Plant Physiol. (1988) 88, 791–793
0032-0889/88/88/0791/03/$01.00/0

Review

Intercellular Communication—Filling in the Gaps

Received for publication March 1, 1988

SALLY MEINERS, ORNA BARON-EPEL, AND MELVIN SCHINDLER*
Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824

ABSTRACT

Coordination and synchrony of a variety of cellular activities in tissues of plants and animals occur as a consequence of the transfer of low molecular weight biosynthetic and signaling molecules through specialized structures (plasmodesmata in plant cells and gap junctions in mammalian cells) that form aqueous channels between contacting cells. Investigations with rat liver demonstrated that cell-cell communication is mediated by a 32 kilodalton polypeptide that forms a hexameric pore structure in the plasma membrane. Following association with the same structure in a contiguous cell, a trans-double membrane channel is created that has been termed a gap junction. In plant tissue, long tubelike structures called plasmodesmata are suggested to serve a similar cell-cell linking function between cytoplasmic compartments. Although morphologically distinct, dynamic observations suggest similarities in transport properties between gap junctions and plasmodesmata. Recent work now provides evidence that these functional similarities may reflect a more profound identity between the paradigm animal gap junction polypeptide (32 kilodalton rat liver polypeptide) and an immunologically homologous protein localized to plant plasma membrane/cell wall fractions that may be a component of plasmodesmata.

Cell-cell communication between contiguous tissue-forming cells is vital for control and coordination of cell proliferation, tissue metabolism, and synchrony (11, 16). The structural element in the physical linkage connecting the cytoplasmic compartment of animal cells is the low-resistance pathway formed by transmembrane protein channels termed gap junctions (11, 16). These structures permit the diffusion-mediated transfer of low molecular weight cytoplasmic components (≤ 1.7 kD as measured with synthetic peptides [16]) that can transmit biochemical signals or metabolically share biosynthetic/energetic precursors (11). The basic unit of the gap junction is the connexon (11, 26), which is composed of six polypeptide chains that form a hexagonally symmetric transbilayer structure containing a single aqueous channel with a diameter of 2 nm (26). A gap junction is formed by the head-to-head association of two connexons between contacting cells (16, 26). Intercellular communication between plant cells has been ascribed to trans-wall tubular structures, termed plasmodesmata, which provide intercellular channels for the movement of low mol wt (≤ 1,000 mol wt) metabolic, biosynthetic, and signaling molecules (8, 10, 23) between contiguous cells. Both gap junctions in animal cells and plasmodesmata in plant cells appear to be the essential structural elements enabling the integration of cellular activity within the synchronous behavior and development of tissues.

The large body of data detailing gap junction-mediated communication in animal cells is contrasted by the limited knowledge of intracellular communication mechanisms in plant tissues. It has been pointed out that the evolutionary development of a cell wall in plants precluded the development of a means for direct communication with vascular systems, *e.g.* blood and lymph in animals, and therefore, that plant cells must rely almost fully on symplastic communication. (Symplast refers to the interconnected protoplasts, all bounded by a continuous plasma membrane [8]). Plasmodesmata have been implicated as trans-cell wall channels providing a low-resistance pathway between plant cytoplasmic/symplastic compartments (10, 23). An important aspect of this research has been the observation that the transport properties of plasmodesmata are strikingly similar to those of animal cell gap junctions (5, 8, 10, 23, 25). Indeed, recent work, using monospecific antibodies that recognize 32-kD rat liver gap junction polypeptide (which migrates as a 27 kD polypeptide on SDS-PAGE gel), has demonstrated a homologous protein of 29 kD (as judged by migration on SDS-PAGE gel) in the cell membrane/wall fraction of soybean (*Glycine max* [L.] Merr. cv Mandarin) root cells (SB-1 cell line) in callus and suspension culture, as well as in whole soybean leaf cell homogenates (17). These findings provide immunological evidence that a gap junction polypeptide may be a central element in cell-cell communication in the tissues of animals and plants.

