Structure and Properties of Ground Substances

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Structure and Properties of Ground Substances[1,2]

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SYNOPSIS. Studies of arthropods and other animals including man have revealed that the interstitium, cytoplasm, and nucleus each contain a matrix or ground substance composed of various biopolymers. The extracellular ground substance consists of chains of glycosaminoglycan molecules which may be linked with hyaluronate to form supramolecular complexes called proteoglycans. The cytoplasmic ground substance contains microtubules, microfilaments, microtrabeculae, and intermediate filaments, and constitutes a movable cytoskeleton. This framework interconnects the cell surface, the various organelles, and the nuclear envelope. The nuclear matrix consists of a peripheral pore complex lamina and an internal matrix.

Glycophorin, fibronectin, and other proteins appear to provide specific linkages between the extracellular and cytoplasmic ground substance. The nuclear matrix has peripheral elements that appear to interact with the cytoskeleton at specific sites.

Intimately associated with the ground substances is a dynamic matrix composed of water and counterions. The structure of the whole system, macromolecules, water, and ions, is being built up from basic laws and principles, enabling quantum mechanical descriptions to be extended to domains containing many interacting components.

INTRODUCTION

Water and ionic regulation, active transport, metabolism, communication, and countless other living processes take place on and within a living structural matrix or ground substance whose properties have been the subject of much research. The first part of this article summarizes the development of our current concepts of the interstitial, cytoplasmic, and karyoplasmic elements of this matrix and the possible relationships between them. Next we consider the association of the matrix with its solvent, counterions, enzymes, and the genetic material. DNA is regarded to be a component of the matrix. Recent research indicates water can form a cross-linked matrix in intimate association with DNA, and it is likely that this arrangement occurs in other parts of the ground substance.

Our symposium concerns ionic regulation in arthropods. This essay synthesizes information on the ground substance obtained from a variety of species from bacteria to man. We begin to develop a general picture of the fabric of the animal body that can help us understand a variety of biological problems.

EXTRACELLULAR GROUND SUBSTANCE

Study of the extracellular ground substance was begun by organic chemists who extracted from tissues "animal gum," "mucin," and, later, "mucoid" and "mucopolysaccharide" (*e.g.*, Landwehr, 1883; Loebisch, 1886; Richards and Gies, 1902; Meyer, 1933).

While the chemists assumed that the mucoid lay between the insoluble fibers of connective tissue, histologists at first had difficulty locating it. Bensley (1934) reviewed the situation and described a remarkable experiment. A suspension of paramecia was injected into the subcutaneous tissue of a guinea pig. The paramecia, vigorously swimming about within the "bulla" or subcutaneous droplet, would suddenly rebound without coming in contact with any microscopically visible structures. None of the protozoans were able to escape into the surrounding spaces. It was also found that connective tissue resists the injection or withdrawal of fluids (*e.g.*, Baitsell, 1915, 1925; Hueck, 1920; Clark and Clark, 1933; McMaster and Parsons, 1939*a*, *b*). And Clark and Clark (1918, p. 234; 1930, p. 115) noted that while the space between individual tissue cells may be

[1] From the Symposium on *Cellular Mechanisms of Ion Regulation in Arthropods* presented at the Annual Meeting of the American Society of Zoologists, 27–30 December 1982, at Louisville, Kentucky.

[2] This essay is dedicated to Dr. Albert Szent-Györgyi in commeration of his 90th birthday.

transparent, it does not show brownian movement, whereas "dancing particles" can always be seen in the lumens of stagnant vessels, in the vacuoles of cells, in the cytoplasm of dying cells, or in edematous tissues. All of this evidence led to the conclusion that between the fibers of the connective tissue there lay an invisible "viscid substance." From its staining reactions, it appeared to be acidic. Its solubility in basic solutions suggested that it might be comparable with the animal gum, mucoids, mucins, or mucopolysaccharides that the chemists had extracted from connective tissue.

We now know that the normal histological and electron microscopic appearance of tissues, in which large vacant spaces occur between the extracellular fibrous structures, is misleading. The ground substance is labile, and is completely extracted during normal tissue fixation. The ground substance can be preserved with the periodic acid-Schiff method (Leblond, 1950) or by precipitation with strongly cationic compounds such as ruthenium red (Luft, 1971), alcian blue (Scott and Dorling, 1965), acridine orange (Saunders, 1964), or lanthanum (Doganges and Schubert, 1964; Overton, 1969; Mayson and Mayes, 1973). These precipitating agents must be added to tissues before or during fixation, or most of the ground substance will dissolve.

The extracellular ground substance is composed of combinations of hyaluronic acid, chondroitin, chondroitin-4 and -6 sulfates, dermatan sulfates, keratan sulfate, heparan sulfate, and/or heparin, collectively termed the glycosaminoglycans. In tissues these molecules can be linked covalently with protein to form proteoglycan. In addition, various structural glycoproteins, of which fibronectin, laminin, and chondronectin are examples, have been identified as components of the ground substance. Together with the insoluble fibers (collagen, chitin, or cellulose) these substances can form composite materials of great versatility. By altering the ratio of fiber to soluble polymer, and their spatial relations, quite different mechanical properties can be achieved. The structure and properties of these substances have been

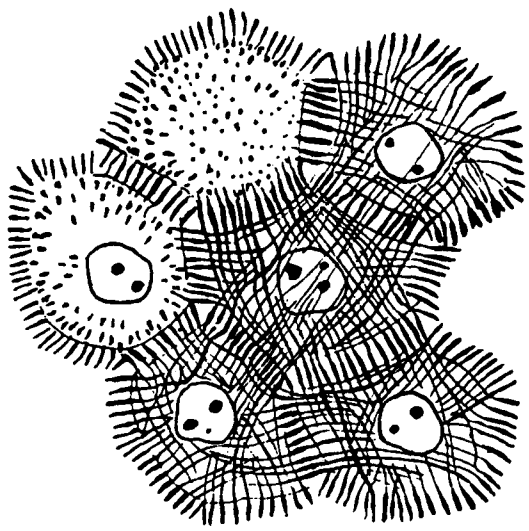

FIG. 1. The cytoskeleton of stratum germanativum as represented in an early histology text (*Bailey's Textbook of Histology*, 12th ed., p. 22).

reviewed (*e.g.*, Brimacombe and Webber, 1964; Katchalsky, 1964; Schiller, 1966; Quintarelli, 1968; Schubert and Hamerman, 1968; Hunt, 1970; Laurent, 1970; Fell and Dingle, 1975; Comper and Laurent, 1978; Oschman, 1978; Chakrabarti and Park, 1980; Hay, 1981*a*, *b*; Berger *et al.*, 1982; Hynes and Yamada, 1982).

