CELL COMMUNICATION

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Preface

Cell communication is a general phenomenon of importance in understanding many biological processes. It interfaces with nearly all disciplines of biology and medicine. From an evolutionary point of view cell interactions provide multicellular organisms with mechanisms for homeostasis and for regulating cellular activities. Some forms of communication require cell contact, whereas others involve transfer through the external milieu.

It is the purpose of this monograph to review a number of examples of cell interactions in several biological systems. The processes described appear different, but the fundamental mechanisms responsible for communication may be quite similar. Although specialized techniques are required for the study of each of the systems, the concepts and approaches to understanding the cellular and molecular bases of these interactions are again similar. Each of the presentations describes experimental evidence on which the current concepts of cell communication are based. It is unavoidable, and probably even desirable, that the special bias of the individual authors is evident in their contributions. Many of the presentations also include new ideas and challenging hypotheses for which compelling evidence is at present lacking. This monograph critically appraises the current status and limitations of our understanding of cell communication and indicates future directions for investigation. We hope it will stimulate workers in other areas of biology to develop new systems and models for exploring cell interactions.

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CHAPTER ONE

Junctions Between Cells

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Cell-to-cell communication, as a general biological phenomenon, consists of both long-range and short-range interactions between cells in both excitable and non-excitable tissues. The short-range interactions require direct physical contact between cells. These interactions are associated with a variety of communication phenomena, such as the immune response, cell fusion, electrical (ionic) coupling, metabolic cooperation, synaptic transmission (chemical), and intercellular adhesion.

Short-range cellular interactions are frequently accompanied by distinct intercellular membrane specializations. These membrane specializations or intercellular contacts can be characterized with physiological and ultrastructural probes into several groups (Table 1). The different groups of intercellular contacts can, in turn, reflect different functional capabilities of various cellular interactions.

The most extensive intercellular contacts that can be distinguished as plasma membrane and intercellular matrix modifications are those that are involved in intercellular adhesion, electrical coupling, metabolic coupling, the chemical synapse, and transepithelial permeability regulation. In this chapter emphasis will be placed on (1) a description of the basic features of the major intercellular contacts; (2) a discussion of their physiological significance; and (3) a careful ex-

Table 1. Summary of Specialized Intercellular Contacts

Contact	Origin	Function	Form	Type
Gap junction	Vertebrate and invertebrate	Intercellular communication; electrical or low-resistance synapse	Plaque	1. Gap junction A (nexus) 2. Gap junction B—arthropods
Tight junction	Vertebrate	Epithelial transcellular permeability regulation; sealing element	Belt or band	1. Zonula and fascia occludens (belt or band) 2. Endothelial cell junction (discontinuous belt or band) 3. Sertoli-Sertoli cell junction (belt)
Septate junction	Invertebrate	Partial permeability barrier; adhesion	Belt	1. Septate junction 2. Zonula continua—arthropods
Synaptic junction	Vertebrate and invertebrate	Chemical synaptic transmission	Plaque	1. Chemical synapse-excitatory and inhibitory 2. Neuromuscular junction
Desmosome	Vertebrate and invertebrate	Mechanical support; adhesion	Plaque, belt, or band	1. Desmosome and hemidesmosome (macula adhaerens) 2. Intermediate desmosome (zonula adhaerens) 3. Fascia adhaerens 4. Septatelike contact

amination of the structural and physiological evidence that has implicated one of the intercellular contacts, the gap junction, in the phenomenon of intercellular communication (ionic and metabolic cell coupling).

DEFINITION OF A CELL JUNCTION

A cell junction is a specialized region of short-range contact between two cells which is associated with a differentiation of the contributing cell surface membranes and/or the intervening intercellular matrix. Cells may maintain a reasonable intercellular distance (up to 200 Å) at these sites, or they may eliminate the intercellular space by a true fusion of their surface membranes. Cell junctions have now been found throughout the metazoan animal kingdom, including both invertebrates and vertebrates, and they are expressed both *in vivo* and in culture.

In both plants and animals the cytoplasmic bridges between cells are the extreme example of cytoplasmic continuity between cells. However, since they are not frequent elements in mammalian organisms (with the exception of germ cell interactions), these structures will not be considered in this chapter.

