LOHAFEX – An Ocean Iron Fertilization Experiment in the Southern Ocean

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Oceans play a key role in shaping global climate by regulating atmospheric concentrations of CO_2. However, fossil fuel burning has swamped the natural carbon cycle and it is a challenge for earth system scientists to investigate how ocean uptake can be enhanced by manipulating natural processes responsible for sequestering CO_2 from the atmosphere. One such technique, ocean iron fertilization (OIF), involves fertilizing certain ocean regions with trace amounts of iron to stimulate the growth of microscopic plant-like organisms known as phytoplankton, which then die and sink. LOHAFEX, an OIF experiment designed to further understanding of this process and its potential was conducted in the Southwest Atlantic Ocean in early 2009. Its results differed significantly from those of previous OIF experiments. The six key findings of LOHAFEX are: (1) Diatoms were conspicuous by their absence due to low ambient silicate levels, and phytoplankton biomass was dominated by small (<10 □m) flagellates; (2) Phytoplankton biomass did not build up beyond 1.7 mg chlorophyll a m^{-3}, presumably due to intense grazing by zooplankton; (3) Although primary productivity almost doubled in response to fertilization, bacterial biomass and production remained low; (4) CO_2 drawdown inside the patch was modest (<15 □atm) and organic carbon accumulated in the surface layer in particulate and dissolved forms; (5) There was little export of particulate organic matter to the deep sea; and (6) Iron fertilization had little effect on the production of other climatically-important greenhouse gases, such as nitrous oxide and ozone-destroying halocarbons.

The LOHAFEX results have two important implications: (1) Although phytoplankton production in the Southern Ocean is iron-limited, supplying iron in the absence of adequate dissolved silicon for diatoms does not support unlimited biomass build-up due to top-down control by grazers. However, bottom-up control due to limitation by other micronutrients, e.g. cobalt, could not be excluded. Cobalt is an essential element required for vitamin B_{12} and its concentrations reached limiting levels at the end of the experiment. (2) Because silicon is at low concentrations over 65% of the Southern Ocean, the potential of OIF as a means to

sequester anthropogenic CO_2 should be substantially smaller than believed so far (1 Gt carbon per year).

Survival of life forms at freezing temperatures

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Psychrophilic or cold loving bacteria are the most predominant, confined to all cold habitats of the world. These bacteria are unique due to their ability to survive at temperatures below the freezing point of water. In order to do so, these cold loving bacteria possess various strategies which facilitate their survival at low temperature. Research activity in my lab has mainly focused on the distribution of psychrophilic bacteria, their survival strategies and their usefulness to the Biotech industry.

Cold-loving bacteria have been characterized from various regions of the world such as the Antarctic, the Arctic, Himalayan glaciers, deep sea etc. As of now more than 1000 different isolates have been characterized upto the species level and several new genera and species of bacteria have been identified. This has been accomplished using both polyphasic taxonomy and the metagenomic approach.

Several strategies and genes required for survival at low temperature have been identified. It has been demonstrated that cold loving bacteria adapt to low temperatures by their ability to modulate membrane fluidity by regulating fatty acid desaturases or by regulating the differential synthesis of polar and non-polar carotenoids. These studies have also led to the identification of a new gene involved in fatty acid desaturase. More recently using transposon mutagenesis, two genes namely Aspartate aminotransferase and tRNA modification GTPase have been shown to be required for growth at low temperature. Several enzymes with optimum activity at low temperature have also been characterized. The studies thus far carried out indicate that psychrophilic microorganisms are universal in their distribution and possess several strategies by which they adapt, grow and divide at temperatures close to the freezing point of water.

Finding one male among many: sound source localization by crickets in complex acoustic environments

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Male crickets use acoustic signals or calling songs as long-distance mate attraction signals. Each species of cricket has a unique calling song, the spectral and temporal features of which are used by females to recognize and localize males of their species. Female crickets must detect, recognize and localize the signals produced by a calling conspecific male, in order to find a mate. Field cricket songs are typically in the frequency range of 3-6 kHz, with wavelengths in the order of several centimetres. This poses a difficult problem for these small animals in terms of localizing sound sources. The problem is even worse in the natural situation of a chorus of conspecific males, where several simultaneously calling individuals can result in interference of song patterns and degradation of available directional cues. How do female field crickets actually manage to locate one male in a chorus? To investigate this, we first explored the natural situation of choruses in the field in terms of male spacing, movement and calling behaviour, together with sound transmission properties close to the ground. All of these define the acoustic environment of female crickets. Using closed-loop phonotactic experiments on freely walking crickets in the laboratory, we explored the rules of sensory processing and motor behaviour and used these in a simulation to produce virtual crickets, whose phonotactic walking paths were comparable with real crickets, including in complex, outdoor acoustic environments.

