CURRENT SCIENCE

Volume 65 Number 7 10 October 1993

NEWS

Mitigating earthquake hazards*

Earthquakes are caused by the sudden release of elastic strain energy which is stored in large volumes of rock masses. These are, in turn, produced by prolonged stresses exerted by the constantly moving outer working parts of the earth's gigantic thermodynamic engine. This outer rocky shell of the earth on which we live is thus forever stressed, and inevitably fragmented into a number of large and small lithospheric caps called plates, which are about 70–150 km thick Jostled about by ascending and descending limbs of massive convection currents rising from the earth's hot interior, these spherical caps strain away from each other astride mantle upwelling rifts that occur as long narrow ridges in the middle of the oceanic crust such as the Carlsberg ridge in the western Indian Ocean. At their far ends, therefore, they are equally hard-pressed against each other so as to fit on the constant-area spherical earth. Elastic strains thus steadily accumulate at their fractured junctions, called plate boundaries, such as the Himalaya, and to a lesser degree – perhaps a few thousandth part or even less – along weak zones in their interior. These are eventually released in catastrophic fractures and slips, thereby causing an earthquake. Thereafter the region prepares for a fresh cycle of long quiet steady accumulation of strain to be terminated by the next quake The source of constant stress in the Indian land mass is likewise furnished by gigantic convection currents underneath. The unabated movement of the Indian plate in response to these forces accumulates 8–10 m of strain along the entire 2400 km long Himalayan boundary in 500–600 years, thereby producing a series of eight to ten great earthquakes, each of magnitude greater than 8 5 from west to east, while a small fraction also accumulates in the weak zones of the otherwise strong peninsular region.

The recent Latur earthquake was the result of this continuing process. Thus, whilst great earthquakes occur mostly at plate boundaries, major and moderate earthquakes are liable to occur also in the interior of the Indian plate, although long periods of quiescence between consecutive earthquakes may give rise to an illusion that the region is aseismic and perfectly stable. Modern scientific understanding of earthquake processes does not as yet tell us how to predict earthquakes with certainty, but it does show the way how seismic hazard in a given area may be estimated quantitatively. These figures when appropriately incorporated into building design codes can lead to safe design of earthquake-resistant structures. We must therefore learn to live with earthquakes on this *terra infirma* by intelligent design of human dwellings and engineering works, for which systematically applied and rigorously implemented modern scientific approaches offer considerable promise.

*Based on a lecture delivered at the National Aerospace Laboratories, Bangalore.

V. K. Gaur, C-MMACS, National Aerospace Laboratories, Bangalore.

Restriction and modification systems: unrestricted frontiers

The FASEB Summer Research Conference on 'Restriction endonucleases and modification methyltransferases: structures and mechanisms', held at Saxtons River, Vermont, USA from July 3 to 8, 1993, focused on the work in progress and some recent findings in this field. Restriction–modification (RM) systems are defence elements employed by bacteria to protect themselves against phage infection. They are made up of: (i) A restriction endonuclease which cleaves foreign DNA at or near specific recognition sites on the DNA, and (ii) A modification methyltransferase which recognizes the same sequence and methylates a specific base within that sequence, thereby protecting the host's own DNA from degradation by the endonuclease.

Ever since the discovery of host-controlled restriction and modification of bacterial viruses in the fifties and the use of restriction enzymes in the early seventies which revolutionized the study of genetics, there has been tremendous progress in this area. Werner Arber, from Biozentrum, University of Basel, Switzerland, a pioneer in this field, dwelt on the historical events leading to the discovery and application of RM systems in biological research in the plenary lecture of this conference. He also spoke on the functional roles of these systems for evolving populations of

microbial cells, where these systems could serve to limit the spread of phage infection. Geoffrey Wilson of New England Biolabs (NEB), Beverly, Massachusetts, USA, presented an overview of the organization and evolution of Type II restriction enzymes, which are most commonly and universally used in biological research. He mentioned that these systems have similar gene organizations, convergent or divergent. Different RM systems can occur in the same bacterial species. Typically, the Type II endonuclease is a dimer of identical subunits, whereas the methyltransferase is a monomer. An interesting feature of these enzymes is that they have insignificant homology at the amino acid level, even though they recognize the same DNA sequence. Isoschizomers (endonucleases of different systems from different species of bacteria that recognize the same sequence of DNA) also do not show any appreciable homology, illustrating the diversity of organization and evolution of these systems[1].

One mode of regulation of these systems was illustrated by Joan Brooks of NEB with a typical example, the *Bam*H1 system, in which this RM system has a controlling 'C' protein element in addition to the modification and restriction enzymes. The 'C' protein, whose gene is situated between the methylase and endonuclease genes, and whose promoter is thought to be located within the coding region of the methylase gene, represses the methylase activity while elevating the endonuclease activity. 'C' proteins are found to be present in other restriction–modification systems such as *Sma*1, *Pvu*II and *Eco*RV. These proteins being highly homologous, are interchangeable among these systems[2].

