fide satellite cells. The three growth factors families discussed here are: Fibroblasts Growth Factor (FGF) family, Hepatocyte Growth Factor (HGF) family and Transforming Growth Factor beta (TGFβ) super family. The TGFβ super family is comprised of several families, here we mostly focus on the main TGFβ growth factors and myostatin. As discussed in the later part of this chapter, there is a general consensus that certain FGFs and HGF are positive regulators that promote satellite cell proliferation and in some instances can also delay differentiation of satellite cell progeny. Studies show that members of the TGFβ super family (myostatin included) may inhibit proliferation and are therefore considered negative regulators-whereas other studies suggest that TGFβ growth factors support proliferation and inhibit differentiation. A general overview of each family of growth factors is first introduced followed by a discussion on specific studies related to myogenesis.

Insulin-like growth factors (IGFs) and platelet-derived growth factors (PDGFs) were also implicated to have a regulatory role in myoblast proliferation but their *direct* effect on bona fide satellite cells has not been demonstrated. IGFs presumably have a dual effect on myoblasts, supporting both proliferation during early stages of myogenesis and differentiation during later stages (Booth, 2006; Florini et al., 1996; Rosenthal and Cheng, 1995; Mourkioti and Rosenthal, 2005). Nevertheless, such a dual effect was observed only in cell lines and long-term proliferating myoblasts, yet it remains unclear if IGFs indeed affect proliferation of satellite cells and their progeny or just play a central role in myofiber hypertrophy (Allen and Boxhorn, 1989; Bischoff, 1986). Platelet-derived growth factors (PDGFs) were originally shown to regulate myogenesis in long-term proliferating myoblasts and myogenic cell lines (Jin et al., 1991; Yablonka-Reuveni et al., 1990; Yablonka-Reuveni and Seifert, 1993; Yablonka-Reuveni and Rivera, 1997; Jin et al., 1993; McFarland et al., 1997). However, unpublished results from our laboratory suggest that rodent satellite cells in isolated myofibers and their progeny do not respond to IGFs or PDGFs. The inconsistency between the two conclusions made with the different culture systems may reflect biological differences between myoblasts undergoing multiple passages in culture to bona fide satellite cells and their immediate progeny. It is however possible that difference in culture conditions-contribute to differing results and that all cell culture models provide physiologically relevant information. Caution should be taken when projecting conclusions from cell line studies onto bona fide satellite cell biology since long-term propagation of cell lines results in alternated expression of growth factor receptors and altered response to growth factors.

## 1.2 The Inside: Interplay Between Transcriptional Loops and Cell Cycle Regulators Govern Satellite Cell Transition from Quiescence to Differentiation

Adult skeletal muscle is composed of mutlinuclated myofibers (fibers) that are established during embryogenesis by fusion of myogenic cells (myoblasts). Typically, in a healthy muscle the myofiber nuclei (myonuclei) are mitotically inactive. Addition

of new myonuclei or formation of new myofibers for supporting muscle growth and repair depends on satellite cells, myogenic stem cells located underneath the fiber basal lamina (Mauro, 1961; Collins et al., 2005; Shefer, 2006). During postnatal growth, activated satellite cells proliferate to form new myoblasts that fuse with the growing myofibers. In mature muscles, satellite cells are typically quiescent, but can be recruited following subtle injuries (e.g., due to exercise) or massive muscle damage (e.g., due to trauma). Satellite cells support muscle integrity by giving rise to progeny that fuse with existing myofibers when minute repairs are needed, and by generating a large pool of progeny cells to form new myofibers upon massive damage (Grounds and Yablonka-Reuveni, 1993; Hawke and Garry, 2001). Since small myofiber injuries routinely occur during daily activity, the need for ongoing repair is essential for muscle maintenance. Therefore, a balance between satellite cell proliferation and differentiation must exist in order to maintain both functional fibers and the satellite cell reservoir.

