Defining a Minimal Motif Required to Prevent Connexin Oligomerization in the Endoplasmic Reticulum*

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In contrast to most multimeric transmembrane complexes that oligomerize in the endoplasmic reticulum (ER), the gap junction protein connexin43 (Cx43) oligomerizes in an aspect of the Golgi apparatus. The mechanisms that prevent oligomerization of Cx43 and related connexins in the ER are not well understood. Also, some studies suggest that connexins can oligomerize in the ER. We used connexin constructs containing a C-terminal dityrosine-based ER retention/retrieval signal (HKKSL) transsected into HeLa cells to study early events in connexin oligomerization. Using this approach, Cx43-HKKSL was retained in the ER and prevented from oligomerization. However, another ER-retained HKKSL-tagged connexin, Cx32-HKKSL, had the capacity to oligomerize. Because this suggested that Cx43 contains a motif that prevented oligomerization in the ER, a series of HKKSL-tagged and untagged Cx32/Cx43 chimera was screened to define this motif. The minimal motif, which prevented ER oligomerization, consisted of the complete third transmembrane domain and the second extracellular loop from Cx43 on a Cx32 backbone. We propose that charged residues present in Cx43 and related connexins help prevent ER oligomerization by stabilizing the third transmembrane domain in the membrane bilayer.

Gap junction channels are formed by a family of proteins known as connexins (1–7). A complete gap junction channel is formed when a connexin hexamer in the plasma membrane of one cell binds to a hexamer in an adjacent cell. Formation of connexin hexamers (oligomerization) occurs in intracellular compartments prior to transport to the plasma membrane. Connexin mutations associated with human disease frequently interfere with proper connexin trafficking and assembly, causing mutant connexins to improperly accumulate in intracellular compartments such as the endoplasmic reticulum (ER)\(^3\) or aspects of the Golgi apparatus (8–10).

Musil and Goodenough (11) first demonstrated that connexin43 (Cx43) oligomerizes into hexamers after exit from the ER. Cx43 differs from most other multimeric transmembrane complexes, which typically oligomerize in the ER as a prerequisite to further transport along the secretory pathway (12). Post-ER oligomerization of Cx43 and other connexins has been confirmed in other studies (13–16). However, there is also evidence suggesting that connexins partially or completely oligomerize in the ER (14, 17, 18). Most of these studies examined the effect of membrane trafficking inhibitors, such as brefeldin A, on connexin oligomerization. In addition, sites of oligomerization have been inferred from rescue studies in which co-expressed wild-type connexins assemble with misfolded connexins and promote their transport to the plasma membrane. The implication of these studies is that oligomerization between the misfolded and properly folded connexins occurred in the compartment where the misfolded connexin was trapped.

As an alternate approach, we have used connexins containing a dityrosine ER retention/retrieval motif, HKKSL, to study early events in oligomerization of Cx32 and Cx43 (19). HKKSL-tagged connexins are retained in the ER in the absence of pharmacologic agents that have the potential to alter ER composition and function (20). We found that ER-retained Cx32-HKKSL had the capacity to oligomerize, whereas Cx43-HKKSL did not (19). This suggests that Cx43-HKKSL contains a motif required to inhibit oligomerization in the ER. Given this possibility, we made a series of HKKSL-tagged Cx43/Cx32 chimeras to define Cx43 motifs that inhibit ER oligomerization.

MATERIALS AND METHODS

Antisera and Reagents—Rabbit anti-Cx43 (21) was generated using His\(_6\)-tagged C-terminal tail constructs as described previously. Rabbit anti-Cx32 and mouse anti-Cx32 were from Zymed Laboratories Inc. (San Francisco, CA), and mouse anti-calnexin and rabbit anti-\(\beta\)-COP were from Affinity Bioreagents (Golden, CO). Monoclonal anti-calnexin was from Chemicon (Temecula, CA). Fluorescent and horseradish peroxidase-conjugated secondary antibodies were from Jackson ImmunoResearch (West Grove, PA). Triton X-100 was from Roche Applied Science. Tissue culture reagents were from Invitrogen. Unless otherwise specified, all other reagents were from Sigma.

