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Structure of the connexin 26 gap junction channel at 3.5 Å resolution

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Gap junctions consist of arrays of intercellular channels between adjacent cells that permit the exchange of ions and small molecules. Here we report the crystal structure of the gap junction channel formed by human connexin 26 (Cx26, also known as GJB2) at 3.5 Å resolution, and discuss structural determinants of solute transport through the channel. The density map showed the two membrane-spanning hemichannels and the arrangement of the four transmembrane helices of the six protomers forming each hemichannel. The hemichannels feature a positively charged cytoplasmic entrance, a funnel, a negatively charged transmembrane pathway, and an extracellular cavity. The pore is narrowed at the funnel, which is formed by the six amino-terminal helices lining the wall of the channel, which thus determines the molecular size restriction at the channel entrance. The structure of the Cx26 gap junction channel also has implications for the gating of the channel by the transjunctional voltage.

Intercellular signalling is one of the most essential properties of multicellular organisms. Gap junctions are specialized membrane regions containing hundreds of intercellular communication channels that allow the passage of molecules such as ions, metabolites, nucleotides and small peptides[1]. A gap junction channel is formed by end-to-end docking of two hemichannels, also referred to as connexons, each composed of six connexin subunits[2]. Connexin is predicted to have four transmembrane helices and two extracellular loops, which are thought to contain a β-strand structure and are an essential structural basis for the docking of two connexons[3]. Gap junctions have crucial roles in many biological processes including development, differentiation, cell synchronization, neuronal activity and immune responses[4,5]. Mutations in connexins thus cause several human diseases, including neurodegenerative diseases, skin diseases, deafness and developmental abnormalities[5,6].

To date, more than 20 different connexins have been identified in the human genome, which have been categorized into α, β and γ isoforms on the basis of their sequence homology. The connexin composition of gap junction channels defines their unique properties, such as their selectivity for small molecules, voltage-dependent gating, and response to Ca^{2+}, pH and phosphorylation[5,7].

Early electron microscopic analyses of gap junctions suggested that channel gating involves a rotation of all six subunits[8,9], and analysis of two-dimensional crystals formed by carboxy-terminally truncated connexin 43 (Cx43, also known as GJA1) resulted in a model for the arrangement of the transmembrane helices and the fold of the connexin protomer[10,11]. Recently, the electron crystallographic analysis of the connexin 26 Met34Ala mutant (Cx26(M34A)) revealed large densities in the pore at the level of the two membranes, which were interpreted as plugs blocking the channel[12]. The structure of Cx26(M34A) was thus assumed to show the channel in a closed state. The structure also suggested that physical blockage by a plug is an essential part of a gating mechanism and is consistent with the physiological studies showing that each connexon can regulate its activity autonomously[13–15]. Electrophysiological studies have demonstrated that gap junctions have several gating mechanisms. At least two regulation mechanisms respond to the transjunctional voltage (V_j), V_j gating (fast) and loop gating (slow)[16]. Gap junctions can also be gated by the membrane voltage (V_m), termed V_m gating, and by chemical factors such as phosphorylation, pH and Ca^{2+}, known as chemical gating[17].

Here we present an atomic structure of the human Cx26 gap junction channel. We find that the four transmembrane helices of a protomer are arranged differently from the previously proposed pseudoatomic model[11], and that several residues associated with non-syndromic hereditary deafness or skin diseases are involved in intra- or intermolecular interactions. We describe in detail the interactions between the two extracellular regions of adjoining connexons. The N-terminal regions of the six subunits line the pore entrance and form a funnel, which restricts the diameter at the entrance of the pore to 14 Å. In conjunction with previous electron microscopy work[12], this finding suggests that conformational changes in the Cx26 N termini play an important part in channel gating, specifically in V_j gating.