At the molecular level, myogenesis of satellite cells is regulated in a highly orchestrated fashion to ensure that specific genes are turned on and off in a temporally organized manner according to genetic blueprints, cell cycle requirements, and environmental factors. The resulting pattern of gene expression yields the terminally differentiated myoblasts that are capable of adding myonuclei to existing myofibers in addition to fusing together to form new myofibers during muscle growth and repair. Both quiescent and proliferating satellite cells express the paired-homeobox transcription factor Pax7 (Collins et al., 2005; Shefer, 2006; Halevy et al., 2004; Seale et al., 2000) as well as Myf5, a member of the family of muscle specific transcription factors (MRFs, that include also MyoD, myogenin and MRF4; (Ludolph and Konieczny, 1995)). The expression of Myf5 in quiescent satellite cells has been demonstrated based on Myf5-lacZ reporter assays and endogenous Myf5 transcript expression (Beauchamp et al., 2000; Zammit et al., 2006; Day et al., 2006). Upregulation of MyoD in activated satellite cells marks the satellite cell's transition into a proliferative phase (Zammit et al., 2006; Yablonka-Reuveni and Rivera, 1994). The onset of myogenin expression marks a commitment of satellite cell progeny to differentiate (Yablonka-Reuveni and Rivera, 1994; Andres and Walsh, 1996). This differentiation commitment is also associated with a decline in Pax7 and Myf5 expression, a withdrawal from the cell cycle and subsequent fusion of myoblasts into multinucleated myotubes (Shefer, 2006; Halevy et al., 2004; Yablonka-Reuveni and Rivera, 1997; Zammit et al., 2004).

The role of Pax7 during satellite cell myogenesis has been under debate ever since its expression in these cells was first identified (Seale et al., 2000). Some studies suggest that Pax7 is required for satellite cell renewal while others proclaim that Pax7 is actually required for satellite cell survival rather than renewal per se (Seale et al., 2000; Oustanina et al., 2004; Casar et al., 2004). Nevertheless, there are solid evidence, indicating that satellite cells typically express Pax7 regardless of the type of parent fiber (i.e., fast versus slow) they are associated with, and that their self-renewed progeny also express Pax7 (and not MyoD), similar to their ancestors (Collins et al., 2005; Shefer, 2006; Day et al., 2006; Zammit et al., 2004).

The role of MRFs as myogenic determination factors during myogenic lineage establishment in early developed, and also as regulators of myogenic differentiation in the adult is well established (Ludolph and Konieczny, 1995; Kassar-Duchossoy et al., 2004). However, the role of MRFs during the life cycle of satellite cells is less clear. It is commonly held that MyoD serves as a master transcription factor that directs activation of differentiation-linked genes (Tapscott, 2005). Continuous MyoD expression in differentiated progeny of satellite cells seems to depend on the extracellular environment. In a serum-replacement based medium satellite cells undergo as little as one or two rounds of proliferation before rapidly entering differentiation, after which their progeny will express myogenin but not MyoD (Yablonka-Reuveni and Rivera, 1997; Yablonka-Reuveni et al., 1999a; Yablonka-Reuveni et al., 1999b). Although MyoD is expressed in proliferating progeny of satellite cells (Shefer and Yablonka-Reuveni, 2006), the actual function of MyoD during myoblast proliferation remains to be determined (see (Wyzykowski et al., 2002) for a proposed role). The findings that Myf5 expression declines when myoblasts enter differentiation, whereas MyoD expression persists well into the differentiation stage, suggest that these two MRFs have different roles during myogenesis of satellite cells (Zammit et al., 2006). Myogenin expression is critical for muscle formation during embryogenesis. However, conditional impairment of myogenin in the adult muscle does not interfere with myogenesis, raising further questions about the actual role of myogenin in adult life (Knapp et al., 2006). Lastly, the role of MRF4 during myogenesis of satellite cells is also unclear, as in different studies its expression was detected before, after or concurrently with myogenin expression (Smith et al., 1993; Smith et al., 1994).

