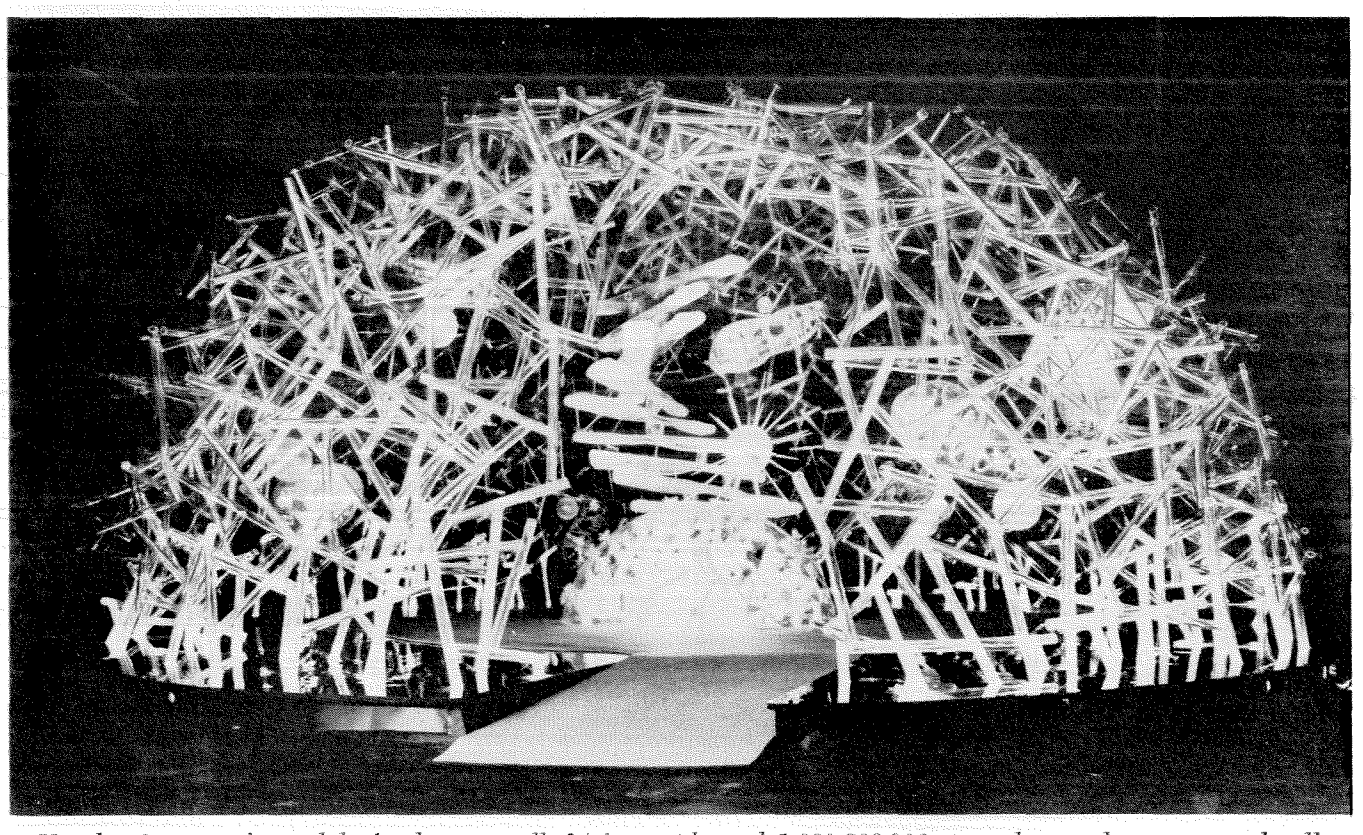


Upjohn Company's model of a human cell, 24 feet wide and 1,000,000,000 times larger than an actual cell.

The Biology of Microsomes

by James Bonner

These are stirring times in the world of biology. We are beginning to find out a little bit about how proteins are synthesized by living creatures.

In recent years we have come to know a great deal about the structure of proteins—as, for example, that proteins consist of amino acids linked into long peptide chains, and that these chains are wound in helical form.

Now we are beginning to understand why individual proteins are different from one another, and why they have different enzymatic activities—even though they are all composed of the same 20 amino acid building blocks. We know that the individuality of a protein resides in part in the sequence in which its amino acid units are put together to form the protein peptide chain. But until recently the mechanisms by which the cell makes its many different enzymatic proteins was totally obscure.

