applications in organic synthesis. Synthesis of various 1,2,4-trioxanes and trioxepanes as simple analogues of artemisinin was the subject matter of the lecture given by Chandan Singh (CDRI, Lucknow).

On the third day, after exploring the Konark and Puri temples, the evening scientific session started with an overview of the natural product research by A. Banerji (BARC, Bombay). This was followed by the presentations by two young scientists T. Rajamannar (SPIC, Madras) and S. Janardhanam (Madras University) on the synthetic aspects of tricyclic systems using vinyl radical cyclization as the key reaction. The post dinner session was illuminated by the talk on organic materials (new organic π donors) by M. V. Lakshmikantham (University of Alabama). Origin of the various molecular mechanics calculations and how to utilize them was illustrated by E. D. Jemmis (University of Hyderabad) in a simplified manner. Subsequently S. Jena (Utkal University, Bhubaneswar) presented the use of computer programmes in organic synthesis.

Photochemistry was the focal theme of the opening session on the fourth morning. First V. Ramamurthy (Dupont, USA) explained various aspects of zeolites followed by their effect on the photochemistry of various substrates. Later A. Ghosh (RRL, Trivandrum) presented a talk on the photodegradable polymers containing a o-nitrobenzyl chromophore. K. Pitchumani continued the topic and discussed the role of cyclodextrins and clays on various photorearrangements. A novel electrochemical mediated 3,3-sigmatropic shifts was unveiled by K. K. Balasubramanian (IIT, Madras) in his lecture. Attempts to construct the molecular houses (calixarenes) was presented by P. Rajkumar (Madras University). In the organometallic front, while A. Sarkar (NCL, Pune) explained the discovery of a new rearrangment reaction in Fischer carbene complexes, S. Sengupta (Jadavpur University, Calcutta) explored the role of palladium catalysts in the coupling of diazonium compounds with olefins. The final evening session was on bioorganic chemistry. In this session V. N. Rajashekaran Pillai (Kerala University, Kottayam) highlighted the discovery of a few efficient polymeric supports for the peptide synthesis. N. Jayaraman (IIT, Kanpur) presented the construction of zinc finger modules and their interactions with DNA. S. Bhattacharya (IISc, Bangalore) explained the complexity of the vesicular topography and their influence on the regulation of the reactivity. The last session of the meeting started with a talk by R. Sankara Subramanian (BPRL, Bangalore) on the enantioselective synthesis of indolizidine alkaloids. Enantiospecific synthesis using enzymes was discussed by N. W. Fadnavis (IICT, Hyderabad) and V. S. Parmar (Delhi University). Finally, S. C. Basa (RRL, Bhubaneswar) presented a talk about the research activities pursued in RRL, Bhubaneswar.

The scientific content presented in the third NOST meeting was very good and the discussions following the lectures were stimulating. Indeed, it is very heartwarming to note, as one of the senior members rightly pointed out, the average age of the participants who made good presentations is quite low, which may portend a bright future for research in organic chemistry in India. Even though this symposium did provide encouragement to local scientists, in future it will be worth exploring the possibility of giving an opportunity to more local scientists at least to attend the lectures (perhaps in an informal manner without registration).

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RESEARCH NEWS

Insights into plasmid biology

Plasmids are autonomously replicating double stranded DNA molecules found in many gram negative and gram positive bacteria. These genetic elements are dispensable for cell viability but they confer advantage in terms of viability under selective conditions such as presence of an antibiotic. The size of naturally occurring plasmids can vary from approximately 4 kb to as large as 250 kb and they can confer such diverse phenotypes on the cell such as drug resistance, degradation of heavy metals, colicin production, virulence factors in case of many pathogenic bacteria and metabolism of various organic compounds. In addition to viruses and phages, plasmids have played an important role in the evolution of molecular biology. Their small size, greater copy number and dispensability for cell viability are the main reasons that these genetic elements have been used extensively in the study of many biological processes such as replication, transcription and translation. Plasmid vectors are one of the most extensively used systems for the expression of prokaryotic and eukaryotic genes. Better understanding of their replication, stability and ability of some of them to survive in different hosts will help in designing more versatile cloning vehicles as well as in gaining insights into the problem of spread of drug resistance.

A meeting was held in Madrid (EMBO workshop on promiscuous plasmids of gram positive and gram negative bacteria — Magalia Castle, Las Navas del Marques, Spain, September 18–22, 1992) to discuss the latest developments in replication, maintenance, conjugal gene transfer and promiscuity of plasmids. Some of the recent advances and interesting aspects of plasmid biology discussed in the aforesaid meeting are briefly presented below.

