

that myocardial infarction induces expression of K_{ATP} channels in cardiac fibroblasts. Fibroblasts were isolated from left ventricles of normal (CON) and infarcted adult rat hearts. Fibroblasts from infarcted hearts were separately isolated from scar (MI-S) and remote (MI-R) regions. Experiments were performed within four days after isolation. qRT-PCR and Western blot were used to determine the expression of K_{ATP} channel transcripts and subunits. The mRNA levels were normalized to actin. The expression of functional K_{ATP} channels was evaluated using the whole cell patch clamp technique in the presence of pinacidil (100 μ M) and glibenclamide (10 μ M). SUR2 mRNA levels were significantly increased in MI-S compared to MI-R (220%; $p=0.006$) and CON (214%; $p=0.013$). Kir6.2 mRNA was elevated in MI-S compared to MI-R (148%; $p=0.025$) and CON (121%; $p=0.022$). The expression of Kir6.1 mRNA was not different in MI-S compared to MI-R or CON. SUR1 mRNA was not detected in MI-S or MI-R ($n=4$). Protein levels for SUR2b (98%) and Kir6.2 (92%; $n=2$) were elevated in MI-S compared to MI-R. In MI-S, pinacidil activated a K^+ current (30.44 ± 7.46 pA/pF at 50 mV; $n=10$) that was inhibited by glibenclamide ($p=0.005$). Pinacidil activate no current in MI-R or CON. Our data demonstrate that K_{ATP} channel subunit expression and pinacidil-induced current is upregulated in cardiac fibroblasts in response to myocardial infarction. These changes are limited to the region within the infarct scar. Modulating K_{ATP} channel function may represent a novel approach for the treatment of myocardial ischemia.

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Voltage-Gated Ion Channels are Involved in the Signaling Pathway of Differentiating Chondrocytes

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Chondrocytes, the matrix-producing cells of cartilage, are non-excitabile cells, yet in their mature form they are known to express voltage-gated ion channels. We investigated the signaling pathway and the involvement of plasma membrane ion channels during the early steps of chondrogenesis.

Our *in vitro* chondrogenesis model system is a high density mesenchymal cell culture, in which chondroprogenitor cells are isolated from limb buds of chicken embryos. These cells spontaneously differentiate into chondrocytes mostly on the second and third days of culturing and secrete a significant amount of cartilage-specific extracellular matrix by the sixth day.

Using whole-cell patch-clamp, RT-PCR and Western blot experiments we detected the presence of voltage-gated Kv4.1 and Kv1.3 K^+ channels and Nav1.4 Na^+ channels in these cells. The expression levels of Kv4.1 and Nav1.4 peaked at the critical days 2 and 3 of differentiation, whereas the level of Kv1.3 showed a gradual increase during the six days. However, unlike the other two channels Kv1.3 was not present in the plasma membrane.

By confocal microscopy we detected high-frequency, short-duration Ca^{2+} transients in these cells that were sensitive to the external Ca^{2+} and K^+ concentrations and tetraethylammonium (TEA, Kv channel blocker). Application of TEA, but not tetrodotoxin (TTX, Nav channel blocker), significantly reduced the frequency of Ca^{2+} transients, cartilage matrix production (assessed by metachromatic staining), cell proliferation, and the protein expression of the transcription factor Sox9, while it did not affect cell viability. Patch-clamp measurements showed that TEA also depolarized cells, whereas TTX had no effect on the membrane potential.

Our results indicate the operation of a complex signaling pathway during early chondrocyte differentiation, which heavily relies on the function of Kv channels to stabilize the membrane potential for adequate Ca^{2+} signaling and the progression of chondrogenesis.

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C. Elegans CLHM-1 expression Induces an Evolutionarily Conserved Voltage- and Ca^{2+} -Dependent Ca^{2+} Permeability

