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MOLECULAR MECHANISMS GOVERNING SKELETAL MUSCLE MYOBLAST FUSION

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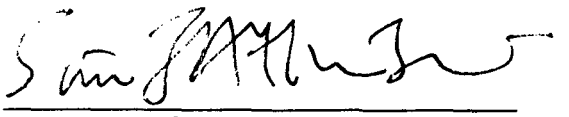

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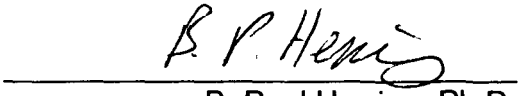

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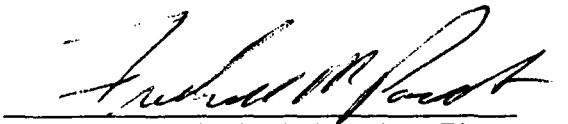

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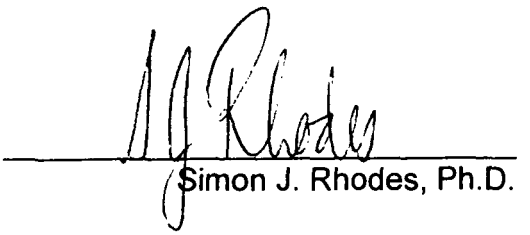

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Abstract

Rui Duan

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Cell-cell fusion is a fundamental process needed for development and formation of multicellular organisms. Myoblast fusion to form multinucleated skeletal muscle myotubes is a well studied, yet incompletely understood example of cell-cell fusion. Despite numerous studies during last three decades, the mechanisms regulating cell-cell fusion remain elusive. Using an *in vitro* skeletal muscle differentiation system, the dynamics of the actin cytoskeleton and the role of its associated motor, nonmuscle myosin heavy chain IIA (NM-MHC-IIA, IIA), in skeletal muscle myoblast fusion were examined (Chapter I). During myoblast pairing and alignment, cortical actin filaments form an asymmetrical wall in aligned cells. As fusion progresses, gaps appear within the actin wall at sites of vesicle accumulation, transmembrane pairing, and fusion pore formation. Inhibition of NM-MHC-IIA motor activity or genetic alteration of IIA bipolar assembly prevents formation of the asymmetric actin wall, the appearance of vesicles to a membrane proximal location, and myoblast fusion. These studies suggest that early formation of an actin wall during myoblast alignment is a critical event for myoblast fusion by supporting membrane alignment and

temporally regulating trafficking of vesicles to the nascent fusion sites during skeletal muscle myoblast differentiation.

The molecules that may directly be involved in plasma membrane fusion during myoblast fusion have also been explored by combining the techniques of cell surface biotinylation, protein fractionation, and mass spectrometry (Chapter II). One of the calcium-dependent, lipid binding proteins, annexin A2, has been identified as an extracellular membrane associated protein that increases during myoblast differentiation. To examine the potential role of annexin A2 in skeletal myoblast fusion, endogenous annexin A2 was depleted in rat L6 myoblast cells using small-interfering RNA (siRNA) mediated knockdown. Depletion of annexin A2 significantly blocked myoblast fusion. In addition, neutralization of the cell surface/extracellular annexin A2 by application of anti-annexin A2 specific antibody to the culture medium attenuated myoblast fusion and myotube formation. These studies suggested that annexin A2 has an important role mediating myoblast fusion.

Patricia J. Gallagher, Ph.D., Chair

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