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**International Scientific Conference on  
50 Years of Biochemistry in Bangladesh:  
Successes and Prospects**

**BOOK OF ABSTRACTS**

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## **FROM THE BIG BANG TO THE DEVELOPMENT OF CONSCIOUSNESS - SELFORGANIZATION OF MATTER**

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### **Abstract**

Soon after the Big Bang, more than 13 billion years ago, matter in the cosmos continuously developed into more and more differentiated structures and meta-structures, led by self-organization processes that control the generation of orderly structures from the cosmic down to the molecular level. Self-organized systems are dynamic and exist far away from the thermodynamic equilibrium. They form dynamic patterns which do not reach equilibrium. Such self-organized processes, forming dynamic patterns, essentially determined the evolution of the universe from the very beginning, from the Big Bang all the way to the development of living structures. Since the beginning of the universe, increasingly complex forms of matter emerged. Controlled by physical laws, matter organized itself into increasingly differentiated and complex structures and superstructures, while the circumstances changed: the volume of space expanded, entropy increased and the average temperature decreased. Self-organization has a dynamic character and determines the formation of orderly pattern in space and time applying to both at cosmic and atomic dimensions and especially to living cells and multicellular organisms. It describes the evolution of dynamic patterns in open systems, far away from the thermodynamic equilibrium. Iliya Prigogine found that the entropy in such self-organized systems aims at a minimum!

Examples are the formation of Galaxies, the creation of elements in the stars as well as the evolution of chemical elements and solids in the cold voids of the space and on the planets. This includes the evolution of living cells, such as the eukaryotes and multicellular organisms. The evolution of life is continuously being influenced by changes of its physico-chemical and biological environment, such as the formation of an oxygen-rich atmosphere on earth through the photosynthesis of cyanobacteria. Self-organization processes also play a key role in the development and regulation of the cellular metabolism. The central role of the hydrophobic effect in water during the formation of self-organizing systems will be discussed as well as its functions, e.g. during the formation of biological membranes, the generation of gradients and microdomains, and the development of differentiated cells and organs.

**PRODUCTION AND SCAVENGING OF REACTIVE OXYGEN SPECIES IN  
CHLOROPLASTS: FUNCTION FOR RELAXATION FROM  
EXCESS PHOTON STRESS**

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**Abstract**

The driving force of plant photosynthesis is solar photon energy, but plants encounter every minute change of solar intensity in addition to diurnal and seasonal ones. When solar intensity (P) is in excess of the photon-utilizing capacity for the fixation of CO<sub>2</sub>(A), photosynthesis is inhibited by oxidative damages with reactive oxygen species (ROS) produced in chloroplast thylakoids by excess photons. Here, the production and scavenging mechanisms of ROS, and the function of scavenging systems to protect from ROS-induced photoinhibition are described.

1. The mechanisms of photoproduction of ROS in the thylakoids under the conditions of  $P > A$  conditions are summarized; reduced species of ROS ( $O_2^-$ ,  $H_2O_2$  and  $-OH$ ) in the photosystem I (PSI), and excited species of ROS ( $^1O_2$ ) in the photosystem II (PSII)
2. Plants equip with the effective scavenging systems of ROS prior to its diffusion to the stroma for suppression of the photoinhibition. The  $O_2^-$  produced in PSI is reduced to water catalyzed by superoxide dismutase and ascorbate peroxidase, and the oxidized ascorbates (monodehydroascorbate and dehydroascorbate) are regenerated catalyzed by the respective reductases using the electron donors derived from water via PSII-PSI. Thus, this system has been referred to as the water-water cycle.
3. Scavengings of ROS by the water-water cycle in PSI and by carotene and tocopherol in PSII are indispensable to protect from photoinhibition by the oxidative damages of the target molecules. In addition, the water-water cycle works as the alternative electron acceptor to dissipate excess electrons under the  $P > A$  conditions and suppresses the photoproduction of  $^1O_2$  in PSII.

**NOVEL DRUGS AND VACCINES AGAINST AIDS PROGRESSION BASED ON  
THE STRUCTURES AND FUNCTIONS OF THE HIV-1 PATHOGENIC  
PROTEINS NEF AND VPR**

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**Abstract**

HIV/AIDS kills more people than any other infectious disease, and causes devastation to the health and economies of the poorest and least developed countries of the world that are least able to afford the currently used retroviral therapy that has had a dramatic effect in reversing AIDS-related morbidity and mortality in the developed countries. While the world eagerly awaits an effective prophylactic vaccine, there is an urgent and on-going need to slow the infection rate and provide relief to the 45 million HIV-infected people. There is, therefore, an urgent need for new drugs and therapeutic vaccines, for the infected population, that are effective, affordable and patient friendly, and that help to slow progression to full blown AIDS.

