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Muscle satellite cells increase during hibernation in ground squirrels

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ABSTRACT

Skeletal muscle satellite cells (SCs) are involved in muscle growth and repair. However, clarification of their behavior in hibernating mammals is lacking. The aim of this study was to quantify SCs and total myonuclei in hibernator muscle during different phases of the torpor-arousal cycle. Skeletal muscle was collected from thirteen-lined ground squirrels, *Ictidomys tridecemlineatus*, at five timepoints during hibernation: control euthermic [CON, stable body temperature (Tb)], early torpor (ET, within 24 h), late torpor (LT, 5+ consecutive days), early arousal (EA, increased respiratory rate >60 breaths/min, Tb 9–12 °C) and interbout arousal (IA, euthermic Tb). Protein levels of p21, Myf5, Wnt4, and β-catenin were determined by western blotting. SCs ($Pax7^+$) and myonuclei were identified using immunohistochemistry. Over the torpor–arousal cycle, myonuclei/fiber remained unchanged. However, the percentage of SCs increased significantly during ET (7.35 ± 1.04% vs. control: 4.18 ± 0.58%; $p < 0.05$) and returned to control levels during LT. This coincided with a 224% increase in p21 protein during ET. Protein levels of Wnt4 did not change throughout, whereas Myf5 was lower during EA ($p < 0.08$) and IA ($p < 0.05$). Compared to torpor, β-catenin increased by 247% and 279% during EA and IA, respectively ($p < 0.05$). In conclusion, SCs were not dormant during hibernation and increased numbers of SC during ET corresponded with elevated amounts of p21 suggesting that cell cycle control may explain the SC return to baseline levels during late torpor. Despite relatively low Tb during early arousal, active control of quiescence by Myf5 is reduced.

1. Introduction

Vertebrate skeletal muscle shows a high degree of plasticity with a remarkable capacity for regeneration and remodeling. Evidence suggests that full regeneration can occur after removing and mincing skeletal muscle and replacing it back into the leg (Studitsky, 1964). Regeneration can occur rapidly in models which destroy the basal lamina such as crush injury (Myburgh et al., 2012). Because myonuclei are post-mitotic, skeletal muscle relies on an adult progenitor cell type called the satellite cell (SC) to replenish and/or increase skeletal muscle myonuclei [recent reviews: (Yin et al., 2013; Brooks and Myburgh, 2014)]. SCs are skeletal muscle stem cells involved in muscle growth and repair. However, their requirement for muscle turnover and hypertrophy is controversial (McCarthy and Esser, 2007; O'Connor and Pavlath, 2007).

SCs are normally in a quiescent state, and express the transcription factor Pax7. Quiescence is not an inactive state, but rather the SCs are under active transcriptional control (Montarras et al., 2013). Alterations in the environment within the SC niche result in changes in their status. Upon stimulation, SCs are activated to exit quiescence, enter the cell cycle and proliferate. Differentiation may ensue depending on the interplay between factors present in the niche environment, receptors and other transmembrane proteins, intracellular signals and nuclear transcription factors (Kuang et al., 2008).

Activated and proliferating SCs express Pax7. In addition, members of the myogenic regulatory factor family (MRFs), specifically myf5 and MyoD, may be expressed during proliferation. Myf5 has been reported to play a particular role in promoting self-renewal of SCs and to promote the return of at least one (self-cell) to the pool of quiescent SCs (Beauchamp et al., 2000).

Activated SCs expressing more MyoD than myf5 continue to proliferate (Grounds et al., 1992). At higher levels, MyoD is involved in myogenic differentiation and SC fusion with adult muscle fibers (Yablonka-Reuveni et al., 1999) so that the total SC pool size may not actually change. In contrast, in the absence of differentiation, the pool of SCs would increase (see Fig. 1 for details).

