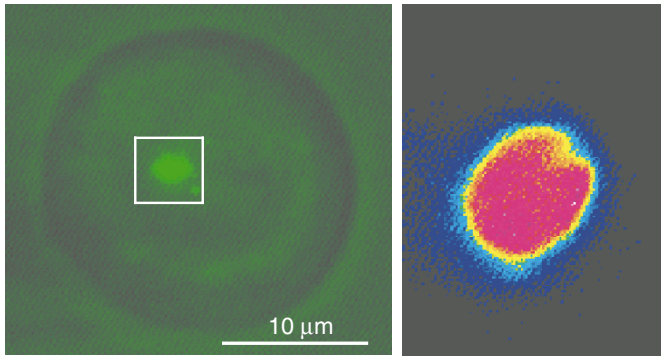
It's good to talk: cell–cell communication by gap junctions



FROM BUKAUSKAS *ET AL. PROC. NATL. ACAD. SCI. USA*

Gap junctions facilitate the exchange of solutes, metabolic precursors and electrical currents between neighbouring cells. They appear as clusters (or plaques) of tightly packed particles, in which each particle is a single channel. Although it was known that the appearance of such plaques is associated with the electrical coupling of two cells, previous methods measured only the average conductance over an entire cell population and not the properties of a single junction.

In a recent study, Bukauskas *et al.* (*Proc. Natl Acad. Sci. USA* **97,** 2556–2561; 2000) used GFP-tagged connexin 43 (Cx43–EGFP) and dual whole-cell patch clamps to investigate the relationship between clusters and junctional conductance (g_j) in cell–cell pairs. When this construct was transfected into cells that were defective in communication, Cx43–EGFP fluorescence was observed throughout, except at areas of cell-cell contact, where punctate staining was present at the cell membrane. Most pairs of cells with large plaques (>0.2 µm in diameter) showed electrical coupling, with g_j values ranging from 11–60 nS, whereas those joined by smaller plaques were frequently uncoupled.

As the intensity of fluorescence in a large plaque is essentially constant (see picture), Bukauskas *et al.* were able to correlate the activity measured across a pair of cells with the activity of a single channel. The fluorescence per unit area within the plaque, together with previous calculations of channel density in plaques, was used to estimate the fluorescence intensity of a single channel. Next, pairs of cells with a single gap junction were identified, and the total fluorescence of each plaque was determined. Using their estimate of single-channel fluorescence intensity, the authors could then estimate the number of channels within the plaque. They also measured the conductance between the pairs of cells. Armed with this information, they calculated the g_j of a single channel within a plaque, and identified three categories. Small plaques (90–330 channels) showed no electrical coupling and had no active channels. Slightly larger plaques (200–400 channels) exhibited weak conductance (g_j values of 0.05–0.7 nS) and contained only 1–2 active channels. Large plaques (≥500 channels), however, possessed 35 or more active channels and had g_j values of ≥4 nS.

These findings were unexpected in several ways. First, it seems that a minimum cluster size, in terms of the number of channels, is required to open a gap junction. Second, only a fraction of channels within a gap junction are active at any given time. Third, in gap junctions above a certain critical size, the proportion of channels that are active does not seem to increase significantly with an increasing number of channels. Thus, gating by gap junctions seems to be an all-or-nothing phenomenon that occurs only when a certain channel concentration is attained, but in which an overlying regulatory step limits the number of channels that are active. These results offer new insights and also raise several questions — how is clustering initiated? What senses when the threshold number of channels has been reached? And how is channel activity regulated so that only a certain proportion of the channels within a junction is active at any one time?

ANGELA EGGLESTON

cate that, in the wing imaginal disc, transcytosis may not be involved in Wg diffusion, and that Shi activity is critical for secretion of Wg. It is worth noting, however, that because the level of expression of Wg-targeted genes was not examined in *shi*-mutant cells, it is possible that Shi also has a role in receiving cells for implementation of the Wg signal.

In another recent study of Wg distribution, Pfeiffer *et al.*[12] have proposed another mechanism underlying the movement of Wg. In the embryo, Wg is responsible for determining the fate of naked regions of cuticle in the epidermis, which incorporates three to four rows of cells anterior to *wg*-expressing cells, and one row posterior to them. Consistent with its function, Wg can be detected in three to four rows of cells anterior to *wg*-expressing cells, and in one to two rows posterior to them. Pfeiffer *et al.* observed that membrane-bound Wg protein could rescue *wg*-null mutants when expression was limited to *wg*-expressing cells. As the membrane-bound Wg protein is not believed to be secreted and the *wg*–Gal4 promoter used in this experiment is not expressed in an area wider than a single row of cells, they investigated whether *wg*-expressing cells moved anteriorly in an epidermal segment. First, they marked a single cell anterior to the parasegment boundary after photoactivation of caged rhodamine (Fig. 1b), and observed a cluster of rhodamine-marked cells anterior to the *wg*-expressing cell. Second, they marked *wg*-expressing cells with either a nuclear or a cytoplasmic β-galactosidase, and found that these non-secreted markers could spread anteriorly. Together, these results indicate that cells originating from the *wg*-expressing domain may be displaced anteriorly, while still retaining the ability to secrete Wg. The anterior movement of originally *wg*-expressing cells may therefore contribute to the patterning of the embryonic cuticle by Wg. However, it is important to note that the inheritance model proposed by Pfeiffer *et al.* is based on Wg-overexpression studies, and it remains to be established whether the amount of endogenous Wg inherited is sufficient for signalling activity.

Since the morphogen-gradient hypothesis was first formulated, positive demonstration that these factors actually diffuse into the extracellular space has been difficult. In the case of Wg, diffusion in the extracellular matrix seems to be the primary mechanism in *Drosophila* imaginal discs. Although extracellular diffusion may be the principal mechanism in cases where Wg acts at a distance of several cells, further mechanisms, such as cell movement, may also function in short-range signalling by Wg in tissues. During development, it is possible that a combination of several mechanisms is used, depending on the cell