We have presented evidence to show that HIV-1 accessory proteins Nef and Vpr could be involved in AIDS pathogenesis. When present in the extracellular medium, Nef and Vpr cause the death of uninfected (bystander) cells, and may, therefore, be responsible for the depletion of immune cells in lymphoid tissues during HIV infection. When present inside the cell they prevent the death of infected cells and could, therefore, contribute to increase in viral load. Neutralisation of extracellular Nef and Vpr should prevent the death of uninfected immune cells and thereby the destruction of the immune system. Neutralization of intracellular Nef and Vpr would be expected to hasten the death of infected cells and help reduce the viral load. Nef and Vpr are, therefore, very important molecular targets for developing therapeutics that slow progression to AIDS.

The primary and 3D structure of the “death domains” of Nef and Vpr have been determined, and these regions have been targeted for the development of novel drugs and therapeutic vaccines. The N-terminal region of Nef and naturally-occurring Bee Venom Mellitin have very similar primary and tertiary structures, and they both act by destroying membranes. Chemical analogues of a Mellitin Inhibitor prevent Nef-mediated cell death and inhibit the interaction of Nef with cellular proteins involved in apoptosis. Naturally occurring Bee Propolis also contains substances that prevent Nef-mediated cell lysis, and increases proliferation of CD4 cells in HIV-infected cultures.

Simple bioassays, based on the pathogenic effects of Nef and Vpr, have been developed in my laboratory (CSIRO, Australia). These can be used in Bangladesh to screen natural product libraries held by Bangladeshi laboratories to discover lead compounds and develop new IP. Bangladeshi scientists could choose to join a drug discovery and development research network being set up in the OIC region to optimize the lead molecules into candidate drugs.

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## SIGNAL TRANSDUCTION BASED DESIGN OF ANTI-CANCER THERAPY

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### Abstract

About fifty years ago, it was first discovered that a class of viruses causes tumors in the rodents. This idea resulted in momentum in research to identify viral genes that cause tumors in humans. With the development of the techniques of molecular biology, scientists discovered viral genes that cause cancer. Later on, it became clear that the viral genes required for tumorigenesis had actually been picked up at some point in the history of virus. One of these genes is the Ras (originally isolated from rats with sarcoma). It was believed that understanding the molecular mechanism of Ras function might eventually lead to the development of therapy for cancer.

Ras is a multieffector signaling molecule that has been implicated in the regulation of many cellular functions including cell growth, differentiation, apoptosis, movement and transformation. Mutations in Ras genes that encode constitutively active proteins are reportedly associated with the development of  $\geq 30\%$  of human cancer. Overexpression of Ras has been implicated in various types of breast cancer and leukemia. Functional activation of a non-oncogenic form of Ras contributes to the molecular pathogenesis of brain tumors and breast cancers. Taken together, these observations suggest that diverse agonists and receptors utilize the activation of Ras as a common mechanism for the progression of cancer. Although three oncogenic Ras GTPases (Ki, Ha and N) have been identified in various types of cancers, the molecular mechanism of their activation of transformation signals was found to be similar. Furthermore, Ras GTPase (Ras) is known to play a key role in normal function and survival of cells. It is important therefore to identify which signaling pathway(s) is utilized for normal function and which signaling pathway(s) is involved in oncogenic signaling. Identification of Ras signaling pathway(s) involved in oncogenesis will be useful in developing a strategy for the treatment of Ras-induced cancer without disrupting its normal function in the cell.

We have shown that the Rho GTPases family (Rho) is required to transduce Ras signals for transformation and we have found that a member of Rho family, Cdc42, plays an important role in the signaling of oncogenic Ras. We have further found that the Cdc42 effector, an activated Cdc42-associated kinase (ACK), is required to transduce Ras signals for transformation. Indeed, our studies indicate that ACK deficiency results in the induction of apoptosis only in v-Ras-transformed NIH 3T3 cells and not the parental NIH 3T3 cells. As further evidence, tyrosine kinase inhibitors, which inhibit kinase activity of ACK *in vitro*, also inhibit growth of v-Ras-transformed cells. Collectively, these results indicate a possible role for Ras-Cdc42-ACK in the survival of v-Ras-transformed cells. Currently, we are designing small molecules by using computer-aided technology to inhibit Ras-Cdc42-ACK signaling pathway with the hope to design a therapy for Ras-induced cancer.