It appears today that proteins are synthesized upon the surfaces of a particular kind of subcellular entity —the microsome. The microsome, in turn appears to be made in the nucleus. The microsome is not only the engine of protein synthesis but also the device whereby the coded information of the chromosome is carried to and utilized by the protoplasm of the growing cell in the synthesis of its individual enzymes.

Our new knowledge of protein synthesis has been made possible by the development during the past 10 years of methods for separating the cell into its component parts. These methods have, in part, been devised at Caltech by Samuel G. Wildman (now professor of botany at UCLA); George Laties, senior research fellow at Caltech; and others.

The individual cellular components, whose structure and nature we know in some detail through electron microscopy, are in general separated from one

Our modest understanding of microsome biology is leading us to an understanding of the problems which lie at the very base of biology

another quantitatively by differential centrifugation. Thus, a plant tissue is first ground at low temperature to rupture the cellulosic material of the cell wall and release the protoplasm. The broken cell walls and other cellular debris are then removed by centrifugation at a few hundred times gravity for a few minutes. The supernatant for this centrifugation may now be spun briefly at higher speeds—perhaps 1,000 to 2,000 times gravity—in order to sediment the relatively large and heavy nuclei. Centrifugation of the supernatant at still higher forces—about 12,000 x g—for a few minutes, results in quantitative sedimentation of the mitochondria, which are rod-shaped particles about 1 micron long, and hence visible in the light microscope.

The supernatant, after removal of the mitochondria, contains still further particulate matter. It may contain lipoprotein membranes—the so-called endoplasmic reticulum—although this is absent in some tissues. It also contains small spherical particles, the microsomes, which are attached to the membranes of the reticulum if this is present. Centrifugation of the mitochondria-free supernatant at forces of 100,000 x g for 30 to 60 minutes results in sedimentation of membrane and microsomal particles together.

In the case of the pea stem (worked on at Caltech by Paul Ts'o, research fellow in biology; Jerome Vinograd, research associate in chemistry; and myself) membranes are absent, and it is possible to obtain the microsomal particles in relatively homogeneous condition. The supernatant which remains after removal of the microsomes contains still further material. It is in this residue that the individual soluble enzymes of the cell—the common everyday garden varieties of enzymes—are to be found.

The microsomal particles prepared by centrifugation are homogeneous in the ultracentrifuge. They appear in the electron microscope as oblate spheroids with a major diameter of 280 angstroms and a molecular weight of about 4 million. They are composed of 40 percent ribonucleic acid (RNA) and the balance of their mass is entirely protein.

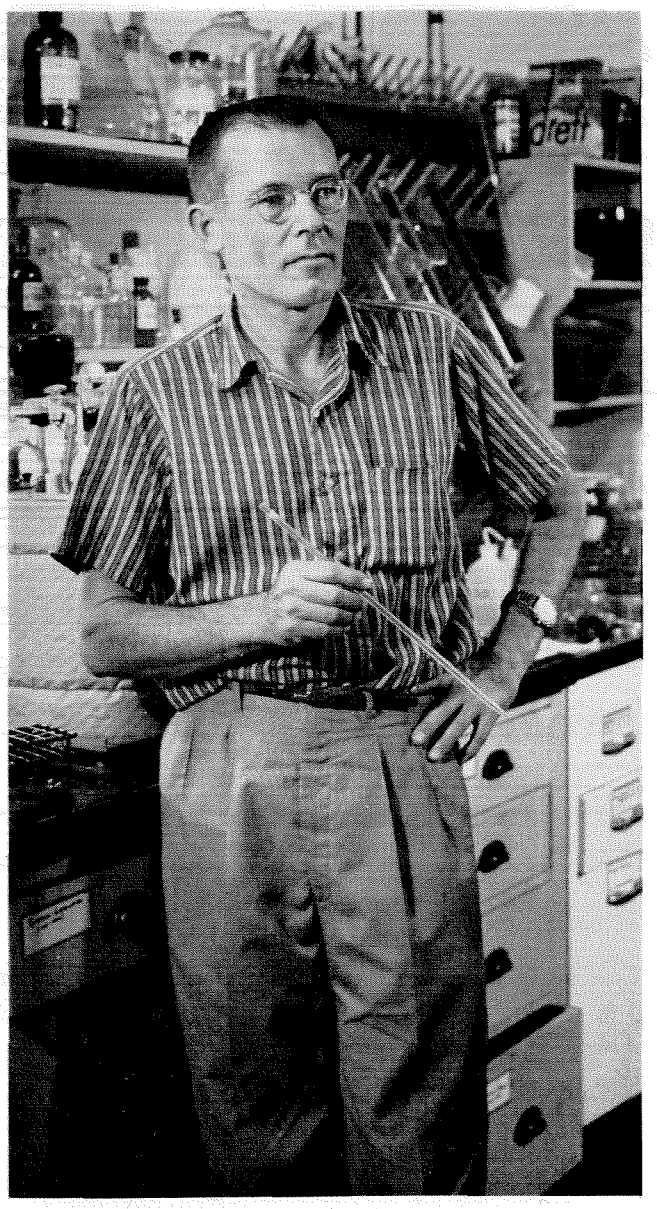
Interestingly enough, microsomal particles appear to be much the same in size, shape and chemical composition throughout a wide spectrum of living creatures. For instance, the microsomes of the pea plant, which we have studied, are very similar to those of yeast, which have been studied at the University of California at Berkeley by Fu Chuan Chao, graduate student, and Howard Shachman, professor of biochemistry. They are also very similar to the microsomes of immature red blood cells (reticulocytes) studied at Caltech by Jerome Vinograd and Howard Dintzis, assistant professor of chemistry.

