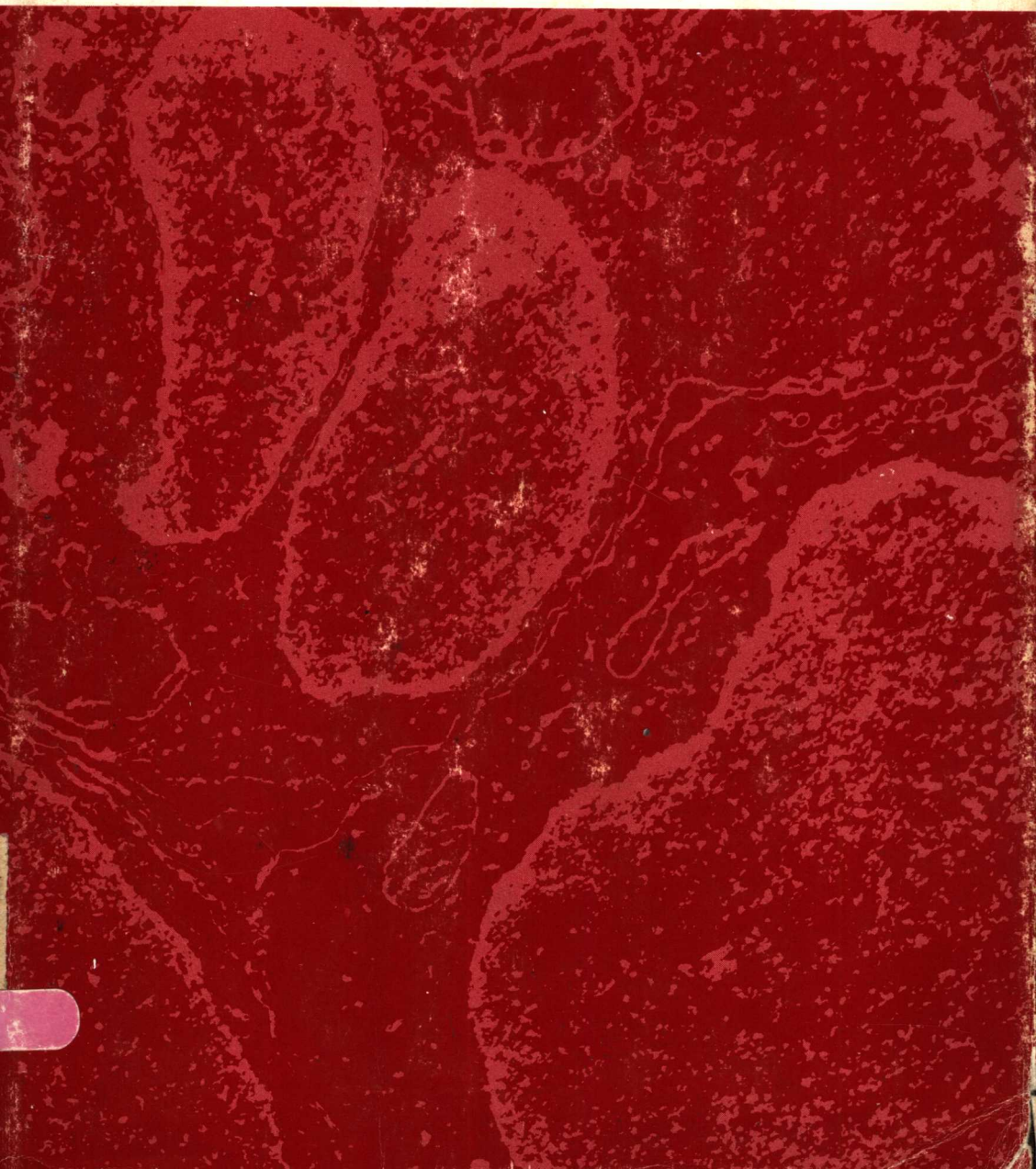
Intercellular Communication

Edited by Walmor C. De Mello



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Preface

Progress made in the last 20 years clearly indicates that the cell surface is an extremely dynamic structure involved in fundamental processes such as cell motility, innervation, and cell adhesion. Of particular interest is the finding that, in several tissues, the cell surface is differentiated at the intercellular region, thereby providing communicating channels between apposing cells.

Although our actual knowledge of the precise structures and mechanisms involved in the complex process of intercellular communication is still scanty, evidence has been presented that ions and molecules diffuse from cell to cell, establishing a physiological continuum. Embryonic differentiation, cell growth, neoplasia, electrical synchronization in nerve and muscles, as well as immune response seem to be related to cell communication.