Members of the myocyte enhancer factor 2 (MEF2) transcription factor family are also involved in myogenesis regulation (Molkentin et al., 1995; Black and Olson, 1998). MRFs and MEF2s function in concert to support the timely expression of muscle specific structural proteins following differentiation commitment. We showed that the transition into the MEF2A-expressing state occurs together with, or shortly after, the onset of myogenin expression in differentiating satellite cells (Kastner et al., 2000; Yablonka-Reuveni and Rivera, 1997). The dual expression of myogenin and MEF2A is soon followed by the expression of sarcomeric myosin (Yablonka-Reuveni and Rivera, 1997). The initial stage of myogenin expression marks myoblast commitment to differentiate. Terminal differentiation of such myogenin-expressing cells involves withdrawal from the cell cycle (Andres and Walsh, 1996; Wang and Walsh, 1996). Cell cycle regulators that are essential for this terminal differentiation include the cyclin dependent kinase cyclin D3, the cyclin-dependent kinase inhibitors p21 and pRb (Andres and Walsh, 1996; Halevy et al., 1995; Kiess et al., 1995; Cenciarelli et al., 1999). pRb is involved in the regulation of both cell cycle withdrawal and the expression of differentiation-linked structural genes (Halevy et al., 1995; Novitch et al., 1999; De Falco et al., 2006). The upregulation of p21, pRb and cyclin D3 in myogenic cells is thought to be governed by MyoD (Halevy et al., 1995; Cenciarelli et al., 1999). Hence, MyoD must be kept in a transcriptionaly inactive form in proliferating cells until appropriate signals

for inducing differentiation are conveyed (Novitch et al., 1999; Song et al., 1998; Kitzmann et al., 1999; Puri et al., 2001; Perry et al., 2001).

An interplay between transcriptional loops and cell cycle regulators during myogenesis is typically investigated in cultures of myogenic cell lines and in some instances using long-term passaged progeny of satellite cells. In such models, myoblasts remain proliferative for a longer time when placed in a serum-rich environment and rapidly differentiate when placed in a serum-poor environment (Yablonka-Reuveni et al., 1990; Yablonka-Reuveni and Rivera, 1997; Clegg et al., 1987; Yaffe and Saxel, 1977; Yaffe, 1969; Rando and Blau, 1994). It is however important to recognize that immediate progeny of satellite cells from adult skeletal muscle typically cannot be stopped from entering differentiation, regardless of medium composition or cell density (Shefer, 2006; Yablonka-Reuveni et al., 1987; Yablonka-Reuveni, 2004). Thus, the ability to stop the differentiation of long-term passaged myoblasts may reflect only a subpopulation of satellite cell progeny (especially when derived from individual myogenic clones). Moreover, cells that undergo long term passaging often transform and become immortal, introducing major variations in regulatory loops compared to founding ancestor cells.

## 1.3 The Outside: Extracellular Cues Regulate The "Built-In" Myogenic Program

### *1.3.1 Defining growth factors and their mode of action via transmembrane receptors*

Growth factors are proteins capable of stimulating cellular proliferation and differentiation. Growth factors stimulate intracellular activities by binding to their transmembrane receptors. Chemokines, cytokines and growth factors are all peptide signaling molecules. Typically, the other groups of signaling proteins are categorized according to the following guidelines: (i) Chemokines – are small protein factors (8–10 kDa) that are released from a variety of cells in response to bacterial infection, viruses and agents that cause physical damage. (ii) Cytokines – are small water-soluble proteins and glycoproteins (8–30 kDa) that are produced by a wide variety of cell types and affect nearby as well as distant cells. The term growth factor is sometimes used interchangeably with the term cytokine. Historically, cytokines were associated with hematopoietic cells and immune system cells. However, some of the signaling proteins of the hematopoietic and immune systems are known today to be common to other cells and tissues as well. (iii) Hormones (which inlcude steroids in addition to peptide molecules) are released from an organ (usually an endocrine gland) directly into the blood stream and affect nearby (paracrine) or distant target cells as they are distributed throughout the body through the blood system (endocrine). In general, hormones that are secreted into the circulation are received by appropriate organs where they produce a specific effect on metabolism. Growth factors typically do not commute via body fluids to their target cells, but rather are produced locally.