Mechanism of replication

Richard Novick (Public Health Research Institute, New York, USA) and Saleem Khan (University of Pittsburgh, Pittsburgh, USA) described the mechanism

of replication of *S. aureus* plasmid pT181. Plasmids from gram negative bacteria replicate by a mechanism similar to chromosomal replication, but the mechanism of replication in case of plasmids from gram positive bacteria is strikingly different and similar to some filamentous phages such as $\varphi \times 174$. These plasmids replicate by an asymmetric rolling circle mechanism and single-stranded molecules are produced as intermediate both *in vivo* and *in vitro*[1]. The staphylococcal plasmid pT181 (Tc^r) has been used as a model system for the study of replication in gram positive plasmids. The replication initiator protein RepC makes a nick at the leading strand origin and replication occurs by covalent extension of 3'-OH end generated at the nick site. Synthesis of RepC is the rate limiting step in the process of replication and this is regulated by a counter transcribed RNA by attenuation of RepC mRNA (ref. 2). The accumulation of active RepC which could result in increased copy number is prevented by modification (possibly by addition of an oligonucleotide to active site tyrosine) of one of the subunits of dimeric RepC resulting in the formation of inactive RepC. The topoisomerase II-like activity and DNA-binding activities associated with RepC can be uncoupled from each other. The replication-initiator proteins from this group of plasmids show extensive homology but can replicate only the cognate plasmid. Through *in vitro* mutagenesis and production of protein chimeras it has been shown that aa 265–aa 270 which are involved in binding to DNA determine the specificity of replication proteins[3].

Partition and stability

The high copy number plasmids such as pBR 322 and pUC 18 are partitioned in a random fashion but in the case of naturally occurring large plasmids such as F, the partitioning of plasmids into daughter cells is an active process. This is made possible by (1) active partitioning in a way similar to centromere of eukaryotic cells, (2) resolution of multimers and (3) killing of plasmid-free segregants. In F plasmid the daughter cells which fail to get a copy of the plasmid are killed by the killer Ccd B protein. Another protein Ccd A acts as an antagonist of Ccd B and thus prevents the killing of plasmid-carrying cells. The differential rates of decay of Ccd A and Ccd B account for the killing action of Ccd B (ref. 4). According to Phillipe Bernard (Universite Libre de Bruxellus, Belgium) the mechanism of killing seems to be through inhibition of topoisomerase II-cleavable complex which in prokaryotes is also inhibited by quinolone group of antibiotics. An Arg462–Cys' mutation in gyr A polypeptide makes the cells resistant to the killing action of Ccd B protein.

Kenn Gerdes (Odense University, Denmark) discussed the regulation of killer systems of RI (*hok/sok*), F (*srn* B) and R438 (*pnd* C) which is mediated through the differential decay rates of unstable antisense RNA and the killer mRNAs. In addition, the full length mRNAs cannot be translated because of presence of anti-Shine-Delgarno sequence present in the 3'-end of the killer mRNAs which can fold back on to the 5'-end of mRNA and inhibit translation. These inactive mRNAs are truncated by 35–70 nucleotides in the 3'-end to give rise to translationally active mRNAs.

Horizontal gene transfers

Transfer of genetic information through the process of conjugation plays an important role in the spread of drug resistance in bacteria. Gene transfer by conjugation occurs both in gram positive and gram negative bacteria, though by different mechanisms. In addition, transfer of genes from a plant pathogen *Agrobacterium tumefaciens* to plant cells can also occur by a process quite similar to conjugation in gram-negative bacteria[5]. Both processes require close cell contact and in both cases single-stranded DNA is transferred from donor to recipient cells. The *A. tumefaciens* plasmid pTiC58 is a large (~150 kb) plasmid which has the genetic information for transfer of genes from donor to recipient bacteria (tra region), transfer of genes from bacteria to plants (vir region) and tumour formation in plants (T-region). The Tra system of *A. tumefaciens* is capable of mobilizing plasmids from other gram negative bacteria (e.g. RSF 1010) which by themselves are not transmissible. The view that the process of conjugation and transfer of plasmid from bacteria to plants are similar is further strengthened by the fact that even the vir region of pTiC58 can mobilize RSF 1010 derivative from donor to recipient cells.

Bacteriodes which is a gram negative anaerobe and is an opportunistic pathogen has a peculiar way of horizontal gene transfer. The region of the chromosome encoding tetracycline resistance (Tc^r elements) is capable of excision from the chromosome, transfer itself into a recipient strain of *Bacteriodes* and get integrated into the chromosome. These Tc elements can also mobilize co-resident plasmids and certain other regions of chromosomes. This process of mobilization has been found to be stimulated by the addition of tetracycline to the medium[6].

The process of horizontal gene transfer by conjugation has been largely responsible for the spread of multiple-drug resistance encoded by R factors among the Enterobacteriaceae. In contrast epidemics of pneumococcal meningitis caused by penicillin resistant *Streptococcus pneumoniae* have evolved resistance to penicillin via the accumulation of mutations in the peptidoglycan-synthesizing proteins namely the penicillin-binding proteins (PBPs). The PBPs identified in *S. pneumoniae* are pBP 1A, 1B, 2X, 2a, and 2b. The physiological role played by different PBPs remains unclear. Studies conducted at Astra Research Centre India and University of Sussex, (UK) have shown that PBP 1A of pneumococcus which is one of the proteins mutated during the development of penicillin resistance in *S. pneumoniae* is completely dispensable for its viability[7].

