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Abstract

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Grant Number:	1R29AR043799-01A1
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PI Title:	ASSOCIATE PROFESSOR
Project Title:	CUTANEOUS GENE REGULATION BY TARGETED OLIGOMERIZATION

Abstract: DESCRIPTION (Adapted from the applicant's abstract): Abnormal gene expression is a dominant theme in human skin disease and may result from a single gene disorder or disturbance of a complex genetic program. Intracellular oligomerization, whether involving transcription factors dimerizing at a specific target gene promoter or the cytoplasmic domains of cell surface receptors cross-linked by cognate extracellular ligand, has emerged as a central mechanism of gene regulation. Recent experimental breakthroughs, such as targeted gene transcription using keratin promoters in transgenic mice, remain limited by unregulated levels of constitutive gene expression through development. In addition, the field of receptor-mediated gene regulation has been clouded by pleiotropic effects of cytokines and cross-reacting receptor ligands. We hypothesize that synthetic ligand-driven oligomerization, where the FK1012 cross linker multimerizes inactive engineered monomeric proteins into biologically active transcription factors or cell surface receptors, can be used to regulate both simple and complex programs of gene expression in keratinocytes. Such an advance will assist generation of powerful new transgenic skin disease models, where primary pathogenic events may be ordered and separated from downstream phenomena, as well as contribute to development of regulated cutaneous gene therapy. Precise regulation of the magnitude of expression of a single target gene in living cells will help apply the quantitative power of biochemistry into complex genetic systems. To accomplish this, we will optimize synthetic ligand-driven transcription in human keratinocytes in vitro by establishing "ON" and "OFF" switches as part of a mechanism to titrate target gene expression through a range from zero to over expression and then utilize this ability to study the keratin intermediate filament network. Second, we will engineer 2 classes of cell surface receptors - growth factor receptor tyrosine kinases and TNF family receptors - to control complex programs of gene expression via synthetic ligand-triggered oligomerization. We will define the subcellular localization requirements for signal transduction initiation by the keratinocyte growth factor receptor and generate a mechanism to trigger keratinocyte proliferation with a pharmacologic stimulus. We will extend our findings with TNF family receptors to define Fas-triggered keratinocyte death at developmental and cell biological levels and to refine a "cell death trigger" responsive to

FK1012. At the end of this funding period, we hope to have achieved precise regulation of simple and complex programs of gene expression in the skin, with future application to cutaneous gene therapy, and to have utilized these advances to address fundamental questions in cutaneous receptor biology.

Thesaurus Terms:
gene expression, genetic model, genetic regulation, intermolecular interaction, keratinocyte, method development, model design /development, protein engineering, protein structure function, transcription factor
FK506, cell death, cell differentiation, cell growth regulation, cytokine, developmental genetics, genetic regulatory element, growth factor receptor, intermediate filament, protein tyrosine kinase, receptor expression, tumor necrosis factor alpha
gene targeting, tissue /cell culture

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