Constructs, Transfection, and Immunofluorescence—Tagged constructs were produced by PCR amplification in a Stratagene Robocycler (La Jolla, CA) using high fidelity DNA polymerase (Roche Applied Science), starting with rat Cx43 or Cx32 cDNA as a template. Cx43/Cx32 chimeras (Fig. 1) were produced by two-step PCR amplification using established protocols (22). In brief, the two halves of a given construct were first produced with the template and primer pairs listed in Table 1. The halves were annealed and amplified using Cx32/Cx32-HKKSL, Cx32/Cx32-HKKSL, or Cx32/Cx43-HKKSL sense/antisense primer pairs as appropriate. The resulting PCR products were cut with KpnI and EcoRI and ligated into a pcDNA3 expression vector, and transformed into bacterial stocks. DNA for transfection was purified from bacteria using the Qiagen Maxiprep kit according to the manufacturer’s instructions. Prior to transfection, cDNA was purified by EtOH precipitation. Cx32/Cx32 sense/antisense primer pairs were used to produce untagged versions of chimeras using the HKKSL-tagged cDNA as a template. For single-site mutagenesis of cysteine to arginine, we used 5'-AGGGACACATGTTGGAGACCTATGGTCATCACG-3' and 5'-GCTGATGAGTGGTCCTCCACAGTGTCCCT-3' as sense and anti-t
sense primers, respectively, with the QuikChange site-directed mutagenesis kit (Stratagene). For single-site mutagenesis of arginine to tryptophan, we used 5′-GGGGCCTTGCTGTGACCATCATCAGCC-3′ and 5′-GGGTAGATGGATAGCTCACAGCAACCAGGCC-3′ as sense and antisense primers, respectively. HeLa cells were transiently transfected with either wild-type or modified connexin cDNAs constructs using Lipofectamine (Invitrogen) and analyzed 48 h after transfection. Transfection efficiencies using this approach were typically >70%.

For immunofluorescence, cells plated on glass coverslips were fixed and permeabilized with MeOH/acetone (1:1) and then washed three times with PBS followed by PBS plus 0.5% Triton X-100 and PBS plus 0.5% Triton X-100 plus 2% goat serum (PBS/GS). The cells were incubated with primary antisera diluted into PBS/GS for 1 h, washed, and then labeled with secondary antisera diluted into PBS/GS. The cells were then washed with PBS, mounted into Mowiol, visualized by fluorescence microscopy using an Olympus X-70 microscope system, and imaged with a Hamamatsu Orca-1 charge-coupled device camera and Image Pro image analysis software (Media Cybernetics, Silver Spring, MD).

Protein Analysis—The techniques outlined below have also been described elsewhere (13, 15, 23). Cells were washed with PBS, harvested into PBS containing protease inhibitors (10 μg N-ethylmaleimide, 1 mM phenylmethylsulfonyl chloride, 2 μg/ml leupeptin, and 1 μg/ml pepstatin) and phosphatase inhibitors (1 mM NaVO₄ and 10 μg/ml NaF), and then passed through a ball bearing homogenizer 100 times (13, 15). The homogenate was centrifuged at 500 g for 30 min, and the resulting postnuclear supernatant was centrifuged at 100,000 g for 30 min, and the resulting postnuclear supernatant was centrifuged at 100,000 g for 30 min using a Sorvall Ultra Pro 80 Ultracentrifuge to obtain a membrane-enriched pellet. To analyze total cell connexin expression, this pellet was resuspended in SDS-PAGE sample buffer, resolved by SDS-PAGE, transferred to polyvinylidene difluoride membrane, and then blocked overnight with 40 μg/ml Tris, pH 7.5, 5% Carnation powdered milk, and 0.1% Tween 20 (Blotto). The blots were incubated with Blotto containing specific antisera, washed, and then further incubated with goat anti-rabbit IgG or goat anti-mouse IgG conjugated to horseradish peroxidase (Jackson Immunoresearch, Malvern, PA). Specific signals corresponding to a given protein were detected with the enhanced chemiluminescence reagent (Amer sham Biosciences) and quantified with a Kodak EDAS system (Rochester, NY).

For detergent solubilization studies, the membrane-enriched pellet was resuspended in PBS plus inhibitors containing 1% Triton X-100 and then incubated for 30 min at 4 °C. The sample was then centrifuged at 100,000 g for 30 min and separated into Triton X-100-soluble and -insoluble pellet fractions. The soluble and insoluble fractions were then resuspended into SDS-PAGE sample buffer and assayed by immunoblot.

