Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Non-Darwinian and Darwinian prokaryotic and eukaryotic evolution - an enigma in cell biology conservation

Hugo Hoenigsberg

Instituto de Genetica Evolutiva & Biologia Molecular,
Universidad Manuela Beltran, Bogota D.C. Colombia
Corresponding author: H. Hoenigsberg
E-mail: hoenisbe@umb.edu.co

Genet. Mol. Res. 2 (3): 279-287 (2003)
Received October 10, 2002
Accepted July18, 2003
Published September 30, 2003

ABSTRACT. Our theory is embarrassingly simple. What made today's prokaryotes and modern cyanobacteria so robust is the fact that in their origin, back in the Archean (3 billion years ago), selection did not play a central role in evolution, it had only a transitory role. Asexual reproduction, mutation, drift and sampling variance in local demes were more important especially when they were accompanied by population catastrophes, where millions perished. Metazoans are generally macroscopic, sexually reproducing, ecologically specialized organisms whose history is full of extinctions and radiations leading to morphological change. On the other hand, prokaryotes, thanks to their origin, avoid extinction because as a group they have slowly evolved as generalists. Specialization appears to be less important than ecological versatility and metabolic unspecialization. Modern cyanobacteria keep on using that strategy.

Key words: Prokaryotes, Evolution, Darwinian evolution, Cell biology conservation

In the beginning

The earliest known prokaryotes are found as microfossils from the Archean in deposits 3.5 billion years old (Schopf, 1992). They were filamentous organisms similar to modern photosynthetic cyanobacteria! It is really an amazing evolutionary adventure of life, which involved the elaboration of complex chemical automatic machinery. For the sake of establishing a comparative narrative to another unicellular microbe let us remember what *E. coli* is (of course a much more complex organic surviver than the organism that fossilized 3.5 billion years ago). We are talking of an organism with 64 possible "words" (DNA codes), each corresponding to a letter in the protein language (or a stop sign), where some "letters" correspond to the same amino acid, with single-strand RNA that functions as adaptors about 80 units long twisted upon itself in a specific manner. The twisting pattern is determined by the sequence of the letters, many of them pairing with each other. This neat piece of machinery has an exposed, unpaired, triplet of RNA letters. The different pieces of adaptors have similar shapes, although with different exposed triplets, as the idea is to "plug" into different complementary triplets. All and all, this works to satisfy the coding mechanism established in the early days by DNA, in order to send its code to the future as an adaptive message for the organism. Although, those early Archean, or closer to us, Proterozoic bacterial deposits during the time of the prokaryote ascendancy, could not have had individuals with all the elements for survival today, such as protein synthesis, complex membrane signaling, more complex ribosomes obsequious to DNA commands, and conflict modifiers for eukaryotic multicellular existence, they had to have sufficient chemical machinery to utilize energy from any source and convert it to organic food for the various metabolic processes of cell biology.

Those unicellular individuals that microfossils reveal to be analogous to photosynthetic cyanobacteria had to have at least an automatic chemical machinery similar to ancient Proterozoic (2500-550 million years ago) microbiological aggregations. The paleomicrobiological aggregations formed thick mats or stromatolites similar to present day cyanobacterial communities in intertidal zones, salt marshes and lagoons, like those reported in Shark Bay, Australia (Berry et al., 1990). For disparate rates and different fates, see Schopf (1994).

The principal organism in the stromatolites, *Entophysalis major*, in order to survive in the midst of natural selection exigencies, necessarily had to have, what we suppose was an already sophisticated receptor and effector mechanism on the cellular surface in order to avoid nondesirable foodstuff, an incipient technique to learn by remembering from disagreeable encounters ways to stay healthy in the face of external osmotic pressure, and enough genetic variability to confront physiologically wise, highly competitive individuals, ready to promote their own messages to the future. Outside a working cytoplasmic suspension and a centralized library (made of DNA), there is a huge ribosome built of both RNA and protein molecules. Is it reasonable to expect to find among the Australian stromatolites organisms with ribosomes like *E. coli*, which has a complex machine, 270,000 atoms strong and about 30,000 of them at work in each one of them (Cairns-Smith, 2000)? Of course not, but we are dealing with a very complex automatic machine already in action and in relatively good functioning condition. The chemical laboratory in those early prokaryotes had to be ready for natural selection to "improve" (can you really improve on a system that must be at top efficiency for conditions prevalent then? The answer is yes!) in those genetically variable DNA-based ancestors of *Entophysalis major*. Even if natural selection had not perfected the machine within the DNA filament to select out