The actions of growth factors are mediated by their receptor specific binding. Growth factor receptors are classified into three major families: (i) tyrosine kinases; (ii) small G-protein-associated receptors; and (iii) serine/threonine kinases. Tyrosine phosphorylation is considered the most characteristic feature of growth factor receptors (Eswarakumar et al., 2005; DiMario, 2002). Binding to thetyrosine kinase receptor causes receptor dimerization, which leads to autophosphorylation of conserved residues in its intracellular domain. Once activated, the receptor functions as a tyrosine kinase inside the cell. A cascade of downstream signaling enzymes carries the signal from the receptor tyrosine kinase domain through cytoplasmic target kinases and into the nucleus. The end targets of the cascade are transcription factors that, once phosphorylated form multi-protein complexes with accessory proteins and bind specific promoter and enhancer sequences of target genes (Naar et al., 2001; Tartakoff, 1994). Nearly all tyrosine kinase receptors described thus far are composed of an extracellular ligand-binding domain, a single transmembrane domain, a region containing the tyrosine kinase activity, and a carboxy terminus extending into the cytoplasm (Perona, 2006). Various studies indicated that at times the receptor may also serve as a vehicle to shuttle its respective growth factor into the cell or nucleus and does not necessarily function to transduce a signaling cascade directly from its intracellular domain (see for example (Haugsten et al., 2005) for the FGF receptor system).

Growth factors that are produced within the same cells that respond to these factors are considered to have an autocrine mode of action. Growth factors that act in a paracrine manner are produced in other sites within the tissue and reach target cells by diffusion for example. Often, one set of cells produces the ligand (e.g., growth factor) while the appropriate receptor is expressed on a separate cell type. For example, within the context of skeletal muscle, we demonstrated that satellite cell progeny express both PDGF-A and PDGF-B but only the surrounding connective tissue cells are able to proliferate in response to PDGF (Kastner et al., 2000 and unpublished results).

### *1.3.2 Extracellular matrix (ECM) and cell surface heparans facilitate growth factor functions*

The complex set of signals conveyed to satellite cells by growth factors is often associated with components of the surrounding ECM, which is adjacent to the basal lamina of the myofiber. The ECM of the muscle tissue is composed of fibroblasts and a complex mesh of several types of collagen, glycoproteins, and proteoglycans. Blood vessels, especially the elaborated network of micro capillaries, also belong to the ECM constituents and affect myogenesis. In addition to serving as a structural scaffold, the ECM, especially the proteoglycan component, regulates cell behavior by interacting with growth factors and by activating cellular signal transduction pathways (Jenniskens et al., 2006; Velleman et al., 2006; Velleman, 2000).

Cell surface heparan sulphate proteoglycans often bind to transmembrane receptors (HSPGs) acting as co-receptors for enhanced binding to the respective

growth factors. HSPGs can be found anchored to the outer membrane surface or in the ECM (Bernfield et al., 1999). HSPGs are able to recognize and bind soluble ligands, and this binding yields high local ligand concentration at the cell membrane proximity that is sufficient to activate signaling receptors (Bernfield et al., 1999; Carrino, 1998). HSPGs are present ubiquitously on cell surfaces and in the ECM of most mammalian cells. Cell surface heparan sulfate (HS) is found mainly attached to two families of proteoglycans: glypicans and syndecans. HS chains found in the extracellular matrix mainly attach to perlecans and agrins (Bernfield et al., 1999). Studies with primary myoblasts cultured on gelatin or Matrigel (Yablonka-Reuveni, 2004; Hartley and Yablonka-Reuveni, 1990) as well as extensive studies with myogenic cell lines demonstrated that the ECM is essential for normal myogenesis, both through direct interactions between ECM molecules with plasma membrane receptors and through modulation of growth factor activities, such as described above (Casar et al., 2004; Osses and Brandan, 2002; Melo et al., 1996).

