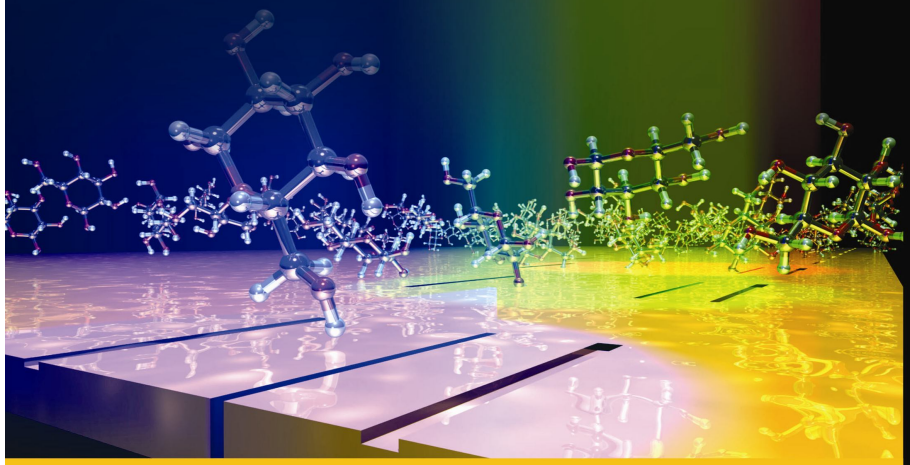
3rd Revised Edition

A Textbook of Molecular Biology

(For B.Sc., B.Sc. (Hons.), Medical and All other Competitive Tests)

Mohan P. Arora
Himanshu Arora



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A TEXTBOOK OF
Molecular Biology

There is no rest for the messenger till the message is delivered

— JOSEPH CONRAD

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Mohan P. Arora
M.Sc., M.Phil., Ph.D.

and

Himanshu Arora
M.Sc. Ph.D.

Edited by
Chander Kanta

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New Delhi : "Pooja Apartment", 4-B, Murari Lal Street, Ansari Road, Darya Ganj, New Delhi - 110 002. Phone: 011-23270392, 23278631; Fax: 011-23256286

Nagpur : Kundanlal Chandak Industrial Estate, Ghat Road, Nagpur - 400 018
Phone: 0712-2738731, 3296733; Telefax: 0712-2721216

Bengaluru : No. 16/1 (Old 12/1), 1st Floor, Next to Hotel Highlands, Madhava Nagar, Race Course Road, Bengaluru - 560 001
Phones: 080-22286611, 22385461, 41138821, 22281541

Hyderabad : No. 3-4-184, Lingampally, Besides Raghavendra Swamy Matham, Kachiguda, Hyderabad - 500 027. Phone: 040-27560041, 27550139

Chennai : New-20, Old-59, Thirumalai Pillai Road, T. Nagar, Chennai - 600 017.
Mobile: 9380460419

Pune : First Floor, "Laksha" Apartment, No. 527, Mehunpura, Shaniwarpeth (Near Prabhat Theature), Pune - 411 030. Phone: 020-24496323/24496333; Mobile: 09370579333

Lucknow : House No. 731, Shekhupura Colony, Near B.D. Convent School, Aliganj, Lucknow - 226 022. Phone: 0522-4012353; Mobile: 09307501549

Ahmedabad : 114, "SHAIL", 1st Floor, Opp. Madhu Sudan House, C.G. Road, Navrang Pura, Ahemdabad - 380 009. Phone: 079-26560126; Mobile: 09377088847

Eranakulam : 39/176 (New No: 60/251) 1st Floor, Karikkamuri Road, Ernakulam, Kochi - 682 011. Phone: 0484-2378012, 2378016; Mobile: 09387122121

Bhubaneswar : 5 Station Square, Bhubaneswar - 751 001 (Odisha).
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Indore : Kesardeep Avenue Extension, 73, Narayan Bagh, Flat No. 302, IIIrd Floor, Near Humpty Dumpty School, Indore - 452 007 (M.P.). Mobile: 09303399304

Kolkata : 108/4, Beliaghata Main Road, Near ID Hospital, Opp. SBI Bank, Kolkata - 700 010, Phone: 033-32449649, Mobile: 7439040301

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Preface to the Third Edition

Earlier published as Molecular Biology, this edition with a bigger title "**A Textbook of Molecular Biology**" has been thoroughly revised and enlarged for students preparing for careers in biology, medicine and related fields. This edition has been designed to establish a conceptual framework that represents a sound approach to learning and facilitates the comprehension of the vast amount of information constituting the field of Molecular Biology. The entire text has been revised with an eye to providing clarity and coverage of the latest cutting-edge topics in Molecular Biology. This text provides students with clearly written straightforward explanations that elucidate difficult, complex topics without over-simplifying presentations.