In cloning and hyperexpression of many of these Type II RM systems in *E.coli*, a major difficulty observed is that stable clones fail to be established in the host strains used. This is because *E.coli* K-12 encodes restriction systems which monitor the origin of invading foreign DNA and determine its fate. One such host-encoded restriction system is the McrBC, (methylated cytosine restriction) which cuts DNA at methylated cytosines occurring at specific restriction site sequences. Work on the McrBC system has revealed that it is an endonuclease which requires GTP as a cofactor. The McrBC system comprises of two genes McrB and McrC. The former encode $McrB_L$, and $McrB_S$ polypeptides, whereas the latter codes for the McrC polypeptide. $McrB_L$ and McrC polypeptides associate to form the mature endonuclease, and the $McrB_L$ subunit is thought to contain the GTP binding domain with flanking DNA recognition domains. All these studies were summarized by Elizabeth Raleigh of NEB[3]. For studies on protein–DNA interactions using RM systems as models, it has become necessary to purify and crystallize the proteins to derive their three-dimensional crystal structures. Three of these enzymes, namely *Eco*R1, *Eco*RV and *Bam*H1 endonuclease proteins have been crystallized and for the former two, the crystal structures of free protein and DNA-bound protein are known. John Rosenberg of the Department of Molecular Biophysics at Yale University, presented his work on the crystal structures and molecular models of the *Eco*R1 endonuclease. It appears that the enzyme binds to its specific DNA in the absence of magnesium, but cleaves it only when magnesium is present. Recognition of DNA at the cognate site requires an extensive network of hydrogen bonds between the amino acid side-chains and the bases of the recognition sites as well as between amino acid residues themselves. Glu-144 is an important residue which positions other residues for specific recognition of the DNA. In particular, a triad consisting of amino acids, Asp-91, Glu-111 and Lys-113, is known to play a role in catalysis. Site-directed mutagenesis at these positions has shown that there is a perturbation of the recognition network resulting in the loss of that catalytic activity. Studies are in progress to elucidate the structure of the protein bound at a non-cognate or 'star' site, GACTTC (ref. 4).

The crystal structure studies of *Eco*RV by Fritz Winkler of Hoffman-La Roche, Basel, Switzerland, have shown that conformational changes occur both in protein and DNA on binding to specific and non-specific DNA, suggesting a structural basis for the strong dependence of efficient catalysis on substrate recognition. The *Eco*RV enzyme is a dimer of identical subunits, each monomer having 244 amino-acid residues, and the protein is of mixed alpha–beta architecture. A large part of contacts between the *Eco*RV monomer and cognate DNA are mediated by two short loops. The active site triad consists of Asp-74, Asp-90 and Lys-92. A magnesium ion fits into the active site pocket and is in contact with Asp-74, Asp-90 and a non-esterified oxygen atom from the scissile phosphodiester group. These observations have been made in part by crystal structure studies of the free enzyme, a specific complex with the decamer GGGATATCC and a non-specific complex with the octamer CGAGCTCC (ref. 5).

Insights into the mechanisms of DNA recognition and cleavage by *Eco*R1 and *Eco*RV were provided by Alfred Pingoud of Zentrum Biochemie at Hannover, Germany. His group has focused on the molecular events taking place between the first non-specific encounter of a restriction enzyme with DNA and specific recognition and cleavage of that DNA. Whereas *Eco*R1 requires magnesium ions for DNA cleavage but not for binding, *Eco*RV requires the ion for specific binding. In both the cases, the enzyme diffuses linearly along the DNA and this movement is retarded when the enzyme comes across one or more sites related to the canonical site. Sequences flanking the recognition site modulate the rate of DNA cleavage, possibly by affecting the flexibility of the DNA backbone. For both enzymes, the reaction mechanism involves an in-line attack by a water molecule on the scissile phosphodiester bond, leading to the inversion of configuration of the phosphorus atom. Experiments involving chemical modifications of the scissile phosphate suggest that *Eco*R1 and *Eco*RV require substrate assistance for catalysis[6].

Taking *Eco*RV as a case in point, Stephen Halford of the Department of Biochemistry, University of Bristol, Bristol, UK discussed how this enzyme, although is able to bind DNA at non-specific sites with the same affinity as it could to its specific site in the absence of magnesium, can cleave its cognate sequence at least a million times faster than any other DNA sequence, in the presence of magnesium. The enzyme shows a much higher affinity for magnesium when bound at the cognate sites than at a non-cognate site. At its cognate site, conformational changes both in the protein and DNA create the correct binding site for the magnesium ion. The residues at the active site are similar to those of *Eco*R1, although the sites in the two enzymes may function differently. It has been found that *Eco*RV requires two metal ions per active site where one may act catalytically and the other may play a structural role[7].