"bad" mutants, catastrophic eliminations of unadaptive mutations (the majority) aided selection, or the "perfected" machine would have gone kaput, with an immense mutational load in a hostile environment. As these are haploid and asexual organisms, mutations had to be the only source of variability. In those early days of prokaryotes, mutations were not a positive element of selection but rather they were the most important instrument of evolutionary change through mass extinctions. New founders emerged from a few survivers out of billions of individuals that died. Those few profited by natural selection's demands for "better" characteristics, and the immediate future was secured once again, and this went on and on. So what we had was selection, once in a while, whenever possible, and mutations and extinctions most of the time! This onerous evolutionary process was necessarily calamitous and conservative.

The centralized machinery of ancient cells with nucleic acid origins must have contained the essential complexity characteristic of primitive prokaryotes. In other words, the most advanced lineages of the prokaryote ascendency in the late Archean and Proterozoic were probably photosynthetic, with DNA messages at the very center, because only they could survive through generations, administering whatever is advantageous to conserve the worn out pieces of the automatic machinery of cell biology. Which in a few words amounts to, DNA making DNA, given primed DNA nucleotides and enzymes; DNA making RNA, also given primed RNA nucleotides and more enzymes; RNA - RNA messages, RNA adaptors, RNA making proteins thanks to amino acids and more enzymes. The proteins (i.e., enzymes) do everything else. For a prokaryote as old as the Archean or even for a paleomicrobiological aggregation from the Australian stromatolites, or from Lyngbya (Oscillatoriaceae) or Paleolyngbya in Siberia (950 million-year-old deposit in Siberia), or from Spirulina (Oscillatoriaceae) or Heliconema (850 million-year-old deposit in Siberia) or Gloeocapsa (Chrococcaceae) or even older deposits such as Gloeodiniopsis (1.55 billion-year-old deposit in southern Russia (for a review, see Schopf, 1992), we must expect a state of a so-so metabolic proficiency very early, although morphologically very slow to evolve thereafter.

The fact that Archean prokaryotes (3.5 billion years ago) survived to similar present day cyanobacteria suggests that these species achieved an amazing chemical feat in only 300 million years (calculating that to support life the Earth that formed about 4.6 billion years ago probably only cooled sufficiently about 3.8 billion years ago) without undergoing morphological evolution to present day forms! In the photos in the book edited by Schopf (1992), there are clear demonstrations that old forms and new ones are very similar, after at least 1.5 billion years.

What happened? Did they reach their optimum external anatomy after those early rapid experiments? As we saw before, there was little help from natural selection. Sporadic interventions of selection were sufficient and efficient by applying themselves to a world with abundant competitive mutants that had survived mass extinctions? Is that enough? This is a law of large numbers, which is equivalent to saying that mathematics acting on a nucleic acid crystal did it most of the time, and selection once in a while!

These early prokaryotes were probably generalists, without a real cell nucleus and membrane-bounded organelles, just like today's prokaryotes. Prokaryotes have their genetic information in the form of a single circular DNA molecule, lying in the cytoplasm; as they do not have a nuclear membrane, transcription and translation occur simultaneously, and there is no processing of messenger RNA transcripts. Genes are in a continuous string, uninterrupted by intron sequences in eubacteria, although introns are present in archaebacteria.

Slowly evolving generalists?

Does the lack of morphological evolution mean that mutations on codes for external phenotype were neutral? Or was selection favoring conservation from the start? Or was external phenotypic optimum achieved from the beginning?

