Regulation and Function of nTrip6, the Nuclear Isoform of the LIM-domain Protein Trip6, in Skeletal Muscle Differentiation
1. Introduction

1.1 Muscle tissue

Muscle tissue is a unique feature of the animal kingdom. Skeletal muscles in higher animals are not only responsible for the posture and locomotion, but also form an essential metabolic organ. Skeletal muscles are composed of myofibers. Myofibers are multinucleated cells that are formed by fusion of single cells, the so-called myoblasts (Mintz and Baker, 1967). Myofibers are the smallest cellular unit of a functional muscle.

Muscle tissue is able to adapt to different physiological needs during the lifetime of an individual and is therefore highly plastic. During development and in the adult body, the mass of the muscle tissue and its functional adaptations fit to its needs. Moreover, adult muscle tissue is constantly damaged and degraded due to muscle overload or trauma. Therefore, a repair mechanism leads to a constant regeneration of functional myofibers. The functional adaptation and regeneration are tightly regulated by different signaling pathways leading to a change in the expression of muscle specific genes. This regulation is directly based on the activity of transcription factors.

1.1.1 Adult muscle regeneration

Adult muscle tissue is constantly damaged and degraded due to muscle overload or trauma. A lack of a repair mechanism would consistently lead to a loss of muscle tissue throughout the lifetime of an individual. In general, after muscle damage two distinct phases can be observed. 1) During the first phase, the degeneration phase, damaged muscle tissue is degraded and removed. 2) In a second phase, new muscle tissue is regenerated in a process called adult myogenesis. The regenerative potential of muscle tissue is based on muscle adult stem cells (Mauro et al., 1961).
1.1.1.1 Adult myogenesis

Adult muscle stem cells contribute to the regeneration capacity of muscle tissue and were first described about 50 years ago (Mauro et al., 1961). Due to their position within the tissue, these cells are called satellite cells. They localize beneath the basement membrane that surrounds the myofiber and the plasmalemma of the fiber. Satellite cells are quiescent cells, thus they do not, or very little, express genes or synthesize proteins. Upon stress such as trauma or weight bearing, satellite cells are activated and they contribute to muscle repair. They proliferate as a population of transient amplifying cells which are called myoblasts. Then, these cells exit the cell cycle and become committed to differentiate (so-called myocytes). Myocytes fuse with existing myofibers, or fuse together to form multinucleated myotubes, which further maturate into myofibers (Figure 1.1).

![Figure 1.1: Adult myogenesis. Upon muscle damage, quiescent satellite cells are activated. An asymmetric cell division gives rise to a new quiescent satellite cell and a myoblast that proliferates. Proliferating myoblasts either differentiate to myocytes or de-differentiate into reserve cells that replenish the satellite cell pool. Myocytes fuse to form multinucleated myotubes.](image)

The pool of cells that is given rise to by the proliferating satellite cells is not entirely assigned to differentiate but some cells will contribute to a new quiescent satellite cell pool. One mechanism by which this is accomplished is asymmetric cell division, where one cell remains as stem cell while the other is committed to differentiation (Figure 1.1) (Conboy and Rando, 2002). Thus, satellite cells are stem cells due to the fact that they give rise to a differentiated cell type and they are able to maintain themselves by self-renewal (reviewed in: Zammit et al., 2006; Le Grand et al., 2007). Another mechanism has been described that contributes to the
replenishment of the satellite cell pool. After proliferation, some myoblasts exit the
differentiation program and revert to an undifferentiated state. These cells are called
reserve cells (Yoshida et al., 1998). Although the mechanism of satellite cell
asymmetric division has been the subject of intense investigation (Troy et al., 2012;
Conboy and Rando 2002; Shinin et al., 2006; Kuang et al., 2008; Cheung et al.,
2012; Le Grand et al., 2009), the mechanisms regulating the formation of reserve
cells is far less understood.

The differentiation process of myoblasts needs to be tightly regulated. Signaling events induce the proliferation of satellite cells, but in order to differentiate,
the cells are withdrawn from the cell cycle. Next to this, specific genes that are
required for commitment and differentiation, then for fusion and further maturation,
need to be expressed at the right time. These programs are controlled by a set of
specific transcription factors.

1.1.1.2 Muscle specific transcription factors

The various steps during muscle regeneration are orchestrated by a network
of transcription factors, which are expressed at specific stages along the
differentiation pathway (Figure 1.2).

Figure 1.2: Muscle specific transcription factors in adult myogenesis. Pax7 is expressed in
quiescent satellite cells, while Myf5 drives the satellite cell activation. MyoD drives the onset of
differentiation in myoblasts and myocytes. Mef2c together with Myogenin regulate late
differentiation.
The paired box transcription factor Pax7 is expressed in quiescent and activated satellite cells, and is lost in dividing myoblasts and committed myocytes. It is needed for the survival and maintenance of the satellite cell population in an undifferentiated state (Seale et al., 2000; Kuang et al., 2008; McKinnell et al., 2008). On the other hand, Pax7 is also needed to induce the expression of Myf5, a muscle regulatory factor (MRF), to drive the initial phase of differentiation (McKinnell et al., 2008).