Spectacular advances have been made in many areas of molecular biology since the publication of the 2nd edition that contribute to our growing understanding of the beauty and wonder of this field. The goal was to design the text in an approachable and readable form that the students can understand and master. In fact, the book was planned to be intellectually gratifying and to convey not only facts, but also a sense to the excitement of modern molecular and cellular biology.

This book is not intended to be encyclopedic in its coverage. Our goal is to present the essential principles, processes, and methodology of molecular biology as lucidly as possible. Recognizing the exceptionally rapid pace of discovery in molecular biology during the past several years, we have sought to weave new knowledge and insights into the fabric of the text while remaining faithful to our central goal of focusing on the essentials of the discipline.

Like the proverbial bride, this edition has "something old and something new" in the sense that we have tried to retain the features of the first two editions that readers have identified as user friendly, yet reorganizing and updating the material and adding new features that we hope will make the text even more useful.

We are indebted especially to Shri U.S. Bhargawa whose words of encouragement and thoughtful comments catalyzed the writing of this edition.

In revising this edition, we have been aided immeascerably by the advice of many students, colleagues and reviewers. They have contributed greately to the accuracy and readability of the book, but they cannot be held accountable for any remaining errors or ambiguities. For those, we take full responsibility. We would like to thank all those people for their help in one way or the other.

The authors express their thanks to their friends and colleagues whose continuous inspiration have initiated them to bring out this textbook. The authors are also grateful to M/s Himalaya Publishing House Pvt. Ltd. for their wholehearted co-operation in publishing of this book.

Suggestions and critism for the improvement of this book would be entertained with great pleasure.

Mohan P. Arora
Himanshu Arora

Preface to the First Edition

Molecular Biology, one of the fast moving fields of science, has undergone a variable revolution over the last two decades leading to major advances in our understanding of all structures and their functions at molecular level. Its range is so wide and vast that it is difficult for a person to know, understand and comprehened in it its entirety. Research has produced a great wealth of information about cells: tens or thousands of research papers having to do with cells are published in scientific journals throughout the world every year. Hundreds of hard decisions had to be made to leave out this or that interesting experimental finding. Too much detail, however, fascinating, makes a long book that can overhelm students and obscure the path of learning the principles of cell function and structure. This book is as short as I could make it without feeling that it had become cryptic. The first draft of the manuscript was twice as long as the final product. The goal of this project has been to provide an introductory level textbook that is clear, accurate, informative and interesting.

The text presents an integrated approach to Molecular Biology, encompassing information on all morphology, physiology and biochemistry. The rationale for this approach is my belief that an accurate and comprehensive picture of current knowledge of the cell can be attained only by utilizing information from several disciplines.

In the present title, the subject matter has been presented in a step by step systematic manner. The selection of material is a problem in such a project and it has been my aim to strike a good balance. I, as a teacher, have found it increasingly important that there be a common language by which information can be imparted to the students. Easy understandable illustrations, correct and latest information are the main features of this book. I have freely consulted other standard books and research papers while preparing the manuscript, so I claim no originality of work.

This textbook represents a group effort, rather than the product of any single individual. Foremost on the list stand the reviewers, whose advice, comments and collective wisdom helped to shape this text into a final form. Their interest in the subject, their concern for the accuracy and method of representation, and their experience with students of widely varying abilities and backgrounds made the review process an educational experience. To these individuals, who carefully recorded their comments, opinions, and sources, I would like to express my sincere thanks and best wishes.

My gratitude is extended to Sh. U.S. Bhargawa who has provided suggestions, comments and support while this project was underway.