Sucrose gradient fractionation was done using postnuclear supernatants solubilized in 1% Triton X-100 for 30 min at 4 °C. The samples were then centrifuged at 100,000 × g for 30 min, and the resulting Triton X-100-soluble fraction was overlaid onto a 5–18% sucrose gradient containing 1% Triton X-100. The samples were then centrifuged at 148,000 × g for 16 h at 4 °C in a Sorvall Ultra Pro 80 centrifuge using an AH-650 swinging bucket rotor. Following centrifugation, 0.2-ml fractions were collected from the bottom of the centrifuge tube at 4 °C and then analyzed by immunoblot.

RESULTS

We previously used a dilysine-based ER retention/retrieval signal as a method to retain connexins in the ER without using pharmacologic agents (19). The application of this approach is shown in Fig. 2. Wild-type versions of Cx43 or Cx32 transfected into HeLa cells are transported to the plasma membrane where they form gap junctions. The addition of an HKKSL motif onto the N terminus of either Cx43 or Cx32 produced proteins that were now retained in the ER, as determined by immunofluorescence microscopy. Nearly all Cx43-HKKSL and Cx32-HKKSL were solubilized by 1% Triton X-100 at 4 °C, in contrast to wild-type Cx43 and Cx32, which had significant Triton X-100-insoluble pools because of incorporation of these proteins into gap junction plaques (13, 24, 25). Also, Cx43-HKKSL did not show the slower migrating phosphorylated forms, which are generated after Cx43 incorporation into gap junctions (26).

We then examined the oligomerization state of connexins present in the Triton X-100-soluble portion using sucrose gradient fractionation (Fig. 2, i–l). Consistent with previous results (13, 16, 26), wild-type Cx43 migrated as two peaks, a low molecular mass peak centered at 9–10% sucrose (light fractions) and a higher molecular mass peak centered at ∼15% sucrose (heavy fractions). In contrast, Cx43-HKKSL was present in only the light sucrose fractions, consistent with a lack of oligomerization by the ER-retained construct. Wild-type Cx32 was predominantly in the heavy sucrose fractions, although there were hints of Cx32 in the light sucrose fractions. Cx32-HKKSL was also present predominantly in the heavy sucrose fractions, suggesting that it oligomerized in the ER.

Because these two ER localized-constructs, Cx43-HKKSL and Cx32-HKKSL, showed differences in oligomerization, we produced a series of HKKSL-tagged Cx43/Cx32 chimeras to identify the minimal Cx43 element required to inhibit oligomerization in the ER. Regardless of the results (13, 16, 26), wild-type Cx43 migrated as two peaks, a low molecular mass peak centered at 9–10% sucrose (light fractions) and a higher molecular mass peak centered at ∼15% sucrose (heavy fractions). In contrast, Cx43-HKKSL was present in only the light sucrose fractions, consistent with a lack of oligomerization by the ER-retained construct. Wild-type Cx32 was predominantly in the heavy sucrose fractions, although there were hints of Cx32 in the light sucrose fractions. Cx32-HKKSL was also present predominantly in the heavy sucrose fractions, suggesting that it oligomerized in the ER.

Of the initial series of HKKSL-tagged chimeras, only Cx43/32a-HKKSL expressed by HeLa cells was present in only the light sucrose fractions (Fig. 3). Removal of the HKKSL tag shifted the sucrose gradient migration pattern from the light fractions to the heavy fractions, suggesting that ER retention was required to prevent oligomerization. In contrast, chimeras containing smaller Cx43 elements migrated to heavier sucrose fractions, suggesting that they had oligomerized in the ER. For these chimeras, there might also be some aggregation, because there were peaks that appeared at ∼20% sucrose. Nonetheless, the general lack of peaks greater than 15% for Cx43/32a-HKKSL is consistent with this chimera being retained in the ER as an apparent monomer.

To further define the Cx43 motif required to inhibit oligomerization, a second series of chimeras was examined. As shown in Fig. 4, Cx32/43/32a-HKKSL, which lacks the first transmembrane domain (TM1) and first extracellular loop (EL1) of Cx43 did not oligomerize in the ER. Consistent with this, a complementary chimera, Cx32/43/32a-HKKSL (Fig. 4f), was present predominantly in heavy sucrose fractions, suggesting that that TM1 and EL1, along with the TM4 and the cytoplasmic tail, did not confer the ability to inhibit oligomerization in the ER. Also, ER localization was required to prevent oligomerization, because removal of the HKKSL tag from Cx32/43/32a-HKKSL shifted the sucrose gradient migration pattern from the light fractions to the heavy fractions.