CYTOPLASMIC GROUND SUBSTANCE

Cienkowski (1863) was one of the first to use the term "ground substance" to describe the "homogeneous" interior of the cell. It was not long before histologists began to resolve various strands, filaments, and fibers extending throughout the cytoplasm (Fig. 1). Our understanding of the nature of this material has been greatly expanded during the last century, and particularly in the last decade. The old concept of "protoplasm" as an amorphous colloidal suspension or "soup" of organelles and inclusions has given way to a more dynamic picture in which strands and arrays of fibrous elements form and reform an exquisitely ordered flexible moving network called the cytoskeleton (reviewed by Brinkley, 1982). One of the most exciting developments during the recent period has been the recognition that muscle-like contractile proteins are present in virtually all cells, including those of plants (reviewed

FIG. 2. Actin filaments in a cultured fibroblast, as revealed by fluorescent antibodies. From Lazarides and Revel, 1979, Scientific American 240, p. 101.

FIG. 3. Isolated cytoplasmic matrix of a HeLa cell. Microfilaments, ribosomes, centrioles, microspikes, and 100 Å filaments are present, while membranes and microtubules are extracted. From Lenk, Ransom, Kaufmann, and Penman, 1977, Cell 10:67–78.

by Pollard and Weihing, 1973; Clarke and Spudich, 1977).

We now recognize that the "structureless" cytoplasmic ground substance in fact contains various filamentous proteins, tubulin, actin, myosin, intermediate filaments, and microtubules. These are able to form polymers, depolymerize, cross-link, intertwine, anchor, bind, elongate, and contract. This gives rise to cell movements, shape changes, cytoplasmic streaming, pigment migrations, pinocytosis, movement of organelles, phagocytosis, secretion, mitosis, the myriad of activities that constitute life. In each case a labile framework seems to be involved. The framework is a dynamic structure maintained by an equilibrium between a cytoplasmic pool of protein monomers and their linearly aggregated polymers, which constitute the various tubules and filaments (Inoué and Sato, 1967).

Vivid demonstrations of the cytoskeleton as a whole system have been made by using fluorescent antibodies to specific cytoskeletal proteins (Lazarides and Weber, 1974; Lazarides and Revel, 1979). Figure 2 shows an example of this, the actin filament array in a "resting" fibroblast in culture. The actin filaments are organized into linear bundles, some of which extend the entire length of the cell.

High voltage electron microscopy reveals an even finer system of microtrabeculae comprising an ordered lattice of slender strands that interconnect membranes, ribosomes, cytoskeletal elements, and other organelles (Wolosewick and Porter, 1979; Ellisman and Porter, 1980).

The cytoplasmic matrix can now be isolated intact, using mild detergents that disrupt the cell membrane and permit the extraction of soluble components of the cell interior (Brown *et al.*, 1976; Lenk *et al.*, 1977; Schliwa *et al.*, 1981). Figure 3 shows the isolated cytoplasmic matrix of a HeLa cell. The cell was lysed with a weak nonionic detergent, Triton X-100. The

isolated cytoskeleton contains 100 Å filaments, microfilaments, ribosomes, centrioles, and microspikes, while most of the cell protein, membranes, and microtubules are extracted.

The feasibility of isolating the cytoskeleton intact may open a new era in studies of cytoplasmic organization. For there is mounting evidence that the various enzymes previously thought to be soluble within the cytoplasm may instead be associated with structure.

In 1965, Green *et al.* reported evidence that glycolytic enzymes may be associated with membranes. These workers noted that the concentration of hemoglobin within the erythrocyte was sufficient to form a highly ordered viscous medium that would severely hinder diffusion of enzymes and substrates. It therefore seemed unlikely that the glycolytic enzymes were simply free within the soluble cytoplasmic phase. The entire glycolytic pathway may be a supramolecular multienzyme complex intimately associated with the cell framework. A complete glycolytic complex can be isolated from *E. coli* as a particulate subunit with a molecular weight of 1,600,000 (Mowbray and Moses, 1976).

The designation of the glycolytic enzymes as "soluble" refers to the ease with which they can be extracted by aqueous solutions. It now appears that in the intact cell weak forces may keep these enzymes associated with particular sites on membranes and on the cytoskeleton (Arnold and Pette, 1968; Mowbray and Moses, 1976; Opperdoes and Borst, 1977; Walsh *et al.*, 1977). This conclusion has been supported by histochemical findings (Takeuchi and Kuriaki, 1954; Fahimi and Amarasingham, 1964). Other enzymes, previously thought to be soluble, may well be associated with cellular structure (reviewed by Masters, 1978, 1979, 1981).

It is not known precisely what the ionic strength, pH, and other properties are within the interstices of the cytoplasmic matrix. However, it has been suggested that under the physiological conditions thought to be present within the muscle cell, 100% of the aldolase may be bound to actomyosin (Arnold and Pette, 1968). Figure 4 shows the binding of aldolase with troponin as visualized by Masters (1981).

