Do Cellular Slime Molds Form Intercellular Junctions?

Intercellular contact specializations such as gap junctions have been thought to play a role in growth and differentiation in many systems. This idea is based on the observation that small molecules can pass directly between the cytoplasms of adjacent cells and on the strong circumstantial evidence that gap junctions may provide for this intercellular permeability (*1*). Gap junctions are found in a wide variety of organisms, and it is reasonable to ask whether they could be associated with the process of differentiation in the cellular slime mold *Dictyostelium discoideum*. In this organism small molecules, such as adenosine 3′,5′-monophosphate (cyclic AMP), play a role in the cell movements which occur during aggregation. In addition, differentiating cells bear antigenic determinants not found on vegetative cells (*2*), and a particular size of intramembranous particles (IMP's) has been implicated in the differentiation process (*3*). These observations suggest a role for cell surface components. However, several studies carried out over a number of years in our laboratories have failed to provide evidence for the existence of gap junctions or, for that matter, any other junctions (occluding, septate, or adhering) in *D. discoideum*.

In our studies we used a variety of fixation procedures at different stages of cell development. The cells were fixed with glutaraldehyde-formaldehyde or osmium [including osmium vapors, see (*4*)], or both. Both fixative concentration and buffer composition were varied. We investigated the cells and slugs with both thin-section and freeze-fracture techniques. Although small gap junctions (less than 0.1 μm in diameter) would be difficult to identify in sections, those involving as few as five to ten IMP's have been convincingly identified in freeze-fracture replicas of other cell systems. We sought evidence for the presence of intercellular junctions at various times during differentiation, from aggregating streams of amoebas to early slug stages; at no time were junctions observed.

Because we are reporting a negative result, we have explored in detail the possibility that junctions do exist in *D. discoideum* but were not detected. A conceivable argument is that typical gap junctions exist in slime molds but did not withstand the various preparative procedures that were used. This hypothesis is not very likely, as the morphology of gap junctions in other systems has been shown to be fairly insensitive to treatment with hyperosmolar salts, EDTA, or proteases, and even during cell lysis (*5*). Another possibility is that the frequency with which gap junctions occur in slime molds is so low as to have escaped detection. The lowest reported value for the area occupied by gap junctions is that calculated for BHK cells, where it is 0.05 percent for those interfaces that contain junctions (*6*). For the slime molds this would mean about 70 junctions per 1000 μm^2, each 0.1 μm in diameter (containing 75 IMP's). If such a low density of junctional material were found only in specialized regions of the cell surface (at the tips of microvilli or at the anterior and posterior ends of the cells where antigenic factors are found in differentiating slime mold cells), the overall density would be even lower, although still detectable (about seven junctions of 75 IMP's per 1000 μm^2). The detection of junctions is complicated not only by the possibility that the areas where junctions occur may be of limited size, but also by the fact that junctions may form only at very specific times and be short-lived, thus further reducing chances of detection.

We conclude that it is impossible to prove the absence of gap junctions. Electrophysiological attempts have also failed to detect low-resistance junctions, largely due to technical difficulties (*7*). We nevertheless postulate that gap junctions do not play a role in slime mold differentiation. This may not be surprising, as slime molds are commonly classified among the fungi, and gap junctions have not been observed in the plant kingdom. Failure to find gap junctions in *D. discoideum* does not lead one to generalize about the role of gap junctions in differentiation.

GEN JOHNSON, ROSS JOHNSON
Department of Genetics and Cell Biology, University of Minnesota, St. Paul 55108

MARCIA MILLER*
JOAN BORYSENKO,† JEAN-PAUL REVEL
Division of Biology, California Institute of Technology, Pasadena 91109

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* Present address: City of Hope National Medical Center, Duarte, Calif. 91010.
† Present address: Department of Anatomy, Tufts University Medical School, Boston, Mass. 02111.

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Synergism Between Trimethoprim and Sulfamethoxazole

In a recent report (*1*) Poe suggests that the observed synergism between trimethoprim and sulfonamide occurs as a result of their simultaneous binding to dihydrofolate reductase. While these data direct attention to factors affecting the binding of diaminopyrimidines to the isolated enzyme, this suggestion overlooks important quantitative aspects of the inhibition of the growth of *Escherichia coli* and cannot be considered an adequate explanation of synergistic inhibition of growth by these two drugs.

The concentration of sulfamethoxazole required in this experiment to enhance the binding of trimethoprim is greatly in excess of that needed to produce synergism in experiments with growing organisms. Bushby (*2*) has shown that the concentration of sulfamethoxazole required to obtain strong synergistic effects on the growth of various *E. coli* strains in the presence of trimethoprim varies between 2000 and 61,000 times lower than the inhibition constant, K_i, of sulfamethoxazole for *E. coli* dihydrofolate reductase as measured by Poe (*1*). We know of no evidence which shows that sulfamethoxazole is concentrated by this organism.

Poe (*1*) quotes from Webb (*3*, pp. 498–500) the conclusion that multiple blockade of a linear sequence of irreversible reactions is theoretically incapable of producing greater inhibition than a single agent alone. From this he argues that sequential inhibition of the biosynthesis of dihydrofolate and its subsequent reduction by sulfamethoxazole and trimethoprim, respectively, cannot account for synergism. However, Webb also states that the operation of an isolated monolinear chain in the cell is probably very uncommon. In fact, dihydrofolate reductase is involved in the recycling of the tetrahydrofolate which is oxidized to dihydrofolate by the action of thymidylate synthetase, as well as the synthe-