Synopsis
Most adult tissues display limited capacities in their ability to respond to damage. Restoration of tissue integrity in adult mammals has been observed to occur by two distinct mechanisms. In tissues such as the liver, damage results in the de-differentiation of cells constituting the tissue, followed by their proliferation and re-differentiation to reconstitute the liver. In many other tissues however, damage results in the activation of a resident population of dormant undifferentiated cells called stem cells. Several organ systems such as the skin epidermis, the intestinal epithelium and the haematopoietic system require continual replacement and therefore possess an active stem cell compartment. However, systems such as the nervous system and skeletal muscle display robust stem cell activity only in response to injury. In skeletal muscle, the multinucleated myofibers represent post-mitotic entities incapable of contributing to the regenerative process. The source for new cells is an anatomically defined population of mononucleated cells that reside between the basal lamina sheath and the plasma membrane of the myofiber. These cells are called satellite cells (SC). In normal healthy muscle, SC are largely mitotically quiescent. Upon injury, activated SC proliferate, differentiate and replace the lost myofiber(s) with which they are associated.

The ability to self-renew and replace the precursor pool justifies the stem cell status conferred on the SC. Thus, the versatility of the SC lies in being able to maintain an undifferentiated and arrested state in normal muscle, which can switch to a myogenically determined state upon activation into the cell cycle, following injury. By contrast, SC self-renewal requires the re-enforcement of the quiescent state in the activated SC. Thus, the key features associated with the divergent choices in SC, are the reversible modulation of myogenic gene expression and cell cycle progression.

The first known event during SC activation that marks the initiation of the muscle differentiation program is the expression of MyoD. MyoD expression signifies the transition from the undifferentiated stem cell to one that is competent to differentiate. Similarly, suppression of MyoD is indicative
of reentry of the activated SC into quiescence. Insights into the mechanisms that regulate MyoD offer the opportunity to address how SC activity is coupled to the myogenic program during activation and self-renewal. The aim of this study therefore was to identify potential regulators of MyoD or mechanisms that modulate MyoD expression.

Previous work in the lab has established the use of a culture model as a means to reversibly regulate the expression of MyoD and the cell cycle. Extensive molecular analyses have validated this system as a suitable model that mimics key aspects of SC quiescence and activation. I have used this system in the initial part of my work to enhance our understanding of MyoD regulation. The in vitro model uses deprivation and restoration of adhesion in the murine myoblast cell line C2C12 as a means to reversibly modulate MyoD and the cell cycle. Taking a cue from the manipulation of adhesion to reversibly arrest or activate myoblasts, the second part of my work focuses on the contribution of adhesion-dependent signaling mechanisms in regulating MyoD expression.

In Chapter 1, I introduce the molecular state of satellite cells during quiescence and activation. An overview of the importance of MyoD expression in the myogenic program and known aspects of the regulation of its expression has been presented. Subsequently, I have briefly compared the in vitro systems to study the cell cycle-stage specific regulation of MyoD. Finally, I describe the contribution of adhesion-dependent pathways that regulate MyoD expression.

In Chapter 2, I describe the methods used in this study

The Results of my investigations are presented in Chapters 3-5.

In Chapter 3, I address the prospect of a muscle specific dimension to the G₀-G₁ transition that might play a role in regulating MyoD expression upon activation from quiescence. Using the technique of Differential Display-
RTPCR, I describe the isolation of 2 cDNAs that are regulated specifically in muscle and are expressed in synchronized myoblasts before MyoD. Preliminary analysis to investigate the role of one of these cDNAs, Cox-2, a gene previously implicated in skeletal muscle regeneration, is also presented. Although this project is still in progress, my data validate the hypothesis that there are early muscle specific events that occur well before MyoD induction and warrant a more detailed investigation as a new source of potential regulators of MyoD.

In Chapter 4, I address the importance of adhesion- a crucial regulator of growth- in regulating MyoD expression. Using the suspension culture model, I have addressed the role of a major adhesion dependent pathway, the Rho pathway and its downstream effector Serum Response Factor (SRF) in regulating MyoD expression. Pharmacological and genetic approaches were used to investigate the pathway leading from RhoA to SRF and MyoD. In addition to confirming and extending the role of SRF in MyoD regulation, evidence provided in this chapter suggests the presence of an alternate pathway downstream of Rho. This novel SRF-independent pathway indicates the preferential use of mDiaphanous 1 (mDia1) an immediate effector of RhoA, in regulating both MyoD expression and cell cycle progression.

In Chapter 5, using gene silencing and over-expression approaches coupled with reporter gene assays, I investigate the mechanism by which mDia1 could be regulating MyoD expression. I provide evidence for the existence of a novel pathway downstream of mDia1 other than the canonical SRF-dependent pathway and show that over-expressed active mDia1 antagonistically regulates these 2 pathways. Finally, I propose a model of mDia1-mediated regulation of MyoD suggesting that the reciprocal regulation of the SRF dependent and independent pathways.

In summary, my study identifies a novel Rho effector in the regulation of MyoD and the cell cycle and a non-canonical pathway in mediating this signaling. My findings suggest that the integrated outcome of the activity of two growth- regulatory transcription factors influence MyoD expression. Not
only does this confirm earlier observations that myogenic gene expression and cell cycle progression are coupled, but also suggests a mechanism for how this coupling may occur. More importantly, my experiments suggest that mechano-chemical signal transduction pathways influence the inseparable processes of lineage determination and cell cycle progression in culture, with implications for the activation of SC residing in a contractile tissue such as skeletal muscle.