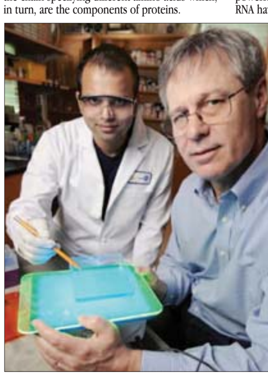
The signature of life in changing times

The basic process, since the time the earth was still young, has stayed intact, says s ananthanarayanan

IT has been shown that amino acids, the basic components of proteins, can self-assemble from available materials in the conditions that prevailed when the earth was still young. So also, essential elements of life, the components of DNA, can arise from basic elements. The appearance of life itself is signalled by its basic, complex signature, which is self-replication through the action of DNA-like structures. Researchers at the Georgia Institute of Technology, Atlanta, report in the journal *Public Library of Science* evidence to show how RNA, which is closely associated with DNA, retained its function through periods when the chemistry of the earth was significantly different from what it is today.

The functions of living things are directed by proteins. And species differ from one another because their body cells create different proteins. Individual cells contain the "instruction set" of what proteins to produce and cells transfer these instructions to offspring during cell division. The great DNA molecule contains the instructions in the form of a series of chains of combinations of only four kinds of components, different parts of the chain specifying different amino acids which, in turn, are the components of proteins.

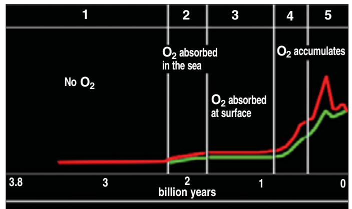


Georgia Tech biology postdoctoral fellow Shreyas Athavale (left) and chemistry and biochemistry professor Loren Williams look at a gel showing the products of a ribozyme reaction that was run with iron instead of magnesium. Under identical anaerobic conditions, the activity of two enzymes was enhanced in the presence of iron, compared to their activity in the presence of magnesium.

In practice, reading the portions of the DNA chain and assembling the proteins requires further process and this is facilitated by RNA, which is related to DNA and similar in many ways. RNA stands for RiboNucleic Acid and DNA is De-oxyriboNucleic Acid — which is to say RNA, with a modification: of taking away two oxygen atoms. RNA can thus perform the functions needed for life and a precursor to DNA. It was perhaps the central actor during the early stages of evolution of the process.

Study of RNA mechanism shows that it directs the synthesis of proteins with the help of the complex shapes that it assumes. The shaping, or folding, in turn is influenced by the specific atoms placed in its structure. It is now known that the presence of magnesium atoms, with their property as a carrier of balancing positive charge, is important to stabilise RNA structure. The presence of a particular kind of atom in RNA structure suggests that the development of life was dependant on particular ambient conditions and access to specific elements in the environment. In this context, the geological record shows that at the time that life, and RNA structure, is known to have arisen conditions on earth were far different from what they are now.

Fossils of the earliest microbe-like objects are of about 3.5 billions years ago. But the earth of three billion years ago had no free oxygen and there was a great abundance of free iron. There was life supported by photosynthesis, yes, but most living things depended on fermentation, with the emission of methane, a powerful greenhouse gas. Could RNA have had a different structure then?



Progress of the accumulation of oxygen

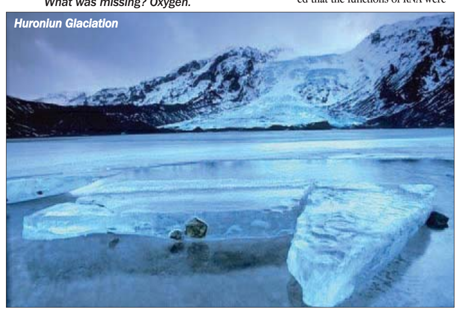
Lava from Kilauea, Hawaii, flows into the steaming Pacific Ocean. The primitive atmosphere of earth was probably made up of carbon dioxide, nitrogen and water, laced with methane, ammonia, sulfur dioxide, hydrogen sulfide and hydrochloric acid. What was missing? Oxygen.

Great oxygenation

The so-called Great Oxygenation Event dates to about 2.4 billion years ago when photosynthesis became the important process on earth and created huge free oxygen. Till this period, the oxygen released by photosynthesis was rapidly consumed by organic matter and in bacteria-aided oxidising of free metals, particularly soluble iron. The geological records of the period indicate huge deposits of oxidised iron — the Banded Iron Formation structures, which show a fixing of free iron into oxides. While oxygen was taken away in this way, life forms were anerobic, or thrived in oxygen-free conditions, deriving energy through fermentation, which generated carbon dioxide and methane. With these gases predominating, there was a marked greenhouse effect and temperatures were high.

The Goe started when oxidisable minerals emitted iron in particular. With oxygen now free to reach the atmosphere, methane got oxidised to water and carbon dioxide and life changed from anerobic to the present, oxygen-based form. Rising photosynthesis consumed carbon dioxide and reducing greenhouse gases meant a falling of temperatures, which triggered the *Huronian Glaciation* some two billion years ago, the most severe Ice Age occurrence on record.