Causes And Consequences Of Transcription-Translation Uncoupling In Bacteria

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That translation occurs co-transcriptionally in bacteria is well known, but it is only in the last few years has the understanding emerged why this should be so. One direct consequence of the uncoupling of translation from transcription in *Escherichia coli* is that the bacterial Rho protein binds the nascent transcript and, in association with factors such as NusG, "catches up" with the elongating RNA polymerase in cis to mediate premature termination of transcription.

We have obtained evidence to suggest that in *E. coli* mutants deficient in Rho or NusG, there is an increased occurrence of R-loops (that is, RNA-DNA hybrids) in the chromosome. Each of the R-loops is generated presumably by re-annealing of a nascent untranslated transcript with the template DNA strand in the negatively supercoiled domain upstream of the transcription elongation complex. The R-loops are ordinarily toxic, because of their expected inhibitory effects on transcription, replication, and recombination. However, R-loops can also confer a survival advantage in mutants defective for RNA turnover, by providing an alternative means of mRNA degradation mediated by RNase H.

In eukaryotes, the steps of mRNA processing (capping, splicing, polyadenylation) and of mRNA export are now known to occur co-transcriptionally. Recent reports indicate that interference with these processes also leads to the generation of deleterious R-loops, in both yeast and vertebrate cells.

Translational control of p53 mRNA

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Tumor suppressor protein p53, the "guardian of the genome" is critical in maintaining cellular homeostasis and genomic stability. Earlier, we have reported the discovery of internal ribosome entry sites (IRESs) within the p53 mRNA that regulate the translation of the full length and its N-terminally truncated isoform, ΔN-p53. The two IRESs regulate the translation of p53 and ΔN-p53 in a distinct cell-cycle phase-dependent manner. We have also demonstrated that polypyrimidine tract binding protein (PTB), an IRES *trans*-acting factor (ITAF), positively regulates the IRES activities of both p53 isoforms by relocating from nucleus to the cytoplasm during stress conditions. Our recent results suggest that the structural integrity of the p53 RNA is critical for the IRES function. We have compared the secondary structure of the wild-type RNA with cancer-derived silent mutant p53 RNAs having mutations in the IRES elements. These mutations result in the conformational alterations of p53 IRES RNA that affects the IRES function. Interestingly, these mutant RNAs failed to bind to some *trans*-acting factors, such as hnRNP C1/C2 (p44). Also, partial silencing of PTB and hnRNPC1/C2 led to decrease in IRES activity and consequent changes in cell cycle. Very recently, we have investigated a naturally occurring C to T single nucleotide polymorphism (SNP) first reported in human melanoma tumors. This SNP is at position 119 in the 5' untranslated region (5'UTR) of p53 mRNA and we demonstrate that it has consequences on the translational control of p53. Introduction of this SNP has led to decrease in cap-independent translation from p53 5'UTR. Interestingly, the 5'UTR with this SNP has shown reduced binding to polypyrimidine-tract binding protein that can be corroborated to its weaker IRES activity. Previously it has been shown that G2-M checkpoint, DNA-damaging stress and oncogenic insult favor IRES-mediated translation. Under similar conditions we demonstrate that this SNP interferes with the enhancement of the IRES activity of the 5'UTR. Taken together, the results demonstrate that natural mutation in the 5'UTR of the p53 mRNA might play a role in translational control of this critical tumor-suppressor gene.

Fluorescence Window to Observe Internal Dynamics in Biopolymers

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The wealth of structural information available in biology has led to the realization that a complete understanding of biological processes requires information on dynamics apart from the knowledge on their high resolution structure. This realization has been leading to an explosion of information on dynamics from a variety of theoretical and experimental methods.

Fluorescence-based methods for extracting information on dynamics have a unique advantage due to their ultra-high sensitivity and selectivity. Furthermore, they cover a wide temporal range of femtosecond to seconds. In our laboratory we have been using various time-domain fluorescence techniques for addressing issues related to dynamics of proteins, protein-DNA complexes, biomembranes and single living cells. Subsequent to obtaining information on dynamics we then look for correlations between dynamics and biological function on these molecular systems. Such correlations, when established on firm grounds, bring out remarkable aspects of mechanistic details. Additionally, information on dynamics often leads us towards structural details which are unavailable from traditional high resolution studies. Several examples from our recent research work will be described in detail for amplifying the above statements.

More specifically, the following issues will be addressed: (i) The mechanism by which the protein MutS recongnize base mismatches in newly synthesized DNA is by sensing the motional dynamics of base-pairs; (ii) Enhancement in the motional dynamics of bases and the backbone is a key contributor to the mechanism of temperature-sensitive translation of RNA ('RNA Thermometer'); (iii) Motional dynamics of protein side-chains and segments are tightly coupled to solvent dynamics and (iv) Site-specific dynamics in a polypeptide provides information on the internal structure of protein fibrils.