Even the amino acid compositions of the microsomes of these different forms are closely similar. All are characterized by high contents of the basic amino acids lysine and arginine, and by high contents of the acidic amino acids glutamate and aspartate.

Research at Caltech by Paul Ts'o and his collaborators has revealed that the microsomal particle is composed of subunits: The microsome contains magnesium ions, and these magnesium ions bind the subunits together to form the intact microsomal particle. If about half of the magnesium is removed by suitable means, the microsome reversibly comes to pieces to form two new particles of masses two-thirds and one-third of the original, respectively (shown on p. 22).

Further removal of magnesium results in further disaggregation of the microsome, each original particle yielding two subparticles with a mass one-third of the original and two subparticles with a mass one-sixth of the original. Each of these microsomal subunits contain ribonucleic acid and protein in the same proportion as the original microsome—that is, they are ribonucleoprotein subunits. Binding of RNA to protein does not concern magnesium but is apparently due to hydrogen bonds.

The microsome is then made up of ribonucleoprotein units, the smallest of which is one-sixth of the



James Bonner, professor of biology and acting chairman of the Division of Biology.

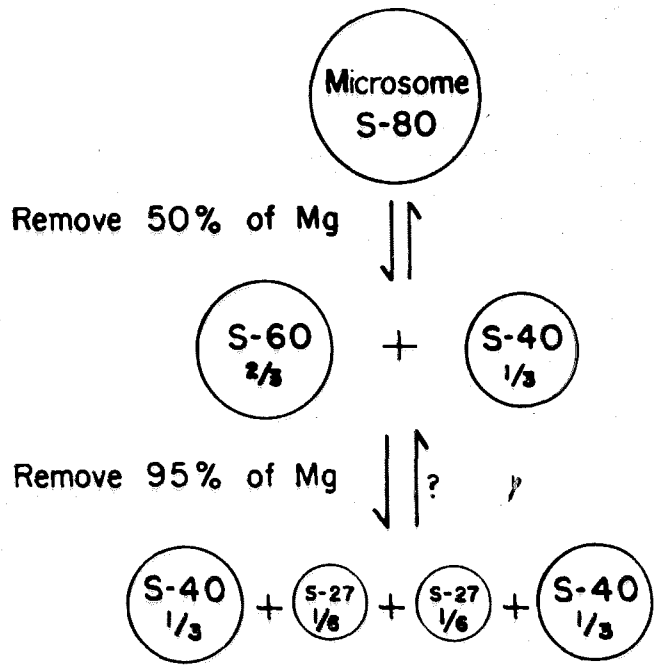
original microsomal particle. The molecular weight of the microsomal RNA appears to be close to that expected on the basis that the one-sixth particle contains but a single giant RNA molecule.