The evolution of life since then is based on oxygen-rich and iron-poor conditions and the form of RNA structure logically provides magnesium, rather than iron at its vital locations. But the question is whether there could, in fact, have been a biological changeover from iron to magnesium, keeping step with the geological overturning of the epoch. Studies have shown that iron was a participant in ancient biological processes. The transition in the presence of iron from soluble salts to being fixed as oxide deposits was accompanied by both biological and geological changes. But iron and magnesium are distinct elements and quite far removed, chemically. Could the delicate RNA structure have functioned to support life if one of its vital components was represented, partly or wholly, by a handy substitute?



The Atlanta study

Shreyas S Athavale, Anton S Petrov, Chiaolong Hsiao, Derrick Watkins, Caitlin D Prickett, J Jared Gossett, Lively Lie, Jessica C Bowman, Eric O'Neill, Chad R Bernier, Nicholas V Hud, Roger M Wartell, Stephen C Harvey and Loren Dean Williams of the schools of biology, biochemistry and the intracellular origins of evolution at the Georgia Institute of Technology, Atlanta, studied the question both on theoretical as well as experimental bases. The molecular environment of the magnesium or iron atoms was determined by X-ray scattering studies. The binding of ionised magnesium or iron to an RNA fragment was then studied, based on known theory, and the energy transfers involved were estimated. The studies indicated that replacement of Mg^{++} with Fe^{++} resulted in no changes that would alter the function of RNA.

State-of-the-art chemical procedures also allow the detailed shape characteristics of complex molecules to be assessed using chemical probes or reactants. The methods, which use the reactivity of specific chemical groups in the RNA structure and test for products of expected chemical reactions, have been employed successfully in work done with the HIV-1 genome. Studies on the same lines again showed that the use of Mg^{++} or Fe^{++} resulted in the same changes, which indicates that iron could take the place of magnesium in the area of interest. Specific studies, in fact, indicated that the functions of RNA were *enhanced* when iron took the place of magnesium!

The possibility of RNA based on iron in place of magnesium, in this context, opens a fresh view of the ancient RNA world. Free iron was, at that time, available in solution and in non-toxic form. Considering RNA based on iron allows us to think of functions for RNA that are wider than with magnesium alone. But it shows a process of a changeover, by selection, to a suitable element, in magnesium, when iron became scarce, and to keep intact the mechanism of cell division and protein replication.

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A matter of opinion

With West Bengal having commenced DDT spraying, christopher gonsalves analyses the double role the insecticide plays ~ between being a boon to preventing vector-borne diseases and a vice on the human system

WITH the monsoon around the corner, the use of DDT (or dichlorodiphenyltrichloroethane, as it is chemically known) in the health sector is always given the go-ahead as an effective and appropriate solution for controlling vector-borne diseases in the country, especially in West Bengal. But while DDT may have immediate positive effects, the long-term effects are equally negative. Since these negative effects span over a long time, it can seem subtle and the way we treat an insecticide like DDT may not be as serious as it should be.

"All districts in West Bengal have started indoor residual spraying. These include North and South 24-Parganas and Noida. The spraying will continue till the end of June," says Souvik Basu, a consultant with the National Vector Borne Disease Control Programme.

"This programme definitely educates spray workers about the dangers of DDT. The state provides them with personal protective equipment that includes aprons, gloves and masks," says Basu, whose organisation is the foremost Central agency for the prevention and control of vector borne diseases. The use of DDT has been banned in agriculture but the NVBDCP uses a regulated amount of it in the health sector to prevent the spread of mosquitoes and sand flies — the latter being notorious for spreading kala azar.

Pradip Malhotra, senior regional director, Kolkata, ministry of health and family welfare, says spray workers are trained intensively on causing the minimum inconvenience to people as well as the environment. However, when workers from the Kolkata Municipal Corporation do the rounds in the city, especially the Elliot Road area, all they are equipped with are canisters filled DDT and no protective equipment. Asked if they are aware of the dangers of their vocation, they say they are but still choose to not wear protective equipment because of the heat.

Pradip Malhotra

Their canisters contain DDT mixed with water. When sprayed, the water soon enough dries and what remains is a thin film of the insecticide over whatever it comes in contact with. Rachel Carson's book, *Silent Spring*, describes in detail the ill effects of DDT on the body. It is fat-soluble and can prove harmful in minute doses. Once it enters the body, it only accumulates. Tests carried out on animals show that DDT inhibits essential heart enzymes and can even lead to the disintegration of liver cells.

Traces of it have even been found in samples of mothers' milk. Carson's book reveals an even more sinister fact that DDT poisoning doesn't start with breast-feeding but from the time the child is in the womb. Experiments on animals show that chlorinated organic insecticides freely cross the placenta barrier and though the amount that passes does so is small, the harm is still immense since unborn and young offspring are more susceptible to poisoning than adults. Avijit Ghosh, NVBDCP project director, says the programme strives to decrease dependency on DDT even if other methods such as insecticide-treated mosquito nets aren't as effective.