FIG. 1. Splice sites for producing Cx43/32 chimera. Untagged and HKKSL-tagged Cx43/Cx32 chimeras were produced using a combination of splice sites (1–5). Vertical lines denote identical amino acids shared by Cx34 and Cx32, and dots denote amino acids with comparable chemical properties.
One feature shared by Cx43/32a-HKKSL and Cx32/43/32a-HKKSL is that they both contain the cytoplasmic loop, TM3, and EL2 domains of Cx43. To determine whether the cytoplasmic loop domain of Cx43 was necessary for preventing Cx43 oligomerization in the ER, we generated a chimera containing the TM3 and EL2 domains of Cx43 on a backbone of Cx32-HKKSL, (Cx32/43/32b-HKKSL). As shown in Fig. 4, Cx32/43/32b-HKKSL expressed by HeLa cells resolved in the light sucrose fractions, suggesting that the cytoplasmic loop domain of Cx43 was not involved in preventing ER oligomerization of Cx43. Most of the Cx32/43/32b-HKKSL migrated in a peak corresponding to monomers; however, there was a shoulder centered at ~12% sucrose, suggesting that some Cx32/43/32b-HKKSL might be partially oligomerized, aggregated, or associated with a putative chaperone. As described above, ER localization was required to prevent oligomerization of this chimera because removal of the HKKSL tag shifted the sucrose gradient migration pattern of Cx32/43/32b to the heavy fractions. Untagged Cx32/43/32b also showed a shoulder at 12% sucrose, which likely corresponds to partially oligomerized and/or misfolded Cx32/43/32b, comparable with Cx32/43/32b-HKKSL.

To determine whether the EL2 domain of Cx43 was necessary for preventing Cx43 oligomerization in the ER, we generated a chimera lacking this domain (Cx32/43/32c-HKKSL). Cx32/43/32c-HKKSL had a sucrose gradient pattern consistent with oligomerization (Fig. 4d), suggesting that the Cx43 EL2 domain was necessary to prevent oligomerization in the ER.

Three of the chimeras tested, Cx43/32a-HKKSL, Cx32/43/32a-HKKSL, and Cx32/43/32b-HKKSL, did not oligomerize in the ER; however, the untagged versions of these chimeras did oligomerize. By immunofluorescence microscopy (Fig. 5), all three of the HKKSL-tagged chimeras showed an ER localization pattern in transfected HeLa cells, as was the case for all of the HKKSL-tagged constructs used in this study. However, Cx32/43/32a, Cx32/43/32a, and Cx32/43/32b showed different patterns of intracellular localization. Untagged Cx32/43/32a and Cx32/43/32a were mistargeted to intracellular compartments, suggesting that they might be misfolded or improperly oligomerized. However, untagged Cx32/43/32b was transported to the plasma membrane as determined by immunofluorescence microscopy, more consistent with proper formation of gap junction channels. Also, the untagged constructs showed a Triton X-100-insoluble pool, which is a hallmark of both gap junction plaque formation (24) and aggregation (27). In contrast, the HKKSL-tagged constructs were predominantly soluble in Triton X-100. Thus, regardless of whether the chimeras were capable of forming gap junctions, HKKSL-tagged constructs were prevented from oligomerizing by virtue of being retained in the ER.

Having determined that the EL2 domain of Cx43 was necessary to prevent connexin oligomerization in the ER, we then examined whether TM3 plays a role in regulating Cx43 oligomerization. The TM3 domain of Cx43 (and Cx32/43/32b) has a charged arginine residue (Arg-153 in Cx43) that is not present in Cx32, which instead has a hydrophobic tryptophan (Trp)
residue. Cx32/43/32bR133W-HKKSL, a chimera that lacks this Arg residue, was not prevented from oligomerizing in the ER, in contrast to Cx32/43/32b-HKKSL (Fig. 6). Thus, the EL2 domain of Cx43 was not sufficient to inhibit connexin oligomerization in the ER. Note that the Cx32/43/32bR133W-HKKSL chimera showed a sucrose gradient pattern with shoulders, suggesting partial oligomerization or perhaps aggregation. Also, the addition of an arginine to a Cx32 backbone had little effect on Cx32 oligomerization because Cx32R133W-HKKSL and wild-type Cx32-HKKSL had similar sucrose gradient profiles.