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Recently Calhm1 was identified as a member of a novel family of integral membrane proteins in neurons and a polymorphism in Calhm1 was linked to certain forms of late-onset Alzheimer's disease. While this suggests a potential role for mutant Calhm1 in disease, the biophysical characteristics and physiological function(s) of the 6 human Calhm family members are unclear. *C. elegans* expresses a single Calhm gene, *clhm-1*, which is 16% identical / 28% similar to human Calhm1. CLHM-1 is expressed in excitable cells, including neurons and body-wall muscles. Loss of *clhm-1* caused an uncoordinated movement phenotype, while over-expression in the body-wall muscles caused embryonic lethality in *C. elegans*. To determine if *C. elegans* CLHM-1 exhibits functional properties similar to human Calhm1, we expressed *C. elegans* *clhm-1* in *Xenopus* oocytes and performed two electrode voltage clamp experiments. Expression of *clhm-1* induced an outwardly rectifying, voltage-sensitive cur-

rent. Voltage-dependent activation was shifted towards hyperpolarized voltages following removal of extracellular Ca^{2+} , suggesting that extracellular Ca^{2+} normally inhibits CLHM-1 function. *C. elegans* CLHM-1-induced currents were relatively non-selective among monovalent cations, with Ca^{2+} selectivity $P_{Ca} : P_{Na} = 3.6$, and with significant Cl^- permeability. CLHM-1 currents were inhibited by gadolinium, ruthenium red and Zn^{2+} , while specific blockers of voltage-gated Ca^{2+} channels, connexins and NMDA receptors had no effect. Our analyses demonstrate that *C. elegans* CLHM-1 exhibits functional properties similar to human Calhm1, indicating that function of the Calhm gene family members as ion channels or regulators of ion channels is evolutionarily conserved from *C. elegans* to humans. Thus, study of *C. elegans* CLHM-1 can be used to further characterize this protein family which may function to regulate intracellular calcium levels and/or cell excitability.

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Vacuolar H^+ -ATPases and Voltage-Gated Proton Channels: Two Electrogenic, Proton-Selective Membrane Transport Mechanisms Co-Existed in Osteoclasts

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The vacuolar-type H^+ -ATPase (V-ATPase) is an electrogenic H^+ pump that is distributed widely in living organisms. Voltage-gated proton channels (H^+ channels) are found in many types of cells including phagocytes. Although their transport mechanisms are distinct, both depend on pH and voltage and share the outcomes, intracellular alkalization (extracellular acidification) and hyperpolarization. So far, V-ATPases and H^+ channels have been studied separately in different cells, using the different approaches under different conditions. V-ATPases and H^+ channels are expressed in the plasma membrane of osteoclasts, multinuclear bone-resorbing cells, which allowed us to investigate the two electrogenic H^+ transfer mechanisms quantitatively under the same conditions. The current-voltage relationships of the V-ATPases are linear and outward currents were maintained even when the extracellular space was more acidic than the cell inside. Protons flowed through the V-ATPase continuously. In contrast, H^+ channels were silent at voltages lower than the threshold. However, once it opened, the H^+ channel current surpassed the V-ATPase current. Consequently, the profile of H^+ efflux was composed of two phases, one dominated by the V-ATPase and the other, by the H^+ channel. Inhibition of V-ATPases by bafilomycin was often accompanied by decreases in the H^+ channel current. These results suggested that the V-ATPase and the H^+ channel work over different ranges of pH- and voltage-gradients across the membrane in single cells and co-regulate the pH environments of osteoclasts.

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Structure-Activity Relationships of Influenza a M2 Inhibitors

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The M2 proton channel of influenza A has become resistant to the traditional antiviral drugs amantadine and rimantadine and is a high-priority target for current rational drug design efforts. Recent studies have revealed the interior of the wild-type channel as the primary binding site for amantadine and related antiviral compounds. We have extracted from the literature a set of 291 amantadine-like compounds with experimentally determined antiviral activity. Generating stereoisomers and alternate protonation states for each of these compounds gave a dataset of nearly 1,000 unique structures for in silico analysis. The binding energy and conformation of each structure were predicted using the AutoDock Vina molecular docking program with a recently determined structure of the wild-type channel. In addition, each structure was characterized by additional features, including molecular weight, charge, surface area, volume, and individual components of the Vina scoring function. Comparisons of these calculated values to the experimentally determined activity of each compound will inform future studies seeking to develop new M2 inhibitors.

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Acid Sensitivity and Amanatadine Block of Influenza a M2 Channels in Folded Bilayers

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Understanding of ion channel function involves assignment of channel transport activities to constituents, to their arrangements and to membrane conditions. One strategy for identifying the structure function relationship is reconstitution of protein in planar lipid membranes. Reconstitution of M2 protein has been reported in the past using lipid vesicles and measuring the proton flux at different pH. Also, single channel conductance measurements were reported based on studies with decane inflated planar lipid bilayers at low pH where amantadine sensitivity was low. The present work reports the proton conductance of Influenza A M2(22-62) in a bilayer (275 pF) folded from