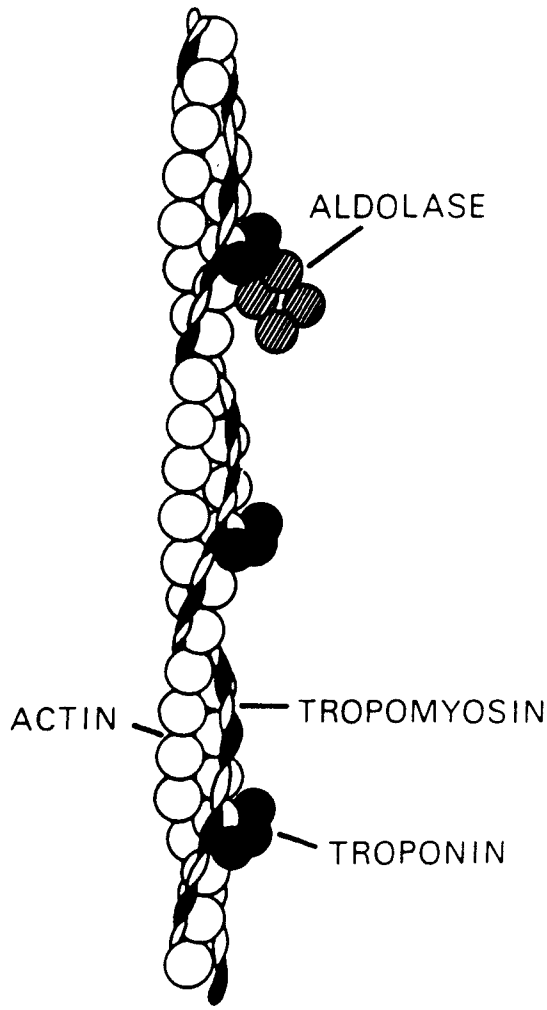

FIG. 4. Binding of a glycolytic enzyme, aldolase, to actin thin filament in muscle. Other glycolytic enzymes (GAPDH, PFK, LDH, PK, etc.) bind as well. After C. J. Masters, 1981, C.R.C. Crit. Rev. Biochem. 11:133.

Association of enzymes with structure has the advantage of increased efficiency and potential for control (Reed and Cox, 1966). It has been suggested that the solvent capacity of the cell may be so limited that it is not possible to adequately solvate all "soluble" enzymes and metabolites as well (Atkinson, 1969). Indeed, even the metabolites may be protein-bound (Sols and Marco, 1970).

Hence a new view of cell structure that is emerging suggests that within the highly ordered ground substance and adsorbed enzymes there is little room for random

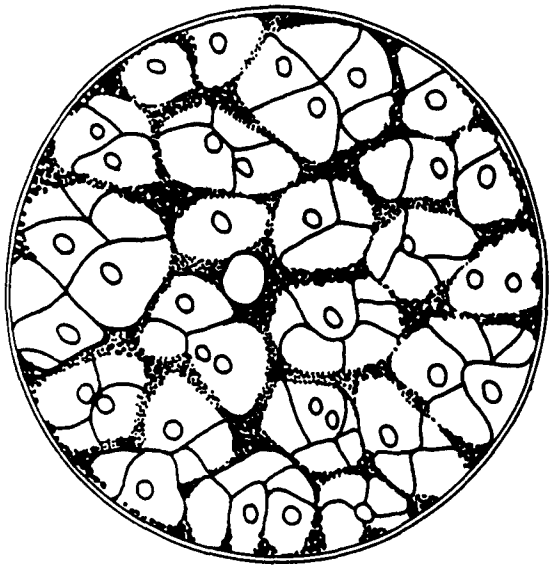

FIG. 5. The nuclear matrix, based on Ramón-Cajal (1933).

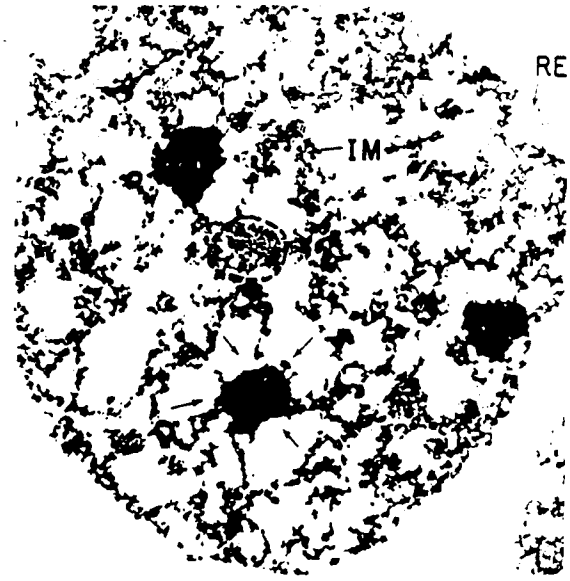

FIG. 6. Isolated nuclear matrix from rat liver. From Berezney and Coffey, 1977, J. Cell Biol. 73:626.

diffusion and convection of metabolites. This may account for the reduced diffusion coefficients for small molecules within the cell interior compared to aqueous solution (Harris, 1957; Harris and Prankerd, 1957; Fenichel and Horowitz, 1963; Dick, 1964; Ling and Cope, 1969). Multienzyme aggregates with restricted diffusion pathways impart economy of structure and function.

In concluding this section, we can say that the cytoplasmic ground substance appears to be organized as a three-dimensional network of microtubules and microfilaments, between which lies an even smaller meshwork of microtrabeculae. Upon this matrix are orderly arrays of enzymes. What is emerging is a new level of supramolecular integration of cytoplasmic structure and function. We shall now see that this picture has a counterpart in the nucleus.

NUCLEAR MATRIX

Histologists have long noted a fibrous scaffold within the nucleus. Figure 5 shows the nuclear matrix as represented in an early text (Ramón-Cajal, 1933). Modern study of nuclear structure has confirmed the presence of a fabric of non-chromatin fibrils that extends throughout the nucleus (reviewed by Agutter and Richardson, 1980). Termed the nuclear matrix, nuclear ground substance, or karyoskeleton, this non-chromatin network provides the immediate environment of the genetic material and the products of transcription. The matrix consists of a peripheral nuclear lamina, a nuclear pore complex, a "scaffold," and a fibrillar, less defined structure extending through the nuclear interior. This complex of structures has now been isolated by removal of chromatin and nuclear membrane phospholipids (Berezney and Coffey, 1977; Adolph, 1980). Figure 6 shows a nucleus isolated from rat liver. Electrophoresis of the nuclear matrix reveals that it is composed of three major and several minor polypeptides.