Cell cycle regulators are also involved in the regulation of SC quiescence vs proliferation vs self-renewal. The cyclin and cyclin-dependent kinase (Cdk) families provide positive regulation of the cell cycle while Cdk inhibitors are involved in negatively regulating the cell cycle (Schafer, 1998; Sherr and Roberts, 1999). Furthermore, SCs in

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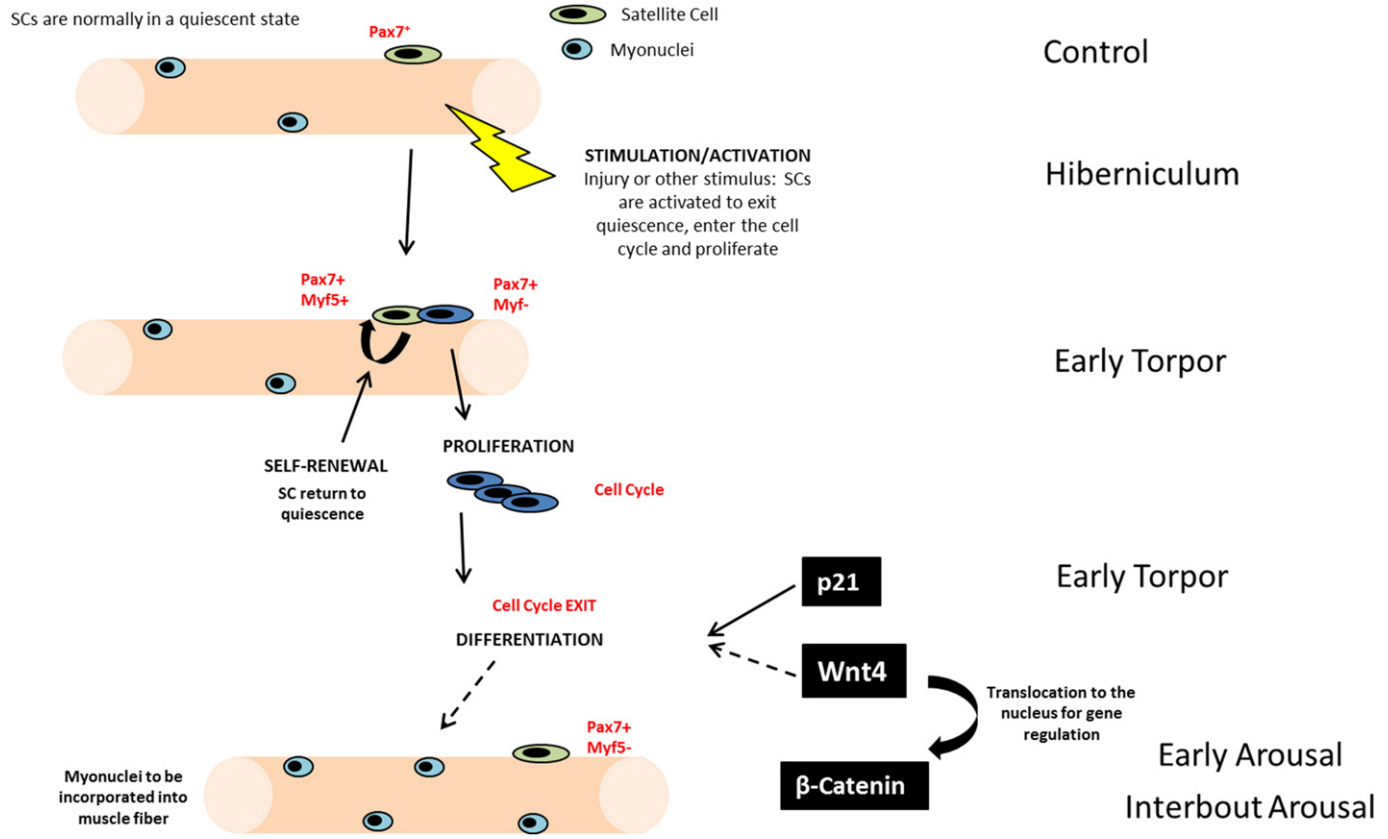