The basic helix-loop-helix transcription factors Myf5, MyoD, and Myogenin belong to the MRFs. These factors drive the differentiation of myoblasts and committed myocytes towards myofibers (Braun et al., 1990; Gayraud-Morel et al., 2007; Weintraub et al., 1989). Although being expressed over several stages of differentiation, the activity of these factors is limited to defined time points. For instance, to drive differentiation, MyoD also cooperates with other transcription factors such as the myogenic enhance factor 2c (Mef2c) that is involved in late differentiation (Molkentin et al., 1995; Li et al., 1994).

Mef2c, belonging to the family of myogenic MADS domain transcription factors (Molkentin et al., 1995), is expressed in cycling myoblasts, in myocytes and also in myofibers. Mef2c is involved in myoblast cell cycle exit, a prerequisite for myocyte fusion (Chen et al., 2000). Yet, Mef2c is also expressed in cycling myoblasts. Thus, not only its expression but also its activity is tightly regulated. For example, the activity of Mef2c is increased prior to fusion through the co-activators Grip-1 and CRAM1 (Chen et al., 2000; Chen et al., 2002). Mef2c is also involved in myoblast fusion. This process is still poorly understood, but a critical step is the initial cell adhesion mediated by surface receptors such as M-Cadherin (Powell et al., 2011; Duan et al., 2012; Leikina et al., 2012; Wang et al., 2012). This is followed by a re-organization of the F-Actin cytoskeleton (Charrasse et al., 2002; Charrasse et al., 2007; Kim et al., 2007), the formation of podosome-like structures and of fusion pores (Sens et al., 2010; Duan et al., 2012). Mef2c is indirectly involved in the initial step of this process. It drives the expression of Myogenin (Edmondson et al., 1992) that is in turn responsible for the expression of M-Cadherin (Hsiao et al., 2010). At later stages, during differentiation and maturation, Mef2c drives the expression of muscle specific genes such as Myomesin I and II, Desmin (Potthoff et al., 2007), the transcription factor Myogenin (Edmondson et al., 1992) as well as genes involved in muscle homeostasis like Glut4 and Myoglobin (Potthoff et al., 2007).
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Thus, the activity of the different transcription factors during differentiation is crucial and orchestrated in a tightly controlled manner. This regulation of translational activity is driven by co-activators and co-repressors (Sartorelli et al., 1997; Sartorelli et al., 2005; Polesskaya et al., 2001a; Polesskaya et al., 2001b; Jeong et al., 2010). Indeed, a miss-regulation of co-activators and co-repressors leads to, for instance, a loss of satellite cells (Seale et al., 2000; Kuang et al., 2008; McKinnell et al., 2008), hampered fusion of myocytes or pre-mature differentiation.

During the last years, more and more co-activators and co-repressors and their regulation were identified, yet not all mechanisms are known. For instance, what mechanisms are involved in coordinating the activity of Mef2c at different stages of the differentiation process? How is the repression and activation orchestrated? Mef2c is of special interest since it is involved at several stages of myoblast differentiation and myocyte fusion. Furthermore, in fully differentiated myofibers, Mef2c is also responsible for the determination and maintenance of the myofiber type identity (see also 1.1.2.3). Thus, it plays fundamental roles in muscle regeneration and maintenance (Schiaffino and Reggiani, 2011).

1.1.2 Muscle fiber types

Myofibers are heterogeneous in their contractile properties such as the force they can generate, their contraction velocity, their resistance to fatigue, and also in their metabolic capacity. According to these properties, fibers are grouped in different fiber types. Type I, or slow myofibers are characterized by a slow contraction velocity, an oxidative metabolism and a high resistance to fatigue. Muscles consisting mainly of type I fibers are red in color. On the other hand, type II, or fast myofibers show a glycolytic metabolism (Pette and Staron, 2000; Bassel-Duby and Olson, 2006). They are capable of rapid contractions but fatigue on a short term. Muscles consisting mainly of type II fibers are also called white muscle tissue due to their light color.

The different fiber types express different sets of genes or gene isoforms coding for proteins of the contractile or the enzymatic apparatus (Chemello et al., 2011) (Table 1.1). For instance, myosin heavy chain (MhC) isoforms are specific for the fiber types and can be used to distinguish between slow and fast myofiber types (Pette and Staron, 2000). Type I fibers express the MhC I (or MhC $\beta$/slow) isoform. Type II fibers are characterized by the expression of MhC II, but in rat, mouse and