This suggested that there may be a role for charged amino acids near the TM3 membrane interfaces in regulating oligomerization of Cx43. As shown in Fig. 6, both the Arg-153 and Gln-173 residues of TM3 were critical to prevent oligomerization of Cx43-HKKSL in the ER. Cx43R153W-HKKSL and Cx43Q173F-HKKSL both had a complex pattern by sucrose gradient fractionation, in which the majority of the protein appeared in a size range larger than monomers yet smaller than hexamers, suggesting partial oligomerization or perhaps aggregation. Also, the addition of an arginine to a Cx32 backbone had little effect on Cx32 oligomerization because Cx32R133W-HKKSL and wild-type Cx32-HKKSL had similar sucrose gradient profiles.

DISCUSSION

We found that the TM3 and EL2 domains of Cx43 were critical to inhibit connexin oligomerization in the ER. Based on homology, these motifs are likely to help prevent ER oligomerization of other connexins, such as Cx46 (13). A role for TM3 in regulating connexin oligomerization is also suggested by the findings of Ahmad et al. (28), who showed that substituting a transmembrane domain of the cystic fibrosis transmembrane regulator for TM3 of Cx32 produced a chimera that did not oligomerize. Consistent with this, most studies suggest that TM3 is the major helix that lines the gap junction channel aqueous pore (29–31) and thus does not correspond to a “classical” transmembrane domain stably integrated into a hydrophobic membrane bilayer. In the case of hemichannels or complete gap junction channels, interactions with other connexins in the complex help stabilize TM3 in the bilayer facing the aqueous environment of the gap junction channel (31). However, monomeric connexins cannot take advantage of these interactions to be stabilized within the ER membrane, so TM3 may require a different conformation.

Charged amino acids present in Cx43 at the two putative membrane/water interfaces of TM3 would be expected to help prevent ER oligomerization of other connexins, such as Cx46 (13). A role for TM3 in regulating connexin oligomerization is also suggested by the findings of Ahmad et al. (28), who showed that substituting a transmembrane domain of the cystic fibrosis transmembrane regulator for TM3 of Cx32 produced a chimera that did not oligomerize. Consistent with this, most studies suggest that TM3 is the major helix that lines the gap junction channel aqueous pore (29–31) and thus does not correspond to a “classical” transmembrane domain stably integrated into a hydrophobic membrane bilayer. In the case of hemichannels or complete gap junction channels, interactions with other connexins in the complex help stabilize TM3 in the bilayer facing the aqueous environment of the gap junction channel (31). However, monomeric connexins cannot take advantage of these interactions to be stabilized within the ER membrane, so TM3 may require a different conformation.
portion of TM3 of an oligomerized connexin, although an equivalent structural model of Cx43 has not yet been produced. However, in a monomeric connexin, the arginine residue could potentially direct TM3 to an alternative conformation where arginine could be localized to the membrane/water interface region, thus stabilizing TM3 in the membrane bilayer. A similar argument can be made for the Cx43 glutamine residue of TM3 (Gln-173). This glutamine is highly conserved by α-connexins, although Cx40.1 and Cx50 substitute histidine for glutamine. By analogy to the structural analysis of Cx32 by Fleishman et al. (31), the polar glutamine residue of Cx43 is likely localized to the luminal membrane/water interface of TM3. Again, Cx32 has an aromatic phenylalanine residue in a comparable position, suggesting that monomeric Cx43 can better anchor TM3 in the bilayer than monomeric Cx32.