Fig. 1. Simplified diagram representing regulation of satellite cells (SCs). SCs are normally in a quiescent state, expressing Pax7. Upon stimulation which can come from injury, hypertrophy stimulus or other means, SCs are activated to exit quiescence and enter the cell cycle. SCs have a number of fates after activation—self-renewal of SC allows at least one cell to return to the pool of quiescent SC thus maintaining the SC pool. SCs expressing more MyoD than Myf5 continue to proliferate. P21 is a cell cycle inhibitor, encouraging SC differentiation. Wnt4 may also be involved in inducing early differentiation acting via β-catenin translocation into the nucleus for gene regulation. Further detail in text.

p21 null mice have deficient differentiation capabilities suggesting a role for p21, another cell cycle inhibitor, in SC differentiation (Hawke et al., 2003).

The Wnt signal transduction pathways are important in skeletal muscle development and regeneration (Münsterberg et al., 1995; Tajbakhsh et al., 1998; Polesskaya et al., 2003; Brack et al., 2007). During canonical Wnt signaling, Wnt ligands bind to Frizzled/low-density lipoprotein-related protein (LRP) receptor complexes which stabilize β-catenin and allow it to translocate to the nucleus and regulate the expression of specific genes (Logan and Nusse, 2004). The genes that are then activated are involved in homeostasis including cell proliferation and differentiation (Nishizuka et al., 2008; Abiola et al., 2009). Recently, the implications of Wnt family member involvement with SC activation and proliferation have been investigated in vitro. In C2C12 cells, Wnt4 was strongly induced during early differentiation of myoblasts (Bernardi et al., 2011). Wnt4 was also shown to inhibit myostatin expression and genetic deletion of the myostatin gene led to inhibition of the SC response to the hypertrophic effect of Wnt4 (Bernardi et al., 2011).

Disuse, disease, weightlessness and hibernation are a few of the conditions that influence skeletal muscle and the SC niche (Brooks et al., 2010, 2011; Andres-Mateos et al., 2012). During hibernation, small mammals undergo long periods of deep torpor where their core body temperature can drop as low as 0–5 °C (Storey and Storey, 2007). During this time there is little or no muscle activity and the animals are in a hypocaloric state (Wickler et al., 1991). When comparing hibernating dormice to euthermic controls, there was no difference in myonuclear numbers suggesting that SCs are inactive during hibernation (Malatesta et al., 2009). However, this does not exclude control of SC cell cycle entry or return to quiescence during different phases of hibernation. Indeed, during mammalian hibernation, levels of Cdk inhibitors were increased during torpor in the liver of hibernating ground squirrels, suggesting an involvement in cell cycle arrest in the torpid animal (Wu and Storey, 2012).

Since changes in the SC environment influence SC status, we hypothesized that SC proliferation may be actively controlled during entry into hibernation and during arousal, despite the inactivity of the hibernators. The aim of this was study was to measure the number of SCs and total myonuclei in hibernator skeletal muscle using immunohistochemistry and to analyze the effect of the different phases of hibernation on selected proteins known to control SCs and indicate their status in relation to the cell cycle.

2. Materials and methods

2.1. Animal collection

Thirteen-lined ground squirrels, *Ictidomys tridecemlineatus*, were used for hibernation studies and the details have been described previously (Bratincsák et al., 2007). Procedures were undertaken during the natural hibernation season in the Northern Hemisphere during January–February. Sampling was done at 5 phases of hibernation (detailed below) at distinct points of the torpor-arousal cycle. All animals had been through a series of torpor–arousal bouts prior to sampling; hibernation bouts were regular, repeatable and rhythmical. For typical hibernation–arousal bouts and more detail see a recent review (Storey, 2010). All animal procedures were approved by the Animal Care and Use Committee of the National Institute of Neurological Disorders and Stroke (National Institutes of Health, Bethesda, MD, USA; animal protocol no. ASP 1223-05).