We now know that the nucleus of a single cell may contain some 10,000 copies of each of 450 non-histone, chromatin-associated proteins (Peterson and McConkey, 1976). One of these proteins is actin (Fukui and Katsumaru, 1979; Bremer *et al.*, 1981).

Glycoproteins are also present within the nucleus (Margolis *et al.*, 1976; Van Ness *et al.*, 1982). One of these glycoproteins appears to be exclusively associated with the nuclear envelope during interphase, but becomes dispersed throughout the cell in prophase when the nuclear envelope is disassembled, only to return to the nuclear

envelope at telophase (*e.g.*, Gerace *et al.*, 1982).

MITOCHONDRIAL MATRIX

The inner compartment of the mitochondrion is filled with a more or less continuous matrix composed of protein and some lipid. It is thought to be an organized semi-rigid system (Lehninger, 1965). The relationship between this matrix and the cytoplasmic matrix is unclear at present.

LINKAGES BETWEEN COMPARTMENTS

We have briefly summarized the discovery and properties of separate extracellular, cytoplasmic, and nuclear ground substances, and mentioned that mitochondria also possess an internal matrix. Let us now focus on the relationships between these matrices, and ask whether they form a continuous network, or if there are missing links or discontinuities at their boundaries.

There is mounting evidence that the cytoskeleton is anchored through the cell membrane to the extracellular material. An important early step was the discovery of trans-membrane glycoproteins (Bretcher, 1971, 1973; Marchesi and Andrews, 1971; Marchesi *et al.*, 1972, 1973). It was soon recognized that the sialic acid-rich extensions of these glycoproteins from the cell surface are the sites where the cell acknowledges extracellular proteins, whether they be antigens, hormones, cell-cell linkers, or anchors to the substrate. Moreover, it is now clear that the cytoskeleton is capable of positioning and repositioning these receptors upon the cell surface (Albertini and Clark, 1975; Nicolson, 1975; Poste *et al.*, 1975; Gabbiani *et al.*, 1977; Koch and Smith, 1978; Chen, 1982; Rapraeger and Bernfield, 1982).

The discovery of fibronectin, chondronectin, and laminin, and their probable roles in linking together the cell surface, basement membrane, and extracellular matrix has begun to fill in the remaining gaps, enabling us to trace the continuity of cytoplasmic and extracellular matrices. Figure 7 shows a recent model of the role of fibronectin, based on Hynes and Yamada (1982). It has been suggested that collagen, laminin, heparin sulfate proteoglycan, and

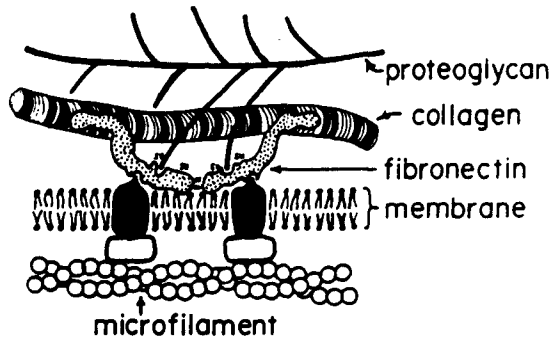

FIG. 7. Hypothetical role of fibronectin in linking cytoplasmic actin microfilament across cell surface to extracellular matrix elements including proteoglycan and collagen. After R. O. Hynes and K. M. Yamada, 1982, J. Cell Biol. 95:374.

fibronectin may form an "integrated complex" that constitutes the basal lamina and its extensions, providing support and adhesion between cells and the connective tissue stroma (Laurie *et al.*, 1982).

In recent reviews, Elizabeth D. Hay (1981 *a*, *b*) has summarized four current models of the trans-membrane arrangements of collagen, fibronectin, proteoglycan, and hyaluronic acid. In addition, Hay has emphasized that these molecules form a continuous system: "It is important to remember . . . that the extracellular matrix and the cell surface form a continuum, in the sense that the adjacent extracellular matrix is a part of the cell and the cell, of the extracellular matrix."

Likewise, Berezney *et al.* (1982) have recently suggested that the nuclear ground substance may be an extension of an overall cell matrix that is distributed throughout the cell, from the plasma membrane to the nuclear interior.

A densely woven perinuclear filamentous network has been identified by polarization microscopy (Blose and Chacko, 1976) and by electron microscopy (Small and Celis, 1978). A spectacular example of a relationship between the nuclear envelope and the cytoskeleton is given by the cellular geodome structure described by Lazarides and Revel (1979) and shown in Figure 8. This structure is composed of actin, tropomyosin, and alpha-actinin. The latter substance is located at the vertices of the network, which appear to be organiz-

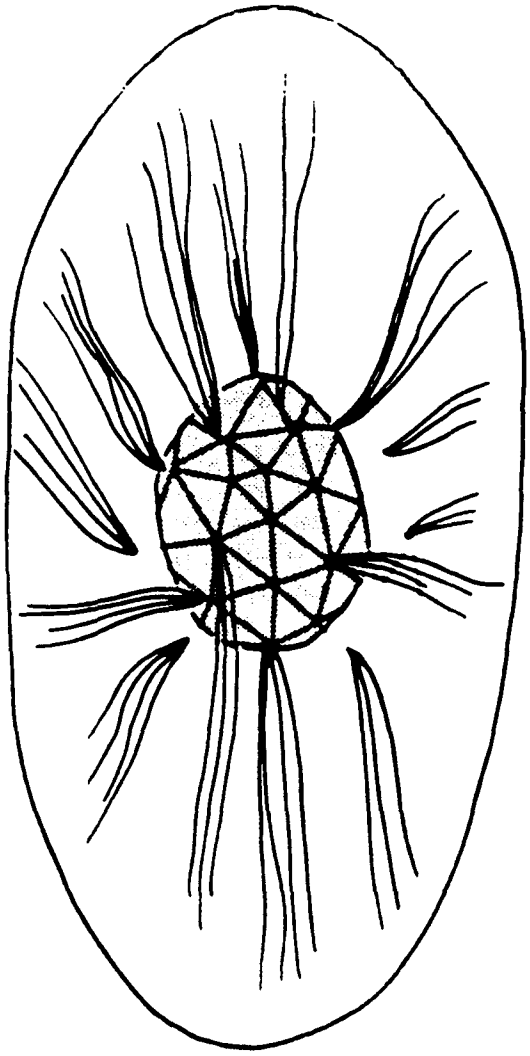

FIG. 8. Cellular geodome structure composed of actin, tropomyosin, and alpha-actinin. From Lazarides and Revel, 1979, Sci. Am. 240.

ing centers where g-actin polymerizes into f-actin filaments to form the cytoskeleton.