I am also grateful to M/s Himalaya Publishing House Pvt. Ltd. who made the entire project possible, and who kept the text, art and production programs on schedule and in relative harmony.

No single individual could expect to produce a flawless textbook of this scope and complexity, and any errors or oversights are strictly my own, rather than those of the reviewers, artists, or editors. In an effort to improve further edition, I would ask that readers with pertinent information, suggestions or comments concerning the organization or contents of this textbook send their remarks addressed to M/s Himalaya Publishing House, 'Ramdoot', Dr. Bhalerao Marg, Gurgaon, Bombay - 400 004.

Mohan P. Arora

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1 ORIGIN OF CELL

The universe was perhaps 15 billion years old when the star that is our Sun came into being. According to current hypothesis, it formed, like other stars, from an accumulation of dust and hydrogen and helium gases whirling in space among the older stars.

The immense cloud that was to become the sun condensed gradually as the hydrogen and helium atoms were pulled towards one another by the force of gravity, falling into the centre of the cloud and gathering speed as they fell. As the cluster grew denser, the atoms moved more rapidly. More atoms collided with each other, and the gas in the cloud become hotter and hotter. As the temperature rose, the collisions became increasingly voilent until the hydrogen atoms collided with such force that their nuclei fused, forming additional helium atoms and releasing nuclear energy. This thermonuclear reaction is still going at the heart of the sun and is the source of the energy radiated from its glowing surface.

The planets, according to current theory, formed from gas and dust moving around the newly formed star. At first, particles would have collected at random, but as each mass grew larger, other particles began to be attracted by the gravity of largest masses. The whirling dust forming spheres continued to revolve around the sun until finally each planet had swept its own path clean, picking up loose matter like a giant snowball. The orbit nearest the sun was swept by Mercury, the next by Venus, the third by Earth, the fourth by Mars and so on out to Neptune and Pluto, the most distant of the planets. The planets, including Earth are calculated to have come into being about 4.6 billion years ago.

EARTH AND THE BIOSPHERE

During the time Earth and other planets were being formed, the release of energy from radioactive materials kept their interiors very hot—when earth was still so hot that it was mostly liquid, the heavier materials collected in a dense core whose diameter is about half that of the planet. As soon as the supply of stellar dust, stones and largest rocks was exhausted, the planet ceased to grow. As earth's surface cooled, an outer crust, a skin as thin by comparison as the skin of apple was formed. The oldest known rocks in this layer are about 3.98 billion years old.

Only 50 kilometers below its surface, the earth is still hot—a small fraction of it is even still molten. We see evidence of this in ocassional volcanic eruption that forces lava through weak points in the earth's skin, or in the geyser, which sprung up boiling water that has trickled down to the earth's interior.

The biosphere is that part of the planet within which life exists. It forms a thin film of the outermost layer, extending only about 8 or 10 kilometers up into the atmosphere and about as far down into the depths of the sea.

WHY ON EARTH?

In our solar system, earth among all the planets is most favoured for the production of life. A major factor is that the earth is neither too close nor too distant from the sun. The chemical reactions on which life—at least as we know it depends—virtually cease at very low temperatures. At high temperatures, complex chemical compounds are too unstable for life to form or survive.

Earth's size and mass are also important factors. Planets much smaller than earth do not have enough gravitational pull to hold a protective atmosphere; and any planet much larger than earth is likely to have so dense an atmosphere that light from the sun cannot reach it's surface.

Many biologists contend that given certain conditions such as energy source, water, a temperature range in which the water can exist in liquid form and a long enough time, the evolution of some forms of life is inevitable. The discovery on another planet of living organisms, no matter how primitive, whose origin was independent of earth, would strongly support this hypothesis.

There are approximately 10^{20} stars in the universe like our own sun that can provide energy for living things. At least 10% of them, according to astronomers, are likely to be surrounded by planetary systems such as our own. If only 1% were to have planets with environments roughly similar to those of earth, that would offer some 10^{18} possibilities for the existence of life in other solar systems.

ORIGIN OF LIFE

No one is certain about the exact time or conditions that sponsored life from non-living sources but there are certain distinctions between the two which allow us to put together a logical sequence of events. In addition, we have some knowledge of the kinds of chemistry that exist in the world today and those which probably existed during primeval times. Laboratory experiments have shown that organic molecules could have arisen **abiogenically**; that is in the absence of life at the time. The first clues to abiogenic synthesis of biologicaly significant organic molecules were presented in 1953 by *Stanley Miller*.