Structure determination of the gap junction channel

Structure determination at 3.5 Å is briefly described in the Methods. The whole structure of each protomer—except for residues 110–124 and 218–226 that correspond to most of the cytoplasmic loop and the carboxy-terminal segment, respectively—was successfully modelled in electron density maps. The amino acid assignment was confirmed by methionine sites and disulphide bonds sites (Supplementary Fig. 1). Of the 226 residues of Cx26, the atomic parameters of residues 2–109 and 125–217 converged well during refinement.

The overall structure of the Cx26 gap junction channel, which is formed by two connexons related to each other by a crystallographic two-fold symmetry axis, is similar in shape and size to that of the C-terminal truncated Cx43 gap junction channel visualized by electron crystallography[10] (Fig. 1a). It is a tsuzumi shape, a traditional Japanese drum. The protomers in each hexameric connexon are related by a six-fold non-crystallographic symmetry (NCS) axis perpendicular to the membrane plane (Fig. 1b). The height of the modelled structure of the gap junction channel without disordered cytoplasmic loop and

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The following is a comment on the published paper shown on the preceding page.

Structure of the Human Connexin 26 Gap Junction Channel

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Introduction

Intercellular signaling is one of the most essential properties of multicellular organisms. Gap junctions are specialized membrane regions containing hundreds of intercellular communication channels that allow passage of molecules such as ions, metabolites, nucleotides, and small peptides (1). A gap junction channel is formed by end-to-end docking of two hemichannels, also referred to as connexons, each composed of six connexin subunits (2). Gap junctions play crucial roles in many biological processes including development, differentiation, cell synchronization, neuronal activity and the immune response (3, 4). Mutations in connexins thus cause a number of human diseases, including neurodegenerative diseases, skin diseases, deafness and developmental abnormalities (4). Here, we present an atomic structure of the human Cx26 gap junction channel. We reveal in detail the structural organization of the dodecameric gap junction channel. The N-terminal regions of the six subunits line the pore entrance and form a funnel. In conjunction with previous EM work (5), this finding suggests that conformational changes in the Cx26 N-termini play a key role in channel gating, specifically in the transjunctional voltage dependent gating (Vj-gating).

Overall architecture of the Cx26 gap junction channel

The overall structure of the Cx26 gap junction channel is a tsuzumi shape (Fig. 1a), a traditional Japanese drum. The height of the modeled structure of the gap junction channel without disordered cytoplasmic loop (CL) and carboxy-terminal tail (CT) is approximately 155 Å. The transmembrane region of the channel is 38 Å thick. TM2 extends about 19 Å from the membrane surface into the cytoplasm. The intercellular "gap" is about 40 Å. Viewed from the top, the channel looks like a "hexagonal nut" with a pore in the centre (Fig. 1b). The diameter of the pore is about 40 Å at the cytoplasmic side of the channel, narrowing to

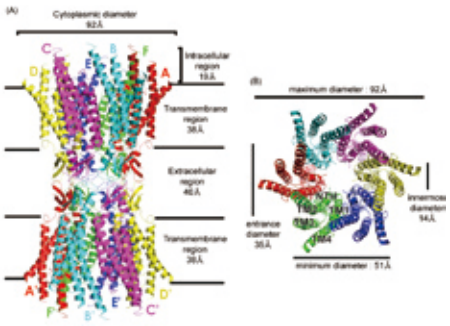

Fig. 1 Structure of the Cx26 gap junction channel. The corresponding protomers in the two hemichannels are shown in the same color. (a) Side view of the Cx26 gap junction channel. (b) Top view of the Cx26 gap junction channel showing the arrangement of the transmembrane helices.

14 Å near the extracellular membrane surface and then widening to 25 Å within the extracellular space. Since 3.5 Å X-ray structure does not show any obstructions along the pore, our structure of wild-type Cx26 appears to be in an open conformation, which is consistent with the used crystallization conditions (neutral pH without any agents which promote channel closure).