Members of the FGF, HGF and TGFβ families are heparin binding growth factors and their function during myogenesis is most likely facilitated by their own or by their corresponding receptors' interactions with HS and HSPGs. Such interactions were suggested to influence various processes including: stabilization of the receptor-ligand complex; protection of the ligand from denaturation; enhancement or reduction of the activity of some members of a growth factor family (or of their alternative splice forms); generating specificity during development, growth and repair; and generating micro-niches with increased concentrations of the growth factors (Bernfield et al., 1999; Roghani et al., 1994; Aikawa and Esko, 1999; Lietha et al., 2001; Ornitz, 2000). Several studies with the mouse myogenic cell line C2C12 revealed that the expression of some HSPGs is differentially regulated during differentiation. For example, synthesis of the protoglycans decorin and glypican is increased whereas the synthesis of perlecan and syndecan-1 is decreased during differentiation (Larrain et al., 1997; Larrain et al., 1997; Olwin and Hall, 1985; Brandan et al., 1991; Brandan et al., 1996). Inhibition of proteoglycan sulfation by chlorate treatment of C2C12 cultures (Osses and Brandan, 2002; Melo et al., 1996), MM14 mouse myoblasts (Olwin and Rapraeger, 1992), or isolated myofibers (Cornelison et al., 2001) affects in vitro myogenesis. Moreover, in vivo administration of synthetic polymers that mimic HSPGs accelerates both regeneration and re-innervation of skeletal muscles (Desgranges et al., 1999; Meddahi et al., 2002).

In recent years much interest has been given to the role of decorin in satellite cell myogenesis and muscle regeneration, in view of its ability to modulate myoblast responsiveness to members of the TGFβ family which in turn affects fibrosis and muscle regeneration (Riquelme et al., 2001; Miura et al., 2006; Fadic et al., 2006; Sato et al., 2003; McCroskery et al., 2005; McFarland et al., 2006). Similarly, there is a growing interest in the role of syndecans in view of the finding that satellite cells express syndecans and that muscle regeneration is impaired in mice lacking certain syndecans (Cornelison et al., 2001; Cornelison et al., 2004).

and subsequent four-kringle domains, and the β-chain contains a serine protease-like domain with no enzymatic activity (Nakamura et al., 1989; Tashiro et al., 1990; Funakoshi and Nakamura, 2003).

For its action, HGF needs to bind to its cell surface receptor, c-met (Naldini et al., 1991; Bottaro et al., 1991). C-met is a heterodimeric receptor tyrosine kinase that was initially discovered as a transforming gene from chemically treated osteosarcoma cells (Cooper et al., 1984). HGF also binds the glycosaminoglycan (GAG) chains of heparan sulfate (HS) and dermatan sulfate (DS) proteoglycans (Lyon et al., 1994; Lyon et al., 1998), although with lower affinity than to the c-met receptor. Evidence suggests that an active ternary complex forms between HGF, c-met and appropriate proteoglycans (Lyon et al., 2002). The primary c-met transcript is translated into a 150-kDa polypeptide that is further glycosylated to give a 195-kDa precursor protein. This precursor is then cleaved into a 50-kDa α-chain and a 145-kDa β-chain, which are linked via disulfide bonds (Comoglio, 1993). The mature c-met heterodimer consists of a highly glycosylated extracellular α-subunit, a β-subunit with a large extracellular region, a membrane spanning segment, and an intracellular tyrosine kinase domain. Upon HGF binding, c-met undergoes autophosphorylation of specific tyrosine residues within the intracellular region of the β chain and ignites downstream signaling (Leshem et al., 2002; Ponzetto et al., 1994; Schaeper et al., 2000; Sachs et al., 2000).

### 3.2 Effect of HGF on Activation, Proliferation and Differentiation of Satellite Cells

HGF is expressed in intact and regenerating muscle (Kastner et al., 2000, Tatsumi et al., 1998; Jennische et al., 1993; Hayashi et al., 2000). Transcripts and protein levels of HGF are increased during the early phase of muscle regeneration, and this increase is proportional to the degree of injury (Suzuki et al., 2002; Tatsumi et al., 2001). Studies demonstrated that HGF is produced by muscle cells in vitro and in vivo and is secreted to the extracellular environment where it is stored in its heterodimeric form. The c-met receptor is expressed by satellite cells and proliferating myoblasts and exogenous HGF promotes satellite cell activation and myoblast proliferation, indicating a direct role of the HGF system in satellite cell myogenesis (Kastner et al., 2000; Yablonka-Reuveni et al., 1999a; Tatsumi et al., 1998; Gal-Levi et al., 1998; Cornelison and Wold, 1997).