METHODS OF CHARACTERIZING CELL JUNCTIONS

Cell junctions usually occur as localized sites of interaction involving small regions of the interacting cell surfaces. Due to their small size, with a few exceptions, cell junctions must be characterized by electron-microscopic examination. With the electron microscope, junctions may be examined in (1) conventional thin-section preparations, (2) material that is treated with an intercellular "stain," or "tracer" substance, (3) freeze-fractured specimens that expose the internal aspects of the junctional membranes, and (4) "negative" stain or shadowed material of isolated (*in vitro*) cell junctions. Physiologically, some cell junctions between intact cells can be studied with microelectrode recording techniques, together with dye injections, and biochemical information can be obtained by studying isolated cell junctions from plasma membrane-enriched subcellular fractions.

TYPES OF CELL JUNCTIONS

Gap Junction

CONVENTIONAL THIN SECTIONS. The gap junction was described in thin sections as early as 1958 (1), and since then there have been varying descriptions of this junction (2–6) due to different tissue sources and preparation (fixation and staining) procedures. From these studies, some of the following names have been applied to this structure: longitudinal connecting surfaces (1), quintuple-layered interconnection (3), external compound membranes (2), nexus (4), and gap junction (6). A detailed discussion of this history has recently appeared (7).

The gap junction was first clearly resolved in its present form by Revel and Karnovsky in 1967. They utilized a preservation procedure (glutaraldehyde, osmium, *en bloc* uranyl acetate staining) that resolved the gap junction (in cross section) into a seven-layered (septilaminar) structure. A seven-layered structure has now been demonstrated in all of the structures described earlier. The seven layers result from the parallel apposition of two 75 Å thick unit membranes (trilaminar in appearance) separated by a 20–40 Å electron-lucent space, or "gap"

(Fig. 1). This structure is 150–190 Å, or a maximum of 40 Å greater than the combined thickness of two unit membranes. The appearance of this arrangement of two membranes separated by a "gap" led to the use of the term "gap junction" to describe this structure (6). The term "nexus," which was initially used to describe a pentalaminar (five-layered) structure (4), is now often used interchangeably with the term "gap junction." It is now clear that the structure originally described as a nexus (pentalaminar) can be preserved as a gap junction (septilaminar) when treated with *en bloc* uranyl acetate staining (6,8–10).

The gap junction with the septilaminar appearance has now been described between cells in both vertebrates (6,8–11) and invertebrates (12–16) as well as between cells in culture (17–20). Due to its presence between nonexcitable as well as excitable cells, this structure is possibly the most frequent cell junction found in animal organisms. Instead of listing the many locations where this structure has been identified, perhaps it is more interesting to consider some places where the structure has not yet been found. This list includes notably mature skeletal muscle cells (myotubes) and circulating blood cells.

TRACERS. An electron-opaque material, colloidal lanthanum hydroxide, was successfully used to demonstrate a penetrable region in the gap junction by Revel and Karnovsky in 1967 (6). This material penetrates or traces a central region of the junction (about 55 Å in thickness) that is slightly larger than the electron-lucent gap. This observation clearly indicates that there is an extracellular continuity (pathway) through the gap region of the junction. Lanthanum penetration has become a characteristic feature of gap junctions that clearly distinguishes them from the occluding pentalaminar structures, which are truly "tight" and represent membrane-to-membrane fusions (6,8–11,13, 16,21). Other substances, such as pyroantimonate and ruthenium red, can also be used to demonstrate the penetrability of the gap junction (11,22). It is also interesting to note that certain high-molecular-weight substances, such as horseradish peroxidase, are excluded from the "gap" region (23); this is presumably due to a size limitation in the extracellular region of the gap.

Revel and Karnovsky also observed that in *en face* view a lanthanum-impregnated gap junction is comprised of a polygonal lattice of 70–80 Å subunits (6). The electron-dense lanthanum outlines the subunits, which have a 90–100 Å center-to-center spacing. The lanthanum also is frequently present as a 15–20 Å dense dot occupying the central region of the 80 Å subunits. These *en face* characteristics have now been found in studies of gap junctions from a variety of different sources (6,8–10,17). A similar membrane polygonal lattice had also been observed prior to the observations of Revel and Karnovsky in 1967. Robertson (2) described a polygonal lattice with 90 Å center-to-center spacing at the electrotonic membrane synapse (goldfish medulla), and Benedetti and Emmelot (24, 25) found a similar polygonal lattice when examining a plasma membrane fraction from rat liver with negative staining. Since 1967 both of these earlier observations have been substantiated as characteristic gap junctions (8,9).