The EL2 domain was also required to prevent Cx43 oligomerization in the ER. The Cx43 EL2 domain near the luminal/extracellular membrane domain of Cx43 has a "WYIYGF" sequence that is well conserved for all human connexins related to Cx43 (α-connexins) (Fig. 5). The tyrosine-glycine-phenylalanine tripeptide (YGF) is particularly well conserved. The comparable region of Cx32 has a "YLLYPG" sequence, which is partially conserved for β-family connexins related to Cx32. The second tyrosine is well conserved among β-connexins, whereas the remaining amino acids tend to be hydrophobic but not conserved. Conceivably, binding of a putative peripheral chaperone to the EL2 of monomeric Cx43 could help stabilize the position of TM3 relative to the membrane bilayer. Whether this is the case remains to be determined. Interestingly, the spacing between the conserved tyrosine (Tyr-158 for Cx32, Tyr-178 for Cx43) and first universally conserved cysteine in EL2 differs for "YGF" connexins (nine amino acids between tyrosine and cysteine) versus β-connexins (10 amino acids between tyrosine and cysteine). This difference in spacing will alter the relative structure of EL2 and thus could promote a specific conformational change for Cx43 versus Cx32. Further, because the conserved glycine (Gly-179) residue has a high degree of flexibility, it might enable the Cx43 EL2 to attain multiple conformations.

Although the EL2 domain is highly conserved for all human α-connexins, four α-connexins (Cx37, Cx40, Cx40.1, and Cx59) lack the critical arginine residue required to prevent Cx43 oligomerization in the ER. Whether α-connexins that lack the critical arginine oligomerize in the ER is not known at present. Interestingly, at least two of these α-connexins, Cx37 and Cx40, form mixed hemichannels with Cx43 as well as with each other (33–37). If Cx37 and/or Cx40 preferentially oligomerize in the ER, then inhibition of Cx43 oligomerization in the ER could be a mechanism to help regulate hemichannel stoichiometry in cells expressing Cx37, Cx40, and Cx43, such as smooth muscle and endothelial cells (38–41). Whether this is the case remains to be determined.

Cx32W133R-HKKSL corresponds to an HKKSL-tagged version of the Cx32 (W133R) variant associated with X-linked Charcot-Marie-Tooth disease (42). We found that this Cx32 construct was capable of oligomerization in the ER. This is in contrast to mutations localized to other regions of Cx32, such as
R142W (within TM3), E186K (at the extracellular interface of the fourth transmembrane domain), and E208K (at the cytoplasmic interface of the fourth transmembrane domain), which produce forms of Cx32 that are incapable of oligomerizing when expressed by PC12 cells (8). Mutant connexins associated with X-linked Charcot-Marie-Tooth disease appear to fall into discrete classes with trapping in either the ER or Golgi apparatus or with partial trafficking to the plasma membrane (8, 9, 43). Although this suggests that the inability to properly oligomerize could cause Cx32 misfolding and mistargeting, our results and the results of Martin et al. (44, 45) suggest that the ability to oligomerize does not necessarily guarantee that Cx32 is functional. In fact, mutant connexins frequently have dominant negative effects by forming inactive gap junction channels with wild-type connexins (46, 47). Also, many X-linked Charcot-Marie-Tooth disease mutants are successfully transported to the plasma membrane, although most of these show mutant amino acids in the C-terminal domain rather than the transmembrane or loop domains (9).

We found that Cx32/43/32b was transported to the cell surface; however, two other constructs, Cx43/32a and Cx32/43/32a, were not. Whether this reflects a difference in proper folding versus misfolding remains to be determined. As mentioned above, aberrant retention in the ER or perinuclear intracellular compartments would be consistent with the potential for connexin misfolding to cause mistargeting of these chimeras (8, 9, 43). The prospect that some chimeras may be misfolded also suggests that some higher molecular mass peaks in the sucrose gradients (e.g., peaks at 12% sucrose) may be because of interactions between the connexin chimeras and chaperones rather than oligomerization per se.

Another key difference between Cx43/32a and Cx32/43/32a as compared with Cx32/43/32b is that Cx32/43/32b contains a compatible Cx32 cytoplasmic loop and C terminus. In contrast, the other two constructs have a Cx43 cytoplasmic loop that may not be compatible with interacting properly with a Cx32 C-terminal domain.
Whether this is the case will require identifying proteins that promote oligomerization upon delivery to the Golgi apparatus. Inhibit oligomerization, then chaperone dissociation might protectively, if one or more putative Cx43 chaperones are required to functional change in Cx43 to promote oligomerization. Alternatively, if there is a difference in pH, calcium concentration, or lipid composition, it might favor a conformational change in Cx43 to promote oligomerization. Alternatively, if one or more putative Cx43 chaperones are required to inhibit oligomerization, then chaperone dissociation might promote oligomerization upon delivery to the Golgi apparatus. Whether this is the case will require identifying proteins that regulate connexin oligomerization.

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