## DEVELOPMENT OF INHIBITORS OF THE DEADLIEST POISON, SEROTYPE A OF THE BOTULINUM NEUROTOXIN

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### Abstract

Botulinum neurotoxins (BoNT) produced by strains of the anaerobic bacteria of the genus *Clostridia*, are the most lethal of all toxins. There are seven distinct serotypes of which serotype A (BoNT/A) is the most toxic. After entering the peripheral neurons, their catalytic endoprotease domains cleave one of several synaptosomal proteins. The process blocks exocytotic fusion of synaptosomes with membranes thereby preventing acetyl choline release in the synapse. This leads to muscle paralysis and eventual death. These neurotoxins are potential biothreat agents, and warrants intervention at both pre-exposure and post-exposure stages. A detailed enzyme kinetic study on many peptides demonstrated that arginine at the P1' position of SNAP-25 is an absolute requirement for the latter to be a substrate of BoNT/A (Schmidt and Bostian, 1987, *J Prot Chem* 16:19). On the other hand the first crystal structure of BoNT/A revealed that the active site and its access route in the light chain (protease domain) is populated by acidic residues (Lacy, Tepp, Cohen, DasGupta and Stevens, 1998, *Nat Struct Biol*, 5:898 (1998)). These two observations led us to reason that arginine or its derivatives, and basic peptides might inhibit the protease reaction by binding to the active site of BoNT/A light chain (LcA), allowing us to design the first structure-based inhibitors. Indeed, we found that D-arginine, D- and L-arginine hydroxamates, and several basic peptides effectively inhibited LcA activity (Ludivico, Smith and Ahmed, *BRCC abstract*, pp. 67, 2006).

To improve the potency and specificity of the basic peptides, we designed various combinations of arginine and lysine and their D-enantiomers in a decamer containing a C-terminal glycine-cysteine pair. We found that the peptide containing L-arginine was the most effective inhibitor of LcA activity. By varying the number of L-arginine residues in a series of larger and smaller peptides, we found RRGc was most inhibitory with a  $K_i$  of 157 nM in a competitive mode. Thus, this highly soluble tetrapeptide in aqueous solutions is by far the best BoNT/A inhibitor described in literature. Determination of the co-crystal structure of RRGc complexed with a catalytically active LcA at 1.6 Å resolution revealed that the inhibitor was bound at the active site such that the second arginine resembled P1' residue of the substrate occupying the expected S1' site on the enzyme (Kumaran, Rawat, Ludivico, Ahmed and Swaminathan, 2008, *J Biol Chem* 283:18883). The crystal structure also revealed that cysteine of the inhibitor was bound in a hydrophobic pocket away from the active site zinc, suggesting a larger, hydrophobic residue in place of cysteine would be better accommodated in LcA. Subsequent co-crystal structures of three more peptides, RRGL, RRGl and RRGm, indeed showed more hydrophobic interactions with LcA. These results provided a structural proof of the effectiveness of our tetrapeptide inhibitors developed in a structure-based approach, and will be the basis of designing more potent inhibitors.

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## CURRENT FOOD AND NUTRITION SECURITY SITUATION IN BANGLADESH

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### Abstract

Bangladesh has made a commendable progress in food production since independence in 1971. Especially remarkable has been the production of rice which tripled between 1971 and 2008, during which time the population has increased by double. Rice availability and consumption has therefore increased, far above the nutritionally advisable amount. On the other hand, the availability and consumption of non-rice foods like fruits, vegetables and animal products is much lower than normal. Prevalence of protein-energy malnutrition among young children has thus been high. However, with continued agricultural and economic growth, Bangladesh has been able to reduce the prevalence of stunting to the level of 36% in 2007 from 68% in 1990. It is expected that the country would be able to achieve MDG1 in child stunting by the year 2015. Overweight, on the other hand, particularly among women, is emerging as a new nutritional problem in the country. Vitamin A deficiency nightblindness, which was prevalent to an extent of 4.1% among under-6 years old children in 1962, is now almost non-existent, thanks to the two-pronged approach – food based strategy through national scale home gardening programmes and the vitamin A capsule supplement as a regular national programme. Similarly, iodine deficiency goiter, which affected nearly half of women and children 10 years ago, has now fallen to below 10%. The national scale Control of Iodine Deficiency Disorders programme is attributed to this great success. Iron deficiency anaemia has remained an ever uncontrollable nutritional problem in Bangladesh and the present status of anaemia among children, adolescents and women of reproductive age is unacceptably high. The National Food Policy (NFP) 2006 addresses this and other food security and nutrition issues very seriously. It is expected that an effective implementation of the Plan of Action of the NFP by the government with technical assistance of the FAO-National Food Policy Capacity Strengthening Programme (NFPCSP) which is currently in place, will bring about further improvements in food security and nutrition situation of the country by 2015.