In organizing this volume, it has been our intention to provide the reader with an actual review of the processes involved in intercellular communication in normal tissues as well as in neoplasia.

We sincerely believe that the opinions and experiences described herein will be of help in establishing new perspectives for the future of this exciting new field of cell biology.

We wish to thank all the colleagues who joined us in the organization of this volume, as well as Plenum Publishing Corporation for making its appearance possible.

Walmor C. De Mello

San Juan

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1. Introduction

Ionic coupling, a widespread mechanism for intercellular communication, is believed to be of major importance in the control of growth and of development (see reviews by Bennett, 1973; and Loewenstein, 1968, 1974a).* The passage of inorganic ions between coupled cells is relatively unrestricted, and in many cases even large molecules such as dyes (Schmidtman, 1925, quoted by Socolar, 1973; Potter *et al.* 1966) and compounds of metabolic significance can be exchanged (Crick, 1970; Gilula *et al.* 1972; Pitts, 1971; Sheridan, 1974a,b). Intercellular coupling probably occurs via a specialized region of the membrane known as a *gap junction. Nexus,* a term originally proposed by Dewey and Barr (1962) in their analysis of cell coupling in smooth muscle, is used synonymously by a number of authors primarily interested in the morphology of this junction (see McNutt and Weinstein, 1973), whereas other terms such as *electrotonic junction* and *electrical synapse* are favored by electrophysiologists. Simionescu *et al.* (1975) have recently suggested the expression *macula communicans* to make the name of this cell junction consonant with the Latin nomenclature for other junctions (Farquhar and Palade, 1963). *Tight junction,* an appellation still used by a few authors, is clearly an

*Due to an oversight the authors listed in the citations in this paper are neither in alphabetical nor chronological order. No meaning should be attached to the order in which various contributions are quoted.

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anachronism from a time when it was difficult to distinguish gap junctions from occluding junctions.*

This chapter deals mainly with the topic of gap junctions in development. The structure and physiology of these junctions have been discussed in a number of reviews (Bennett, 1973; DeHaan and Sachs, 1972; Gilula, 1974b; Goodenough, 1975; Loewenstein, 1968, 1972, 1973, 1974a,b; McNutt and Weinstein, 1973; Martinez-Palomo, 1971; Overton, 1974; Pappas, 1973; Revel, 1974; Satir and Gilula, 1973; Socolar, 1973; Staehelin, 1974) and are therefore not covered in exhaustive detail. New data on the molecular components of the junction are reported, as well as evidence from various systems bearing on the possible involvement of cell coupling in development.

2. Gap Junctions as Mediators of Cell Coupling

2.1. Morphological Correlates of Coupling

Demonstration of electrotonic coupling is frequently considered presumptive evidence for the existence of gap junctions between involved cells. Conversely, morphological identification of gap junctions is commonly used as a substitute for obtaining functional information concerning the extent of coupling in a given system. A convincing correspondence between the presence of gap junctions and the existence of cell-to-cell coupling, however, has only been demonstrated in a few instances; the data consist chiefly of close physical and temporal correlations between the ultrastructural presence of gap junctions and the appearance and disappearance of coupling measured electrophysiologically. The evidence for the role of gap junctions in intercellular communication must therefore still be considered circumstantial, especially since there is no detailed understanding of the way in which gap junctions might allow coupling between cells.

Robertson (1963), investigating the fine structure of the electrical synapse in the Mauthner cells of the goldfish brain, described a specialized intercellular contact which he thought represented the site of electrical transmission (Robertson *et al.,* 1963); we would now call what he saw a gap

*The term tight junction should be reserved for the occluding junction or zonula occludens and related structures which play a role in controlling transepithelial permeability; occluding junctions in thin sections are characterized by punctate cell contacts, where the outer leaflets of the apposed cell membranes are fused (Farquhar and Palade, 1963). In preparations that are freeze-cleaved, the occluding junction forms an apical belt seen as a meshwork of interconnecting ropes.

