“CENTRAL FOLD” OR TRUE JUNCTIONAL PROFILE?

Recently, in an interesting paper entitled “In vitro formation of gap junction vesicles,” Daniel Goodenough presented evidence of critical importance to the question of the existence of cell-to-cell channels within the intramembranous particles of the gap junction. The evidence is based upon Dr. Goodenough’s presentation of a folded junctional profile dried on a grid and negatively stained (4; Fig. 5) in which “slender channels approximately 2 nm in diameter appear to span the full 15-nm thickness of the junction profile.” The interpretation of these channels as cell-to-cell channels depends upon the identification of this profile as a fold in the edge of a flattened gap junction vesicle as is suggested in the text (reference 4, p. 221-223).

We considered, however, the possibility that this folded profile might represent a fold in the center of a flattened gap junction vesicle rather than at an edge, for the following reasons: (a) this fold is not readily identifiable as being at the edge of a gap junction vesicle; (b) it is straight, rather than curved as are most of the edge folds illustrated in Fig. 3 of reference 4; and perhaps more importantly (c) it is discontinuous, each end appearing to taper and merge into the surrounding flattened membrane sheet. We also noted that this profile was approximately twice the width of a true gap junction profile, as would be expected of a central fold in the top layer of a flattened gap junction vesicle.

Although the magnifications of Fig. 5 (4) have now been corrected (5) so that the width of this profile and the periodicity of “superposed junctional subunits” approximate these parameters for a true junctional profile, we feel that these measurements would also approximate the expected values for the same parameters of a central fold in a single junctional membrane sheet (or hemi-junction). This possibility is supported by the appearance of apparent central folds of usual junctional width (16-18 nm) in isolated gap junction preparations presented by Benedetti and Emmeloit (1) and by Goodenough (3) and by the occurrence of single membrane profiles (hemi-junctions?) in stained thin sections of isolated gap junctions both before (3; Fig. 6) and after (4; Fig. 1) trypsin treatment.

If the profile illustrated in Fig. 5 (reference 4 and the accompanying letter) represents a central fold in a single gap junctional sheet, the paired junctional elements implied to be in two separate membranes would be in the same membrane, and the channels would span the recess of a fold rather than a true intercellular gap.

We believe that consideration of the following points may be relevant to the question we have raised in this letter. (a) What is the frequency of central folds in these preparations? Is it possible that stereo pairs of these profiles might help to clarify this question? (b) What is the frequency of single, unpaired membranes in isolated gap junction preparations? Is it possible that some of these single membranes might represent hemi-junctions? (c) With respect to the reference to the occurrence of profiles similar to that illustrated in Fig. 5 (4) in micrographs exhibited by N. B. Gilula (2), it has occurred to us that Gilula’s preparations were not trypsinized, and nonvesicular, and therefore lacking the edge folds described in the present report (4). (d) This issue might be resolved by the presentation of a low power micrograph giving an overview of the profile in Fig. 5 (4) which might clarify the topology of this fold.

Our commentary on these profiles does not necessarily question the concept of intercellular channels within the subunits of the gap junctional particles but does raise what we feel are important considerations of interpretation of negatively stained preparations of isolated gap junction membrane.

REFERENCES

1. BENEDETTI, L., and P. EMMELOT. 1968. Hexagonal array of subunits in tight junctions separated from isolated rat liver plasma membranes. J. Cell Biol. 38:15-24.
2. GILULA, N. B. 1974. Isolation of rat liver gap junctions and characterization of the polypeptides. J. Cell Biol. 63(2, Pt. 2):111a. (Abstr.)
3. GOODENOUGn, D. A. 1974. Bulk isolation of mouse hepatocyte gap junctions. J. Cell Biol. 61:557-563.
4. GOODENOUGH, D. A. 1976. In vitro formation of gap junction vesicles. J. Cell Biol. 68:220-231.
5. Correction. 1976. J. Cell Biol. 69:519.

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Channels traversing two junctional membranes and intervening “gap”

Larsen et al. have provided an interpretation of the gap junction profile presented in Fig. 5 (1; and herein). Half-junctions folded back upon themselves have never been seen in thin-sectioned material. It is possible that such a fold may occur during negative staining. If a half-junction has indeed formed under conditions of negative staining, Fig. 5 (1) reveals that the single junctional membrane has “reannealed,” with the extracellular portions of the connexons in register. In this case, the image in Fig. 5 (1) would still represent slender channels traversing two junctional membranes and the intervening 2-nm stain-filled “gap.” Thus, although the junction may have split and reformed during specimen preparation, the micrograph in Fig. 5 (1) and the low magnification view submitted with this communication (Fig. A) may still be interpreted as a negatively stained profile of a gap junction, apparently spanned by thin, 2-nm diameter hydrophilic channels.

Reference

1. Goodenough, D. A. 1976. In vitro formation of gap junction vesicles. *J. Cell Biol.* 68:220-231

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Figure 5 At high magnification, the details of the negatively stained folded junctional profile can be seen. At a fold, the two stain-excluding junctional membranes are separated by the stain-penetrated gap (large arrow, G) which at favorable planes of tilt show the expected periodicity resulting from superposition of the rows of junction subunits (connexons). Occasionally the connexons are exactly aligned, permitting visualization of the electron-dense core in the center of each connexon in profile view (arrows labeled P). In profile, the inset shows that these stain-penetrated cores appear as slender channels, approximately 2 nm in diameter, which appear to span the full 15-nm thickness of the junction profile. × 266,000; inset, × 540,000. (From Goodenough, D. A. 1976. *J. Cell Biol.* 68:220-231).
Figure A This figure shows the entire field of negatively stained gap junction vesicles contained in the original electron micrograph. The fold illustrated in the inset and in Fig. 5 (1) may be seen in this low power view at arrow 4. In addition to the unambiguous profiles seen at the edges of each of the gap junction vesicles, several junctional profiles which overlie the centers of vesicles may be seen (arrows 1-4). Arrow 1 illustrates a profile resulting from the folding of a vesicle edge back towards the vesicle center. The origins of the folds at arrows 2, 3, and 4 are less straightforward, originating perhaps from puckering of the vesicle surface as it collapses, perhaps from a vesicle within a vesicle (an image common in thin sections), or perhaps from the experimentally unsupported speculation of Larsen et al. All conceivable origins result in a junction profile, however, such that the slender channels indicated in the inset (arrows) may still be interpreted as spanning the full junction thickness. The open arrow indicates nonjunctional membrane. × 162,000; inset × 540,000.