The molecular weight of this giant RNA molecule, approximately 280,000, may well place an upper limit on the amount of information which can be contained within the microsome. Thus, the elementary RNA chain of the microsome is about 900 nucleotide units in length. Current coding theories suggest that a sequence of at least 3 nucleotide residues are required to determine the position of each amino acid in a peptide chain—supposing, of course, that RNA does somehow determine amino acid sequence. Accordingly, an RNA chain 900 nucleotides in length could code in sequence no more than 300 or so amino acids. A protein 300 amino acids in length would have a molecular weight of about 30,000. This is indeed the average molecular weight of the mixture of cellular proteins. It is apparent then that each microsome contains information sufficient to make only one or, at most, a small number of kinds of proteins.

It was first suspected that the microsome has a role in protein synthesis because of the fact that when C^{14}-labeled radioactive amino acid is supplied to living tissues it appears most rapidly in the microsomal fraction—a fact first noted at Caltech by Henry Borsook, professor of biochemistry, and his group in 1950. Although the microsomes of living tissue become labeled very rapidly in the presence of labeled amino acid, they do not become highly labeled and they reach a plateau within a short period of time. At this plateau, or steady-state level of labeling, about one-tenth to one-half percent of the amino acid of the microsome has become labeled. This suggests immediately that some small portion of the total microsomal protein is capable of rapidly incorporating amino acid, the remainder of the microsomal structure being relatively inert.

In addition, the labeling of microsomes in the steady state is transitory. Labeled amino acid, once incorporated into the microsome, may be washed out again if the labeled amino acid is replaced by unlabeled. This is not true of the incorporation of labeled amino acid into the proteins of the soluble cytoplasm, for example. The kinetic evidence available is in agreement, then, with the hypothesis that microsomes somehow assemble amino acids into growing peptide chains and finally into protein molecules, which are then shed from the microsome to appear as soluble protein.

Where do little microsomes come from? Cellular particles such as nuclei, chloroplasts and mitochondria multiply by division but this does not appear to be



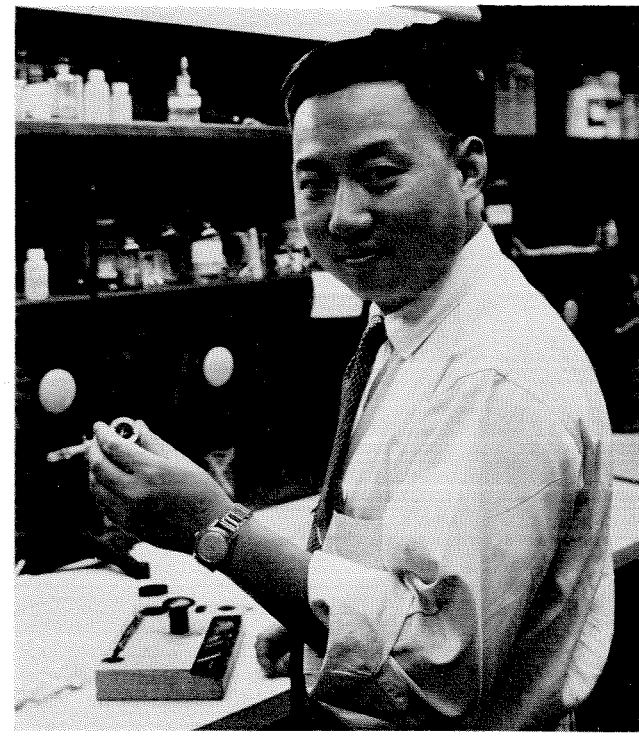
When microsomes are robbed of their magnesium they come apart into fragments. S is the unit for measuring the rate of sedimentation in the ultracentrifuge.

the case for microsomes. On the contrary, microsomes appear to be synthesized within the nucleus. Thus, with the electron microscope, objects resembling microsomes can be seen within the nucleus. In addition, it has been possible to isolate particles physically and chemically identical with microsomes directly from preparations of purified nuclei.

