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Membrane Specializations in Skeletal Muscle Cells

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The neuromuscular junction (NMJ) and the myotendinous junction (MTJ) are two important membrane specializations of vertebrate skeletal muscle cells. At the NMJ, acetylcholine receptors (AChRs) are clustered in response to motor innervation. This clustering of AChRs ensures the rapid transmissions of signals from nerve to muscle to elicit contraction. The AChRs are stabilized at the postsynaptic membrane by a complex of cytoskeletal proteins. The MTJ, on the other hand, is the interface between the contractile apparatus and the extracellular matrix in the form of the tendon apparatus. The sarcolemma at the MTJ exhibit extensive membrane invaginations, which distribute contractile force to a large membrane area. Thus, the MTJ is structured to ensure the integrity of the sarcolemma during force transmission from the myofibrils to the tendon. This specialization is also marked by an extensive array of cytoskeletal proteins.

Our lab has been interested in the cellular and molecular mechanisms involved in the development of these specializations. Using light microscopy, including conventional and confocal fluorescence microscopy, a number of proteins have been localized to these specializations, These include both structural proteins, such as talin, vinculin and dystrophin, and proteins with enzymatic activities,

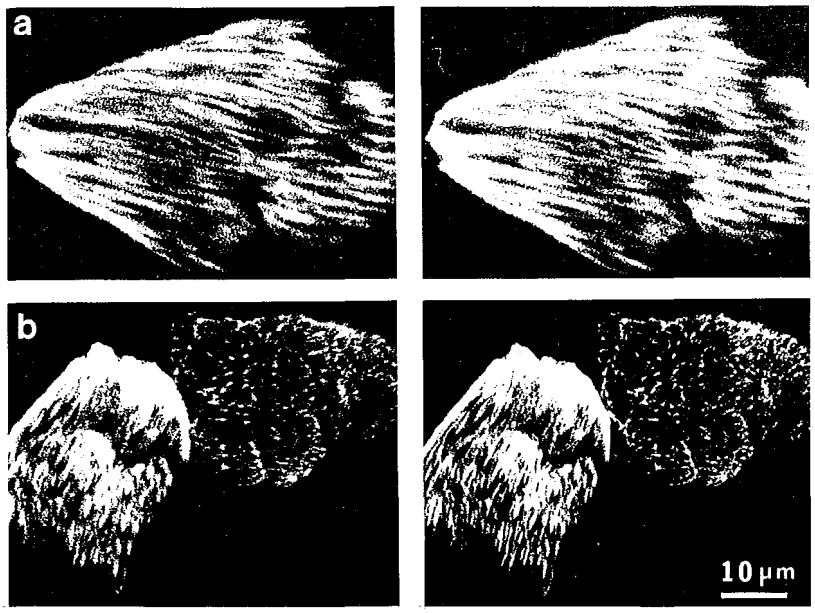

Fig. 1. Confocal stereo views of the myotendinous junction (MTJ). Dissociated single *Xenopus* myotomal muscle fibers were labeled with an antibody against dystrophin which is highly concentrated along membrane invaginations at the MTJ. These invaginations are seen as longitudinal streaks at the end of muscle fiber. (a) A longitudinal view. The sample was optically sectioned at 2 µm intervals and a stack of 23 images were superimposed. (b) Longitudinal and end-on views. The sample was optically sectioned at 1 µm intervals and the stack contained 41 images.

such as focal adhesion kinase (FAK). However, the distribution of these proteins at these two specializations is often non-overlapping. For example, FAK is localized at the MTJ but not at the NMJ. On the other hand, AChRs and the postsynaptic 43K protein are only clustered at the NMJ. Thus, these proteins can be used as markers for these two types of specializations.