Enzymes other than Type II systems were discussed by William Jack of New England Biolabs. His group while attempting to clone the DNA polymerase gene

from the hyperthermophile archaebacterium '*Thermococcus litoralis*' (commonly known as Vent Polymerase, used in certain PCR applications), discovered the presence of two in-frame intervening sequences in the DNA, the second of which codes for a 'homing' endonuclease. The mature polymerase protein is produced by the recently discovered phenomenon of protein-splicing, and not by RNA splicing to remove introns. Site-directed mutations of the splice junctions at the protein level either abolish or decrease the rate of splicing reaction. Pulse-chase experiments demonstrate the initial appearance of a precursor followed by appearance of the spliced products and the intron-encoded endonuclease protein. Other experiments show that protein splicing and the 'homing' endonuclease activity are independent of each other[8].

The poster sessions covered a wide range of topics related to RM systems. Eric Fisher, of the University of Illinois, Urbana-Champaign, USA, presented his work on the isolation of mutants of *Eco*R1 endonuclease with enhanced specificity for the methylated GAATTC sequence of the DNA. Cloning, expression and purification of the recombinant *Bam*H1 enzyme was presented in a poster by M. A. Mukund of Astra Research Centre India, Bangalore in which overexpression was achieved using the dual compatible plasmid strategy. Wolfgange Wende of Medizin Hochschule, Hannover, Germany, described the construction of fusion proteins of the enzyme *Eco*RV, with different N-terminal tags, such that heterodimers of the protein could be made for purification by a rapid affinity chromatography procedure. Maria Yebra of the Department of Chemistry, Wayne State University, USA presented her work on a rapid, sensitive and quantitative method to detect restriction enzyme activity, which involves the incubation of an oligonucleotide substrate DNA (having its 5′ end radiolabelled and its 3′ end tagged with Biotin) with a chosen enzyme. One of the products would be radioactive and could therefore be quantitated, after its separation from the other (biotinylated) fragment on a streptavidin-agarose column.

Future directions of enquiry in this expanding field point towards investigating the structural aspects of protein–DNA interactions in the restriction and methylation reactions, identification of the catalytically important amino acids in these proteins, and elucidation of their reaction mechanisms.

1. Wilson, G. G., *Nucleic Acids Res.*, 1991, **19**, 2359–2566.
2. Brooks, J. E. *et al.*, *J. Bacteriol.*, 1992, **174**, 7194–7201.
3. Raleigh, E. A. *et al.*, *J. Mol. Biol.*, 1992, **225**, 327–348.
4. Rosenberg, J. M., *Curr. Opin. Struct. Biol.*, 1991, **1**, 104–113.
5. Winkler, F. K., *Curr Opin. Struct. Biol.*, 1992, **2**, 93–99.
6. Pingoud, A. *et al.*, *Biochemistry*, 1992, **31**, 3727–3732.
7. Halford, S. E. *et al.*, *Biochemistry*, 1991, **31**, 8743–8753.
8. Perler, F. B. *et al.*, *Proc. Natl. Acad Sci. USA*, 1992, **89**, 5577–5581.

M. A. Mukund, Astra Research Centre India, Bangalore.

The measure of science and scientists

A report recently released by the Philadelphia-based Institute for Scientific Information (ISI), USA, (*New Scientist*, 1993, **138**, 12–15) regarding detailed analysis of the work of scientists, has kicked off a debate among scientists in the West. Commissioned by a science magazine, *New Scientist*, published from the UK, the report is a study of scientists' published work, especially citation-analysis of their publications during a period of ten years. Though the analysis was specifically carried out with reference to AIDS research, the results have a bearing on all areas of science. The report suggests that a lot of work is generally redundant, mediocre and rarely read by scientists. In turn, the analysis indicates as to which science (and scientists) should be supported.

Detailed analysis of individual scientist's publications brings out quite a few surprises. The more important results are: funding of scientific projects is often continued for years without proper evaluation; good science often emerges from small countries and laboratories with modest funds where researchers are working on well-defined areas. At a time when public funds for S&T are scarce, such an analysis can provide good insights into a laboratory's work, help a scientist in planning the experiments and publishing judiciously, and assist the donor in coming to a right decision regarding financing.

Largely, these points are true in the Indian context too. With about 2,000 Indian scientific journals, work in approx. 20 is internationally indexed and cited. And for the work published abroad by the bulk of our scientists, according to the report, India finds no place among the first fifteen countries with a high citation index.

Objectives of the study

Economic crunch in most of the countries of the world has led to decreased funding of S&T. This, in turn, has led to quite a few changes, the extreme case being the closure of laboratories or discontinuation of on-going programmes. In this exercise, questions being increasingly asked are: what is the importance and the impact of the on-going science programmes?

Here the time-tested methods which have been relied upon are peer review, the ability to raise funds for a project and citation analysis. Scientific works which are quoted maximally by colleagues in their publications (i.e. receive a large number of citations) are often considered as the more influential papers. While there are occasional misgivings regarding the relative fairness of the first two criteria, the reliability of citation index can become a highly contentious issue. This is specially true for scientists in India and other developing countries and has been dealt in great detail by several eminent scientists (see *Current Science*, 1991, **61**, 25 July and 1992, **63**, 10&25 November). In brief,