Palaeobiochemisty—Bridging the gap between the living and dead

Development of very sensitive molecular biological techniques like polymerase chain reaction (PCR) and cloning on one hand, and of computer graphics and cinematographic techniques on the other, have allowed imaginations to soar to heights, unthinkable just a few decades ago. The result is that the 'Jurassic Park' and the like are no more only in the realm of science fiction but have raised exciting possibilities in the field of palaeontology which with the conventional approaches had not remained as informative and useful as it used to be.

Molecular biological studies of fossil and geological materials have great potential—not only such studies may provide very definitive and vital information about evolution of living forms but also have immense applied values, particularly in the search of fossil fuels etc. Considering this potential and considering the fact that in India, molecular biologists and palaeontologists/geologists generally do not understand each other's 'language', the Department of Science and Technology, New Delhi, sponsored a 'brain-storming' session on palaeobiochemistry at the Centre of Advanced Study in Geology, Panjab University, Chandigarh on 9 and 10 December 1993, with Ashok Sahni as the coordinator. A base paper by Sahni provided the background for the discussion. The meeting was attended by many leading geologists/palaeontologists representing various universities and institutes and a number of molecular biologists.

Molecular biological studies on fossil material depend on the availability of organic remains. Most of the organic remains are degraded beyond recognition during the process of fossilization. The carbon derived from organic remains of the organisms is identifiable in coal, lignites, petroleum crudes, etc. However, these do not allow any information about the original macromolecules. Nevertheless, in spite of all odds against them, some biomolecules have been identified in Pre-Cambrian remains (500 million years). On the other hand, certain conditions of fossilization, for example the entombment of the organisms in amber (derived from certain plant resins[1]) or a continued burial in frozen state, provide a remarkably good preservation of even macromolecules like DNA. Under certain conditions, some structural proteins, like collagen, can be better preserved physically rather than chemically so that even though amino acid analysis fails, electron microscopy of the fossil material allows rather detailed comparison with extant collagens[2]. Radioimmunoassays have also been applied to detect small quantities of proteins from bones, teeth or skins of extinct animals[3].

However, the most exciting and exotic possibilities of applications of molecular biology to palaeontology are with the fossil materials that have preserved the nucleic acids. Since DNA carries the genetic information, comparison of the DNA base sequences in extinct and extant relatives allows a direct visualization of the process of evolution that may have actually occurred millions of years ago. In the recent past several reports have appeared which show feasibility of studies in which DNA from organisms extinct millions of years ago could be characterized[4,5]. At the heart of such dream come true achievements lies the remarkably simple technique of polymerase chain reaction, which attracted last year's Nobel prize in Chemistry[6].

The polymerase chain reaction, or PCR in short, depends on the unique property of an existing DNA molecule to act as the template for synthesis of its replicas with the help of a DNA polymerase and short complementary 'primer' sequences. With the discovery of DNA polymerase that can withstand high temperatures (enzyme is isolated from thermophilic bacteria), several cycles of DNA synthesis can be carried out without the need of supplying fresh enzyme for every cycle of denaturation and reannealing of the DNA duplex and the primers. This results in exponential increase in the amount of new DNA synthesized. Since the process of replication has a very high fidelity, the large number of DNA molecules resulting from PCR remain identical to the original template. As a result, one can start with very minute quantities of DNA (in picogram or even smaller quantities) and generate sufficiently large amounts for a variety of analytical and preparative approaches. An important component of the PCR approach is the short primer sequences that need to flank the region of DNA that one wants to amplify. Our understanding of genomic DNA in multicellular organisms has revealed the repetitive occurrence of a variety of short sequences at short intervals along its length—this has been the basis of application of DNA fingerprinting and also provides a rationale for hoping to amplify genomic DNA from suitable organic remains of a wide variety of fossil organisms.

With all the simplicity of molecular biological techniques, there remain grave dangers of artifacts. These were elaborately discussed at the brain-storming session. The fossil material has all the chances of being contaminated with DNA of a variety of extraneous origins, either during the process of fossilization, or during the long interval between its fossilization and discovery by the worker or during the interval between its discovery and processing for PCR etc. The power of PCR amplification would work as effectively (if not more effectively!) on the contaminating DNA and one may end up chasing an extant and undesirable DNA rather than the real palaeontological stuff.

Thus molecular biologists who take up such studies would need to worry about the very high probability of amplifying non-target DNA. At the same time, palaeontologists and geologists would also need to be careful in selecting the material for molecular biological studies. The first thing that needs to be done is to identify possible sources of fossils that have a good probability of retaining original DNA, e.g. amber-entombed fossils (amber deposits are known to be present in India). Equally important is to define specific questions in relation to such fossil material—whether one wants to study evolution of certain specific gene/s or one wants to make more general global studies on changes in genomic and/or organelle (mitochondria, chloroplasts, etc.) DNA during the course of time in related taxa. The discussions during this meeting indicated that the existing information on the availability of fossils of kinds suitable for molecular biological studies in India is not sufficiently specific and detailed. Therefore, it was decided that this must be first accomplished before meaningful

approaches in palaeobiochemistry or molecular palaeontology can be taken up. However, a related approach that was discussed at the meeting and which could possibly be taken up almost without much delay relates to what may be called as **molecular archaeology**, i.e. molecular biological studies with the human and other organismal remains known to be present in a number of archaeological sites—these studies may provide very interesting and significant data for archaeology, anthropology, demography, history, etc. In these studies also, one would need to be very careful about the contaminations that may happen at the site or in transit.

Another important step that was decided upon in the meeting was to organize a laboratory workshop jointly by the Department of Geology, Panjab University and the Institute of Microbial Technology (IMTECH), Chandigarh, where interested geologists/palaeontologists can learn the relevant molecular biological techniques and the molecular biologists get familiar with fossil materials and the vagaries of extracting DNA from such sources. In any case the session was a good meeting point for specialists working with as diverse material as the living and the dead.

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