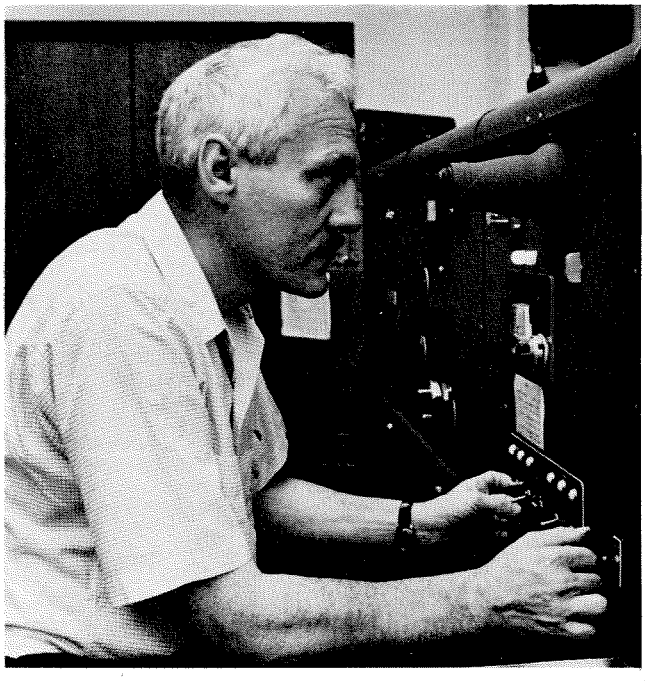
This has been done by Paul Ts'o and Clifford Sato at Caltech and also by Soyozo Osawa and his group at Nagoya University. Particles identical with the cytoplasmic microsomes in molecular weight, RNA content and other characteristics have been prepared from such isolated nuclei.

Microsomes are then contained in the nucleus. Are they in fact synthesized within the nucleus? It has been known for some years that the nucleus is the seat of active RNA synthesis and that much of the RNA of the cell is formed there. Cells which do not contain nuclei do not possess the ability to form RNA, or at least they form RNA sluggishly. Cells which contain nuclei possess the ability to form RNA abundantly. If tissues containing nuclei are supplied with labeled precursors of either RNA or protein, the label is recovered in the nuclear microsomes at relatively high levels of activity, as would be expected if microsomes are synthesized within the nucleus.

And, finally, the matter has been approached directly in the laboratory of Alfred E. Mirsky, a former Caltech research fellow who is now a member of the Rockefeller Institute. In the experiments of Mirsky, V. G. Allfrey and Syozo Osawa, the synthesis of RNA and of protein was studied in isolated nuclei from the thymus gland. In these experiments, it has been shown that ribonucleoprotein (material which we now believe to be in part nuclear microsomes) is formed in the nucleus, and that the formation of such material takes place only in nuclei containing intact DNA (deoxyribonucleic acid), intact chromosomal material. Treatment of the isolated nucleus with the enzyme DNAase, which destroys DNA, abolishes the ability of the nucleus to synthesize ribonucleoprotein.



Paul Ts'o, research fellow in biology and leader of the Caltech group studying microsome biology.



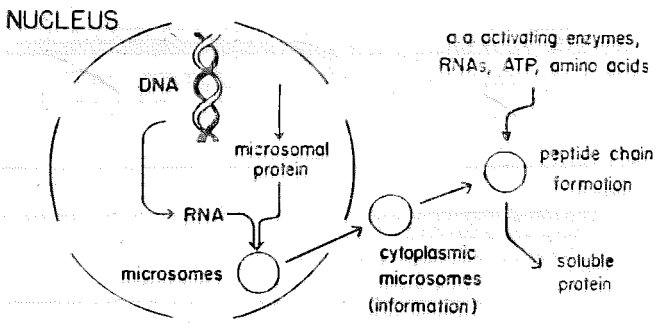
Jerome Vinograd, research associate in chemistry, at the analytical centrifuge.

How do the microsomal particles, once made in the nucleus, escape into the cytoplasm? Experiments with amoebae by L. Goldstein and Walter Plaut, research fellows at the University of California in Berkeley, have shown that such escape does take place.

In these experiments, nuclei containing labeled RNA were transplanted to unlabeled cytoplasm of a second amoeba. The RNA from the labeled nucleus escaped to and filled the cytoplasm of its host. No experiment has as yet been done, however, which directly shows the movement of microsomal particles from nucleus to cytoplasm. Such an experiment, difficult as it would be, is a logically essential one.

When tissues which are actively synthesizing RNA are supplied with labeled amino acid the microsomes may attain very high levels of labeling. In such microsomes, the structural protein of the particle itself becomes labeled.