Eukaryotes from Prokaryotes

We cannot begin to document the numerous evolutionary studies aimed at analyzing species relationships, but we can examine viewpoints concerning the origin of eukaryotic cellular organization. We have a good idea of similarities and differences between prokaryotic and eukaryotic cell organization and chemistry, and we can make at least three basic points at the start:

1. Prokaryotes existed for about 2 billion years before eukaryotes appeared, according to the history of life preserved in the fossil record.
2. There are basic similarities that clearly show common descent for prokaryotes and eukaryotes, for example, they possess the same genetic codon dictionary, their genetic material is DNA, there is a similar ribosomal machinery for protein synthesis; there are common metabolic pathways and enzymes catalysis and various other features.
3. The air contained only traces of molecular oxygen about 2 billion years ago, and became essentially oxidizing to present a level of about 21% O_2 as per 700 million to 1 billion years ago. Estimates concerning oxygen are derived from studies of fossils and rock formation of the known age.

From these observations, we may conclude that prokaryotes were ancestral to eukaryotes, and that the great evolutionary divergence that led to eukaryotes took place while the earth was relatively

but not entirely anaerobic. Since prokaryotes are for more ancient than eukaryotes and since there are fundamental similarities that are unlikely to have arisen independently by chance, we can safely say that eukaryotes must have evolved from prokaryotic ancestors.

Origin of the Eukaryotic Cell

Eukaryotic cells have membrane bounded compartments while prokaryotic cells have only plasmalemma and in some cases one or more types of infolding of this membrane. These infoldings are usually enzymatically distinct from the remainder of the plasmalemma. Because there is a basic organizational difference between compartmented and non-compartmented types, we must look for explanations of permanent membrane systems that are physically separate from the plasmalemma in eukaryotes. Most of the formal hypothesis include the infolding and eventual separation of infoldings from the common plasmalemma source. As these internalized membranes became functionally distinct and as they came to enclose particular sets of molecules and reaction systems, they eventually assumed the special qualities and appearance we see today in the nucleus, ER, lysosomes, microbodies and other compartments.

There are different views, however, concerning the origins of mitochondria and chloroplasts in particular. Each of these organelle types is unique in having its own portion of DNA, RNA and ribosomal machinery. According to one point of view, these organelles evolved through membrane infolding separation differentiation just like other eukaryotic compartments, but with the difference of having captured some pieces of cellular genetic apparatus which then became a part of organelle construction and function. According to the opposite view, mitochondria and chloroplasts originally were prokaryotic organs in their own right, but later became functioning parts of their host organism. Their genetic material, therefore, is the remnant of the system originally present in their free living ancestor.

There are common features, however, held by both schools of opinion about mitochondria and the origin of chloroplasts. In each theory, it has been postulated that the primary modification in the prokaryotic ancestors of eukaryotes was loss of rigid confining cell wall and acquisition of mobile cell surface. This amoeboid prokaryote could move around by creeping locomotion, but most importantly it could evolve into the ingesting organism. Various materials, including foods, would enter the cell by **endocytosis**. This would be an adaptive change, since it would allow additional source of food and therefore provide an anaerobic organism with a larger supply of fuel for growth and reproduction. Beyond this point, there are major differences in two evolutionary sequences. The two principal hypotheses explaining mitochondria and chloroplast origins are 1. endosymbiosis and 2. internalized membrance differentiation.

ENDOSYMBIOSIS

The earth's atmosphere contained only 1% of O_2 1.3 to 1.4 billion years ago, the time when the first eukaryotic fossils appeared in the fossil record. There must have been some prokaryotic organisms therefore which used oxygen in aerobic respiration. If such respiring bacteria were ingested by an amoeboid prokaryote, and if these bacteria persisted unharmed as symbiotic partners with their host cell, the result would be a respiring 'amoeboid' prokaryote host. This would be beneficial to the host, allowing more efficient energy extraction on during food break-down, and it would be beneficial to bacteria because they would have available readymade food provided in host cells. Such a mutually beneficial association is called **symbiosis**. The bacteria are endosymbionts, housed inside their permanent host cell.