MAMMALIAN GAP JUNCTIONS—BIOCHEMISTRY AND DYNAMIC CHARACTERIZATION

Structural Characterization. The connexons comprising gap junctions have been found in most metazoan phyla and have been demonstrated in most vertebrate tissues. (Exceptions are adult skeletal muscle, lymphoid cells, and many nerve cells [2, 4, 12). Recently, invertebrate tissues have also been shown to possess morphologically distinct, gap junction-like structures following isolation and electron microscopic analysis (6). Gap junction material isolated by a number of procedures from a variety of sources contains, upon analysis by reducing SDS-PAGE, a predominant polypeptide with reported molecular masses of 26 to 29 kD (equivalent to the 32 kD rat liver polypeptide as determined by sequencing of cloned cDNA) (2, 4, 12). Other reports by various groups show the presence of a 16-kD polypeptide in invertebrates (6) and a 43-kD polypeptide in heart (2). Considering the range of tissues examined, it may not be inappropriate to suggest either that a variety of gap junction proteins exist or that tissue specific processing of a larger gap junction polypeptide occurs post-translationally. Indeed, work by Paul (19) and by Kumar and Gilula (15) both demonstrate that *in vitro* translation of the RNA products of the cDNA from human and rat liver produces a ~32-kD protein. It is now becoming clear by comparative biochemical and immunological studies that immunological and sequence homologies exist between the paradigm rat liver 32 kD junction protein and junctional proteins present in other tissues and organisms (2, 15, 19). Considering that the amino acid sequence has recently been determined for

both human and rat liver junctional proteins (15, 19), these structures currently supply the bulk of information concerning polypeptide chemistry. The NH_2-terminal of both rat and human junctional polypeptides contains a majority of hydrophobic amino acids, while the COOH-terminal (suggested to contain the cytoplasmic residing fragment) possesses many charged and hydrophilic amino acids. There are two consensus sites for N-linked glycosylation, but the polypeptide is apparently not glycosylated (19). There appear to be two cAMP-dependent Ser phosphorylation sites and several Tyr phosphorylation sites (15). This is significant given observations that channel state, open or closed, may be controlled by phosphorylation events (28).

Functional Characterization. Past suggestions that gap junctions were indeed the structural elements of an intercellular communication system have rested on predominantly correlative observations relating to transport (3, 11, 16). More recently, both immunological (22, 27) and channel reconstitution experiments (22) have provided the direct link between the connexon polypeptides and the site of a trans-bilayer communication channel. Warner *et al.* (27) demonstrated that affinity purified anti-rat liver gap junction antibodies could block dye transfer and electrical coupling between the progeny cells when microinjected into an identified cell of the 8-cell stage embryo of *Xenopus*. In a similar manner, anti-rat 32 kD polypeptide irreversibly blocked intracellular junctional conductance and dye permeability following injection into pairs of rat ventricular myocytes, hepatocytes, and cultured sympathetic neurons (13). Using purified gap junction polypeptides inserted into bilayers, Spray *et al.* (22) demonstrated similar ionic permeabilities and control properties when compared to isolated pairs of rat hepatocytes. In the light of these direct demonstrations of gap junction-mediated transport, work has now focused on examining how pH, Ca^{2+}, alcohols, and tumor-promoting substances can specifically affect gap junction polypeptides to modify the channel permeability.

Developmental Significance. Gap junctions have been described as routes by which information is transmitted for the control of embryonic development and differentiation (3, 21, 27). Gap junctions may permit chemical gradients to develop and so influence cell behavior in such a manner that a spatial patterning of cell types develops (3). These gradients could control tissue growth and polarity. In two tests of the role of gap junctions in embryonic development, developmental abnormalities were induced in tadpoles by the injection of anti-rat liver gap junction polypeptide antibodies into early amphibian embryos (27), and a patterning process in *Hydra* was interfered with by injection of anti-rat liver gap junction polypeptide antibodies (7).