NEGATIVE STAIN. Negative staining with heavy metal salts, such as sodium phosphotungstate, ammonium molybdate, and uranyl acetate, has provided some useful complementary information about the gap junction. Since gap junctions are portions of subcellular plasma mem-

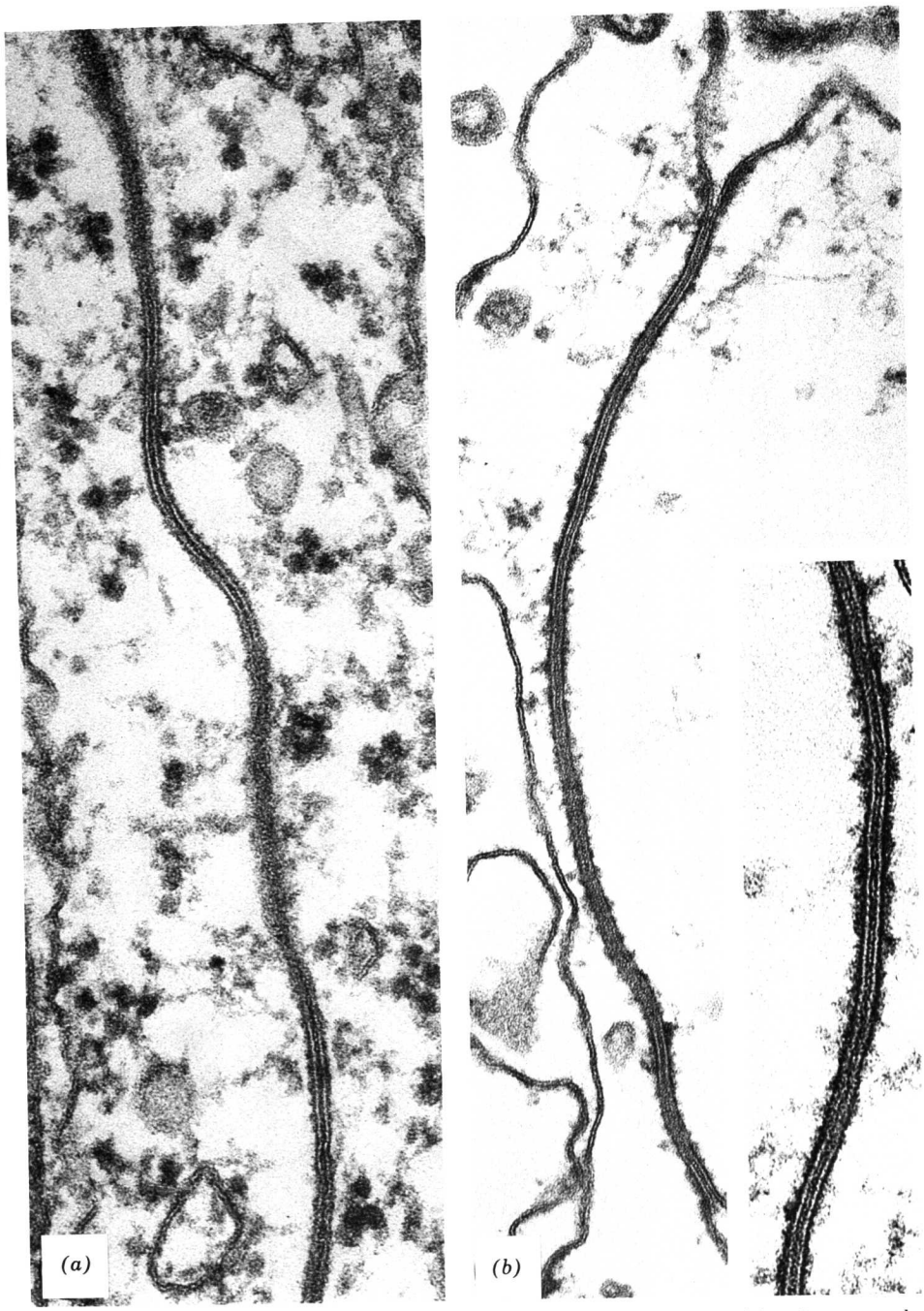

Fig. 1. Thin-section appearance of the gap junction between intact cells and in plasma-membrane-enriched subcellular fractions. (*A*) Extensive gap junction between Don hamster fibroblasts in cell culture. The gap junction is present in both transverse and slightly oblique planes. ×180,000. (*B*) Gap junction in an enriched plasma membrane subfraction from rat liver. The junction can be further purified from the nonjunctional plasma membranes present in this fraction. ×105,600. During the isolation procedure, the structural integrity of the gap junction is maintained as indicated by its appearance in thin sections (inset): two closely apposed junctional membranes are separated by an electron-translucent 20–40 Å space, or "gap." The isolated liver gap junction frequently has a discontinuous layer of electron-dense material associated with the cytoplasmic surfaces of the junctional membranes. ×160,000.

brane preparations, they can be observed in these samples with the negative-staining techniquie. Benedetti and Emmelot originally observed a polygonal lattice of subunits in a membrane preparation from rat liver (24). They initially felt that this lattice was a component of the general plasma membrane; however, they later (25) equated this array with the lattice that was described by Revel and Karnovsky. Later Goodenough and Revel (9) conclusively demonstrated that the negative-stain polygonal lattice of subunits was directly related to the thin-section image of a septilaminar gap junction. The negative-stain technique is now an important probe for assaying gap junctions in isolated membrane preparations (Fig. 2). In this regard it is interesting to note that the polygonal lattice is more easily visualized with negative staining after the membranes have been treated with detergent, such as deoxycholate (9,25) or sarkosyl (26). In the case of deoxycholate treatment, the lattice is still present even though the thin-section appearance of the gap junction may be altered from a septilaminar structure to a pentalaminar one (disappearance of the gap) (9,25).

