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Introduction

All the cells of a given organism possess an identical genotype, the literal sequence content of their entire genome. The unique phenotypes (observable characteristics) displayed by neurons, osteoblasts, hepatocytes, etc. within an organism derive from differential gene expression, protein synthesis, and activation. Over the last several decades, numerous studies have demonstrated the critical and cooperative role of extracellular stimuli in the development and maintenance of cellular phenotypic characteristics and functions. Growth factors, hormones, and extracellular matrix adhesion molecules have been shown to influence cell survival, division, migration, and differentiation. In general, these effects are mediated through transmembrane receptors that couple extracellular ligand binding to activation of intracellular enzymes. Subsequent signal transduction cascades of cytoplasmic enzymatic reactions ultimately culminate in the activation of transcription factors, proteins that bind to regulatory sequences within DNA, activating or inhibiting transcription of specific genes.

The application or removal of externally applied mechanical loads has long been recognized to have profound effects on the macroscopic structure and biomechanics of bone, cartilage, muscle, and tendon/ligament. Mechanobiology clearly functions at the cellular level with numerous *in vitro* studies using isolated cells and 3D culture models to demonstrate loading-induced changes in cell proliferation, gene expression, and biomechanical properties. However, compared to growth factor or integrin-mediated signaling, relatively little is known about the molecular mechanisms by which cells detect external mechanical stimuli, transduce these inputs into changes in intracellular biochemistry, and ultimately alter levels of gene expression and protein synthesis/activation to achieve functional changes in cellular/tissue properties. The effects of mechanobiology have been most widely observed in connective tissue and muscle, specifically with respect to regulation of extracellular matrix metabolism and tissue biomechanics. An improved understanding of mechanotransduction will provide opportunities for the development of novel strategies to manipulate ECM production that may be particularly important in the treatment of various fibroproliferative disorders and chronic wounds, as well as tissue engineering of musculoskeletal and cardiovascular tissues.

The objective of this chapter is to provide researchers from traditional engineering disciplines entering the field of mechanobiology/mechanotransduction with an introduction to the theoretical principles and practical techniques of experimental molecular biology. The first section examines analysis of mRNA expression levels with a focus on real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR), including RNA isolation, qualification/quantification, controls, normalization, and quantitative data analysis. The second section introduces antibodies

and their application in analysis of protein expression using immunohistochemistry, Western Blotting, and enzyme-linked immunosorbant assay (ELISA). The final section presents a brief introduction to the use of various types of inhibitors for “loss of function” assays that are useful in identifying causal roles of specific signaling mediators in mechanotransduction, including recently developed techniques of RNA interference.