Cytoplasmic Matrix: Old and New Questions

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Conferences on the nature of protoplasm have not been in vogue for perhaps some 30-odd years, in part for reasons that I wish to discuss, but, with the turning of time's kaleidoscope, new patterns of organization have now emerged and a new synthesis is perhaps possible. Cell biology is passing from an analytical phase in which each component of the cell was isolated and characterized apart from other components to a new phase in which the integration of these components to give recognizable cell behavior once again is commanding considerable attention. In his introduction to this supplement, Porter points out that these questions on the cytoplasmic matrix and the integration of cellular function are a part of our heritage that extends back at least 100 years and were the subject of intensive experimental inquiry in the first few decades of this century. At least, we can confront the old questions with new perspectives and perhaps find important new questions to ask of the cell.

Our heritage has two aspects, the first and older one being a view of the cytomatrix based on physical chemistry. Porter reminds us of the colloidal and coacervate chemistry of the cytoplasm. This is the view of protoplasm as a thixotropic gel and a return to explanations of cell behavior based on sol-gel transformations, for example. The other view of the cytomatrix is structural, and although it echoes ancient ideas, it is based primarily on the high-voltage electron microscopy of cell whole mounts as practiced in the last decade or so. Porter refers to this basic structural organization of the cytomatrix as the microtrabecular lattice (MTL). The MTL connects or entraps all formed structures in the cell and influences, as we shall see, the most intimate properties of molecules in the cytoplasm. The cytoplasm has two phases: cytosol and cytomatrix. In the recent past, only the cytosolic fraction of the cytoplasm has been considered, but the papers presented in this supplement show us that a new biochemistry of the cytomatrix is rapidly being developed.

The major filament systems of the cytomatrix are the subject of the articles by Stossel, Steinert et al., Olmsted et al., and Pollard et al. The manner in which these elements are interconnected and their spatial specification within the cell concern Vallee et al., Tucker, Tiwari et al., Tilney and Tilney, Mangeat and Burridge, Miller and Solomon, Moo-}

**How Many Proteins Comprise the Cytomatrix?**

The selective extraction methods discussed in the articles by Fey et al. and Fulton, for instance, have been used, together with two-dimensional gel analysis, to distinguish between polypeptide components of a cell that are part of the cytomatrix, operationally defined as the detergent-insoluble cell residue, vs. other fractions of the cell. Paine's discussion of localization of about 100 polypeptides in the cytoplasm of a single Xenopus egg beautifully illustrates how this approach is applied. In a similar tour de force, Bravo and Celis (1) have catalogued about 1,350 polypeptides in the HeLa cell. All of these have been given numbers and are reproducibly identified in the gels. For each polypeptide, a catalogue of characteristics can be developed, including the distribution between cytoplasm and nucleus, the fraction remaining upon Triton extraction, whether the polypeptide is found during different stages in the cell cycle, and so on. An important conclusion is that the major polypeptides on such electrophorograms, and hence the major proteins of the cell, include actin, α- and β-tubulin, α-actinin, and vimentin, i.e., the cytoskeleton proteins of the major fibrous systems of the cell. As we have learned over the years and as is reinforced in this supplement, it is important to pay attention to these major cytoskeletal proteins, and a large body of work has been devoted to characterizing these components. It is rather surprising that there are only half a dozen or so of these proteins in the eukaryotic cell. Possibly clathrin, the coat protein of the coated vesicle, and spasmin, the Ca²⁺ contractile protein, are

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1 Abbreviations used in this paper: MAP, microtubule-associated protein; MTL, microtrabecular lattice.
to be included in this list for some cells, and the intermediate filament protein perhaps varies among cell types. These proteins are the building blocks of the cytomatrix and as such are relatively stable evolutionarily.

But where is trabeculin? Evidently the MTL that connects the major fibrous systems of the cytoplasm has a regular structural element. One possibility, of course, is that this structural element corresponds to a single polypeptide. If this were so, we might identify this polypeptide by its abundance in the detergent-treated cytoskeleton, as indeed we do with the other major fibrous systems that I have just mentioned. Cursory inspection of the abundance of polypeptides in the HeLa cell cytomatrix shows that this possibility is unlikely, although it is not ruled out completely.