The reasons for the apparent slow pace of morphological evolution in those days is unknown; neither do we know how much genetic variability was there for their robust physiology and biochemistry. They were tough organisms, similar to cyanobacteria. The stasis that appears in the fossil record can be attributed to the absence of a sexual cycle or a parasexual process or other mechanisms that generally promote heterozygosity. It is unlikely that they were all homozygous clones, due to the fact that they went through climatic changes, constant osmotic changes and intertidal influences that required variability. The sources of spurts of genetic change would be drift and sample variance, plus individual mutations within large populations, passively and widely distributed. Extinction may also be precluded under such conditions. In this environment evolutionary change was slow. Thus, if they went through catastrophic eliminations that reduced numbers to a great extent, producing isolated populations, genetic drift should be included among the instruments of evolutionary change, with mutations, when bottlenecks appeared.

The survival strategies of those early inhabitants of stromatolites could have been different from what occurred in bacterial and metazoan species. Whereas cyanobacteria have persisted for some 2 billion years, metazoan species on average persist, according to some accepted estimates, for only 4 million years. If prokaryotes were slowly evolving generalists, as it seems, they had to have an eventual selective mechanism working sporadically on perfecting each of the elements of the internal chemical machinery. The stasis in the morphological characteristics of prokaryotic evolution could have been an important achievement to "concentrate" on genetic codes for slowly evolving internal physiological optima (physiological robustness). To ensure survival, specialization might have been less important than ecological versatility and biochemical unspecialization. Indeed modern cyanobacteria show those generalist features. They are strong; some species are known to photosynthesize at very low levels of illumination and yet they can survive climatic and other extreme conditions, such as high temperatures or freezing; other species can live in anoxic lakes or other extreme conditions, aggravated by sudden changes.

Physiological stability rather than evolvability?

A few polymorphic loci in eukaryotes are enough to account for a great deal of plasticity. And a whole range of environmental conditions, even extreme ones, can be addressed with a few polymorphic loci that have pleiotropic effects. It is theoretically arguable that a very complex machine that resulted from internal genetic selection acting for millions of years on few polymorphic loci, of the kind we are proposing here, cannot be changed if those loci affect important pieces in the machine elsewhere. In the geometry of evolution (Conrad, 1990), we read that there are characteristics that allow organisms to evolve quickly and still maintain fitness. The sense of the article is that a system can satisfy two apparently conflicting conditions - that it be physiologically stable and also be easily changed in evolution. Many authors in population genetics have maintained that changes in the conditions of the environment necessarily mean a change in the physiology. Moreover, they go on to say that genetic stability and

evolvability cannot go hand in hand; in fact they claim that the real gist of Darwinian selection is that genetic stability means living in a stable environment. Nevertheless, the norm of reaction of a genotype admits control of the phenotype without changing specific gene loci. In other words, physiological stability does not imply genetic stability. Organisms can suffer genetic change (even chromosomal aberrations in animal and plant systems) without observable phenotypic change under normal conditions.

There are many kinds or degrees of fitness: 1) the optimum fitness, in a fitness landscape, means that through a specialized highly adaptable and physiologically stable phenotype an organism, or its lineage group, does not change because changing means moving away from the optimum, and that in turn means that lower reproduction and survival rate will render the lineage less competitive. In this case, the organism survives and competes successfully as long as the environment remains fixed. 2) When the organism gets to a not-so-high an optimum in the fitness landscape, it implies that the lineage has acquired a few genotypes (polymorphic) and in the case of metazoa a few alternative embryological landscapes to cope with several environments, without specializing as the best in the Darwinian sense. Such is the case of many that did not abandon their evolvability. 3) When the organism acquires biochemical stability (or developmental stability in the case of metazoa) that permits (as was the case for Archean prokaryotes, which were successful for many billions of years with the same external phenotype, even in highly changeable conditions) that they optimize their generalist features for the sake of slow evolvability. It is theoretically possible for generalists to have physiological adaptation when they shift their proteins in their third dimensions in the face of extreme conditions. It is possible that at the beginning of life, when sexuality was not developed in Archean prokaryotes, lineages of homozygous genomes (clones) were physiologically unstable or that their physiology responded in obedience to internal mandates of stability and slow evolvability.