HGF was also suggested to play a role in preventing proliferating satellite cells from differentiating; this inhibitory effect may occur via the involvement of the basic helix loop helix protein Twist and the cyclin-dependent kinase inhibitor p27 (Leshem et al., 2002; Tatsumi et al., 1998; Anastasi et al., 1997; Zeng et al., 2002). Nonetheless, the pattern of satellite cell proliferation on isolated rat myofibers did not support the notion that HGF delays satellite cells differentiation (Kastner et al., 2000, Yablonka-Reuveni et al., 1999a). In-vivo administration of HGF to injured mouse indeed revealed enhancement of satellite cell proliferation and delayed differentiation. However, such sustained HGF administration resulted in

impaired regeneration (Miller et al., 2000). The latter study further exemplified the difficulties associated with controlling muscle regeneration by supplementation of growth factor. The interplay between myoblast proliferation and differentiation is a complex process that requires an optimal spatial and temporal milieu of multiple growth factors, each present in the right amount at the right time.

In vitro and in vivo data demonstrate that release of nitric oxide synthase from the basal lamina, in response to myofiber stretch or damage, leads to the production of nitric oxide. Nitric oxide then activates matrix metalloproteinases, which in turn can cause release of HGF from its association to HSPGs, making HGF available for binding to the c-met receptor and to activate satellite cells (Anderson, 2000; Tatsumi et al., 2006; Tatsumi et al., 2002; Yamada et al., 2006). In addition to this autocrine/paracrine mechanism that provides HGF from cellular source near by satellite cells, an endocrine delivery of HGF to the injured muscle was also suggested based on the rapid upregulation of HGF in the spleen following muscle injury (Suzuki et al., 2002).

Aside from the effect on proliferation and differentiation, HGF is also involved in promoting satellite cell migration to the site of injury, via activation of the Ras-Ral pathway, as demonstrated by the in vitro chemotactic activity of this factor in primary myogenic cultures and the C2C12 cell line (Bischoff, 1997; Suzuki et al., 2000). Taken together, these data demonstrate the pleiotropic role that HGF probably plays during muscle regeneration by boosting the proliferating myoblast population due to its mitogenic and chemotactic activities. These may be important for accomplishing a threshold myoblast density needed to start the fusion phase.

## 4. THE TGFβ SYSTEM AND ITS ROLE IN MYOGENESIS OF SATELLITE CELLS

### 4.1 The TGFβ Superfamily and its Receptors: Overview

The TGFβ superfamily consists of more than 40 members, such as TGFβs, bone morphogenetic proteins (BMPs) and growth differentiation factors (GDFs) (Shi and Massague, 2003). One of the more recently discovered members of the GDF family, named GDF8 or myostatin, is a negative regulator of embryonic and postnatal skeletal muscle growth that functions to maintain a proper muscle mass (Dominique and Gerard, 2006). The diverse TGFβ ligands share a common sequence and some structural features but elicit different cellular responses during pre- and postnatal development as well as in disease. Members of the TGFβ superfamily are known to participate in the regulation of various biological processes, such as tissue homeostasis, cell-cycle progression, differentiation, reproductive function, motility, adhesion, neuronal growth, bone morphogenesis, wound healing, and immune surveillance (Attisano and Wrana, 2002; Chang et al., 2002; Massague, 2000; Massague, 2000).

TGFβs, the prototype members of this superfamily, are released from cells as an inactive complex where their active domain is masked by a propeptide, termed