PLANT INTERCELLULAR COMMUNICATION— PLASMODESMATA AS TRANS-WALL PERMEABLE CHANNELS

Organization and Evidence for Transport Channel in Plasmodesmata. Plasmodesmata are tubular structures that penetrate the cell walls of higher plant cells, algae, and some fungi, apparently serving to connect the cytoplasmic compartment of contiguous cells (8, 10, 23, 25). In higher plants, they have a diameter of ~60 nm and in thin sections appear to be composed of a central cylindrical membrane containing a central channel surrounded by 6 to 9 doughnut-like structures (10). This structural unit is called the desmotubule and is situated in contact with the plasma membrane, which traverses the cell wall to form a continuous sheath between cells. All structural information to date describing plasmodesmata consists of transmission electron microscopy of fixed sections (8–10, 20, 23, 25). This has led to a variety of models and precludes a more exact characterization of their structure. Plasmodesmata, as yet, have been neither isolated nor biochemically characterized. It is, however, believed that both an aqueous and a membrane transport pathway (connecting endoplasmic reticulum) exist in these structures (1, 8, 10, 23, 25). Over the whole wall surface, the frequency of plasmodesmata may vary from 1 to $10/\mu m^2$ depending on plant type (8, 9, 20). This density is considerably greater in pitfields, which are specialized areas of high plasmodesmatal content. On a total cell basis, even the smallest meristematic cells have between 1,000 and 10,000 connections with their neighbors (8). The best evidence for the role of plasmodesmata as trans-wall, low-resistance pathways is: (a) they appear to have an aqueous channel that spans the cell wall (8, 10, 23); (b) transport of plant cellular substances between cells is proportional to the plasmodesmatal frequency (8, 10, 20, 23); and (c) intercellular transport appears not to exist between cells not connected by plasmodesmata (18).

Functional Analysis of Intercellular Communication in Plants. Plasmodesmata have been suggested to serve as the structural element for continuity between the membrane-bound cytoplasmic network that is continuous throughout the plant, termed the symplast (8, 10, 23, 25). The evidence for symplastic transport is that: (a) compounds move between plant cells without being exchanged with molecules in the external solution; (b) ionic and metabolic coupling occurs between plant cells; and (c) chemicals that do not pass across membranes may be injected into plant cells and subsequently spread to surrounding cells (5, 8, 10, 23, 25). Employing the latter method, a number of groups using a variety of fluorescent dyes have established that hydrophilic molecules $\leq$ 1000 mol wt may be transported between cells (1, 5, 10, 23–25). This transport can be reversibly inhibited by Ca^{2+} and 12-*O*-tetradecanoylphorbol-13-acetate (TPA), a tumor promoter (1). That there may be developmental control of plant cell-cell communication is suggested by the observation that dye movement occurs between guard mother cells and young guard cells, but stops midway through development as cells begin to swell before a transport channel opens (18).

PLASMODESMATA AND GAP JUNCTIONS— DIFFERENT ARCHITECTURE, SAME BRICK?

Functionally, plasmodesmata appear to be very similar in activity to animal gap junctions. The similarity in molecular size discrimination, ionic transport properties, and control by pH and/or Ca^{2+} is striking (1, 3, 8, 10, 11, 16, 21). Structurally, however, there appear at first glance to be considerable architectural differences between plasmodesmata and gap junctions. The gap junction comprised of two connexons must only span a 2 nm gap between cells, while the plasmodesma traverses the thickness of cell walls 70 to 90 nm. Considering other biological structures that can form vastly different organizations even though the same structural element is employed, *e.g.* actin or tubulin, it may not be inappropriate to speculate that plasmodesmata may be comprised of connexon-like elements stacked in a concentric fashion to form a trans-wall aqueous channel. That such connexon-like elements may exist in plants is suggested from our observation that when soybean (*Glycine max* [L.] Merr. cv Mandarin) root cell (SB-1 cell line) homogenates prepared from suspension or callus culture were electrophoresed on SDS polyacrylamide gels and transferred to nitrocellulose paper, the resulting immunoblot with monospecific anti-rat liver gap junction antibody yielded two polypeptide bands migrating at M_r 29,000 and 48,000. This is to be compared with two polypeptides migrating at M_r 27,000 and 48,000 observed for rat liver gap junction when immunoblotted in a similar fashion (17).

In his book, *Plant and Planet*, Huxley (14) points out that Charles Darwin once wrote in his diary, "prove animals like plants." The discovery of a gap junction-homologous polypeptide in plants may be a step on the road to that vision by suggesting the exciting possibility that gap junctions may be an evolutionary modification of plasmodesmata.