We must distinguish therefore between two kinds of protein synthesis. We have, on the one hand, synthesis of the structural protein of the microsome. This apparently takes place within the nucleus and leads to high levels of microsomal labeling. Synthesis of protein by microsomes, on the other hand, occurs outside of the nucleus. This is a process in which microsomes



Microsomes are synthesized within the nucleus. They escape to cytoplasm. There they participate in protein formation.

become labeled rapidly, to be sure, but in which only a small proportion of the microsomal protein is labeled in the steady state.

The microsome then is a component of the protein synthesis mechanisms. But it is only one link in the chain. We now know that protein synthesis consists of a series of events. The nature of these events has been elucidated by work on animal tissues done by former Caltech research fellows Richard Schweet (now at the City of Hope), and Paul Zamecnik (now at the Massachusetts General Hospital) and his group –including Mahlon Hoagland, Elizabeth Keller and others; and by work on plant systems at Caltech done by George Webster (now associate professor of biochemistry at Ohio State University), John Clark, Jr. (now instructor of biochemistry at the University of Illinois), Paul Ts'o, and others.

In this sequence of events, amino acids are first converted into activated forms which we call AMP-acyl-amino acid complexes. This is the step of amino acid activation. It appears that both plant and animal tissues contain separate amino acid-activating enzymes for each of the 20 individual amino acids which compose proteins.

Amino acid, once activated, is next transferred to an acceptor which is soluble cytoplasmic RNA. This was first discovered at Caltech by Robert Holley (now of the Agricultural Research Service at Cornell University), and was studied in detail by Mahlon Hoagland, and by Richard Schweet and his group.

Richard Schweet's research also indicates that there are individual soluble acceptor ribonucleic acids, one for each of the individual activated amino acids. Transfer of RNA-acyl-amino acid complex to the microsomal surface appears to be the next event in this catenary sequence. Amino acid is somehow transferred to the microsomal surface, there to be incorporated into peptide bond linkage with other similarly activated and transferred amino acids.

It appears probable that the microsome contributes to this process by acting as the long-postulated template, and by ordering in proper sequence the amino acids which are being assembled in the growing peptide chain.

This is suggested by the experiments of Howard Dintzis, which show that microsomes of red blood cells, which manufacture hemoglobin, receive activated amino acids and assemble them in the relative abundances characteristic of hemoglobin. Then, too, the work of Richard Schweet, Hildegarde Lamfrom, and Esther Allen shows that immature red cell microsomes make hemoglobin even if wedded to activating enzymes and soluble RNA of liver. Clearly, it is the microsome which contributes information to the process of amino acid assembly into protein.

It has already been noted that the store of information as to the appropriate sequence which can be contained within one microsome is finite and in fact small. While a single microsomal particle may contain the information necessary to assemble a few hundred amino acids in proper sequence, it can hardly be imagined to contain the information necessary to assemble more than this small number. It appears quite probable, therefore, that the individual microsome is concerned with the synthesis of one individual kind of protein. Although they appear similar in structure, the microsomes actually seem to be different from one another—each containing, in RNA code, information appropriate to the synthesis of a particular kind of protein.

Genetic information

Interestingly enough, the number of nucleotides in the elementary RNA chain of a single microsome corresponds approximately to the number of nucleotides estimated by geneticists to be contained in the DNA of a single gene. The attractive possibility presents itself that each individual gene sends out its information to the rest of the cell in the form of a single species of microsome—that each microsome contains in RNA language the message contained in DNA language in a single gene. Indeed, the working hypothesis and rallying cry of the microsome biologist today is "one gene, one microsome, one enzyme."

We know today, at least, that the problem of how proteins are synthesized is a problem which can be solved. The mechanism by which energy is made available for peptide bond formation is known. Microsomes appear to be the engines of protein synthesis. Microsomes appear also to be the agency by which the information contained in the DNA of the chromosomes is transmitted to, and utilized in, the synthesis of soluble cytoplasmic enzymes.

An understanding of the complex processes of differentiation itself may ultimately flow from our increasing knowledge of microsome biology. Differentiation may well consist merely in enrichment or impoverishment of the cell in particular kinds of microsomes at the expense of others. Our modest understanding of microsome biology is giving us understanding of problems which lie at the very basis of all biology.