A more likely possibility is that the MTL contains many polypeptides. There are two alternatives implied by this possibility. The first is that the trabeculae are homogeneous, even though their individual structure is complex. The radial spokes of ciliary axonemae have these characteristics. Presumably, each spoke contains the same arrangement of 16 or 17 polypeptides. The functions of the individual polypeptides in a spoke perhaps vary, but obviously the cell has invested considerably energy in producing such a distinctive arrangement, and the entire structure seems involved in the control of microtubule sliding within the axoneme. The spokes are discussed further by Porter as examples of microtrabeculae.

An attractive feature of this conception of the microtrabecular lattice is that it suggests that the more or less uniform structural element of the lattice also has a well-defined, uniform chemical composition. This "homotrabecular lattice" would not be identified as readily by examining a list of polypeptides in the Triton cytoskeleton, because the abundance of the component polypeptides of the lattice would be perhaps only 5–6% of that of the major cytoskeletal proteins. Although the possibility of a homotrabecular lattice element exists, this conception seems too confining, at least to many who participated in this conference.

The most attractive alternative, discussed in one guise or another in many of the articles presented here, is that the MTL has a highly heterogeneous composition. For instance, some trabeculae may be microtubule-associated protein (MAP) 2 and connect microtubules to intermediate filaments, as discussed by Vallee et al., or other MAPs connecting microtubules and microfilaments as discussed by Pollard et al.; some may be spectrin or spectrinlike proteins, forming a lattice such as is seen in the erythrocyte cytoskeleton; some may be similar to the 120,000-dalton actin binding protein discussed by Condeelis; and so on. In this "heterotrabecular lattice," the trabeculae share the morphological property of being rodlike molecules, and their size is relatively constant because of the ways in which rodlike proteins are generated by folding of amino acid chains, rather than because of compositional uniformity. Some trabeculae would be single polypeptides; in others the connection would comprise many polypeptides. If this is not the correct conception of the MTL, the large number of accessory polypeptides associated with the main cytoskeletal elements would not be integral parts of the lattice and would have to be integrated into our concepts of the cytomatrix in some other way. This does not seem to be merely a question of semantics. It would be interesting to know whether some major subunit, or subunits, forms the MTL.

How Regularly Organized Is the Cytomatrix? Does It Have Repetitive Units of Organization?

These questions are closely related to the questions of cytomatrix composition. The organization of the cytomatrix could be extremely irregular if the composition were highly complex and the relationship between elements or major fibrous systems highly variable. On the other hand, the organization of the cytomatrix could be very regular, with only small local perturbations, if the trabeculae were of homogeneous, or nearly homogeneous, composition. Such a regular order is perhaps implied by the work of Branton (2) and his colleagues on the erythrocyte. The paradigm presented in that work is that if we carefully first define the molecules involved and second define the appropriate protein-protein interactions one by one, then self-assembly, with its regular geometrical constraints, will yield the molecular network that determines the cytomatrix. The exact placement of macromolecules need not matter in this meshwork, in part because all interactions will be essentially equivalent. In such a network, Branton argues, global properties will be determinant. Primarily, we will need to know the materials science features of the network, for example the elastic and shear moduli involved, and how these change in terms of global changes in pH or divalent cation concentration.

With both the irregular cytomatrix and the very regular cytomatrix, determined by rigorous or relaxed self-assembly principles, it is the physical chemistry aspects of protoplasm of which we must be aware. Although no one will gainays the importance of these global properties, we would not do justice to the past 30 years of cell biology nor to the overwhelming preponderance of evidence presented here to let this picture stand. A crucial feature of the organization of the cytomatrix, found as the theme of most of the articles of this supplement, is the specific placement of critical organizational elements within the cell. Important aspects of the organization of the cytomatrix are determined by the specific placement of, for example, microtubule-organizing centers, as discussed in the articles by Pickett-Heaps et al., Tiwari et al., Tucker, Sandoval et al., and Karsenti et al.; by microfilament-organizing centers, such as Tilney and Tilney consider; and by local membrane differentiation, for example the recruitment of clathrin and actin to specific membrane sites, as discussed by Condeelis, Geiger et al., Mangeat and Burridge, and Rodewald. This model of the cytomatrix proposes that there are general regularities within its organization but that "positional information" and site-specific local variation often are of paramount importance. In their discussion of hair cell morphogenesis, Tilney and Tilney present an exquisite example of the degree of positional information used to generate cell structure. A cytomatrix possessing a high degree of site-specific organization would, as a corollary, have the ability to respond locally. An aspect of such a cytomatrix would be that it is dynamic without being chaotic.