The theory was first proposed in the late 1800s, but has been revived most recently by *Lynn Margulis*. Since 1967, she has presented an extensive exposition of the endosymbiosis theory. In

her view, the respires bacteria eventually evolved into mitochondria of the eukaryotic cells. The DNA and other genetic apparatus in modern mitochondria are therefore considered to be formerly free living endosymbiotic bacteria. Respiratory enzymes are essentially similar to mitochondrial membranes and in modern bacterial plasmalemma. A further similarity between mitochondria and bacteria is their size and shape, if we accept this particular model of the mitochondrion.

Margulis proposed that the second endosymbiosis took place some time afterwards, leading to more effective locomotion for the respiring amoeboid prokaryote. In this case, it is postulated that spiral shaped bacteria called **spirochetes**, became incorporated as **endosymbionts**. These were ultimately modified to become cilia and flagella in eukaryotic descendants. Spirochetes have an unusual ultrastructure and there are certain protozoa that move about locomotor action of their own symbiotic spirochetes. This modern observation led to the suggestion for the past evolutionary endosymbiosis.

The third endosymbiosis has been suggested by *Margulis* to have occured after eukaryotes had evolved; one or more of these ancient, respiring, flagellated eukaryotes ingested blue green algae. These algae eventually evolved into modern chloroplasts. The genetic machinery in modern chloroplasts is therefore explained as being the remains of genetic system originally present in the endosymbiotic bluegreen algae. Eukaryotes that did not happen to establish a symbiotic relationship with blue green algae remained non-photosynthetic, ultimately giving rise to various size of **protists**, some of which in turn diverged to produce fungi and animals. Eukaryotes with chloroplasts gave rise to photosynthetic protisis, algae and green plants.

There are numerous modern examples of symbiotic associations between different organisms. In many cases, it is possible to show that each symbiotic partner can live independently of other, but that mutual benefit keeps the partners together under natural conditions. The scruffy lichens that grow on rocks and trees and in relatively living barren zones are actually composed of algae and a fungus. *Paramecium bursaria* is a ciliated protozoan that harbours a population of unicellular green algae in symbiotic association. Assorted cellulose digesting protozoa live in gut of termites, providing the essential digestive capacity in these wood-eating animals. There are examples of blue green algae that live symbiotically in protozoans and other host cells, and such algae usually lose their rigid cell wall when they keep up residence in their hosts.

In an experiment in which chloroplasts from flowering plants infecting the mouse cells grown in culture, the chloroplast could be recovered undamaged some days later. All together, **symbiosis** is a well known natural phenomenon often involving algae as one of the partners. There is no case, however, of a prokaryote which houses a symbiont organism. The endosymbiosis theory is attractive in certain ways in explaining why mitochondria and chloroplasts have a genetic apparatus, alone among all the types of eukaryotic organelles. In addition, the theory also explains particular similarities between prokaryotes and these two kinds of organelles by the accepted doctrine of common descent. For example, they all have naked DNA duplex molecules that are circular in form and that contain all the genes of the system in single molecule. Their ribosomes respond similarly to various antibiotic inhibitors of protein synthesis while eukaryotic cytoplasmic ribosomes respond differently to the same drugs. Taking these and other factors into consideration, many people have accepted endosymbiosis as the evolutionary explanation for mitochondria and chloroplasts. The rationale for flagellar origin, however, is less well accepted.

Internalized Membrane Differentiation

There have been some various suggestions concerning the origin of one or other eukaryotic membranous compartments, particularly in relation to mitochondria. In the mid 1970s, *T Cavalier-*

Smith presented a relatively comprehensive scheme for eukaryotic cell development during evolution. He suggested that the prokaryotic ancestor was blue green alga that had developed a mobile cell surface capable of **endocytosis** and other surface activities. One of the first membranous compartments to appear would be **lysosomes**, since powerful digestive enzymes would be advantageous in handling ingested solid foods. These enzymes would first digest the cell itself if not seqestered behind a membrane, so only mutations leading to lysosomal compartments would be highly advantageous to a cell with a mobile surface.