LITERATURE CITED

1. BARON-EPEL O, D HERNANDEZ, L-W JIANG, S MEINERS, M SCHINDLER 1988 Dynamic continuity of cytoplasmic and membrane compartments between plant cells. J Cell Biol 106: 715–721
2. BEYER EC, DL PAUL, DA GOODENOUGH 1987 Connexin 43: a protein from rat heart homologous to a gap junction protein from liver. J Cell Biol 105: 2621–2629
3. CAVENEY S 1985 The role of gap junctions in development. Annu Rev Physiol 47: 319–335
4. DERMIETZEL R, A LEIBSTEIN, U PRIXEN, U JANSSEN-TIMMEN, O TRAUB, K WILLECKE 1984 Gap junctions in several tissues share antigenic determinants with liver gap junctions. EMBO J 3: 2261–2270
5. ERWEE MG, PB GOODWIN 1984 Characterization of *Egeria densa* leaf symplast: response to plasmolysis, deplasmolysis and to aromatic amino acids. Protoplasma 122: 162–168
6. FINBOW ME, T ELDRIDGE, J BUUHJENS, NJ LANE, J SHUTTLEWORTH, JB PITTS 1984 Isolation and characterization of arthopod gap junctions. EMBO J 3: 2271–2278
7. FRASER SE, CR GREEN, HR BODE, NB GILULA 1987 Selective disruption of gap junctional communication interferes with a patterning process in Hydra. Science 237: 49–55
8. GUNNING BES 1976 Intercellular Communication in Plants: Studies on Plasmodesmata. Springer-Verlag, New York, pp 1–13
9. GUNNING BES, JE HUGHES 1976 Quantitative assessment of symplastic transport of pre-nectar into the trichomes of *Abutilon* nectaries. Aust J Plant Physiol 3: 619–637
10. GUNNING BES, RL OVERALL 1983 Plasmodesmata and cell to cell transport in plants. Bioscience 33: 260–265
11. HERTZBERG EL, TS LAWRENCE, NB GILULA 1981 Gap junctional communication. Annu Rev Physiol 43: 479–491
12. HERTZBERG EL, RV SKIBBENS 1984 A protein homologous to the 27,000 dalton liver gap junction protein is present in a wide variety of species and tissues. Cell 39: 61–69
13. HERTZBERG EL, DC SPRAY, MVL BENNETT 1985 Reduction of gap junctional conductance by microinjection of antibodies against the 27 kDa liver gap junction polypeptide. Proc Natl Acad Sci USA 82: 2412–2416
14. HUXLEY A 1987 *Plant and Planet*. Penguin Books, Harmondsworth, Middlesex, England, p 16
15. KUMAR NM, NB GILULA 1986 Cloning and characterization of human and rat liver cDNAs coding for a gap junction protein. J Cell Biol 103: 767–776
16. LOEWENSTEIN WR 1979 Junctional intercellular communication and the control of growth. Biochim Biophys Acta 560: 1–65
17. MEINERS S, M SCHINDLER 1987 Immunological evidence for gap junction polypeptide in plant cells. J Biol Chem 262: 951–953
18. PALEVITZ BA, PK HEPLER 1985 Changes in dye coupling of stomatal cells of *Allium* and *Commelina* demonstrated by microinjection of Lucifer yellow. Planta 164: 473–479
19. PAUL DL 1986 Molecular cloning of cDNA for rat liver gap junction protein. J Cell Biol 103: 123–134
20. SAUTER JJ, S KLOTH 1986 Plasmodesmata frequency and radial translocation rates in ray cells of poplar (*Populus* × *Canadensis* Moench 'robreta'). Planta 168: 377–380
21. SHERIDAN JD, MM ATKINSON 1985 Physiological roles of permeable junctions: some possibilities. Annu Rev Physiol 47: 337–353
22. SPRAY DC, JC SAEZ, D BROSIUS, MVL BENNETT, EL HERTZBERG 1986 Isolated liver gap junctions: gating of transjunctional currents is similar to that in intact pairs of rat hepatocytes. Proc Natl Acad Sci USA 83: 5494–5497
23. TERRY BB, AW ROBARDS 1987 Hydrodynamic radius alone governs the mobility of molecules through plasmodesmata. Planta 171: 145–157
24. TUCKER EB 1982 Translocation in the staminal hairs of *Setcreasea purpurea*. I. A study of cell ultrastructure and cell-to-cell passage of molecular probes. Protoplasma 113: 193–201
25. TUCKER EB, RM SPANSWICK 1985 Translocation in the staminal hairs of *Setcreasea purpurea*. II. Kinetics of intercellular transport. Protoplasma 128: 167–172
26. UNWIN PNT, G ZAMPIGHI 1980 Structure of the junction between communicating cells. Nature 283: 545–549
27. WARNER AE, SC GUTHRIE, NB GILULA 1984 Antibodies to gap-junctional protein selectively disrupt junctional communication in the early amphibian embryo. Nature 311: 127–131
28. WIENER EC, WR LOEWENSTEIN 1983 Correction of cell-cell communication defect by introduction of a protein kinase into mutant cells. Nature 305: 433–435