Latency Associated Peptide (LAP). They have little or no biological activity until LAP is cleaved by a furin-like endoproteinase (Dubois et al., 1995). Members of the TGFβ superfamily signal through transmembrane receptors that have a cytoplasmic serine/threonine kinase domain. The TGFβ receptors are divided into two subfamilies, type I and type II, which interact together to initiate TGFβ signaling. Both receptor types are glycoproteins of approximately 55 kDa and 70–85 kDa, respectively. Their extracellular region contains about 150 amino acids, including 10 or more cysteines that determine the folding of this region. A unique feature of the type I TGFβ receptors is a highly conserved intracellular region, composed of 30 amino acids, located upstream to the cytoplasmatic kinase domain named GS domain for its SGSGSG sequence (Wrana et al., 1994). Binding of a TGFβ ligand induces the type II receptor kinase to phosphorylate multiple serine and threonine residues in the TTSGSGSG sequence of the cytoplasmic GS region of the type I receptor, leading to its activation (Wrana et al., 1994; Souchelnytskyi et al., 1996). Binding of the ligand triggers the assembly of a receptor complex and thus initiating the phosphorylation of signaling transducers of the SMAD protein family; once phosphorylatied, SMADs migrate into the nucleus, where they assemble to form protein DNA binding complexes that control gene expression. (Massague, 2000). TGFβ type III receptor was also described (Cheifetz et al., 1988; Wickert et al., 2004). It is a large (250–350 kDa) transmembrane proteoglycan with a large extracellular domain and a 43 amino acid residue cytoplasmic domain. The cytoplasmic domain of the Type III receptor lacks an obvious signaling motif and the receptor may not be involved directly in signal transduction. The Type III receptor binds TGFβ2 with the highest affinity. Other TGFβ isoforms also bind the Type III receptor, but with lower affinities. Cellular responsiveness to TGFβ2 appears to be dependent on the presence of the Type III receptor, which can interact with the signaling receptor complex. In addition to the transmembrane Type III receptor, a soluble form of the receptor is secreted by some cell types (Venkatesha et al., 2006). The physiological role of this soluble receptor remains to be determined.

### 4.2 Effects of the TGFβ Family on Proliferation and Differentiation of Satellite Cells

The TGFβ family comprises of three typical members (1, 2, and 3). Two additional members, TGFβ4 and TGFβ5, were found in chicken and xenopus, respectively but (but not in mammalians). Selective TGFβs were shown to regulate myogenesis of adult-derived myobasts. It is generally accepted that TGFβs suppress myogenic differentiation. Nevertheless, some studies indicate a positive effect of TGFβ on mammalian myoblast proliferation while in other instances they were shown to suppress proliferation (Allen and Boxhorn, 1989; Cook et al., 1993; Hathaway et al., 1991; Quinn et al., 1994; Hathaway et al., 1994). Importantly, addition of TGFβ1 to isolated myofiber cultures resulted in a drastic reduction in the number of proliferating satellite cells both in the absence or presence of FGF2 (Yablonka-Reuveni and Rivera, 1997; Bischoff, 1990). These findings clearly

demonstrate that TGFβ1 suppresses proliferation of bona fide satellite cells. Differently, administration of TGFβ1 to C2C12 myogenic cell line, or to the muscle tissue, initiated fibrosis (Li et al., 2004). Taken together, the latter studies indicate that TGFβ might directly affect satellite cell myogenesis within their niche, but when its physiological levels are increased it may contribute to muscle pathology. The correlation between elevated expression levels of TGFβ in the mdx mouse, model of human Duchenne muscular dystrophy (Zhou et al., 2006), further supports involvement of TGFβ in this muscle pathology. In contrast, there is no evidence for increased TGFβ expression in the laminin alpha 2 (merosin)-deficient dy mouse, which shows progressive muscle fiber necrosis and ineffective muscle regeneration (Sakuma et al., 2000).

Clearly, there is a need for more studies on the role of TGFβ during myogenesis of satellite cells. We demonstrated that freshly isolated myofibers express high levels of TGFβ1 transcripts and it is conceivable that age-associated changed in this factor within the context of the myofiber could be involved in reduced performance of satellite cells in old age (S. Kastner and Z. Yablonka-Reuveni, unpublished studies). A gene array study of myogenic cells propagated for long term in culture demonstrated alterations in the expression level of many genes directly or indirectly involved with the TGFβ signaling pathway (Beggs et al., 2004). This study suggested that with age, myogenic progenitors acquire the paradoxical phenotype of being both TGFβ-activated based on overexpression of TGFβ-inducible genes, but resistant to the differentiation-inhibiting effects of exogenous TGFβ. Additionally, over expression of TGFβ-regulated genes, such as connective tissue growth factor, was proposed to play a role in increasing fibrosis in aging muscle (Beggs et al., 2004). The caveat that comes with this study is that cells passaged for long term to amplify sufficient cells for the study, were not necessarily free of contribution of muscle connective tissue cells. Thus, results can be affected by the contribution of gene expressed by non-myogenic cells. If such contribution is higher in preparations from aged animals, it can lead to the conclusion that that myoblasts from old age mice undergo alterations with regard to the TGFbeta signaling system. Nevertheless, it is clear that studies on the role of the TGFβ system of the satellite cells are greatly needed. However, the research effort on the role of TGFβ during myogenesis has been shifted toward myostatin upon the discovery of this presumably muscle-specific member of the TGFβ superfamily.