In prokarytoes, the DNA molecule is attached to the inside surface of plasmalemma. This would be a safe place in a cell with an active cell surface, since the DNA could be expelled by **exocytosis** or be taken into the cell interior by **endocytosis** and perhaps be digested there by lysosome interactions. Mutations leading to detachment of DNA from the cell membrane would be adaptive, but segregation or replication DNA during cell divisions require some rigid attachment systems. In place of cell surface, *Cavalier Smith* proposed an evolution of a microtubular system which serves to hold the DNA molecules taut during their separation into new cells. This system of DNA and microtubules eventually became enclosed in a nuclear envelope, and differentiated as an internalized system that is physically separate from the plasmalemma. Other membrane differentiation led to cytoplasmic compartments including mitochondria, thylakoid-containing chloroplasts, ER and others.

According to this theory, there is no reason to postulate a different origin for any of the organelles in eukaryotes, or to require a sequence of endosymbioses occuring in a particular order and at particular times in eukaryotic evolution. Compartmentation is an effective means for sequestering coordinated reactions and components and once there was some internalized membrane, there would eventually be others during eukaryote evolution. *Cavalier-Smith* postulated that chloroplasts were lost in some eukaryotic lineages, which we see today as non-photosynthetic groups of organisms. Modern photosynthetic groups presumably arose from eukaryotes which retained their chloroplast compartments.

Evolutionary Relationships

As we have divided all cellular life into prokaryotes and eukaryotes, it seems appropriate here to ask that how these two groups of life forms are related. Prokaryotes are generally accepted as the more ancient kinds of organisms which gave rise to eukaryotic descendant forms during evolution. We might infer that prokaryotes are the ancestral types since they are much simpler in structure and organization than the eukaryotes. The most convincing evidence, however comes from the fossil records. Prokaryotes have been found in deposits well over 3 billion years old, while the earliest record we have for eukaryotes is about 1.3 billion years old. Since a fossil record provides a reasonable sampling of former life, we judge that prokaryotes are ancestral and eukaryotes are descendants of ancient prokaryotic lineages. Each group has continued to evolve through all these cons of time and modern species are of course different in many details from their ancient ancestors, but sufficient distinction remains even now to clearly recognize the two subdivisions of cellular life.

There is considerable similarity between particular molecules and particular functions in prokaryotes and eukaryotes, which implies that they are related rather than being unique forms of life that appear entirely independent of one another. All cellular life is characterized by having DNA as genetic material, a ribosomal machinery for protein synthesis enzymes that influence chemical reaction rates in various metabolic pathways which are similar or even identical, and

other features that point to a common ancesty for modern cellular life. The particular lineages, however are the subjects of lively controversies.

R.H. Whittakar has proposed a detailed lineage for five kingdoms of organisms which he recognizes. Although this is only one proposal out of several that are under consideration at present, it is a scheme that takes many observations into account. The prokaryotic **monera** includes species that are **heterotrophic** and others that are **autotrophic**. Heterotrophs derive energy and carbon based building blocks for growth from their breakdown of complete organic foods, autotrophs are able to get energy either from light or from inorganic chemicals and to derive carbon from CO in air, using these to make organic compounds in metabolism. The most familiar prokaryotic autotrophs are the photosynthetic bacteria and the blue green algae. The heterotrophic bacteria absorb all organic nutrients in solution from their environment. The simplest and probably the most ancient types of eukaryotes are in the Kingdom Protista which include heterotrophs and autotrophs. The autotrophs are photosynthetic, but among the heterotrophs, some only absorb organic nutrients and others have the ability to ingest solid foods rather than attaining their needs only from materials that move across the cell boundary in dissolved form.

Wittakar and others have proposed that different kinds of protists give rise to new eukaryotic lineages which are represented today by species belonging to the three remaining kingdoms of eukaryotes : plants, animals and fungi. Except for a few species, plants are photosynthetic. Fungi are heterotrophic and absorb nutrients in solution from their environment. Animals are heterotrophic and almost all ingest solid foods. The invention of mobile cell surface which is typical of animal cells may well have been the key factor that contributed to that ability to obtain solid foods by ingestion larger quantities of food can be taken in per unit time by ingestion than by absorption which may have been advantageous during eukaryotic evolution which ultimately led to animals as a novel life form.