Could the capacity of proteins to incorporate variability of sequence within stable conserved three-dimensional structures be the origin of the conserved phylotypic stage of development of the body plan of phyla?

I suppose that we should begin this section with the prokaryotic conservation that the fossil record advertises so profusely (Schopf, 1992). A highly conserved prokaryote probably initiated the trend, preserved as microfossils of cyanobacteria (for mind-boggling photographs, see Schopf, 1992).

Eukaryotes probably emerged from prokaryotic ancestry about 1.6-2.1 billion years ago (Knoll, 1992). The evolutionary diversification of prokaryotes or pre-eukaryotes has involved a profound compartmentalization, with inventions such as organelles and many more complex structures suspended in the colloidal cytoplasm. Now, about 60 types of eukaryotes can be distinguished on the basis of their cellular organization (Patterson and Sogin, 1992; Patterson, 1994, 1999). One of these lines (the opisthokonts) have some million species of animals and fungi, another (the Viridaeplantae) is composed of the green algae and the land plants. The majority of the lines of eukaryotes are considered in a group of their own, the paraphyletic, of mostly unicellular organisms, referred to as the protists. The amazing question is, how did microbial life, which started out with a relatively simple unicellular prokaryote with just a seemingly "simple" chemical machine suspended in an unorganized internal milieu with only a nucleic acid circular filament that coded for proteins in physiological stable functions, opt for a slowly evolv-

ing generalized life-form and manage to survive violent tidal movements, extreme temperature fluctuations and even more extreme changes in osmotic pressures?

We have offered before that in this early start, organisms went through an amazing non-Darwinian evolution, with spurts of change produced by catastrophic eliminations, mutations, drift and sample variance, coupled with very large numbers and bottlenecks. If we have "punctuated equilibrium" (Gould and Eldredge, 1977) with an evolution that does not take place incrementally, but rather in spurts, then "punk-eq" departs significantly from Darwinian competition among organisms (Hoenigsberg, 2002).

The jump to eukaryotic organization was immense; whatever provoked it for a continuous period of millions of years had to start out with an already well-built, chemically complex machine. Let us just summarize what a eukaryote is: the eukaryotes are distinguished from prokaryotes by the structural complexity of the cells, which have many functions segregated into autonomous compartments of the cells, the organelles, and the cytoskeleton. The most evident organelle in most cells is the nucleus, and from it came the name of eukaryotes. Most cells have a single nucleus, but some have thousands, and others like our red blood cells have none, though they derive from cells with nuclei. Nuclei contain the central government of the cell with its genetic material, but other codes of the genome are located in mitochondria and plastids (when present). The nucleus is bounded by a membranous envelope, and the nuclear envelope is part of the endomembrane system, which extends to include the endoplasmic reticulum, dictyosomes (Golgi apparatus), and the cell or plasma membrane that covers the whole cell. The nuclear envelope is perforated by nuclear pores, which allow compounds to pass on to the surrounding cytoplasm. Some protists are so specialized that they have more than one kind of nucleus; one of them is used to keep a copy of the central genome for reproduction, and another is used to amplify some genes to regulate certain activities.

The cytoskeleton in eukaryotes is comprised of many proteins. The major ones are tubulin (in microtubules) and actin (in microfilaments) and hundreds of interacting proteins that take part in transportation and in the skeletal architecture of cells. The cytoskeleton supports the membranous organelles. The cytoskeletal architecture plays an important role in supporting metaphase chromosomes and other elements of cell division in mitosis and meiosis.