There are now indications that the nuclear ground substance includes a microtrabecular lattice, much like that found in cytoplasm (Berezney *et al.*, 1982).

Little is known of the relationship between the mitochondrial matrix and the cytoskeleton. A recent review suggests that this is "a promising hunting ground for future researchers" (Ernster and Schatz, 1981).

RELATIONSHIPS WITH WATER AND IONS

It is well known that the physical and chemical properties of macromolecules are profoundly influenced by their aqueous and ionic environment. Much work has been done to clarify the nature of these interactions. One approach has been to begin to build up models of the matrix of relationships from quantum mechanical principles. The results indicate that solvent and counterions may be structured as well.

A few years ago this building up of structure would have been impossible, because the application of quantum principles to molecular structure was limited by the huge computations required to deal with the wave equations for structures containing more than a few atoms. Advances in computer technology have now made it possible to model complex systems. Larger and faster computers are not the whole story, for a conceptual breakthrough has occurred as well. This advance involves the appropriate use of statistical mechanics to extend the atomic and molecular orbital models to many atom systems and to large numbers of mutually interacting molecules. Some 20 years of research have gone into the development of a set of models such that the output of one model constitutes the input for the successive one.

Let us examine some of the results from computational chemistry that are relevant to the study of the living matrix and its relationships. The molecule that has been given most detailed treatment is DNA. Since DNA is intimately associated with the nuclear matrix (Berezney *et al.*, 1982), it is appropriate to consider it as part of the ground substance.

The usual representation of the DNA molecule (Fig. 9), reproduced in countless texts, is physically impossible. A structure such as this would fly apart due to the electrostatic repulsion between the highly charged phosphate groups. What holds the molecule together in nature are the counterions and water molecules that associate with and neutralize the charged residues.

DNA or any other molecule has a certain degree of flexibility. Its parts are vibrating, folding, and twisting in response to thermal perturbations. There are two ways of dealing with thermal motion of a molecule. The simplest is to determine an average or equilibrium or energetic minimum molec-

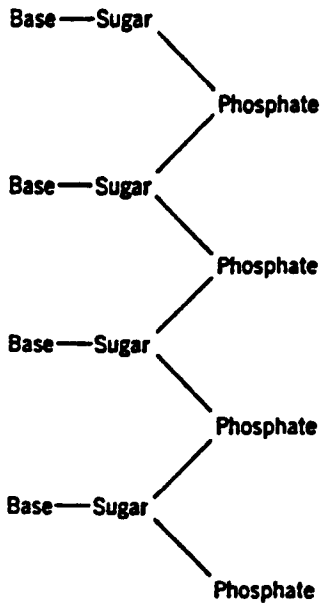

FIG. 9. The nucleic acid backbone of DNA. The bases are side chains on the repeating sugar-phosphate polymer chain. As represented here without the counterions, this structure is unstable due to the mutual repulsion of the highly charged phosphate groups.

ular structure. A more difficult but more realistic technique is to use molecular dynamics. Here the atoms comprising a molecule move about under the influence of their own kinetic energy and the forces exerted on them by surrounding atoms. With this more dynamic approach, the simulation never converges on a single conformation. Instead the molecule continuously changes and moves about in space. In practice one begins with the first approach, at low temperature, to estimate the starting coordinates of the molecule. The temperature is then raised in steps until the atoms reach the velocities appropriate to body temperature.

It is also necessary to consider polarization phenomena. The various atoms within a molecule can be visualized as clouds of electrons that are readily polarized by the strong electric fields of counterions or the highly polar water molecule. When a counterion or a water molecule approaches a protein or a nucleic acid, the shapes of the electron clouds within the molecule can be altered. This in turn affects the electrical properties of the approaching counterion or water molecule. Thus it is necessary when introducing a relationship to recalculate the energy structure of the system again and again until a final induced dipole moment is determined. These approaches have been reviewed by Levitt (1982).

Enrico Clementi and his colleagues at IBM have used as many as four large computers to develop quantum mechanical and thermodynamic models of molecules of increasing complexity. Beginning with single atom systems and amino acids, the studies have progressed to considerations of nucleic acid bases, base pairs, a DNA single helix, and finally the DNA double helix (see Corongiu and Clementi, 1981*a*, *b*, for references). Recent results are for a B-DNA fragment of 12 base pairs, *i.e.*, two more than needed to reproduce a full turn of the double helix. Included are the corresponding sugar and phosphate units. The modeled DNA fragment is placed within a cylindrical space 36 Å high and 29 Å in diameter. Monte Carlo simulations are used in which counterions and water molecules are added to the system, one at a time, to see what position they will take up in relation to the DNA molecule. Each time another ion or water molecule is added to the system, the DNA structure must be recalculated to accommodate the influence of its new neighbor. The probable locations of counterions and water molecules are determined for 2,000,000 different possible conformations of the DNA helix at 300°K.

Let us first examine the models of counterion distribution (Clementi and Corongiu, 1982). Figure 10 shows the probability distribution of the Na counterions in a view down the axis of the DNA molecule. This ionic distribution occurs in the presence of 400 water molecules, which are not shown here. The counterion concentration is such that one counterion is available for each phosphate group.