## 4.3 The Role of Myostatin (GDF8)

### *4.3.1 Myostatin regulates muscle mass*

Myostatin, a negative regulator of embryonic and postnatal skeletal muscle growth, functions to maintain a proper muscle mass during development and in adult life-(Dominique and Gerard, 2006; McPherron and Lee, 1997; Kambadur et al., 1997; McPherron, 1997; Schuelke et al., 2004; Carnac et al., 2006). A myostatin deficiency results in an enhanced muscular phenotype that is maintained throughout life,

resulting in reduced age-linked muscle atrophy (Wagner, 2005; Siriett et al., 2006). The production and effect of myostatin is generally held to be skeletal muscle-specific during pre and postnatal growth (McPherron and Lee, 1997), however, myostatin mRNA or protein have been detected in other tissues of vertebrates, including mammary gland (Ji et al., 1998), adipose tissue (McPherron and Lee, 1997) and in plasma (Gonzalez-Cadavid et al., 1998) as well as in multi-tissues in the fish (Ostbye et al., 2001). Loss of myostatin activity in cattle, mice, and humans leads to a profound phenotype of muscle overgrowth, associated with increased fiber numbers and size (McPherron and Lee, 1997; Schuelke et al., 2004; Grobet et al., 1997; Nishi et al., 2002). Myostatin null animals and transgenic mice overexpressing signaling inhibitors of myostatin such as follistatin and myostatin propeptide, exhibit increased muscle mass that results both from increased number of muscle fibers, and/or larger than normal fibers (Lee and McPherron, 2001; Yanget et al., 2001).

Injured muscle lacking functional myostatin, exhibits improved regeneration and reduced fibrosis, while over expression of myostatin leads to reduced muscle and wasting (cachexia) (Cornelison et al., 2001; Wagner, 2005; Reisz-Porszasz et al., 2003; Jespersen et al., 2006). Animal models with constitutive over- or under-expression of myostatin do not permit direct evaluation of myostatin role in adult life, as the observed mass increase could be a consequence of events taking place during muscle histogenesis and prenatal development. Nevertheless, conditional gene targeting approach exploiting the cre-lox system, demonstrated that *postnatal* inactivation of the myostatin gene is sufficient to cause a generalized muscular hypertrophy of the same magnitude as that observed for constitutive myostatin knockout mice (Grobet et al., 2003). Additionally, the increased expression of myostatin associated with muscle atrophy after periods of muscle inactivity and upon the induction of cachexia in mice, by systemically administered myostatin, also provides evidence for a role of myostatin in adult muscle (Zimmers et al., 2002; Carlson et al., 1999; Wehling et al., 2000; Morley et al., 2006).

Ablation of myostatin function was also shown to ameliorate the dystrophic phenotype in certain myopathies. In the mdx mouse model of Duchenne muscular dystrophy, deletion of the myostatin gene or treatment with a myostatin dominant-negative polypeptide enhanced muscle mass and reduced disease severity (Wagner et al., 2002; Bogdanovich et al., 2002; Bogdanovich et al., 2005). In contrast, loss of myostatin activity in the dyW/dyW mouse model of laminin-deficient congenital muscular dystrophy, a more severe and lethal disease model, did not improve all aspects of muscle pathology (Li et al., 2005). Genetic manipulation or antibody-mediated inhibition of myostatin function in a model of limb-girdle muscular dystrophy (mice lacking delta-sarcoglycan), improved muscle mass, regeneration, and reduced fibrosis. However, this improvement was achieved only during the phase of postnatal growth but not in adults (Parsons et al., 2006). Altogether, the aforementioned in vivo studies suggest that myostatin inhibition may benefit muscle function in dystrophic and atrophic conditions.

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