FREEZE-FRACTURE. Studies on gap junctions that are frozen and then fractured to expose internal membrane components have provided important information about the structural characteristics of the gap junctional membranes. These studies have also been instrumental in providing a more comprehensive view of the form and distribution of gap junctions between cells in tissue and in culture.

The freeze-fracture process exposes two complementary internal membrane components or fracture faces (26,28–31). With the cell surface plasma membrane, these two fracture faces can be distinguished on the basis of their relationship to the cytoplasm and the extracellular space. They are commonly referred to as (1) an inner membrane fracture face (face A), which is adjacent to the cytoplasm; and (2) an outer membrane fracture face (face B), which is adjacent to the extracellular space. Therefore a single unit membrane is split into two components (faces A and B) when fractured. In the last few years the terms "face A" and "face B" have emerged as the popular convention for referring to the two junctional membrane fracture faces (7,32).

Freeze-fracturing dramatically demonstrates that the gap junction is a uniquely specialized region of the plasma membrane (9–11,14,18,20,26,31). As seen in Fig. 3, gap junctions are generally present as segregated domains or localized regions in plaques which are distinctly differentiated from regions of nonjunctional membrane (see also Fig. 9). Gap junctions possess a characteristic polygonal arrangement of homogeneous 70–80 Å particles on face A with a 90–100 Å center-to-center spacing. A 20–25 Å central dot or depression is frequently present in the center of these junctionl particles (10,14). Face B of gap junctional membranes contains a polygonal arrangement of pits, or depressions, which have a similar packing. Freeze-fracture observations have indicated that gap junctions can exist as large plaques (9–11,26, 33), as small plaques (11,18,20,33,34) or as a single band or strand of particles (35). At present there is no documented information that precisely defines the minimal size of a detectable gap junction. Theoretically there should exist a gap junction containing only a single particle that is matched by a similar particle in the adjacent cell membrane. The packing of face A particles within a gap junctional plaque can vary significantly. These variations usually include a homogeneous polygonal packing (liver and