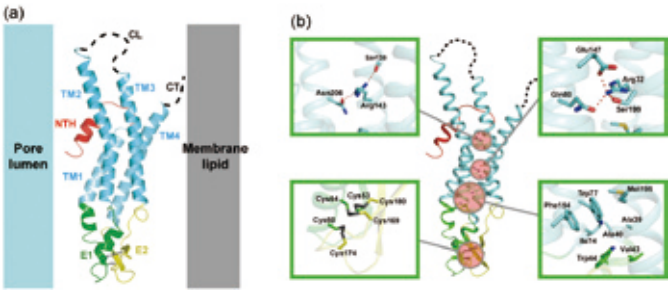

Fig. 2 Structure of the Cx26 protomer. (a) Cx26 protomer drawn in ribbon representation and colored differently with its region. (b) Interactions which stabilize the protomer structure.

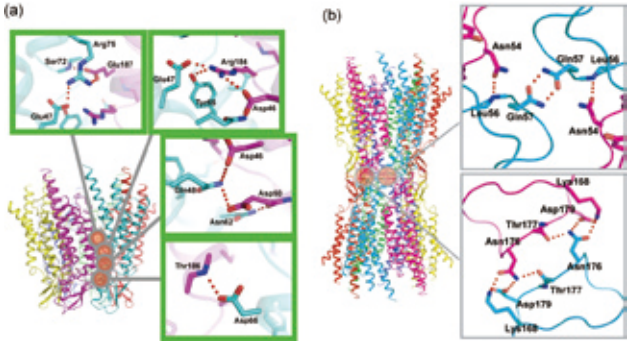

Fig. 3 Molecular architecture of the Cx26 gap junction channel. (a) Inter-protomer interactions which stabilize the hexameric connexon. (b) Intercellular interactions between two connexons.

Structure of the Cx26 protomer

The protomer has four transmembrane (TM) segments (TM1-4), two extracellular loops (E1 and E2), CL, an amino-terminal helix (NTH), and CT (Fig. 2a). Cx26 forms a typical four-helix bundle in which any pair of adjacent helices is anti-parallel. TM1 and TM2 face the interior, whereas TM3 and TM4 face the hydrophobic membrane environment. The major pore-lining helix TM1 is inclined, so that the pore diameter narrows from the cytoplasmic to the extracellular side of the membrane. The extracellular loop E1 contains a 3_{10} helix at the beginning and a short α-helix in its C-terminal half. E2, together with E1, contains a short anti-parallel β-sheet and stretches over E1, forming the outside wall of the connexon. Six conserved cysteine residues, three in each loop, form intra-molecular disulfide bonds between E1 and E2 (Figs. 2a,b). Most of the prominent intra-protomer interactions are in the extracellular half of the transmembrane region (Fig. 2b).

Structural organization of the hexameric connexon and the intercellular junction

The inter-protomer interactions in the hexameric connexon are mostly located in the extracellular half of transmembrane helices TM2 and TM4 and in the extracellular loops (Fig. 3a). Most of the residues involved in intra- and inter-protomer interactions are conserved within the connexin family, and mutations of these residues are associated with deafness and skin diseases (6). The mutations are likely to interfere with

the proper folding and/or oligomerization of connexins, thus resulting in defective channels.

Our structure also revealed the interactions between the two adjoining connexons of the gap junction channel, which involve both E1 and E2 (Fig. 3b).

Architecture of the channel interior

The permeation pathway of a gap junction channel consists of an intracellular channel entrance, a pore funnel and an extracellular cavity (Fig. 4a). The intracellular channel entrance has a diameter of 40 Å and a cluster of basic residues make a positively charged environment. The funnel surface is lined by N-terminal residues Asp2, Trp3, Thr5, Leu6, and Ile9 (Fig. 4b). Because the funnel forms a constriction site at the cytoplasmic entrance of the pore, the size and electrical character of the side-chains in this region should have a strong effect on both the molecular cut-off size and the charge selectivity of the channel. Glu42, Asp46 and Asp50 face the pore interior and create a negatively charged path with a diameter of 20 Å, approximately at the height of the extracellular membrane surface (Fig. 4a). Along with the pore funnel, this region is also likely to contribute to the size restriction and possibly to the charge selectivity, considering the pore diameter and the charge character.