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## **50 YEARS OF BIOTECHNOLOGY AND GENETIC ENGINEERING**

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### **Abstract**

The fundamental science of biotechnology and genetic engineering can look back on an impressive history of the last fifty years. Of particular significance, though, has been the uninterrupted success story of the ideas and products generated through this technology that touched upon the quality of life around the world more than they ever have before.

However, an understanding of the genetic material of all living things which had been and still is crucial in the phenomenal progress of this discipline has witnessed the rise and fall of dominance of the different players of this technology. In the family of genetic material, RNA had long been the poor cousin of DNA. DNA made up the genes, the master instructions of life, while RNA merely conveyed those instructions to other parts of the cell. It is now becoming clear to us that the DNA molecule had been enjoying undue prominence. Instead of being just a vehicle for DNA's commands, as scientists have long held, we are now learning that RNA issues its own commands and alter what genes do in the next generation.

It had been hypothesized that the Central Dogma provides for the notion of genetic determinism, the belief that genes "control" the character of life. But in the span of fifty years, evidence against the Central Dogma has piled up to such an extent that rumblings of challenging the dogma can be heard. In the last few decades we have witnessed a revolution launched by full genome sequencing. This has resulted in paradigm shift in life sciences. Past experiments were hypothesis driven, hypotheses were evaluated to complement existing knowledge. However present experiments are data driven, we now discover knowledge from large amounts of data.

**FIFTY YEARS OF BIOCHEMISTRY AND MOLECULAR BIOLOGY:  
ADVANCES IN AGRICULTURE AND PLANT BIOTECHNOLOGY**

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**Abstract**

Agricultural advances are essential, particularly in developing countries, where increase in food production is imperative to feed the mouths of the rising population. Matters have been made worse by natural disasters like floods and extremes in temperature. In Bangladesh, we are faced with salinity in the South, drought in the North and flash flooding in the plains. Depletion in nutrient content of soil is also becoming a persistent problem in specific areas, while in others mineral toxicities prevail. With the sequence of essential crops like rice available now, DNA markers are being used to efficiently breed crops tolerant to abiotic and biotic stresses, like submergence tolerant Swarna and submergence tolerant BR11. In collaboration with IRRI and BRRI, we are developing salt tolerant versions of mega rice varieties BR11 and BRRIdhan 28, using marker-assisted backcrossing. Genetically transformed crops started appearing commercially in the early 90's, because of the inherent plasticity of the plant cell which allows a single transformed cell to be regenerated into a complete plant. In the developed countries, the targeted traits were insect resistant crops produced by inserting the Bt gene and herbicide tolerance. Although the first abiotic stress tolerant crops were reported in the late 90's, these proved to be only partially tolerant. Some genes were however shown to confer salt tolerance and flowered under salt stress by transformation with vacuolar Na/H antiporter and Helicase genes. Using some of these genes, we have transgenic rice, whose yield performance under salt stress is being tested. Due to recent sequence information, many transcription factor families or regulatory proteins have been identified which are involved in stress regulation. Transformation of rice with these regulatory proteins have now for the first time produced saline and drought resistant plants which are capable of setting grains under severe stress conditions. Crops with increased micronutrient contents have now also been produced and research is being done to identify transporters which can uptake minerals even under soil deficient conditions.