Conclusions

Plasmids are dispensable for cell viability but confer certain phenotypes on the organisms which help them to adapt to extreme environmental conditions. These genetic elements play an important role in the process of horizontal gene transfer and evolution of genomes since many of the naturally occurring plasmids are self-transmissible and also some of the genes present on the plasmid are in the form of transposons which help in spreading the genetic information from plasmids to chromosomes and vice versa. The fact that bacterial genes can be transferred to

plants is being exploited for genetic manipulation of plants for creating better varieties[5].

The study of mechanism of replication, molecular basis of plasmid stability and conjugal transfer has helped in understanding the basic biological processes involving replication, maintenance and horizontal transfer of plasmid genes and also their utilization in biotechnology.

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Aspersing aspirin: Salicylate-inducible antibiotic resistance

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One outcome that results from the exposure of microorganisms to antibiotics in their environment is the selection of resistant mutants. The origin of such mutants can be traced back ultimately to random errors in DNA replication. Another possible outcome is the emergence of variants which are genetically wild type, but phenotypically adapted to the adverse environment. In such cases the resistance is not selected but is induced. The distinction between selection and induction may not always be clear-cut. The possibility that the wild type may be programmed to 'induce' adaptive mutations under certain stresses is again attracting serious scientific attention[1]. Conversely, one can imagine the selection of mutants that are more efficient at inducing the tolerance phenotype. The problem then is to understand how different inducible resistance mechanisms get turned on. One instance in which this problem has resulted in a tale with surprising twists is the induction by salicylate of antibiotic resistance in gram-negative bacteria.

The first report of salicylate-inducible antibiotic resistance was that in 1985 by Judah L. Rosner, who showed that *Escherichia coli* K-12 cells become significantly tolerant to chloram-phenicol, tetracycline, ampicillin and nalidixic acid in the presence of millimolar concentration of chemorepellents such as salicylate, acetate, acetylsalicylate (aspirin), benzoate, dimethyl sulfoxide and 1-methyl 2-pyrrolidinone[2]. The cells reverted completely when returned to chemorepellent-free medium. It is striking that these antibiotics have diverse modes of action and that their structures are not related to that of the tolerance inducing chemorepellents.

In 1987 Sawai *et al.*[3], reported that growth in the presence of salicylate drastically reduced the OmpF porin content of the outer membrane of *E. coli*, *Klebsiella pneumoniae* and *Serratia marcescens*. Since the OmpF porins are largely responsible for permeation through the outer membrane of low-molecular-mass (<600 Da) hydrophilic molecules (including the antibiotics memtioned above), it was reasonable to infer that the resistance phenotype induced by salicylate was a consequence of reduced uptake of the antibiotics due to the loss of OmpF porins from the outer membrane. Thus the original question of how salicylate induces antibiotic resistance became sharpened into the more focussed one of how salicylate reduces OmpF porin expression.

Using various *ompF-lacZ* fusion strains, Rosner *et al.*[4] addressed this latter question by examining the effects of salicylate on the transcription and translation of the *ompF* gene. The *lacZ* gene codes for the enzyme β-galactosidase whose activity can be easily assayed. If promoterless *lacZ* sequences are fused to the *ompF* promoter and this fusion is introduced into a strain that is deleted for the normal *lacZ* locus, then β-galactosidase activity in this strain provides a measure of the *ompF* promoter's activity. Such *ompF-lacZ* fusions are called transcriptional fusions. If the promoterless *lacZ* sequence also lacks the ribosome binding site and is fused downstream of the *ompF* translation start site in the correct reading frame to yield an *ompF-lacZ* fusion protein with β-galactosidase activity, then the *ompF-lacZ* fusion is called a translational fusion and the β-galactosidase expression is a measure of both transcriptional and translational activity of *ompF*. Rosner *et al.*, found that salicylate had no effect on β-galactosidase activity from the transcriptional fusions, but two translational fusions showed 12- to 15-fold decreases in β-galactosidase activity in the presence of salicylate. From these results they could conclude that salicylate reduced *ompF* expression via a post-transcriptional effect.

OmpF porin expression was previously shown to be subject to post-transcriptional regulation by the product of the *micF* locus[5]. The *micF* locus does not contain translational open reading frames but codes for a micRNA. micRNA is the acronym carefully chosen by Mizuno, Chou and Inouye for messenger RNA interfering complementary RNA (i.e., RNA molecules with sequence complementarity to transcripts of particular genes and therefore able to bind as an antisense RNA and prevent the translation of bound transcripts). Rosner *et al.*[4], therefore constructed *micF-lacZ* fusions, and used them to show that salicylate induced *micF* transcription. Since *micF* transcripts inhibit the translation of *ompF* transcripts one