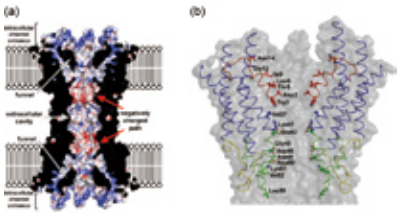

Fig. 4 Pore structure of the Cx26 gap junction channel. (a) A vertical cross-section through the gap junction channel, showing the surface potential inside the channel. The channel features a positively charged channel entrance, negatively charged path, and pore funnel. (b) Pore-lining residues in a Cx26 gap junction channel. The main chain is depicted as a thin ribbon and side chains facing the pore as balls and sticks.

Pore funnel and implications for the voltage-dependent gating mechanism

The short amino-terminal helices (NTHs) of the six protomers form the funnel (Fig. 5a), and their very high crystallographic temperature factors indicate conformation change in this domain. Asp2 hydrogen-bonds with the main-chain amide of Thr5 of the neighbouring protomer. The Asp2 and Thr5 residues on neighbouring NTHs at the bottom of the funnel form a circular girdle, which stabilizes the funnel structure (Fig. 5a). Trp3 forms hydrophobic interactions with Met34(TM1) of the neighbouring protomer, which draws the NTH to the inner wall of the channel. One of the most frequent deafness mutations is Met34Thr, which decreases electrical current, but forms structures indistinguishable from wild-type gap junctions (7). This mutation would indeed disrupt the interaction of NTH with Trp3, which would cause the funnel to detach from the inner wall of the pore, resulting in a narrower funnel. This notion is supported by recent EM studies that showed a prominent density, which is referred to as "pore plug", in the centre of the pore in Cx26Met34Ala (5), which was decreased in the N-terminal deletion mutant Cx26Met34Ala-del2-7 (8).

A cytoplasmic movement of the N-terminal portion, where

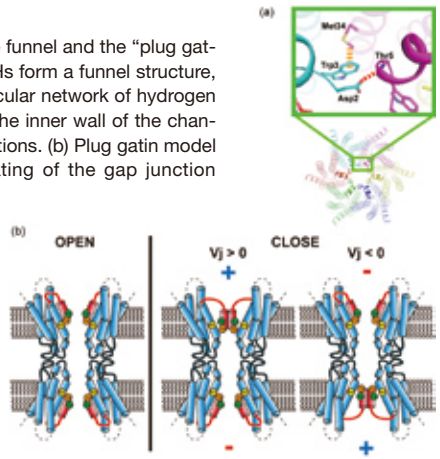

Fig. 5 Structure of the pore funnel and the "plug gating model". (a) The six NTHs form a funnel structure, which is stabilized by a circular network of hydrogen bonds and is attached to the inner wall of the channel by hydrophobic interactions. (b) Plug gatin model for voltage-dependent gating of the gap junction channel. (left) Model of the Cx26 gap junction channel in the open state, where transjunctional voltage (Vj) is zero. (right) Model of the Cx26 gap junction channel in the closed or subconductance state, where Vj is not zero.

the voltage sensor is believed to reside, has been suggested to initiate voltage-dependent gating (9, 10, 11). While the EM structure may not exactly represent a physiological closed state, it is conceivable that an inside positive Vj would cause an inward movement of Asp2, thus disrupting the interactions between Asp2-Trp5 and Trp3-Met34, which could function as a trigger for gating in response to a change in Vj.

The structure in this work could suggest a speculative Vj-gating model, in which the N-termini play the major role in sensing Vj within the conductive pore and in forming the plug to close the pore (Fig. 5b). The structure in this work strongly suggests that the plug detected in the EM structure is composed of the assembly of Cx26 N-termini. However, we do not rule out a possibility that the invisible cytoplasmic loop or the C-terminus might have contribution as a component.

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