Figure 11 shows a comparable side view of the DNA helix. Note that in both of these illustrations the counterions are not

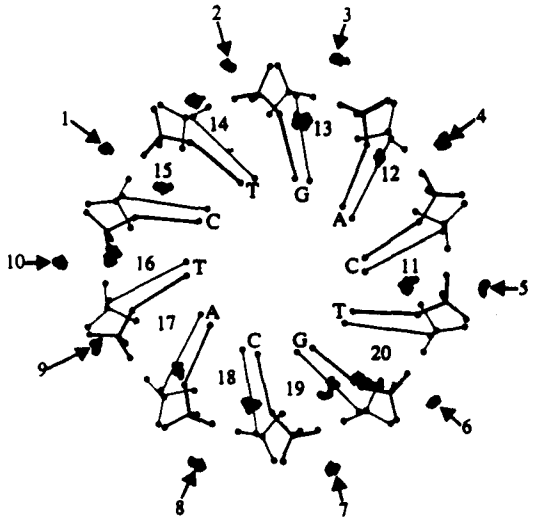

FIG. 10. Probability distribution of Na counterions in relation to a model of the DNA molecule, here viewed down its axis. This is the ionic distribution in the presence of 400 water molecules, not shown, and with counterion concentration sufficient to make one counterion available for each phosphate group. From E. Clementi, 1981, IBM Journal of Research and Development 25:324.

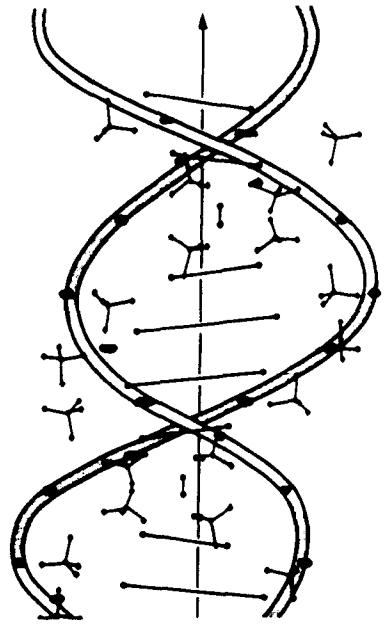

FIG. 12. Same as Figure 11, except that the counterions are drawn as part of a double helix-like structure. From E. Clementi, 1981, IBM Journal of Research and Development 25:325.

described as points, but as irregularly shaped volumes that represent their most probable locations.

Finally, Figure 12 shows the same situation except that the counterions are drawn as belonging to a double helix-like structure. This is a real structure in the sense that it represents the mutual interactions of the counterions with each other and with the phosphates of the DNA. The two ionic helices have different structures, one extending farther from the DNA axis than the other. The counterions of the inner helix are coordinated to the two phosphates and the bases of one strand, whereas the ions of the outer helix are coordinated mainly to the phosphates of the other strand.

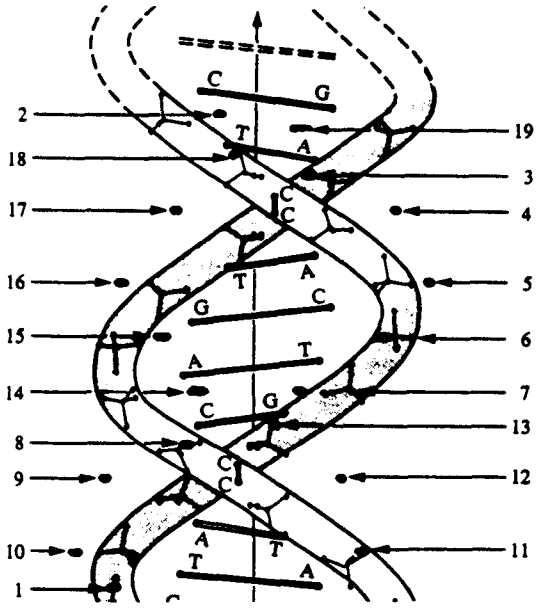

FIG. 11. Comparable side view of DNA helix and associated counterions. From E. Clementi, 1981, IBM Journal of Research and Development 25:325.

A fascinating aspect of these results is a predictable charge transfer from the phosphate and sugar groups to the bases as a result of the field of the counterion. This charge redistribution could convert the DNA molecule from an insulator to a weak semiconductor (see also Clementi, 1971; Ladik and Suhau, 1980). Such charge transfers occur widely in biological systems (*e.g.*, Szent-Györgyi, 1960). An implication is that biological systems may have the potential to serve as models for electronic devices (Clementi, 1981). Likewise, the

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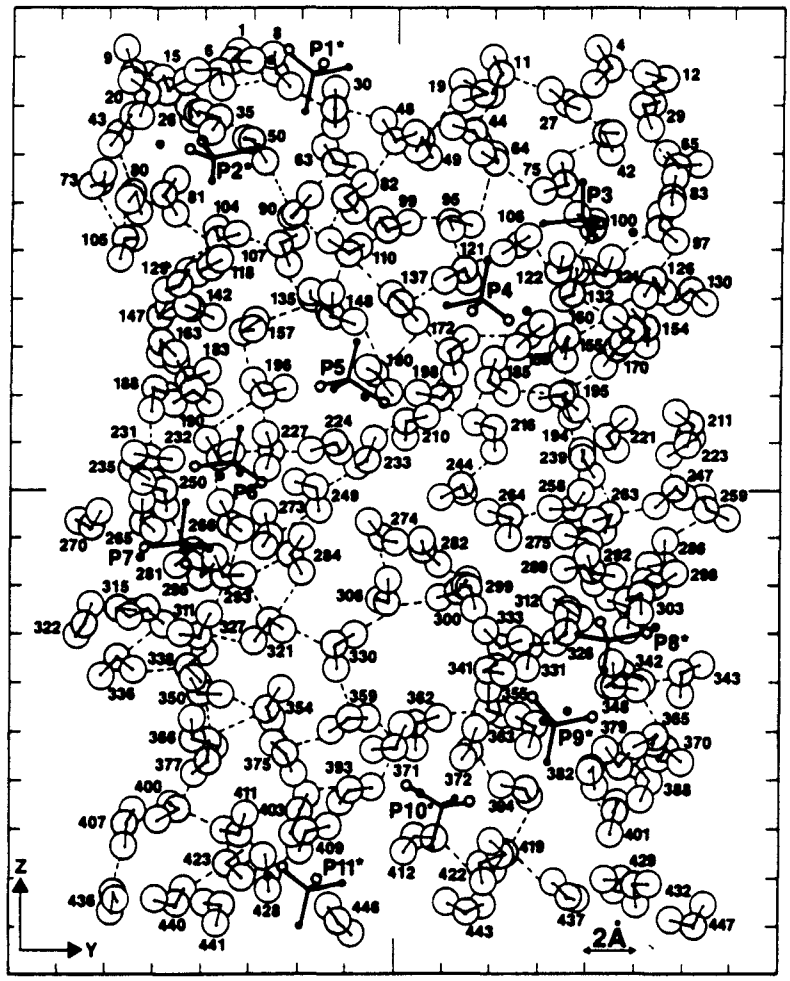

