*Commentary*

# Gap Junction Voltage Dependence: A Clear Picture Emerges

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All vertebrate gap junction channels display voltage-dependent gating. Historically, voltage-dependent behaviors have been characterized as being transjunctional voltage (Vj) and membrane voltage (Vm) dependent. The transjunctional voltage dependence of gap junction channels composed of two identical hemichannels, each composed of identical connexins subunits (a homotypic channel) is usually symmetric and is often referred as Vj-dependent gating. Vj steps of equal amplitude but opposite polarity will result in junctional currents that are mirror images of each other in terms of magnitude and kinetics (Harris et al., 1981; Spray et al., 1981). Figure 1 in Oh et al. (2000) illustrates such symmetric (slow) Vj dependence, as well as the characteristic steady state "residual" junctional conductance.

It is interesting to note that the first demonstration of voltage dependence in cell-to-cell communication is best considered to be a subset of Vj dependence. It is the rectifying or asymmetric voltage dependence first observed in the crayfish rectifying synapse by Fursphan and Potter (1959), where the kinetics are quite rapid, which gave rise to the term fast Vj dependence. Similar rapid asymmetric effects of voltage on junctional conductance have been described in vertebrate gap junctions as well. It is usually observed in heterotypic gap junction channels. Heterotypic channels are composed of two hemichannels of different connexin composition. The hemichannel of one cell can be composed of Cx32, for example, while the adjacent cell contributes a hemichannel composed of Cx26. Many such combinations of connexins produce rectifying or asymmetric Vj dependence. This fast Vj dependence is thought to be embodied in the rectification of single channel conductance (Bukauskas et al., 1995). That is, single channel conductance of heterotypic gap junction channels is dependent on Vj and its polarity. Gap junction channels composed of hemichannels that contain more than one type of connexin are called heteromeric forms and display a variety of behaviors intermediate between those of homotypic and heterotypic forms (He et al., 1999; Brink et al., 1997).

At the single-channel level, two types of gating that can account for the behavior of macroscopic junctional conductance are typically observed. At the single-channel level, the term Vj gating refers to rapid transitions between a fully open state and a subconductance state. A second form of gating in hemichannels has been provisionally termed loop gating and is characterized by a series of step-like transitions between the open and closed states with a time course of many milliseconds (Trexler et al., 1996). Rather than closing to a stable subconductance state, closure follows a series of stepwise transitions declining to a truly nonconductive state. (see Figure 2 in Trexler et al., 1996, and Figure 7 in Oh et al., 2000) The term loop gating was coined because of the resemblance of these stepwise gating transitions to those ascribed to hemichannel docking by Bukauskas and Weingart (1994), which presumably involves interactions between the extracellular loop domains of connexins. Both of these gating motifs can be observed in multichannel records of gap junction channels using dual whole-cell patch clamp (Bukauskas and Weingart, 1994). But the dual whole-cell method, while innovative, has a number of technical limitations. Paramount among them is a poor signal-to-noise ratio and an even poorer time resolution due to the introduction of whole-cell capacitance.

One last development in understanding the evolution of the gap junction channel gating story is that Paul et al. (1991) showed that Cx46 was able to function as a hemichannel or half-gap junction channel. Single oocytes injected with mRNA for Cx46 displayed large outward membrane currents at inside positive voltages. In both the attached and the excised patch mode, it is possible to observe single channels whose behavior mimics the macroscopic phenomena, as subsequently demonstrated by Trexler et al. (1996). Unfortunately, however, not all connexins appear to readily gate under the same conditions that make Cx46 hemichannels gate in single cells or when undocked or unapposed.

With this background, let us turn to Oh et al. (2000), in this issue of *The Journal*. First, the authors have used a Cx32Cx43 chimera, originally constructed by Pfahnl et al.(1997), that forms functional hemichannels. The rationale for using this construct is based in the observation made in oocyte pairs by Verselis et al. (1994) that the second amino acid of the $NH_2$ terminus was a determinant of gating polarity. The authors therefore constructed a variant of the Cx32Cx43E1chimera in which the second amino acid, which is normally neu-


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tral, is replaced with a negatively charge moiety (Cx32N2ECx43E1). Coinjection of oocytes with mRNA for the two chimera yields heteromeric hemichannels that contain some connexins that gate positively when expressed as homomers and others that gate negatively when expressed as homomers. The observation that the heteromeric hemichannels of Cx32Cx43E1 display "bipolar" gating has revealed a fundamental property of gap junction channel-voltage gating. The bipolar gating refers to the fact that for either polarity the hemichannel will gate to a subconductive state, unlike the homomeric counterparts that only gate to subconductive states at the appropriate single polarity.

Even when injecting the two mRNAs at ratios of 1:20 where, according to the binomial distribution, ~30% of channels will contain five of one connexin chimera and one of the opposite type, the hemichannels still exhibit bipolar gating. This leads to the notion put forth by Oh et al. (2000) that a single connexin subunit can initiate gating, and that Vj gating can arise from or be initiated by structural changes in a single connexin rather than from a more "macroscopic" conformational change involving all six subunits.

The adept use of the oocyte expression system and careful quantitative analysis by Oh et al. (2000) have given new insight into the nature of the Vj gating mechanism for the connexin protein family and lead us further along the gating trail. Apparently, only one connexin needs to respond to voltage to initiate closure.

A sticking point among gap junctionologists has been whether the hemichannel is an appropriate tool in understanding the biophysics of gap junction channels. In fact, some have questioned the relevance of such studies based on experiments that show that the sum of two hemichannels cannot equal the whole (White et al., 1994). The important question thus becomes, is the gating consistent between hemichannels and gap junction channels? The answer is yes. Cx46 and the chimeric Cx32 hemichannel show Vj gating and loop-like gating. At inside positive voltages, gating of Cx46 hemichannels was mainly to a long-lived substate(s) similar to what is typically described for gap junction channels. Similar evidence is provided by Oh et al. (2000) and, in addition, demonstrates that mutations in the $NH_2$ terminus affect Vj dependence the same way in hemichannels and cell–cell channels. Furthermore, mutations that affect the polarity of Vj gating do not affect loop gating, providing evidence that the two gating mechanisms, Vj gating and loop gating, are molecularly distinct.

The article by Oh et al. (2000) is one in a series by this group of investigators that uses sound biophysical methods of analysis and site-directed mutagenesis to demonstrate the nature of connexin gating. It bridges the gap between macroscopic description and underlying molecular mechanism, and articulates a cleaner picture of the intricacies of voltage-dependent gating in gap junction channels.

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