The positional information involved in cytomatrix organization may even extend to the specification of polypeptide localization at the point of synthesis. The prevalent idea that polysomes come to rest in unspecified positions in the cytoplasm and that their polypeptide products diffuse as monomers or small proteins through the cytosol to give a uniform distribution and concentration almost certainly needs serious modification and perhaps is fundamentally incorrect. The
papers by Fey et al., Lasek et al., Masters, and Fulton all provide clues indicating that the synthetic system and its polypeptide end products are more carefully positioned in the cytomatrix than has been supposed. Measurements of protein diffusion through the cytoplasm by a variety of techniques, as discussed in the articles by Mastro and Keith, Paine, and Parsegian and Rau, show that the diffusion of these substances is limited or slow compared to that of ions, amino acids, and sugars—small molecules that indeed freely diffuse in the cytosol. The conclusion to be drawn from this work is that there is enough surface within the cytomatrix and its associated membranes to affect diffusion of proteins within the cytoplasm. Cellular components may be separated by being in different membrane-bounded compartments, but within the contiguous matrix of a single membrane-bounded compartment, local separation can also be achieved by protein-protein interaction. This is defined by Masters as "selective affinity compartmentation." Histone localization in chromatin is a well-studied example of this phenomenon. As discussed by Lasek et al., axonal transport is another particularly good example of the movement of proteins by binding to or incorporation into the cytomatrix at one site for later deployment at another site, in this case far from the site of incorporation.

What Is the Interaction Scheme of the Cytomatrix? How Does This Lead to Functional Integration? What Is the General Algorithm for Positional Information in the Cytomatrix? How Is This Altered or Distorted in Specific Cases?

There seems to be a reasonably common set of organizational principles based on a reasonably common set of macromolecules that developed early in eukaryotic cell evolution and that embodies and defines the positional arrangements within the cytomatrix. Detailed examples of evolutionarily important positional arrangements are provided for a variety of differentiated cells in this supplement; for many cells, the centriole provides a key focus for such information, as Kar Sprint et al. and others indicate here. My co-workers and I (3) discuss the integration of the cortical cytomatrix in relation to centriolar structures more fully elsewhere. This approach taken from comparative cell biology has also been somewhat neglected lately, but it too may prove valuable in the current context.

Four features of the construction of the cytomatrix that must be incorporated into an interaction scheme leading to an explanation of cell shape determination, morphogenesis, or motility are (a) a common set of macromolecular building blocks, (b) general regularity of protein-protein interaction and assembly, (c) important local spatial variation, including specific placement of critical organizational macromolecular elements, and (d) dynamic features, including rearrangements in response to stimuli.

In Fig. 1, I attempt to define an interaction scheme for major groups of proteins that are involved in the functional integration of the cytomatrix, as developed by the papers in this supplement. I propose calling this development the "dynamic heterotrabecular model" of the cytomatrix in the hope that it will provide the sort of clarification that the fluid mosaic model has for the cell membrane. It is important to remember that the protein-protein interactions embodied in this scheme have geometrical, as well as biochemical and biophysical, consequences. For example, certain interactions will occur only at the ends of cytoskeletal polymers, others will require or produce parallel arrangements of such elements and interact only with their lateral surfaces, still others may require orthogonal dispositions of elements. These differences imply that different specific macromolecules mediate the interactions. In turn, if these elements are all structurally part of the MTL, the rich heterotrabecular lattice that I have discussed above would be invoked.

