Receptors and Recognition

Series B Volume 2

Intercellular Junctions and Synapses

Edited by
J. Feldman, N. B. Gilula and J. D. Pitts

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CHAPMAN AND HALL

A Halsted Press Book

John Wiley & Sons, New York

First published 1978
by Chapman and Hall Ltd.,
11 New Fetter Lane, London EC4P 4EE

© 1978 Chapman and Hall Ltd

Typeset by Josée Utteridge-Faivre of Red Lion Setters
and printed in Great Britain
at the University Printing House, Cambridge

ISBN 0 412 14820 X

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Receptors and Recognition

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About the series

Cellular recognition – the process by which cells interact with, and respond to, molecular signals in their environment – plays a crucial role in virtually all important biological functions. These encompass fertilization, infectious interactions, embryonic development, the activity of the nervous system, the regulation of growth and metabolism by hormones and the immune response to foreign antigens. Although our knowledge of these systems has grown rapidly in recent years, it is clear that a full understanding of cellular recognition phenomena will require an integrated and multidisciplinary approach.

This series aims to expedite such an understanding by bringing together accounts by leading researchers of all biochemical, cellular and evolutionary aspects of recognition systems. The series will contain volumes of two types. First, there will be volumes containing about five reviews from different areas of the general subject written at a level suitable for all biologically oriented scientists (Receptors and Recognition, series A). Secondly, there will be more specialized volumes, (Receptors and Recognition, series B), each of which will be devoted to just one particularly important area.

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Preface

Intercellular communication is a fundamental requirement of complex multicellular organisms. This book describes two forms of such communication in animal tissues. One, the nervous system, provides a wide-ranging network of specialized cells connected by chemical synapses. The other allows direct communication between adjacent cells through permeable intercellular junctions (gap junctions).

The functional significance of the chemical synapse is clear – it provides a mechanism for the transfer of electrical signals from one cell to the next. However, the functional significance of the gap junction is less clear. Gap junctions contain channels which directly connect the cytoplasms of coupled cells and these channels are freely permeable to small cellular ions and molecules but are impermeable to cellular macromolecules. In some instances (e.g. in heart muscle), gap junctions behave as electrical synapses and, by ion transfer, permit the propagation of electrical impulses through coupled cell populations. Gap junctions though, are abundant in non-excitable tissues, where they are usually larger and more numerous than in nervous tissues. They may be necessary for the co-ordination of cellular activity and proliferation during embryonic development and in adult tissues.

Chemical synapses connect excitable cells in specific patterns but the mechanisms which determine specificity are not understood. The patterns of communication between cells through gap junctions are mostly undefined and it is not known if they are governed by rules of specificity.

This book has been planned to draw attention to the similarities and differences, in both structural and functional terms, between chemical synapses and gap junctions. The basis for the book originated at a meeting held in Cambridge, England in 1976 when research workers in the different fields were brought together to exchange ideas. A small number of authors were subsequently invited to review the important topics covered at the meeting in an attempt to provide a more cohesive and useful description of the current status of knowledge than would have been possible in a collection of shorter papers from all fifty or so participants.

The editors hope that these reviews, written in this form and at this time, will stimulate further research and point towards areas of joint interest to neurobiologists, developmental biologists, cell biologists and biochemists.

September, 1977

J.D. Feldman
N.B. Gilula
J.D. Pitts

Contents

1 Structure of Intercellular Junctions

N. B. GILULA

Acknowledgements
The author has received support from the Irma T. Hirschl Trust, U.S.P.H.S. Grant HL 16507, and an R.C.D. Award (N.I.H.) HL 00110.

Intercellular Junctions and Synapses
(*Receptors and Recognition,* Series B, Volume 2)
Edited by J. Feldman, N.B. Gilula and J.D. Pitts
Published in 1978 by Chapman and Hall, 11 New Fetter Lane, London EC4P 4EE
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1.1 INTRODUCTION

Specialized sites of cell-to-cell contact are generally referred to as intercellular junctions. Since the regions of intercellular contact are very small, they cannot be clearly resolved by light microscopy. Therefore, our appreciation of these specialized contacts has paralleled the development of appropriate techniques for electron microscopy. Virtually all of the major classes of cell junctions had been described on the basis of thin-section electron microscopic observations by 1970. At that time, the number of studies on cell junctions were limited because:

(1) the thin-section features of cell junctions were strikingly similar in different tissues and different organisms;
(2) 'classic' thin-section descriptions of the major junctions had already been provided; and
(3) it was difficult to expand the appreciation for structural details and function-related properties that had to be derived from studies on 'static' images in thin-sections. With the development of the freeze-fracture technique, it became possible to characterize the internal membrane modifications that correspond to the sites of intercellular contact. This technique was initially utilized to study various features of general membrane structure but, by 1970, it became an important approach for characterizing the structure of cell junctions. In addition, it became equally useful for identifying and quantitating junctional membranes in a variety of tissues. Since that time, there has been a significant increase in our appreciation of cell junctions, both in terms of their structure and function. In many respects, the freeze-fracture technique has provided a more 'dynamic' Gestalt about functional properties than was available previously through 'static' thin-section information alone.

The purpose of this chapter is to provide a representative overview of the state of affairs in the area of cell junction structure. Since our current knowledge of junctional structure has been intimately associated with the development and application of the freeze-fracture technique, much of the information has been generated since 1970. Several reviews that extensively deal with general and esoteric structural details of cell junctions are currently available (McNutt and Weinstein, 1973; Gilula, 1974b; Staehelin, 1974; Overton, 1974; Weinstein *et al.*, 1976; Griepp and Revel, 1977). These reviews should be utilized as resources to supplement the brief treatment of this area included in this chapter.

1.2 GAP JUNCTIONS

The gap junction has perhaps received the most attention in the past 10 years because it is present in most metazoan animals, and it has been strongly implicated as a structural pathway for cell-to-cell communication. There are two major types of gap junctions that have been characterized: one of these is present in most animal phyla, with the exception of Arthropoda, while the other has been extensively found in arthropod organisms. The two types of gap junctions have been treated separately in this chapter since their structural characteristics are distinctly different and, in turn, their physiological properties may be significantly different.

1.2.1 Ultrastructural features of non-arthropod gap junctions

The gap junction was resolved in its present form by Revel and Karnovsky in 1967 (for review of early history see McNutt and Weinstein, 1973). It is currently synonymous with the structure that was called the nexus by Dewey and Barr in 1962. In thin-section electron microscopy, the gap junction can be detected as a unique apposition between adjacent cells. At the site of contact, the junction can be resolved as a seven-layered (septilaminar) structure (Fig. 1.1). The entire width of the

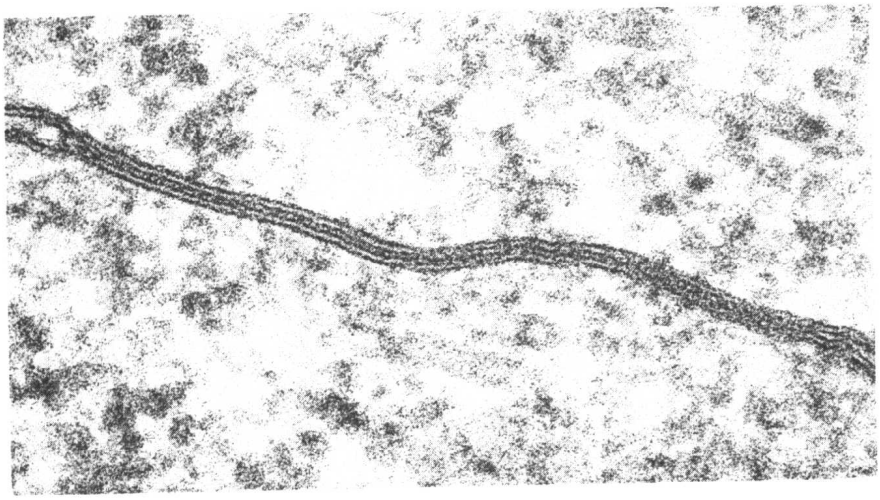

Fig. 1.1 Thin section appearance of a gap junction between insect cells (TN cell line) in culture. The junctional membranes are separated by a small 2–4 nm space or 'gap'. x 153 900.

septilaminar structure is 15–19 nm, or about 2–4 nm greater than the combined thickness of two 7.5 nm unit membranes. The septilaminar image represents the parallel apposition of two 7.5 nm unit membranes that are separated by a 2–4 nm

'gap' or electron-lucent space. This thin-section appearance led to the use of the term 'gap junction' to describe this structure. In many tissues the gap junction appears as a pentalaminar structure, and this created some confusion in the literature with another type of cell junction, the tight junction. Currently, practically all gap junctions can be resolved as septilaminar structures when they are treated with uranyl acetate staining *en bloc* (Revel and Karnovsky, 1967; Brightman and Reese, 1969; Goodenough and Revel, 1970; McNutt and Weinstein, 1970).