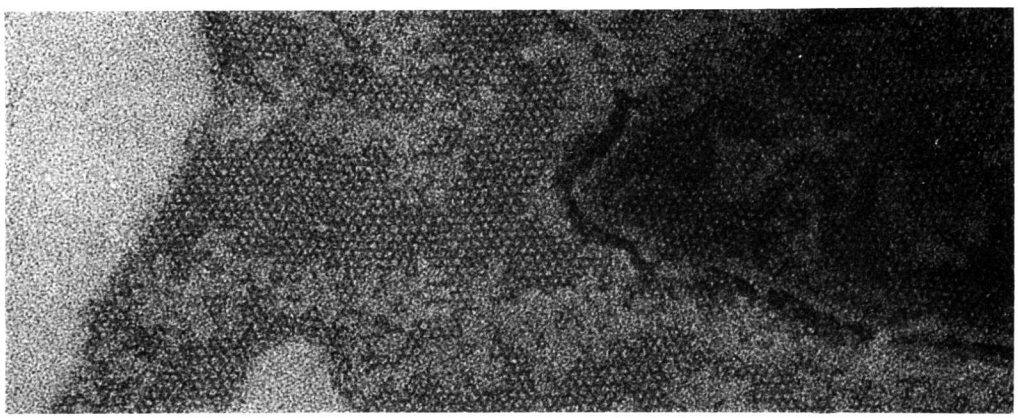

Fig. 2. Negative-stained gap junction from isolated rat liver plasma membranes (phosphotungstic acid at pH 7.0). The polygonal lattice of the gap junction is frequently discontinuous when "stained" at a low temperature (4°C). An electron-dense dot is present in the center of some of these polygonal subunits. ×160,000.

pancreas) (Fig. 3), an interrupted packing where there are small particle aggregates separated by smooth regions within a plaque (myocardium, certain cells in culture, ciliary epithelium of the eye, and adrenal cortex; see Fig. 9), and a single strand or two of aggregated particles (between photoreceptor cells in the vertebrate retina) (35). In general, the gap junctions that are present between cells in any specialized region of an organ are strikingly similar with regards to size, distribution, and packing characteristics. Gap junctions usually exist as individual (isolated) junction elements, but they can also be associated with another junction element, the tight junction (11,26,33).

CHEMICAL AND PHYSICAL PROPERTIES. There have been a limited number of observations on the physicochemical properties of gap junctions. These observations have been made on (1) junctions in intact tissue and (2) more recently on isolated junctions *in vitro*.

Studies on intact tissue have revealed that the gap junctions are generally insensitive to treatment with proteolytic enzymes or divalent-cation chelators, such as ethylenediaminetetraacetic acid (EDTA) (36–39), but they are affected by osmotic or tonicity changes (36,40,41). The gap junctions can be opened, or "unzipped," by hypertonic (sucrose) treatment (40–42), and the junctional membrane fracture faces are not detectably altered during this process (42). Tissue dissociation into single cell populations using proteolytic treatments disrupts the normal interactions of cells without affecting the integrity of the gap junctions (38). In this case the gap junctions are present as the entire 150–200 Å thick complex, which is attached to only one cell. In general, gap junctions display a remarkable resistance to physical or mechanical stress.

Enzymatically, no endogenous activity has been found to be consistently associated with gap junctions either *in vivo* or *in vitro*. However, there have been two separate reports that an ATPase reaction product can be localized cytochemically at the gap junction in intact myocardium (43) and in isolated gap junctions from rat liver (44). Due to the