Since many functions of the metabolism are carried out within the membrane-bound organelles, it is important for the cellular general frame that the organelles include endoplasmic reticulum, dictyosomes (Golgi apparatus), lysosomes and peroxisomes. Other membrane-bound organelles include chloroplasts (in plants, algae and organisms that have developed symbiotic associations with plastids), mitochondria and hydrogenosomes. Protists (mostly microbial eukaryotes) have membrane-bounded organelles not found in most other eukaryotes, such as contractile vacuoles and extrusomes. Non-membrane-bound organelles include cytoskeletal elements (those made up of tubulin, or filamentous structures sometimes incorporating actin), contractile systems (actin-myosin systems and spasmin/centrin organizations of various sizes), or other motility devices (mitotic spindles, myonemes, cilia, flagella).

The protista

Although the protists are eukaryotes, they represent a paraphyletic group, which are not animals, fungi or green plants. Those that have classified protists can identify about 60 types, but the relationships among these lineages are not clear (Doolittle, 1995). It is estimated that

there are some 200,000 named species of protists. Some of the accepted groups contain only one or a few genera or species, however, others include an enormous variety of diverse organizational types (including multicellularity). A good example of protists is the stramenopiles, which embrace a quantity of photosynthetic activity almost as vast as that of plants, and includes fungus-like organisms (Oomycetes), parasitic protozoa (Opalines and Blastocystis), free-living protozoa (heliozoa and flagellates) and various unicellular (Chysophytes) and multicellular algae (Kelp and other brown algae). Traditionally the best-known protists are the following non-monophyletic adaptive groups: Flagellates, Amoebae, Algae, and Parasitic Protists.

Evolution is made up of selective and non-selective mechanisms

The evolution of multicellular forms is the result of changes in development. It is generally accepted that to get to multicellular forms prokaryotes first evolved to the stage of eukaryotes, where certain cellular characteristics were acquired, like a nuclear membrane, a more complex cellular membrane with many different types of molecular signals, several pumps to equilibrate osmotic pressure and several internal organelles to carry on transcription and translation of RNA messages destined to assemble polypeptides. Most of these functional compartments are effectively isolated thanks to lipid membranes, across which most materials move selectively. It is highly probable that this compartmentalization of the cell interior, plus other accompanying biophysical and genetical phenomena functioning in the cytoplasm, were, in the early days of Vendian, Precambrian or Proterozoic days some 1000-550 million years ago, prerequisites for the development of complex multicellularity, which originated developmental patterns from which 32 Phyla diversified (Brusca and Brusca, 1990). There is a molecular phylogeny of metazoa that testifies to a monophyletic origin (Muller, 1995). Moreover, the homeobox-containing genotypes in the most primitive metazoa (Seimiya et al., 1994) indicate a single eukaryotic origin that later diversified into different developmental phyletic patterns (Wolpert, 2002), or maybe a Vendobionta sister of Eumetazoa (Buss and Seilacher, 1994). Experiments in cloning *Giardia lamblia* suggest an origin of eukaryotic cells and of endoplasmic reticulum (Gupta et al., 1994), and protein phylogeny of the same *Giardia lamblia*, a mitochondria-lacking protozoa, gives a robust estimation of early divergencies of eukaryotes (Hashimoto et al., 1994). From what we have seen in this paper, we can say that conservation, from prokaryotes on to cellular processes and cellular behavior in the development of metazoa, is a general principle in cellular biology.

Should the explanation for conserved protein function be what Gerhart and Kirschner (1997) consider: "1) conservation as a reflection of optimality; 2) conservation as a reflection of multiple functions producing extensive pleiotropic effects if they changed or 3) conservation as a reflection of the tolerance of a protein function to changes in structure". There are operational ceilings of protein and nucleic acid codes that pertain to the biophysical or to the chemical realms where natural selection cannot reach. Physiological stability is nothing else than a point of no return for optima. Certain aspects of conservation are steps away from selection, or better still, places where selection cannot reach unless erosion of optimal adaptive peaks previously accomplished by selection set in to produce defects and eventual death. Conservation of protein function need not be accompanied with amino acid replacement. The conservation of three-dimensional structure hemoglobin allows for a wide variety of sequences and modulations of the oxygen-binding sites at various altitudes in populations living in high mountains (like in Bogota,

