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Review

Structure and closure of connexin gap junction channels

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ABSTRACT

Connexin gap junctions comprise assembled channels penetrating two plasma membranes for which gating regulation is associated with a variety of factors, including voltage, pH, Ca^{2+}, and phosphorylation. Functional studies have established that various parts of the connexin peptides are related to channel closure and electrophysiology studies have provided several working models for channel gating. The corresponding structural models supporting these findings, however, are not sufficient because only small numbers of closed connexin structures have been reported. To fully understand the gating mechanisms, the channels should be visualized in both the open and closed states. Electron crystallography and X-ray crystallography studies recently revealed three-dimensional structures of connexin channels in a couple of states in which the main difference is the conformation of the N-terminal domain, which have helped to clarify the structure in regard to channel closure. Here the closure models for connexin gap junction channels inferred from structural and functional studies are described in the context of each domain of the connexin protein associated with gating modulation.

1. Introduction

Most multicellular organisms have established intercellular communication mediated by gap junctions. These junctions comprise assemblies of channels in which two hemichannels are docked head-to-head with each other, and a patch of channel clusters is referred to as a gap junction plaque. The term "gap" refers to the narrow clearance of 2–4 nm between the two adjoining cells [1]. The central pore of the channel allows for the transfer of small signaling molecules with relatively low specificity, and the adjacent cells are coupled together electrically as well as chemically.

Genetic studies have revealed that the molecular component of vertebrate gap junction channels is connexin (Cx) [2–4], which contains four-pass transmembrane domains (TM1–TM4), two extracellular loops (EL1, EL2), cytoplasmic N- and C-terminal domains (NT, CT), and a single cytoplasmic loop (CL). Over 20 members of the connexin family with the same topology have been identified, and these differ mainly in the lengths and sequences of cytoplasmic loop (CL) and carboxyl terminal domain (CT) [5]. Because gap junction channels autonomously form channel clusters, and sometimes a two-dimensional (2D) crystal lattice, great effort has been devoted to developing an isolation protocol for rodent liver gap junctions [6–10], and electron microscopy (EM) and X-ray diffraction studies performed in the 1970s led to a dodecameric connexin model of the gap junction structure [11].

Along with these structural studies, functional electrophysiology and dye permeability studies determined that cytoplasmic pH and calcium ions modulate gap junction channel activity [12–14]. Around the same time, electrophysiology findings revealed that junctional voltage regulates the intercellular coupling of connexin channels [15], and a model with charge-sensing residues lying within an aqueous pore was proposed [16]. It is now accepted that connexin channel activities are also modulated by various chemical factors, such as amino sulfonates, phosphorylation, lipophiles, cyclic nucleotides, and others [17]. Comprehensive structural models that rationally account for the conformational changes corresponding to these gating modifications, however, are currently insufficient. Traditional and recent structural studies are reviewed here along with the gating movements inferred from functional studies.

2. Three-dimensional structures of connexin gap junction channels; historical overview

The first three-dimensional (3D) reconstruction of connexin was generated by electron crystallography with negatively-stained

Abbreviations: Cx, connexin; Vj, transjunctional voltage; NT, amino terminal domain; CT, carboxyl terminal domain; CL, cytoplasmic loop; EL, extracellular loop; TM, transmembrane helix; EM, electron microscopy; Cx32*Cx43E1, Cx32 chimera in which the EL1 of Cx32 is replaced with the EL1 of Cx43; MD, molecular dynamics

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rat liver gap junctions [18] and cryo EM [19], providing a model for open and closed rearrangement by tilting the subunits in response to the Ca^{2+} concentration [19,20]. This model, referred to as the subunit rotation model, is based on the high degree of cooperativity between subunits (Fig. 1(A)) [21]. First, at least all six subunits in a hemichannel (connexon) simultaneously assume an identical conformational change in a concerted manner. Second, although rearrangement of the subunits in one hemichannel tangential to the channel centers around the extracellular docking surface, the allosteric conformational change of the apposed hemichannel is not negligible. Therefore, it is thought that two docked hemichannels move in conjunction with each other. Structural studies with isolated native gap junctions, followed by EM for split gap junctions in a cryo-negative preparation, revealed six protrusions on the extracellular surface [22]. After recombinant expression of connexin in cultured mammalian cells was established [23,24], electron crystallography revealed a 3D structure of a truncated Cx43 mutant at 7.5 Å resolution [25]. While this structure clearly demonstrated 24 transmembrane helices, thereby validating the models of hexameric hemichannels and dodecameric junction channels, it was unclear whether the channel was open or closed because of the wide pore pathway despite the addition of oleamide, a potential blocker for gap junction channels [26], to the crystallization buffer.