This interaction scheme focuses on the major assembly proteins, the abundant proteins of the cytomatrix. It implies that each of the assembling proteins that form the major fibrous systems of the cytomatrix must be stringently shaped to interact with a multiplicity of other proteins, including proteins that control the polymerization, bundling, and functional properties of the assembling protein. Perhaps this accounts for the relative stability of these proteins in evolution. Nevertheless, unexpectedly, one or another of these major systems can be missing in groups of cells in the eukaryotes and some substitutions have taken place during cell evolution. For example, Tiwari et al. remark that there is no intermediate filament system in cells of higher plants; it is also well known that ciliates may have restricted actin localization and use spasin or spasmnilike proteins rather than actin to produce cellular contractility.

The tubulin and actin families of proteins provide the best illustrations of the specifics of the interaction scheme of Fig. 1. The "polymerization control" proteins that affect the assembly or disassembly of microtubules and microfilaments, which include proteins that cap the ends of the filaments, accelerating or decelerating polymerization events, have been the subject of a number of investigations and are well represented in this supplement. However, our appreciation of what I have chosen to call "bundling proteins" and the capacity of these proteins to organize the cytomatrix is a newer thematic development. The bundling proteins may link polymers of the same sort together. Microtubule is linked to microtubule
in the heliozoan axopod, as Tucker reminds us, and also in the cilium and mitotic apparatus. Microfilament is linked to microfilament in the microvillus and elsewhere by proteins such as fimbrin, as discussed by Mooseker et al. Bundling proteins may also link dissimilar elements, such as microtubules to intermediate filaments, microtubules to microfilaments, microtubules to intermediate filaments, or microfilaments to clathrin. The bundling proteins then form a distinctive set that can be matched in a one-to-one manner with the heterogeneous trabeculae and with the variety of geometrical interactions between cytomatrix elements. In this way our picture of the MTL seems reasonably consistent.

The third set of proteins interacting with the major assembly proteins I have called “interaction proteins” to emphasize their role in the functional interactions of the cytomatrix. The functional interaction that has attracted the most attention of the authors of this supplement is cell motility. This is regulated by the two known mechanoenzymes: myosin for the microfilament system, dynein for the microtubule system. There may be unknown mechanoenzymes as well. It seems clear from the articles by Pickett-Heaps et al. and Stearns that our present ideas fully account for neither chromosome motion during mitosis nor motility in the erythrophore. We are learning that the known mechanoenzymes are localized within specific cells at critical times, but we still do not know which, if either, of these molecules is involved in the movement of membranes. Mechanoenzyme localization is only one aspect of cytomatrix-membrane interaction. Other interaction proteins, such as talin, discussed by Mangeat and Burridge, or vinculin, discussed by Geiger et al., seem particularly important in mediating interactions between integral membrane proteins and the cytomatrix. Perhaps the motion and sorting of transmembrane proteins and membrane-bounded organelles in the cytomatrix is less a question of mechanochemistry and more one of membrane attachment.

Finally, the effects of the polymerization-control proteins, the bundling proteins, and the proteins responsible for functional interactions seem to be mediated either directly by pH and Ca²⁺ or indirectly via regulatory proteins. Means et al. remind us that the enzymes that require Ca²⁺-CaM or cAMP for activation are particularly important in such regulation. The effect of these interactions is probably posttranslational modification of the appropriate control protein, normally by phosphorylation or dephosphorylation. After a local change in ionic environment, mediated by transmembrane proteins, the cascade depicted in Fig. 1 works first via modification of regulatory protein activity and then by posttranslational modification of the three groups of proteins that interact to change the state of major fibrous elements in the cytomatrix. The dynamic heterotrabecular model of the cytomatrix suggests that, in this way, the cell can respond in an integrated fashion to an environmental stimulus. Whether the major assembly proteins of the cytomatrix are themselves covalently modified in this cascade is unclear.

From this conference we have arrived at a state in our study of the cytoplasmic matrix and the integration of cellular function comparable to the memorable meeting on tissue fine structure held at Arden House in 1956 that produced the first supplement to what is now The Journal of Cell Biology and ushered in the period of classic study of that subject. Hopefully, the old and new questions of this conference, briefly reiterated in this article, may provide a comparable impetus.

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