the Himalayas, La Paz, etc.). Goldberg (1995) recalls that the parasitic nematode Ascaris produces a hemoglobin that binds oxygen with 200 times more affinity than mammalian myoglobin. The small amount of oxygen sequestered in the gut serves for the epoxidation of squalene in sterol biosynthesis. Ascaris globin shares only 10-15% sequence homology with vertebrate globins, although it has the same three-dimensional structure (Gerhart and Kirschner, 1997). There is nothing strange about globin in the clam *Lucinia pectinata*, which lives entirely off a symbiotic bacterium in its gill. Crystallographic analysis at a 1.5-Å resolution (Rizzi et al., 1994, quoted by Gerhart and Kirschner, 1997) demonstrates that the three-dimensional structure of the polypeptide backbone of the clam's sulfide-reactive hemoglobin (the bacterium uses the oxidation of hydrogen sulfide as energy) can be superimposed almost exactly on that of sperm whale myoglobin. However, the sulfide-reacting hemoglobin of the clam has only 18% homology with vertebrate globins.

Comparative biochemistry of globins shows that there is a wide sequence difference among them in the animal kingdom that does not affect their physiology in the different unrelated taxa. Nevertheless, at particular positions, even small changes in amino acid sequence can have significant effects on physiology. To be able to adapt to extreme muscular exertion at very low oxygen concentration over Mount Everest at heights of 9000 m, the bar-headed goose (*Anser indicus*) has replaced proline with alanine on the alfa chain. Its sibling species at lower altitude (*Anser anser*) does not have to do that to its globin structure. There are important works (Clementi et al., 1994) on hemoglobin functions that demonstrate that under extreme life conditions conserved protein structures work just fine. In other papers on the adaptation of bird hemoglobins to low oxygen pressure (Gillespie, 1991; Jessen et al., 1991), proline to alanine mutations are reported that cause, even in humans, an increase in oxygen affinity. Almost insignificant replacements in overall protein sequence and structure are enough to produce adaptive changes. Therefore, not all genetic changes in the genome variability can be used to exemplify evolution. The evolution of the hemoglobin molecule seems to have responded to both neutral and selective specific physiological modifications that have allowed reversible binding of oxygen to heme, without revoking the conserved structure of protein.

In this essay we maintain that there are genetic and chemical optima that constrain selection in its creative role. Selection cannot go further! It must stop or the automatic chemical machinery will become detrimental to cell biology. Moreover, we have discussed that non-Darwinian evolution preceded the Darwinian part of evolutionary change and that there is still much to be learned from those catastrophic massive destructions of early Archean life (including the ten-kilometer-wide rock that hit us in the Cretaceous) that constitutes a challenge to natural selection as the only guiding force of organic evolution.

REFERENCES

Berry, P.F., Bradshaw, S.D. and **Wilson, B.R.** (1990). *Research in Shark Bay - Report of the France-Australe Bicentenary Expedition Committee*. Western Australia Museum, Perth, Australia.

Brusca, R.C. and **Brusca, G.J.** (1990). *Invertebrates*. Sinauer, Sunderland, MA, USA.

Buss, L.W. and **Seilacher, A.** (1994). The Phylum Vendobionta - a sister group of the Eumetazoa? *Paleobiology 20*: 1-4.

Cairns-Smith, A.G. (2000). *Seven Clues to the Origin of Life*. University Press, Cambridge, UK.

Clementi, M.E., Condò, S.G., Castagnola, M. and **Giardina, B.** (1994). Hemoglobin function under extreme life conditions. *Eur. J. Biochem. 223*: 309-317.

Conrad, M. (1990). The geometry of evolution. *Biosystems 24*: 61-81.

Doolittle, R.F. (1995). The origins and evolution of eukaryotic proteins. *Philos. Trans. R. Soc. Lond. B. Biol. Sci. 349*: 235-240.