junction. Shortly thereafter, Barr *et al.* (1965) showed that immersion in hyperosmotic sucrose solutions causes reversible uncoupling between heart muscle cells with concomitant disruption of gap junctions. They concluded that this result "argues strongly that the nexuses are the low resistance connection between the cells." Further support for this hypothesis comes from the work of Asada and Bennett (1971) and Pappas *et al.* (1971) on the giant axons of crayfish. Using low chloride solutions, they note a disappearance of gap junctions temporally correlated with electrophysiological measurements of increased coupling resistance; gap junctions reappear when coupling is reestablished upon return of the axons to physiological saline solutions. Loss of gap junctions coincident with uncoupling also follows mechanical injury. Some gap junctions are still found, however, after immersion in low calcium solutions, a treatment which results in moderate increases in coupling resistance. Rash and Fambrough (1973), using chick myogenic cells, found a close temporal correlation between the appearance of electrotonic coupling at the onset of myoblast fusion and the transient presence of gap junctions (Rash and Staehelin, 1974). Although these observations and others like them seem to point to gap junctions as the site of electrical coupling, the existence of other junctional specializations which might also be involved cannot be completely ruled out (Revel *et al.,* 1971).

Some of the best evidence that ionic coupling is indeed achieved via gap junctions is found in the study (Gilula *et al.,* 1972) of "metabolic cooperation" between Chinese hamster fibroblasts deficient in the ability to incorporate purines, and fully competent cells of the same type. By autoradiography, they show that deficient cells can incorporate purines when ionically coupled with fully competent cells; in these instances, gap junctions can also be identified by thin sectioning and freeze-etching. Where deficient cells do not survive in mixed culture with competent cells, no ionic coupling can be demonstrated, and no gap junctions are found.

Azarnia *et al.* (1974), while investigating the possible role of cell coupling in growth control, made hybrids between human skin fibroblasts which show coupling, gap junctions, and normal growth, and malignant derivatives of mouse L cells which do not couple, show no gap junctions, and no density-dependent inhibition of growth. The hybrids are initially growth inhibited, but with gradual loss of human chromosomes some revert to a state of uncontrolled growth; these heterokaryons concurrently lose the ability both to couple and to form gap junctions.

In their study of formation of gap junctions, Johnson *et al.* (1974) combine electrophysiological measurements of reaggregating Novikoff hepatoma cells with electron microscopic observations of freeze-cleaved

cells. Coupling is not found until particle aggregates are present, and increases gradually together with an increase in aggregate size.

Further confirmation of the role of gap junctions in mediating cell coupling comes from studies of heart muscle, where synchrony between beating cells has been used as a tool to study formation of gap junctions. Goshima (1969, 1970) and Hyde *et al.* (1969) have shown that synchronously beating mouse myocardial cells are electrically coupled even via heterologous cells, and claim that gap junctions are present between them on the basis of thin sections. Studying the acquisition of synchrony between pulsating chick embryo myocardial cells, DeHaan and Hirakow (1972) demonstrated gap junctions in thin sections of synchronously beating pairs of cells. Gap junctions have also been found in freeze-cleaved specimens of intact embryonic chick heart and of beating reaggregates of heart cells (Griepp, Bernfield, and Revel, unpublished).

Despite the rather convincing accumulation of data suggesting that gap junctions mediate ionic coupling, under special circumstances cell-to-cell coupling probably can exist in the absence of gap junctions (Bennett, 1973). It is even conceivable that coupling could occur simply by extensive apposition of nonjunctional membranes (Katz, 1966), but such a high degree of overlap would be necessary that the absence of large areas of membrane juxtaposition between coupled cells is actually a good argument for the existence of specialized junctions.

Although gap junctions are frequently the most obvious mediators of cell coupling, in some instances tight junctions are also involved. Bennett and Trinkaus (1970), for example, have shown electrotonic coupling in *Fundulus* blastulae, which form an essentially closed system sealed off by tight junctions. Septate junctions are probably not involved in cell-to-cell coupling despite early suggestions that arose from the belief that they were the only membrane specializations present between cells of many invertebrates (Gilula *et al.*, 1970; Weiner *et al.*, 1964; Bullivant and Loewenstein, 1965); since then, gap junctions have been discovered between coupled cells linked by septate junctions (Flower, 1971; Rose, 1971; Hudspeth and Revel, 1971). In conclusion, although cell-to-cell coupling can be achieved by several different mechanisms, gap junctions are probably responsible for intercellular exchanges in most systems.