The gap junction structure in an obviously closed state was derived from 2D crystals of Cx26 with a single point mutation from Met to Ala at position 34 (Cx26M34A) [27,28]. This mutant is a derivative of the hereditary hearing-loss mutant M34T [29], and its permeability to dye tracer is significantly decreased, if not completely blocked, indicating that it favors a closed state [30]. The 3D structure of Cx26M34A reconstructed at 10 Å resolution (a vertical resolution of 14 Å) revealed an aggregate density, termed a plug, in

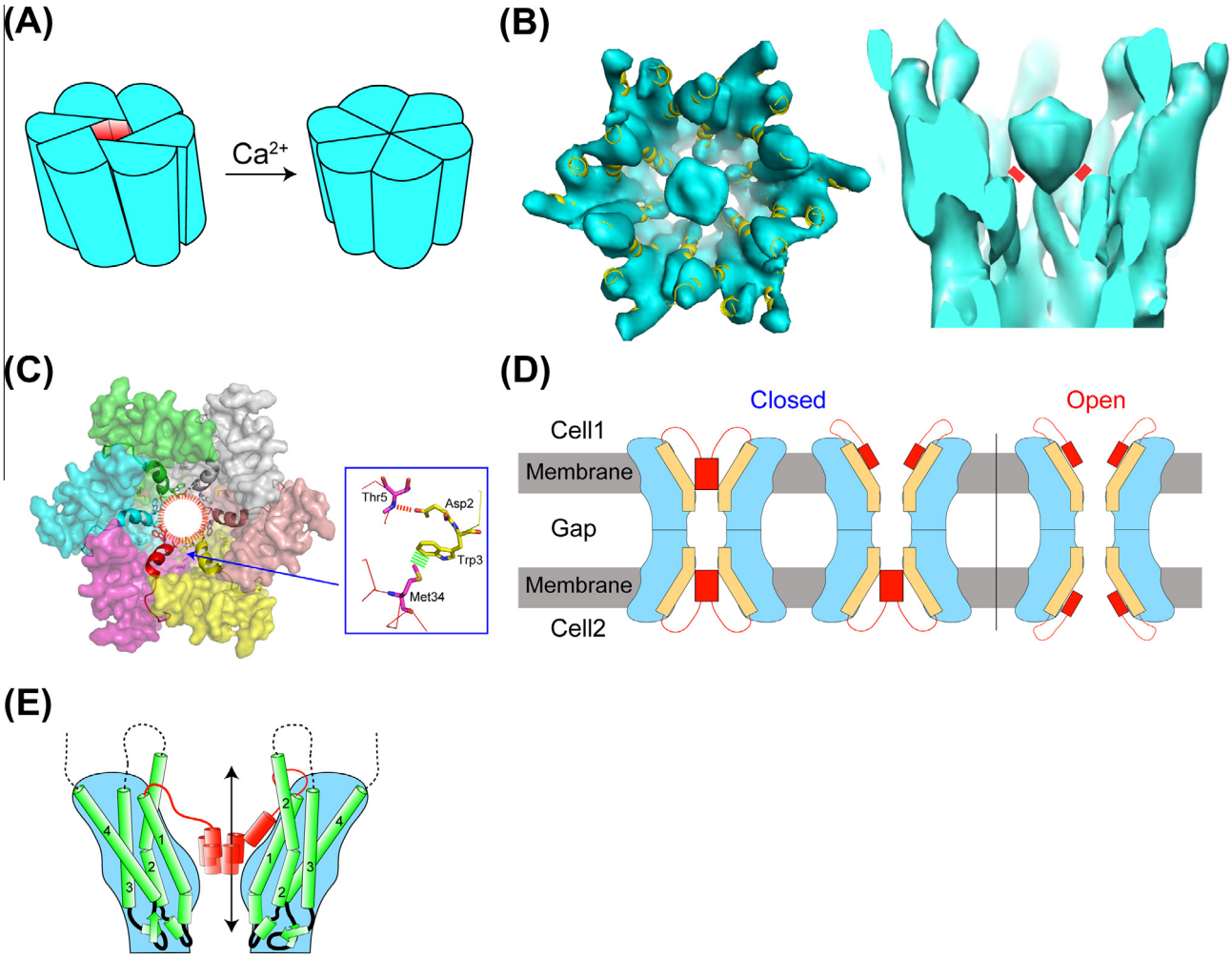

Fig. 1. Gating models inferred from structural studies of connexin gap junction channels. (A) Subunit rotation model [19]. Ca^{2+} induces the gating transitions of the gap junction channel between open and closed configurations. Tilted subunits in a hemichannel assume a tangential displacement upon exposure to Ca^{2+}. The subunits move inward and narrow the pore near the cytoplasmic membrane surface. (B) 3D structure of a Cx26M34A mutant corresponding to a hemichannel portion in a surface representation contoured at 1.0σ reconstructed by electron crystallography [27]. (left) Top view shows the density in the vestibule plugging the pore pathway. The helix ribbon model is shown next to the Cx26 X-ray structure (PDB code: 2ZW3) [32], fitted into the EM map where the NT portions are eliminated for a different conformation. (right) Side view reveals that the constriction between the plug and TM1 is less than 6 Å (shaded in red), which would not permit any transfer of hydrated ions. (C) Top view of Cx26 X-ray structure [32] demonstrates the N-terminal arrangements in an open conformation. The six NT helices form a pore funnel stabilized by a circular network of hydrogen bonds (broken circle in orange). The residues relevant to the interactions for the funnel formation are shown in stick model. (Inset) Detailed representation of the interactions that stabilize the pore funnel, which involve the hydrogen bond between Asp2 and Trp5 (orange dotted line) and the hydrophobic interaction between Trp3 and Met34 (green dotted line). (D) Schematic representation of hypothesized plug gating mechanism of gap junctions. Each hemichannel (cyan) has its own plug formed by an assembly of NTs, and can regulate its channel activity autonomously. The gap junction is open only when the N-termini (red) in both hemichannels assume an open conformation (modified from [27] and [32]). (E) For hydrated ion permeation of the plug-gated structure, the movement of one N-terminal helix would be sufficient to create an 8 Å space to pass through.