FIG. 13. Relationship of Na-DNA with water molecules, from Monte Carlo simulation. Here 447 water molecules, approximating the condition of 95% relative humidity, are shown in their equilibrium positions. Dotted lines show hydrogen bonds that join water molecules into cross-linked filaments that lie both parallel to the DNA axis and along the helix. From G. Corongiu and E. Clementi, 1981, Biopolymers 20:2460.

properties of electronic devices may provide insights into biological functions.

Now let us examine the relationship of Na-DNA with water molecules (Fig. 13). Here the counterions are kept at fixed positions during the addition of the water molecules, a reasonable approximation because of the very strong attraction of the phosphates. The illustration shows the addition of 447 water molecules, approximating a condition of 95% relative humidity.

Of considerable interest is the discovery that the water moleules associated with the DNA are held in positions that allow them to associate with each other by means of hydrogen bonds. In the illustration hydrogen bonds are shown as dotted lines, and are only indicated when the oxygen-oxygen distance between two water molecules is equal or smaller than 3.5 Å, and if the oxygen-hydrogen distance is smaller than the corresponding oxygen-oxygen distance.

The water molecules enclose the phosphate groups and also form cross-linked filaments that extend across the grooves, parallel to the DNA axis, as well as from phosphate to phosphate along the helix. These water filaments are statistically stable and meaningful structures that are likely

to influence the dynamic and temperature-dependent properties of the DNA molecule. Moreover, it is thought that protons can be transferred preferentially along the filaments.

The image presented by Figure 13 represents an average configuration. In a living system the water filaments would be constantly forming and reforming. The fact that a structure has a short lifetime does not mean that it lacks significance. The active form of an enzyme, for example, may have a short lifetime, yet it is this structure that has the most biological impact.

This model of solvent structure is a simulation based on a set of mathematical relationships, *i.e.*, an *ab initio* model. It has resulted in a prediction, the filaments, which may be tested by comparison to experimental data. Corongiu and Clementi (1981*a*) have compared their simulations with experimentally determined absorption and desorption isotherms. The comparison is made through a tedious process: water molecules are subtracted (as would occur with a decrease in relative humidity) and the Monte Carlo simulations are reperformed, allowing the water molecules to rearrange themselves. When this is done, the isotherm has a nicely sigmoidal shape comparable to the experimental data. It appears that the sigmoidal shape of the curve is the result of an hysteresis that can be accounted for in part by the fact that when one water molecule is removed the DNA structure rearranges. This in turn induces a rearrangement of the remaining solvent structure. Hence the highly ordered texture of the solvent is intimately connected with the DNA structure and stability.

CONCLUSIONS

This essay has summarized some aspects of our understanding of the extracellular, cytoplasmic, and nuclear matrices or ground substances. The discovery of glycophorin, fibronectin, and related substances has provided a structural basis for the suspected link between the cytoplasmic and extracellular ground substances. It has also been suggested that the nuclear matrix is a continuation of the cytoplasmic matrix. If this proves correct, it is possible that the ground substance forms a more or less continuous supramolecular network extending throughout the animal body.

The concept of a molecular fabric of the animal body, extending into all of its nooks and crannies, is certainly not new. Two hundred years ago, Haller (1779) described an extracellular fibrous matrix that is now known as the connective tissue: "This web-like substance in the human body is found throughout the whole, namely, wherever any vessel or moving muscular fiber can be traced, without exception. The principal use of the fabric is to bind together contiguous membranes, vessels, and fibers . . . ." And Lamarck (1809) noted that all of the organs in animals without exception are enveloped in this material. The well-accepted concept of the connective tissue as a continuous system throughout the animal body applies with equal force to the ground substance matrix, which forms a finer reticulum within the intervals between connective tissue fibers, and appears to link them, via the cell surface, to the cytoplasmic and, most probably, the nuclear matrix.

Figure 14 summarizes some of the relationships between extracellular, cytoplasmic, and nuclear ground substances. There is emerging a detailed description of the continuity of this fabric from tissue to cytoplasm to nucleus. The continuity across the cell surface is well documented, although details are still being worked out. Figure 7 presented one model of the role of fibronectin in linking the cytoplasmic actin filaments to extracellular collagen fibers. Hay (1981*a*, *b*) presents several other models.

The relationship between the cytoplasmic and mitochondrial matrices is an open question. Likewise one would like to have more details of the arrangement at the nuclear-cytoplasmic interface.