To understand the development of these specializations, we have experimented with non-cellular stimuli to induce their formation. One of the most effective stimuli to induce NMJ and MTJ is latex beads. Using cultured Xenopus muscle cells as a model, we found that both NMJ-type specialization, as evidenced by clustering of AChRs, and MTJ-type specialization, as evidenced by FAK localization, can be induced at bead-muscle contacts. By coating beads with different endogenous and exogenous molecules, we were able to make inference into the signal transduction processes. Beads coated with heparin-binding growth factors, such as basic fibroblast growth factor and the newly discovered heparin-binding growth-associated molecule (HB-GAM), were most active in inducing AChR clustering. Heparan-sulfate proteoglycan (HSPG) is a ubiquitous component of the basement membrane of a variety of cell types. Recent studies have shown that an important function of the HSPG is to serve as storage sites for certain growth factors. Factors that are bound to the HSPG are essentially immobile and thus are ideal candidates for pericellular signaling. In fact, our confocal microscopy on cultured *Xenopus* muscle cells has demonstrated that HB-GAM is localized at the cell surface in association with HSPG. This suggests that extracellular matrix-associated molecules may be the endogenous ligands for the induction of these membrane specializations. Our results are consistent with the notion that these matrix-bound ligands are locally presented to the muscle cell surface to effect the development of NMJ and MTJ.

Since the receptors for these growth factors are usually receptor tyrosine kinases (RTKs). We used phosphotyrosine antibodies to probe the signal transduction process initiated by bead presentation. Our results have shown a concentration of phosphotyrosine labeling at bead-muscle contacts as one of the earliest detectable changes. This is consistent with the notion that beads induce the local activation of RTKs at the contact site, leading to tyrosine-specific phosphorylation of substrate proteins. To identify RTKs and their substrates, we have begun to isolate specializations that are induced by beads. By removing beads from muscle cell surface, pieces of membrane with their associated specializations are often isolated in high purity. This is evidenced

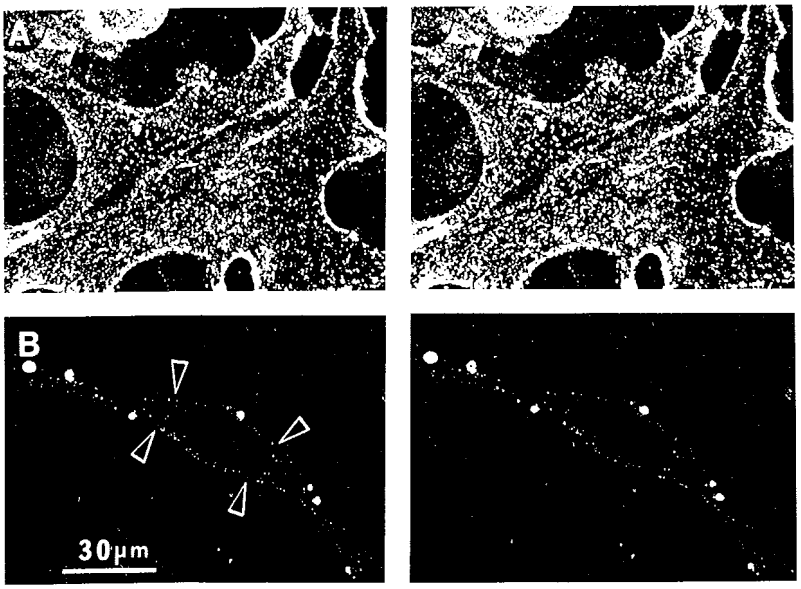

Fig. 2. Confocal stereo images of HB-GAM distribution on the surface of cultured *Xenopus* myotomal muscle cells before (A) and after (B) heparinase treatment. HB-GAM is present on the entire cell surface. Heparinase removes a significant amount of this protein from the cell surface. The arrowheads in B point to residual HB-GAM staining after enzymatic treatment. The large fluorescent objects around the cell periphery are autofluorescent yolk granules released from damaged cells during dissociation.

by fluorescence microscopy of AChR patches that are left on beads upon their removal from cells. When these bead-associated membrane patches are subjected to biochemical analysis, We found both structural proteins, Such as AChR and syntrophin, and kinases, such as mitogen-activated protein kinase, are components of the bead-associated specialization. We are hopeful that this analysis may lead us into the identification of essential components of the signal transduction complex in the induction of these sarcolemmal specializations in skeletal muscle cells.