"DDT comes from the Hindustan Insecticide Limited factory in Rasayani, Madhya Pradesh. Last year, it supplied approximately 70,000 tons but this year it is down to 60,000 tons," says Ghosh. Separate consignments are ordered for the districts, where these are stored in public health centres and then distributed in smaller quantities to various localities. The consignments are sealed and transported by truck.

That, though, is where caution presumably ends because spray workers never give residents prior notice of their arrival. Malhotra says most people do not have any time to take precautionary measures keep vessels and food safe from DDT.

Transcripting actively

tapan kumar maitra explains the amplification process of rRNA genes and rDNA

ALL organisms have multiple copies of rRNA genes. While a few copies are present in bacteria, in eukaryotes the rDNA is highly repetitive. In the case of *Xenopus*, each nucleolar organiser contains 450 rRNA genes that are tandemly repeated along the DNA molecule (ie, head-to-tail) and are separated from each other by stretches of spacer DNA, which is not transcribed. These linear repeats of genes can be visualised clearly in electron microscopy. These rRNA genes are actively transcribed and the nascent RNA chains are spread perpendicularly to the DNA axis. Each gene is transcribed into a long RNA molecule (which varies in size from 40S to 45S, depending on the species) that will eventually be processed, giving rise to 18S, 28S and 5.8S RNA. Since each gene has a fixed initiation site (promoter) and a fixed termination site, the transcripts adopt the characteristic "Christmas tree" or "fern leaf" configuration.

Nucleolar rRNA genes are transcribed by RNA polymerase I, and these molecules (about 100 per gene)

The Nucleolus 327

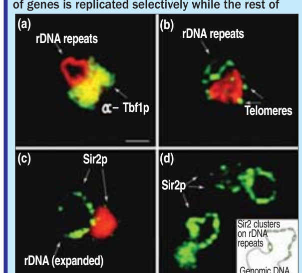
REPETITIVE rDNA CISTRONS IN VARIOUS ORGANISMS

Organism	% rDNA	DNA cistrons per Haploid genome
E.Coli	0.42-0.65	8-22
B. Subtillis	0.38	9-10
Hela cells	0.005-0.02	160-640
Drosophilla (wild-type)	0.27	130
Xenopus (wild-type)	0.2	450

can be visualised at the origin of each nascent RNA chain. The genes coding for 5S RNA are not located in the nucleolas and, therefore, are transcribed normally in the *Xenopus* anucleolate mutant. The 5S genes are also present in multiple copies; *Xenopus* has 24,000 5S genes that are located in the tips (telomeres) of most chromosomes, as shown y *in situ* hybridisation of ^{3}H-5S RNA to chromosome preparations.

As with rDNA in the nucleolus, the 5S genes are arranged in tandem repeats separated by segments of spacer DNA. Tandem repeats of genes separated by spacers seem to be a general organisational feature of the eukaryotic genome. The 5S RNA is transcribed by RNA polymerase III and is then transported to the nucleolus, where it is incorporated into the immature large ribosomal subunits.

Gene amplification is the process by which one set of genes is replicated selectively while the rest of



the genome remains constant. The clearest example of such amplification is seen in the rDNA of amphibian oocytes, such as those of *Xenopus*, whose eggs are extremely large cells (1.2 mm in diameter) that accumulate large numbers of ribosomes (10^{12}) for use during early development. Oocytes must synthesise all these ribosomes using the genes contained in a single nucleus, and they achieve this by amplifying the number of rDNA genes about 1,000-fold.

Amplification takes place in very small oocytes which are in the early stages of meiotic prophase. During *pachynema*, excess DNA begins to accumulate on one side of the nucleus, forming a cap that can be stained. This cap incorporates 3H- thymidine intensely (while the chromosomes do not) and by the end of the amplification process it contains 25pg of DNA, an amount equivalent to two million rRNA genes. As the oocytes grow, the extra DNA is accommodated in 1,000-1,500 extra-chromosomal nucleoli. The amplified DNA is not inherited by the embryo but is lost in the course of development.

The newly synthesised DNA is indeed rDNA. This can be shown by an *in situ* hybridisation procedure in which 3H-ribosomal RNA is hybridised to cytological preparations of *Xenopus* oocytes that have been previously treated with alkali to denature the DNA. The hybridised material can then be located by autoradiography.

This shows that RNA hybridises to the cap of amplified DNA. Unlike the genes coding for the large ribosomal RNAs, the 5S genes are not amplified during oogenesis. Oocytes meet the increased demand for ribosomes by activating all 24,000 5S genes.

The amplification of genes at specific developmental stages is not a common event. The only instance in which it is known to occur, other than in oocyte rDNA, is in the DNA puffs of insects. In both cases, the amplified material is not passed on to future generations.

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