2.2. Structure of Gap Junctions

2.2.1. Fine Structural Appearance

One of the initial observations of gap junctions was made by Karrer (1960), between cardiac muscle cells. Most early descriptions, such as those of Dewey and Barr (1962, 1964) in smooth muscle cells, show intercellular

membrane contacts at which the extracellular space is extremely reduced or even absent, a structure resembling the tight junction of Farquhar and Palade (1963). Using potassium permanganate as a fixative, Robertson (1963) shows an array of subunits within membranes at the electrical synapse. A similar array is noted by Benedetti and Emmelot (1968a,b) in negatively stained preparations of a membrane fraction enriched in intercellular junctions isolated from rat liver. With uranyl acetate staining before dehydration, junctions in heart, liver, and elsewhere are seen actually to have a narrow space, a gap 2 nm wide between adjacent cell membranes; this narrow space allows gap junctions to be distinguished from true tight junctions (Revel and Karnovsky, 1967). Neutral preparations of lanthanum ("colloidal lanthanum") fill the gap and delineate sets of hexagonally packed substructures some 6 nm in diameter, with a center-to-center spacing of about 9 nm. These structures are electronlucent but contain an electron-dense core approximately 1.5 nm wide.

First Kreutziger (1968) and then many others (Bullivant, 1969; Goodenough and Revel, 1970; McNutt and Weinstein, 1970; see Staehelin, 1974) showed that the junctional elements seen in thin sections can more reliably be recognized by freeze-cleaving. In most cases the gap junction appears as a collection of large membrane particles on the A face (or the cytoplasmic leaflet of the fractured cell membrane) and a matching area of pits on the B face.* The dimensions of the particles and their spacing correspond closely to what can be found in sectioned or negatively stained material. A common but not quite universal feature of freeze-cleaved gap junctions is a particle-free halo or rim (McNutt and Weinstein, 1970) surrounding the aggregated particles.

2.2.2. *Variations in the Appearance of Gap Junctions*

While gap junctions in general seem to have a common structure, there are a number of variations, some minor but others so substantial that the identification of some suspicious structures as gap junctions is difficult. At one end of the spectrum are gap junctions which, on freeze-cleaving, reveal A-face particles with a normal appearance and distribution but no pits on the corresponding B face (Bellairs *et al.*, 1975; Hastings and Enders, 1975): whether or not this has any functional significance is not established. Another variant is a junction in which membrane particles in the characteristic array are found on the B face instead of remaining with the A face (Flower, 1971; Gilula and Satir, 1971; Johnson *et al.*,

*Branton *et al.* (1975) have suggested adoption of a new nomenclature in which the symbol PF denotes the A or cytoplasmic face and the symbol EF, the B or external face of a plasma membrane.

1973). The distribution of membrane particles between the A and B faces of membranes is characteristic for a given tissue as described by a "partition coefficient" (Satir and Satir, 1974).

Another variation in the appearance of gap junctions has been described by Staehelin (1972) and Perrachia (1973a,b), and consists of a widely spaced hexagonal array of membrane particles which are somewhat larger than usual. Perrachia and Dulhunty (1974) have suggested that the pattern of particles may change with the physiological state of the junction, with a regular array seen in uncoupled junctions and a loosely or irregularly spaced pattern in junctions which are functional.

A number of odd-shaped arrays have been described in the literature. Probably some of these represent gap junctions of peculiar shape or size (Raviola and Gilula, 1973; Albertini *et al.,* 1975; Decker and Friend, 1974; Hudspeth and Yee, 1973), but in other cases it is not so easy to determine whether or not one is dealing with a real gap junction (Revel and Brown, 1976; Benedetti *et al.,* 1974). Not all geometric particle patterns seen on cell surfaces are part of junctional arrays. For example, rectangular arrays of intramembranous particles (Kreutziger, 1968; Staehelin, 1972), originally thought to represent a variant of the gap junction, cannot have anything to do with cell coupling since they are found in parts of the cell membrane which do not contact other cells (Rash *et al.,* 1974).

One of the problems of great importance, particularly in developmental studies, is how to define a gap junction, particularly when it is very small. Although recognized by Hyde and his collaborators (1969) very early, this difficulty was not always perceived as clearly by other investigators and contributed to the confusion between tight and gap junctions. It would seem safest to use as many available techniques as possible to document the presence of gap junctions. Freeze-etching is probably the most reliable method, but even with this technique arrays of membrane particles should not be considered junctions unless membrane apposition is occurring. If close contact between membranes can be demonstrated, tiny clusters of closely packed particles surrounded by particle-free halos can be postulated to represent junctions, although it may not be possible to prove that a suggestive grouping of less than five or six particles is not just a matter of chance.

2.2.3. *Models of Gap Junction Structure*

It is now generally agreed that gap junctions contain structures which span the phospholipid bilayers of each of the adjacent membranes and extend across the intercellular space. The elements which bridge the space probably form the wall of a hydrophilic channel through which ions can