Gerhart, J. and **Kirschner, M.** (1997). *Cells, Embryos, and Evolution*. Blackwell Scientific, Inc., MA, USA.

Gillespie, J.H. (1991). *The Causes of Molecular Evolution*. Vol. 2. Oxford Series in Ecology and Evolution. Oxford University Press, New York, NY, USA.

Goldberg, D.E. (1995). The enigmatic oxygen-avid hemoglobin of Ascaris. *BioEssays 17*: 177-182.

Gould, S.J. and **Eldredge, N.** (1977). Punctuated equilibria: The tempo and mode of evolution reconsidered. *Paleobiology 3*: 115-151.

Gupta, R.S., Aitken, K., Falah, M. and **Singh, B.** (1994). Cloning of *Giardia lamblia* heat shock protein HSP70 homologs: implications regarding origin of eukaryotic cells and of endoplasmic reticulum. *Proc. Nat. Acad. Sci. USA 91*: 2895-2899.

Hashimoto, T., Nakamura, Y., Nakamura, F., Shirakura, T., Adachi, J., Goto, N., Okamoto, K. and **Hasegawa, M.** (1994). Protein phylogeny gives a robust estimation for early divergences of eukaryotes: phylogenetic place of a mitochondria-lacking protozoan, *Giardia lamblia*. *Mol. Biol. Evol. 11*: 65-71.

Hoenigsberg, H.F. (2002). Beyond Darwinism. In memoriam of Stephen J. Gould. *Genet. Mol. Res. 1*: 372-375.

Jessen, T.H., Weber, R.E., Fermi, G., Tame, J. and **Braunitzer, G.** (1991). Adaptation of bird hemoglobins to high altitudes: demonstration of molecular mechanism by protein engineering. *Proc. Nat. Acad. Sci. USA 88*: 6519-6522.

Knoll, A.H. (1992). The early evolution of eukaryotes: a geological perspective. *Science 256*: 622-627.

Muller, W.E. (1995). Molecular Phylogeny of Metazoa (animals): monophyletic origin. *Naturwissenschaften 82*: 321-329.

Patterson, D.J. (1994). Protozoa: Evolution and systematics. In: *Progress in Protozoology* (Hausmann, K. and Hülsmann, N., eds.). *Proceedings of the IX International Congress of Protozoology*, Berlin 1993. Gustav Fischer Verlag, Stuttgart, Jena, New York, pp. 1-14.

Patterson, D.J. (1999). The diversity of eukaryotes. *Am. Nat. 154* (Suppl): S96-S124.

Patterson, D.J. and **Sogin, M.L.** (1992). Eukaryote origins and protistan diversity. In: *The Origin and Evolution of Prokaryotic and Eukaryotic Cells* (Hartman, H. and Matsuno, K., eds.). World Scientific Pub. Co., NJ, USA, pp. 13-46.

Rizzi, M., Wittenberg, J.B., Coda, A., Fasano, M., Ascenzi, B. and **Bolognesi, M.** (1994). Structure of the sulfide-reactive hemoglobin from the clam *Lucina pectinata*. *J. Mol. Biol. 244*: 86-99.

Seimiya, M., Ishiguro, H., Miura, K., Watanabe, Y. and **Kurosawa, Y.** (1994). Homeobox containing genes in the most primitive metazoa, the sponges. *Eur. J. Biochem. 221*: 219-225.

Schopf, J.W. (1992). The oldest fossils and what they mean. In: *Major Events in the History of Life* (Schopf, J.W., ed.). Jones and Bartlett, Boston, MA, USA, pp. 29-63.

Schopf, J.W. (1994). Disparate rates, differing fates: tempo and mode of evolution changed from the Precambrian to the Phanerozoic. *Proc. Nat. Acad. Sci. USA 91*: 6735-6742.

Wolpert, L. (2002). *Principles of Development*. Oxford University Press, Oxford, USA.