Molecular dynamics simulations for the study of deafness-related M34T and C169Y mutations of connexin 26.

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Connexins (Cx) are tetraspan transmembrane proteins that form hexameric assemblies in the plasma membrane known as hemichannels or connexons. When two hemichannels from adjacent cells dock and join, they may form an intercellular gap junction channel which spans the two plasma membranes and allows the exchange of cytoplasmic molecules. Virtually all cells in solid tissues are coupled by gap junctions, thus it is not surprising that mutations in connexin genes have been linked to a variety of human diseases, including cardiovascular anomalies, peripheral neuropathy, skin disorders, cataracts, and deafness. Mutations of the GJB2 gene encoding the connexin26 (Cx26) gap junction protein, which is widely expressed in the inner ear, are the primary cause of hereditary non-syndromic hearing loss in several populations. In this work, we used molecular dynamics to study two different single point natural mutations: the deafness-associated single aminoacid substitution of methionine34 (M34) in the first transmembrane helix (TM1) with a threonine (T), and the substitution of cysteine169 (C169) in the second extracellular loop (EL2) with a Tyrosine (Y). The former natural mutation ensues in the production of mutant Cx26M34T channels that are correctly synthesize and assembled in the plasma membrane. However, mutant channels overexpressed in HeLa cells retain only 11% of the wild type unitary conductance[1]. According to the published X–ray model of the human wild type Cx26 (Cx26WT) gap junction channel[2], M34 interacts with W3 of the N-terminal helix (NTH) belonging to an adjacent connexin(Figure 1). The six NTHs fold inside the pore and the M34-W3 hydrophobic interactions stabilize their position at the cytoplasmic mouth. We extend and rationalize those findings by comparing Cx26WT and Cx26M34T mutant channels in silico, using molecular dynamics simulation[3,4](Figure 2). Our results indicate that the quaternary structure of the Cx26M34T hemichannel is altered at the level of the pore funnel due to the disruption of the hydrophobic interaction between M34 and tryptophan3 (W3) in the NTH. These structural alterations significantly increase the free energy barrier encountered by permeating ions, correspondingly decreasing the unitary conductance of the Cx26M34T hemichannel. Our results accord with the proposal that the mutant resides most of the time in a low conductanc e state.

The latter natural mutation, previously classified as a polymorphism, ensues in the production of mutant Cx26M34T and has been identified as causative of severe hearing loss in two Qatari families.We have analyzed the effect of this mutation using a combination of confocal immuno fluorescence microscopy and molecular dynamics simulations. At the cellular level, our results show that the mutant protein fails to form junctional channels in HeLa transfectants despite being correctly targeted to the plasma membrane. At the molecular level, this effect can be accounted for by disruption of the disulfide bridge that Cys169 forms with Cys64 in the wild type structure(Figure 3). Lack of the disulfide bridge in the Cx26C169Y protein causes a spatial rearrangement of two important residues, Asn176 and Thr177. In the Cx26WT protein, these residues play a crucial role in the intramolecular interactions that permit the formation of an intercellular channel by the head-to-head docking of two opposing hemichannels resident in the plasma membrane of adjacent cells[2]. Our results elucidate the molecular pathogenesis of hereditary hearing loss due to the connexin mutation and facilitate the understanding of its role in both healthy and affected individuals.

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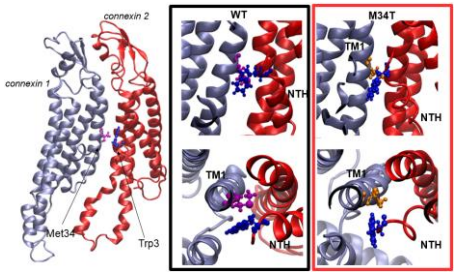

Figure 1. On the left two adjacent wild type connexins are shown in ribbons representation. The two residues highlighted in ball and stick representation are Met34 (purple) and Trp3 (blu). On the right are shown details of the hydrophobic interaction between these two residues for Cx26WT and Cx26M34T proteins.

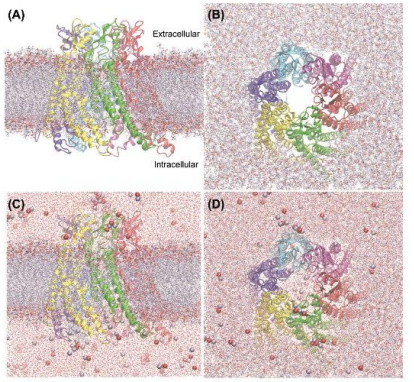

Figure 2. Cx26WT connexon model in a realistic computational environment. (A,B)The connexon is shown embedded in a phospholipid bilayer. (C,D)Also water molecules and ions (shown as colored spheres) were included.

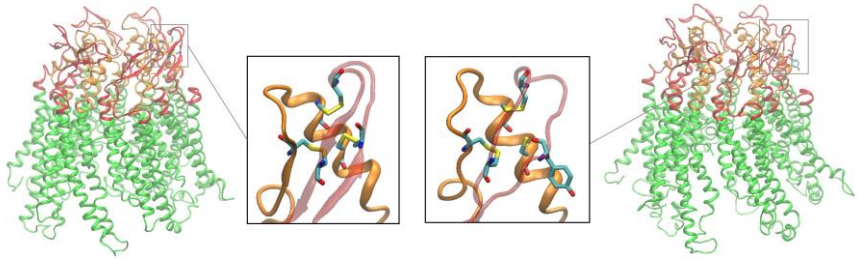

Figure 3. Cartoon representation of Cx26WT (left) and Cx26C169Y (right) hemichannels. The six connexins composing the hemichannels are drawn in ribbon, the extracellular loops (EC1 and EC2) are shown in orange and red (respectively). The insets show details of a single connexin; residues 169 and 64, which in the wild type structure are linked by a disulfide bond, are drawn in licorice representation.