All parts of the ground substance are intimately associated with counterions and water molecules. The structure of the matrix is profoundly influenced by those relations. And the counterions and water interact with each other as a result of their

B

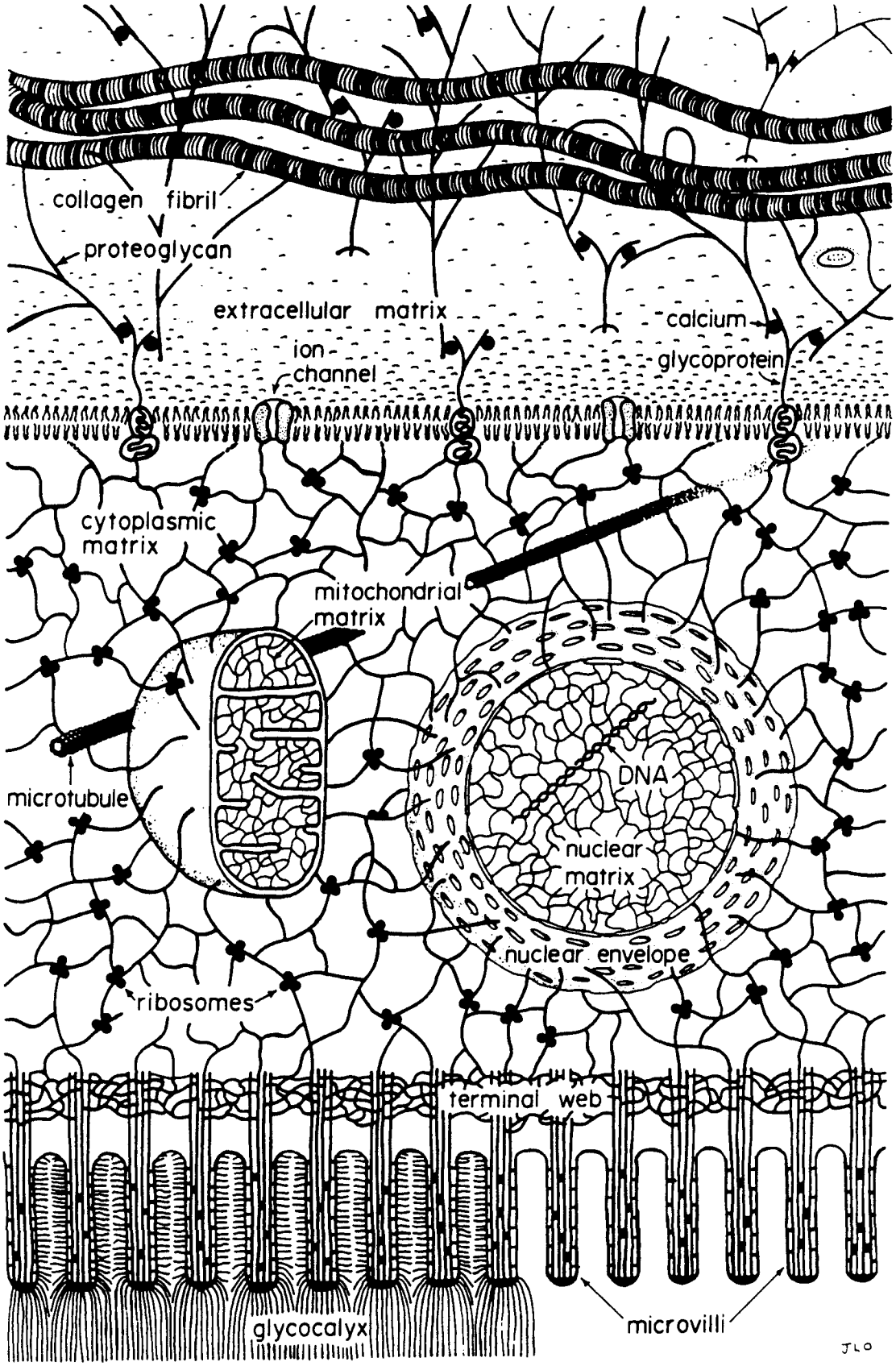

FIG. 14. Summary of the distribution of ground substances in the nucleus, mitochondria, cytoplasm, and extracellular matrix.

association with the ground substance. It appears that water and ions may form a matrix as well.

The association of the ground substance with enzymes previously designated as "soluble" indicates that metabolic pathways may be structured upon the membranes and ground substance fabric. We have come a long way from the time when a cell was regarded as a bag full of protein and electrolyte solutions. Enough structure has been discovered to virtually fill the bag, and one wonders if there remain any open channels through which diffusion and convective flow can occur.

The picture that is emerging has had a long history, during which the focus of biological thought has shifted from one component of the living organism to another. For example, Picken (1960) has recounted the urgent discussion that long ago took place around the question of the relationship between cells and the ground substance in which they are embedded. Schwann (1839) had suggested that the extracellular matrix is the basis of life, and that cells form within it "according to definite laws." After 2 decades of debate, Virchow (1859) concluded differently: the extracellular matrix is "in definite dependence on the cells" which had become "the truly elementary units that characterize everything that lives." The cell then became the fundamental "atom" of life. None of the intricate chemical processes of the living state could take place, it was thought, except within a cell. This idea was shattered by the discovery that fermentation could be performed by a cell-free extract of dissolved molecules and enzymes. There soon arose a molecular prejudice: living matter, being built of molecules, must have as its basis a set of molecular reactions.

A classical physicist looking at this picture might suggest that both the cellular and molecular views do not consider the realm of electrons and protons, obvious candidates for the fundamental building blocks and units of energy in nature.

A modern physicist might offer a totally different perspective based on quantum theory. This could be characterized as a systems view in the sense of general systems theory (von Bertalanffy, 1968; Laszlo, 1972). According to this perspective, any system, whether an atom or an organism, is less a machine made up of numerous parts than a dynamic whole whose parts are interrelated.

From the systems perspective, a search for fundamental units is replaced by study of the web of relations between the various parts of the whole. Heisenberg (1958) stated it this way: "The world thus appears as a complicated tissue of events, in which connections of different kinds alternate or overlap or combine and thereby determine the texture of the whole."

These concepts, summarized in a recent book by Capra (1982) can be profitably applied to the molecular fabric of the animal body that is emerging from the research summarized here. The interconnected network shown in Figure 14 has no fundamental unit, no central aspect, no part that is primary or most basic. For the functioning of the whole network depends upon the integrated activity of all of the components.

Hence the study of organs, tissues, cells, molecules, atoms, and charged particles all contribute equally to our knowledge of the living state. One way to integrate this information is to build up a picture of living matter that includes the great contributions and discoveries of the various disciplines.

The ground substance matrix provides one point of departure for the integration of physiological, ultrastructural, and molecular discoveries with quantum mechanical principles. One wonders the extent to which the cooperative, integrative, communicative, transductive properties of the ground substance may serve to order and integrate the rapid and subtle activities of the living system.

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