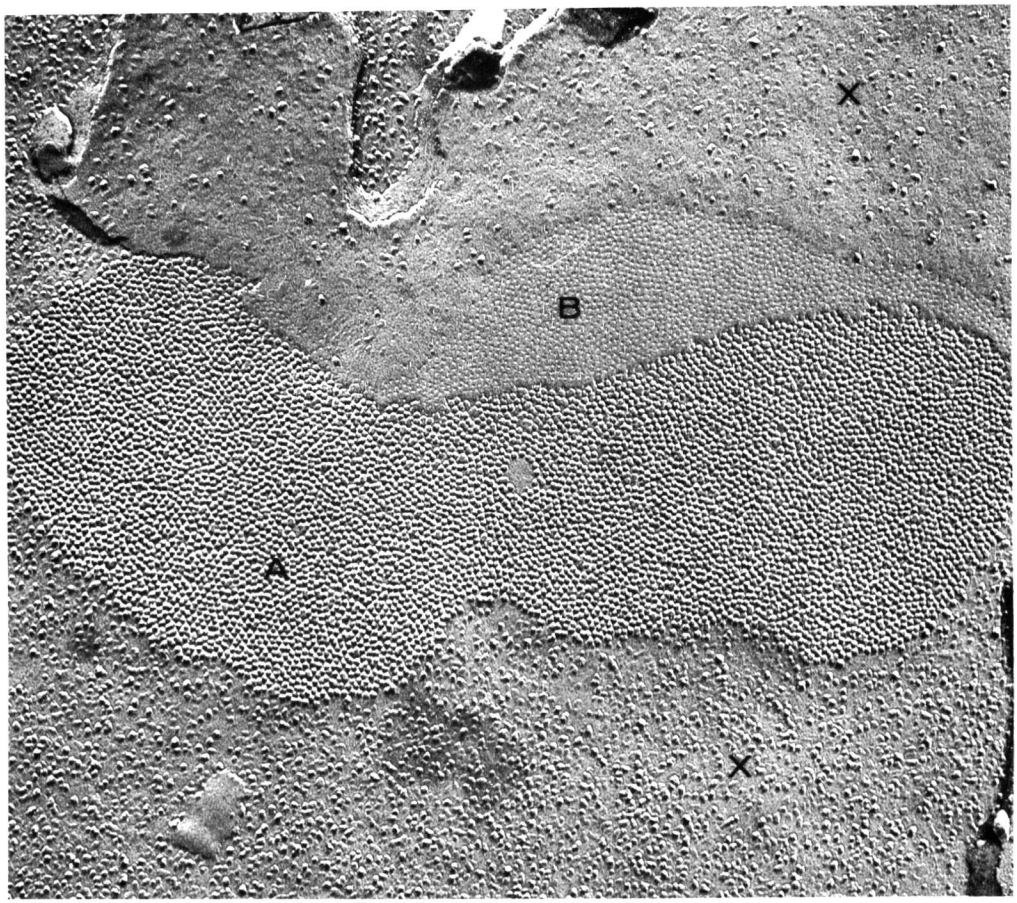

Fig. 3. Freeze-fractured gap junction from intact mouse liver. The freeze-fractured gap junction possesses a unique membrane differentiation that is characterized by a polygonal arrangement of membrane particles on fracture face A (indicated by the letter "A") and a polygonal array of complementary pits or depressions on fracture face B indicated by the letter "B"). Fracture face A is synonymous with the term "inner membrane fracture face," which corresponds to the cytoplasmic leaflet of a plasma membrane; fracture face B is synonymous with the term "outer membrane fracture face," which corresponds to the extracellular leaflet of a plasma membrane. Note that the gap junction is present as a plaquelike region that is segregated from regions of nonjunctional plasma membrane (X). The nonjunctional membrane fracture faces are characterized by a random distribution of a heterogeneous (size) particle population. ×96,000.

rather spurious results with cytochemical ATPase localizations, these observations must await further substantiation with other methods. Other cytochemical probes on gap junctions have produced negative results so far. These include the absence of colloidal iron hydroxide binding at low pH (45), the absence of concanavalin A binding (45), and the absence of cationic derivatized ferritin binding (45).

Gap junctions are normal components of subcellular plasma membrane fractions (Fig. 1*B*), and this fact has led to several structural and biochemical observations on *in vitro* gap junctions. Three different studies have been focused on gap junctions from rat and mouse liver.

Benedetti and Emmelot were the first to identify the gap junctional hexagonal lattice in plasma membrane preparations from rat liver (24,25). They used these preparations to study a variety of effects on the gap junctional lattice observed with negative staining. In summary, they found that the lattice image was temperature dependent (enhanced at 37°C), neuraminidase treatment did not affect the lattice spacing, and papain or trypsin caused a reduction in the lattice spacing (46). Benedetti and Emmelot also utilized a brief detergent treatment (1% deoxycholate) to isolate an enriched gap junctional fraction (25). Along with the gap junctions, this fraction contains a significant amount of amorphous material, thus hindering biochemical analysis.

Goodenough and Revel have used a variety of chemical probes to determine the biochemical content of the isolated mouse liver gap junctions, using the presence of the 20–40 Å gap as an indicator of structural integrity or intactness (9,39). In two separate studies they reported that the 20–40 Å gap can disappear after (1) extraction with 60% acetone; (2) treatment with 1% deoxycholate; or (3) treatment with phospholipase C (from *Clostridium welchii*) at 1 mg/ml. They also found that the 20–40 Å gap had a remarkable resistance to treatment with (1) 0.02*M* EDTA; (2) 6 *M* urea; or (3) Pronase (1 mg/ml).

