stem cells represent only about 10% of satellite cells and are found, with the other satellite cells, between the cell membrane and the extracellular basal lamina of mature myofibers. Satellite stem cells express Pax7 but not Myf5 (this is designated $Pax7^{+}/Myf5^{-}$) and can divide asynchronously to produce two types of cells: another $Pax7^{+}/Myf5^{-}$ stem cell and a $Pax7^{+}/Myf5^{+}$ satellite progenitor cell that differentiates into muscle (Figure 3). The $Pax7^{+}/Myf5^{-}$ stem cells, when transplanted into other muscles, contribute to the stem cell population there (Kuang et al. 2007).

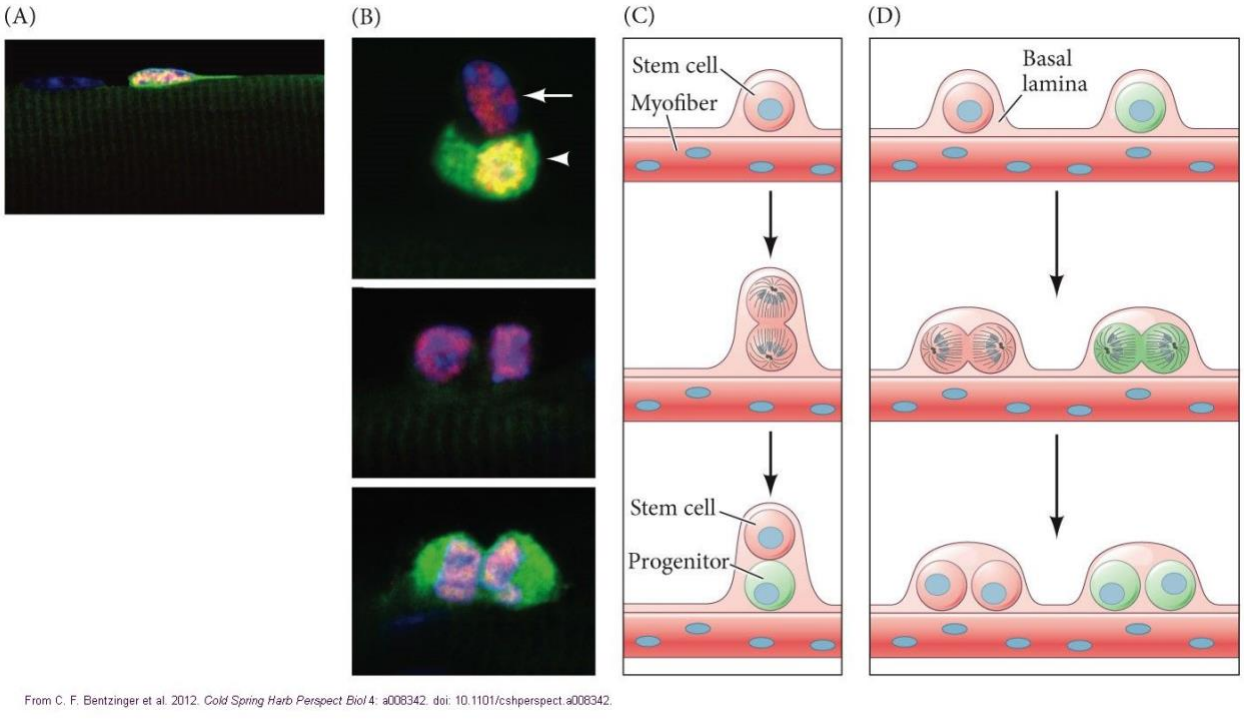

Figure 3 Satellite cells and muscle growth. (A) Satellite cells (stained with antibodies to the Pax7 protein) reside between the myofiber cell membrane and the basal lamina. (B) The top photograph shows asymmetrical cell division of a satellite

stem cell and the distinction between the daughter cell keeping Pax7 (stem cell; red) and the daughter cell downregulating Pax7 and expressing Myf5 (progenitor cell; green). The bottom two photographs show symmetrical division, with stem cells and progenitor cells making more stem and progenitor cells, respectively. (C) Diagrammatic representation of the asymmetrical cell division shown in the top photograph in (B). (D) Diagrammatic representation of the bottom two photographs in (B). (After Bentzinger et al. 2012; photographs courtesy of F. Bentzinger and M. A. Rudnicki.)

The factor responsible for the asymmetry of this division appears to be miRNA-489, which is found in quiescent stem cells. Upon division, miRNA-489 remains in the daughter that remains a stem cell but is absent in the cell that becomes part of the muscle. MiRNA-489 inhibits the translation of the message for the Dek protein, which becomes translated in the daughter cell that differentiates. Dek is a chromatin protein that promotes the transient proliferation of progenitor cells (Cheung et al. 2012). Thus, miRNA-489 maintains the quiescent state of an adult muscle stem cell population.

Mechanotransduction in the musculoskeletal system

We know that physical forces generated by exercise cause muscles to enlarge. Exercise stimulates protein synthesis in the muscle cells, and each nucleus in the multinucleate fiber appears to have a region around it where protein synthesis is regulated (Lai et al. 2004; Quaisar et al. 2012). If physical stress continues, the force appears to cause the muscle satellite cells to proliferate and fuse with the existing muscle fibers. Indeed, endurance exercise has been shown to increase the number of satellite cells in the elderly (Shefer et al. 2010). Insulin-like growth factor, acting as an autocrine secretion from muscle cells, is a candidate for causing such muscle growth (Yang 1996; Goldspink 2004; Sculthorpe et al. 2012), but how this factor or any other is induced by stress may involve induction by other factors, such as TGF-β (Pumklin et al. 2015).

Also, in a way that is not yet understood, the tension produced by weight-bearing loads activates production of TGF-β2 and 3 in the tendon cells (Maeda et al. 2011). Indeed, mice lacking these genes completely lack tendons. The TGF-β pathway (through the Smad2/3 transcription factors) continues to activate the gene for the transcription factor Scleraxis after the initial FGF signaling; in turn, Scleraxis activates the genes responsible for forming the extracellular matrix. Moreover, TGF-β produced by the developing tendon may recruit cells from the cartilage and muscle to make the bridge between these three tissues (Blitz et al. 2009; Pryce et al. 2009).