The precise clarification of the gap junctional structure in thin sections relied on the use of electron-opaque material, or tracer substances, that are able to fill the extracellular space. Currently, colloidal lanthanum hydroxide, pyroantimonate, and ruthenium red can all be utilized for this 'tracing' or 'staining' purpose (Revel and Karnovsky, 1967; Payton *et al.*, 1969; McNutt and Weinstein, 1970; Martinez-Palomo, 1970; Friend and Gilula, 1972a). The tracer substances are capable of penetrating a central region of the junction that corresponds to the location of the 'gap'. This fact clearly indicates that there is an extracellular continuity through the gap region of the junction, and it can be utilized as the basis for distinguishing between a tight junction (pentalaminar) and a gap junction (septilaminar). In oblique or *en face* views of tracer-impregnated gap junctions, it is possible to visualize a unique polygonal lattice of 7–8 nm subunits. The tracer outlines the subunits, which have a 9–10 nm center-to-center spacing, as a result of penetrating the regions of the lattice that are continuous with the extracellular space (Revel and Karnovsky, 1967). A 1.5–2 nm electron-dense dot is frequently present in the central region of these subunits, and it has been difficult to understand the manner in which the tracer material gains access to this internal region of the subunits. A similar lattice was described by Robertson (1963) at the site of an electrotonic synapse in a study that preceded the use of the tracer approaches. When gap junctions have been examined in detergent-treated isolated membrane fractions with negative stain procedures, a similar polygonal lattice of subunits has been observed (Benedetti and Emmelot, 1965, 1968; Goodenough and Revel, 1970; Goodenough and Stoeckenius, 1972; Goodenough, 1974, 1976) (Fig. 1.2).

The freeze-fracture technique has been utilized to obtain important complementary information about the gap junctional structure. Whereas the thin sections provide information about the relationship of the unit membranes and the intervening 'gap', the freeze-fracture procedure provides detailed information about the internal content of the junctional membranes. In general, specialized membranes, such as those present at the sites of cell junctions, have significant internal membrane structural modifications (for review, see McNutt and Weinstein, 1973; Staehelin, 1974). The freeze-fractured gap junctional membranes contain two complementary membrane halves or fracture faces (Fig. 1.3). The cytoplasmic or inner membrane half (fracture face P) contains a polygonal lattice of homogeneous 7–8 nm intramembrane particles. The extracellular or outer membrane half (fracture face E) contains a complementary arrangement of pits or depressions. In many instances, a 2–2.5 nm dot is detectable in the central region of these junctional particles. These

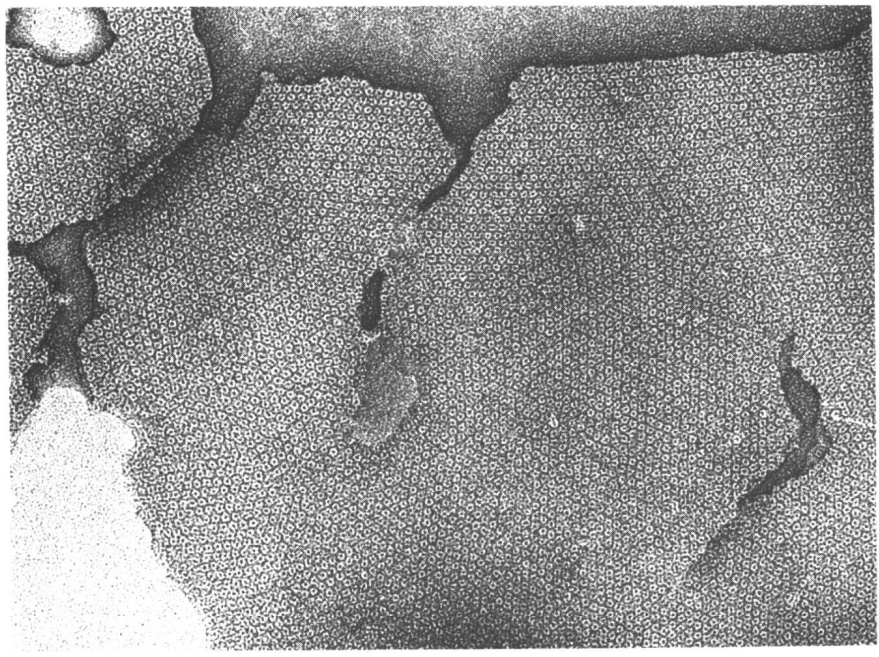

Fig. 1.2 Isolated gap junctions treated with negative stain. The isolated junctions are comprised of 8–9 nm particles that contain a central electron dense 1.5–2 nm dot. This central dot is a possible location of the low-resistance pathway or channel. x 179 550.