1. *Continuous dissolution of structure during the unfolding of a small protein. Santosh Kumar Jha, Deepak Dhar, G. Krishnamoorthy and Jayant B. Udgaonkar Proc. Natl. Acad. Sci. USA (2009) 106, 11113-11118.*
2. *Characterization of the heterogeneity and specificity of inter-polypeptide interactions in amyloid protofibrils by measurement of site-specific fluorescence anisotropy decay kinetics. Anjali Jha, Jayant Udgonakar and G. Krishnamoorthy (2009) J. Mol. Biol. 393, 735-752.*
3. *Protein Folding, Unfolding and Aggregation Process Revealed by Rapid Sampling of Time-Domain Fluorescence. Saswata sankar Sarkar, Anoop Saxena, Nihav Dhawale, Jayant B.Udgaonkar, and G. Krishnamoorthy. Reviews in Fluorescence (2009), ed. C.D. Geddes, Springer, pp 281-301.*
4. *Exploration of the Correlation between Solvation Dynamics and Internal Dynamics of a Protein. Anjali Jha, Kunihiko Ishii, Jayant B. Udgaonkar, Tahei Tahara and G. Krishnamoorthy. Biochemistry (2011), 50, 397-408.*
5. *Reduced Fluorescence Lifetime Heterogeneity of 5-Fluorotryptophan in Comparison to Tryptophan in Proteins: Implication for Resonance Energy Transfer Experiments. Saswata Sankar Sarkar, Jayant B. Udgaonkar and G. Krishnamoorthy. J. Phys. Chem. B. (2011) 115, 7479-7486.*
6. *Motional Dynamics in Proteins and Nucleic Acids Control Their Function: Revelation by Time-Domain Fluorescence. G. Krishnamoorthy, Current Science (2011), in press.*

Systems-based understanding of Chemical complexity in Nature

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The genome sequencing projects have revealed an unanticipated variety of metabolic and cellular capabilities. Although the molecular basis whereby enzymes generate functional diversity remains to be explored, these sequences present an opportunity to undertake genome-based approaches to dissect complex biochemical pathways. Our group is interested to delineate mechanistic as well as regulatory aspects of metabolic and chemical diversity of novel metabolites, which provides distinctive advantage to these organisms. In *Mycobcaterium tuberculosis*, we are dissecting lipid metabolic network both in terms of understanding biosynthesis of novel metabolites and lipid degradation pathways during mycobacterial infection and dormancy. In another system, we are investigating into the biochemical mechanisms involved in melanin synthesis and the underlying melanocyte-keratinocyte interactions in context of vitiligo. Our studies thus decipher the core principles influencing genetic networks and metabolic pathways underlying the emergence of chemical diversity in living systems.

Structural model for an editing-defective aminoacyl-tRNA synthetase

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Aminoacyl-tRNA synthetases (AARSs) charge tRNAs with their cognate amino acids for protein synthesis thereby establishing the rules of the genetic code. Although, generally very accurate, AARSs can mischarge wrong amino acids onto tRNA and these errors are corrected by a separate module called editing domains which hydrolyze non-cognate aminoacyl-tRNA pairs. Mutations in proofreading modules have been show to lead to disease conditions including neurodegeneration. We earlier solved the structure of the editing domain of an archaeal threonyl-tRNA synthetase from *Pyrococcus abyssi* (Pab-NTD). This domain showed a striking structural homology to D-aminoacyl-tRNA deacylases (DTDs), which remove D-amino acids charged onto tRNA, leading us to propose a model of perpetuation of homochirality in proteins. Pab-NTD hydrolyzes serine mischarged on $tRNA^{Thr}$ through a substrate-assisted water-mediated catalytic mechanism. Our recent structural and biophysical work revealed that even cognate threonine can bind in the pocket designed for serine in variation to the textbook 'Double-Sieve Model'. However, this binding leads to elimination of a 'catalytic water' critical for hydrolysis suggesting that functional positioning of substrate rather than steric exclusion is critical for cognate/non-cognate discrimination. The active site in this protein can be divided into three pockets i.e. adenine-binding, aminoacyl-binding and the core. In our ongoing efforts to understand this protein, we have perturbed the active site by making mutations in these pockets. Our structural and biochemical studies on these mutants elucidate the intricate network of interaction in the substrate-binding pocket wherein perturbation even away from the aminoacyl-binding pocket leads not only to loss of cognate/non-cognate discrimination but also relaxation of enantioselectivity. The study also delineates the role of RNA in proofreading and presents a structural model for an editing-defective AARS.