In a recent report Goodenough and Stockenius have isolated a preparation of mouse liver gap junctions that has an exceptionally high degree of purity (26). This purification is based on a collagenase digestion, a brief treatment with the detergent sarkosyl NL-97, a brief ultrasonication, and finally a sucrose gradient. This junction preparation has only three detectable protein components on sodium dodecyl sulfate–polyacrylamide gels; the most prominent component has a molecular weight in the range of 20,000. A thin-layer chromatography profile of this material indicates the presence of some neutral lipid and some phospholipid (tentatively phosphatidylcholine and phosphatidylethanolamine). A hexagonal lattice with 86 Å center-to-center spacing was also reported from low-angle X-ray diffraction studies on both wet and dried junctional preparations.

Evans and Gurd have recently isolated an enriched gap junctional preparation from mouse liver based on a resistance to the detergent N-laurylsarcosinate (47). Although they have obtained a substantial amount of chemical information on this preparation, it is difficult to determine what portion of their information directly applies to the gap junction, since there is a significant amount of amorphous material within the preparation. In a separate study they have also reported that the detergent-resistant fraction has a slow degradation rate in relation to other membrane components; this information was obtained by administering radioactive leucine (double-label technique) to intact mice and then following the fate of the labels in the membrane fractions (48).

The most recent study on isolated gap junctions is focused on the "synaptic discs" or electrical synapses in the goldfish medulla (49). In this study Zampighi and Robertson found that divalent-cation chelators (EDTA and EGTA) produce discontinuities in the polygonal lattice. This effect is also accompanied by fragmentation of the junction in some instances. Also the fragmentation effect can be enhanced by applying 0.3% deoxycholate in the presence of a divalent-cation chelator.

A VARIATION—GAP JUNCTION B. A structural pleomorphism has recently been established for the gap junctions in a

variety of arthropod tissues (32,50–54). As seen in Fig. 4, in thin sections the arthropod gap junctions appear quite similar to the "conventional" gap junction (12,13,54–58), even though the intercellular "gap" is slightly larger than normal (about 30–40 Å). In lanthanum-treated preparations the polygonal lattice of subunits has slightly larger dimensions that the conventional gap junction (13,54,55,58).

In freeze-fractured tissue three basic structural differences can be observed in the arthropod gap junction (Figs. 5 and 6):

1. The gap junctional membranes contain two complementary fracture faces: the A face (inner or juxtacytoplasmic membrane fracture face), which contains pits or depressions; and the B face (outer membrane fracture face), which contains junctional membrane particles.

2. The gap junctional particles on the B fracture face are large and often heterogeneous in size; the particles are 110 Å or larger in diameter, and they are frequently present as fused aggregates of two or more particles.

3. The gap junctional particles are generally present in an irregular, nonpolygonal, packing.

These freeze-fracture characteristics clearly distinguish the arthropod gap junction from those described in other organisms so far. This arthropod junction has been termed gap junction B due to the disposition of the junctional membrane particles on fracture face B (32,50, 51).

The gap junction B pleomorphism is now clearly established for arthropods; however, too little information is available on other invertebrate gap junctions to be able to extend this pleomorphism at present. It is interesting to note that molluscan gap junctions are similar to the conventional structure, or gap junction A (14,59). Therefore it will be of evolutionary interest to characterize the gap junctions of annelids and other closely related phyla in the future. Other cell junctions, such as the tight and septate, already provide taxonomic distinction between invertebrate and vertebrate tissues; hence it may also be possible that a pleomorphism may exist in the gap junction that is associated with an evolutionary divergence.

The arthropod junctional pleomorphism is perhaps most significant with regards to the physiology of intercellular communication. The original observation of electrotonic coupling (60), as well as a large body of subsequent information concerning dye and macromolecular intercellular transfer (12,55,61,62), has been made on arthropod tissues. The junctional pleomorphism may indicate that the physiological properties of these junctions may be uniquely or qualitatively different from those associated with gap junction A. At any rate it is important to note this difference when one attempts to apply the physiological phenomena from arthropod tissues to other systems.

Tight Junction

CONVENTIONAL THIN SECTIONS. The tight junction is practically a ubiquitous structure between vertebrate epithelial cells (5,7–9,11,33). The structure is characterized by a true fusion or union of the membranes of adjacent cells. At the site of fusion the membranes are usually 140–150 Å thick. A tight junction may exist as a beltlike structure or as an isolated band. In thin sections it has been demonstrated that the tight junctions are capable of excluding or occluding the diffusion of large molecules between cells (5,9,63). In 1963 Farquhar and Palade