fracture face characteristics and membrane particle dispositions have now been documented as a constant feature of most non-arthropod gap junctions that have been examined (Goodenough and Revel, 1970; Chalcroft and Bullivant, 1970; McNutt and Weinstein, 1970; Friend and Gilula, 1972a; Staehelin, 1974). The junctional membrane lattices can exist in a variety of pleiomorphic forms, but the variations surround a single theme – a plaque-like or localized (focal) contact between interacting cells. Gap junctions are usually present as oval or circular plaques; however, a variety of forms, including linear strands (Raviola and Gilula, 1973) have been reported.

In general, the gap junction represents a unique paracrystalline lattice that is comprised of 7–8 nm particles or subunits that can be visualized with at least three independent techniques; tracer-impregnated thin section, freeze-fracturing and negative staining. Although the lattices are very attractive from a structural standpoint, it has been difficult to relate the size and arrangement of gap junctional units with a functional state. Thus far, gap junctions have been identified *in vivo* as aggregates of 2–3 particles as well as large plaques containing hundreds of particles.

Fig. 1.3 Freeze-fracture image of gap junctions between granulosa cells in a rat ovarian follicle. The junctional membranes contain a polygonal lattice of intramembrane particles on the P fracture face and a complementary arrangement of pits on the E fracture face. x 64 260.

The gap junctions are structurally resistant to treatments with proteases and other agents that are used to dissociate intact tissues. When tissues are dissociated by such treatments, the gap junctions are retained as intact complexes on the single dissociated cells (Muir, 1967; Berry and Friend, 1969; Amsterdam and Jamieson, 1974). This response indicates that the gap junctional membranes are tightly bound into a cohesive unit or complex, and the binding is not simply explained by divalent cation salt linkages. With time, the gap junctional remnants are either ingested by the cells or re-utilized for establishing contact between single cells.

At the present time, there has been only one satisfactory procedure for 'splitting' or separating the gap junctional membranes in intact tissues. This procedure involves the perfusion of tissues with hypertonic sucrose solutions (Barr *et al.*, 1965, 1968; Dreifuss *et al.*, 1966; Goodenough and Gilula, 1974). In intact mouse liver, the junctional membranes are separated by this treatment somewhere in the central region of the extracellular 'gap' (Goodenough and Gilula, 1974). The separated junctional membranes still contain the characteristic particle lattices in freeze–fracture replicas, and the particles appear to be more tightly packed when the membranes are separated. Furthermore, the junctional membrane particle lattices respond to this treatment as intact domains rather than as a membrane sector comprised of independent particles or units. In essence, the interactions between junctional membrane particles are strengthened, if anything, by this treatment, while the interactions between the two junctional membranes are definitely weakened to result in separation. Although this treatment results in a radical disruption of the gap junction, it must be considered relatively mild or physiologically significant since the entire process can be easily reversed by simply replacing the hypertonic sucrose with a normal salt solution.

1.2.2 Ultrastructural features of arthropod gap junctions

Gap junctions have been described in a variety of arthropod tissues with both thin-section and freeze-fracture techniques (Flower, 1972; Peracchia, 1973b; Johnson *et al.*, 1973; Satir and Gilula, 1973; Gilula, 1974b; Dallai, 1975). The structural features of the arthropod gap junctions are sufficiently different from non-arthropod gap junctions to be considered as a unique structural variation.

In thin sections, the arthropod gap junctions are quite similar to non-arthropod gap junctions (Fig. 1.1), although the intercellular 'gap' is slightly larger (about 3–4 nm) (Payton *et al.*, 1969; Hudspeth and Revel, 1971; Rose, 1971; Peracchia, 1973a). Also, in lanthanum-impregnated specimens, the subunit lattice has slightly larger dimensions (Hudspeth and Revel, 1971; Johnson *et al.*, 1973; Peracchia, 1973a). In freeze-fracture replicas, the structural differences in the arthropod gap junctions are very striking (Fig. 1.4). The gap junctional membranes contain two complementary fracture faces: the inner membrane half (fracture face P) contains pits or depressions; and the outer membrane half (fracture face E) contains a plaque-like arrangement of intramembrane particles. The junctional membrane

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