How Do Cells Regulate the Intercellular Communication Mediated by Gap Junctions?

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Essentially all animal cells communicate with their neighbors via "gap junctions" (GJs), which are collections of cell-to-cell channels, formed in vertebrates by "connexin" proteins. These plasma membrane channels allow small molecules to pass from cytoplasm to cytoplasm, thus providing a form of intercellular communication that is required for normal cell growth control, development and a variety of cellular responses to external and internal signals/events. In the last ten years we have learned much about the role of GJs through work on targeted gene disruptions in mice and analysis of human mutations in different connexins. For example, a number of human diseases are now known to be caused by connexin mutations. We will briefly review these findings, which emphasize the qualitative differences between connexins. Another key concept in the field is that quantitative variations in GJs also have a significant effect on junctional communication. Quantitative differences are caused by changes in: a) connexin synthesis, b) GJ assembly, c) the gating of GJ channels and d) the degradation of connexins. This presentation will address the regulation of GJ assembly, both positive and negative mechanisms. However, the focus will be on the enhancement in GJ assembly seen with cAMP treatments of cells expressing connexin43 (Cx43). Enhanced assembly can occur within one hour without an increase in cellular levels of Cx43. However, cAMP-mediated increases in assembly can be blocked by trafficking inhibitors (e.g., brefeldin A) and by agents that disrupt microtubules. Thus, the enhanced trafficking of Cx43 to the plasma membrane appears to be a critical feature of cAMP-mediated increases in assembly and communication. That is, the enhanced assembly results from a redistribution of Cx43 within the cell. The role of microtubules in assembly has been studied with freeze-fracture TEM and with intracellular dye injection methods to study junctional permeability. Our model for microtubule-based transport of connexins involves membrane vesicles with connexin oligomers that associate (directly or indirectly) with motor proteins (e.g., members

of the kinesin family) to move over microtubules in an ATP-dependent manner. We are taking three different approaches to studies of connexin trafficking: a) microscopic examination of the trafficking exhibited by "tagged connexins", b) functional assays for the "delivery" of connexin oligomers (i.e., "hemichannels") to the plasma membrane and c) biochemical assays (biotinylation) for delivery. The microscopy involves tagging Cx43 with green fluorescence protein (GFP) for fluorescence microscopy on living cells and, in the near future, will involve tetracysteine tagging of Cx43 for thin-section TEM analysis. The presentation will concentrate on the microscopy and the functional assays. Our work takes advantage of Cx43 mutations that fail to respond to cAMP and appear to be defective in trafficking. One of these mutations (Ser364Pro) has been reported in the hearts of young children with major defects in heart development. Our hypothesis is that the lack of enhanced GJ assembly during the early stages of heart development, e.g., in response to agents that increase cAMP levels, leads to insufficient GJ communication at a critical time and contributes to the observed developmental heart defects. This underscores the importance of the cell communication that is mediated by GJs, structures identified in the electron microscope over 35 years ago.