## **DIETARY PATTERN, NUTRITIONAL STATUS, ANAEMIA AND ANAEMIA-RELATED KNOWLEDGE IN URBAN ADOLESCENT COLLEGE GIRLS OF BANGLADESH**

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### **Abstract**

Dietary pattern, nutritional status and prevalence of anaemia were investigated in 65 adolescent college girls of Dhaka aged 15-19 years. A 7-day food frequency questionnaire was used to investigate the dietary pattern. Nutrient intake of the participants was assessed by 24-h recall method. Habitual dietary pattern indicated poor intake of milk, liver, leafy and root vegetables and the dietary data revealed a deficit of 1.97 MJ/day (471 kcal/day) in energy. Mean intakes of carbohydrate and fat were lower than RDA; while protein, iron, vitamin A and vitamin C intakes were much higher. Anthropometric data indicated that 63% of the girls were stunted (height-for-age <95% of NCHS reference values) and 45% were underweight (weight-for-age <75% of NCHS reference values). The prevalence of anaemia (Hb <12 g/dl) among the participants was 23%. About 17% had low serum iron (<40 µg/dl), 23% showed evidence of iron-deficient erythropoiesis (TS <15%) and only 8% had vitamin C deficiency (<0.29 mg/dl). Significant positive correlations were observed between haemoglobin and serum iron, TS and plasma vitamin C, while there was a negative correlation with serum TIBC. Consumption frequency of banana, fish and liver was found to have significant impact on haemoglobin and iron levels of the participants. About 65% of the participants had correct knowledge about the causes of anaemia; while 72.3% and 80% respectively, knew about the prevention and treatment of anaemia. Surprisingly, 73.8% of the participants were not aware about the sources of iron-rich foods. These results indicate an overall poor nutritional status of the urban adolescent college girls in Bangladesh, demanding the need for appropriate nutrition interventions, including nutrition education, to overcome the problem.

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**NKX2-5 IS A TRANSCRIPTIONAL ACTIVATOR OF ETSRP71, WHICH PROMOTES AN ENDOCARDIAL FATE IN THE DEVELOPING EMBRYO**

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**Abstract**

The overall goal of our study was to decipher transcriptional networks that promoted cardiac morphogenesis. We generated an Nkx2-5 enhancer-EYFP transgenic mouse and utilized FACS analysis to isolate wildtype and Nkx2-5 null cardiac progenitors from single, age-matched embryos at distinct developmental stages (E7.75, E8.25 and E9.5). Transcriptome and QRT-PCR analyses were used to identify Ets-related protein71 (Etsrp71), a transcript that was significantly downregulated in the Nkx2-5 null cardiac progenitors. The biological function of Etsrp71 during cardiac morphogenesis is unknown. Using molecular and biochemical techniques, we established that Etsrp71 is a novel downstream target of Nkx2-5. We observed an evolutionarily conserved Nkx2-5 responsive element (NKE) in the Etsrp71 promoter and verified the binding of Nkx2-5 to this site using electrophoretic mobility shift, mutagenesis and ChIP assays. Using RT-PCR and in situ hybridization techniques, we further established that Etsrp71 is transiently expressed in the endocardium of the developing heart (E7.75-E9.5) and is extinguished during the latter stages of development and in the adult heart. These spatial and temporal expression results were further supported using transcriptional assays. We observed that Nkx2-5 activated the Etsrp71 gene in a dose-dependent fashion and mutation of the NKE significantly abrogated the transcriptional activation. To further confirm that Etsrp71 is an Nkx2-5 direct downstream target, we engineered an Nkx2-5 inducible ES/EB system and have shown that upon induction, Nkx2-5 can upregulate the endogenous Etsrp71 expression. We further engineered an Etsrp71 inducible ES/EB system and using FACS, QRT-PCR and immunohistochemical techniques, we demonstrate that induction of Etsrp71 promotes an endocardial fate. Finally, using a gene disruption strategy, *Etsrp71* mutant embryos lacked endocardial/endothelial lineages and were nonviable. Tie2 expression is essentially absent in the Etsrp71 null heart and initial studies support the conclusion that Etsrp71 is a direct upstream transactivator of *Tie2* gene expression. Our results uncover a novel functional role for Nkx2-5 and define a transcriptional network that specifies an endocardial/endothelial fate in the developing embryo. These results further extend the Nkx2.5-Etsrp71-Tie2 transcriptional network and support our hypothesis that Nkx2-5 transactivates Etsrp71, which promotes an endocardial fate in the developing embryo by regulating its downstream target genes.

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**FROM MARKER TO GENE: CHARACTERIZATION OF A PUTATIVE  
LDLP GENE LINKED TO LOW TEMPERATURE TOLERANCE IN JUTE**

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**Abstract**

Jute is an important cash crop and a versatile natural fiber. In recent years, researchers are becoming interested in improving the jute genotype through molecular approaches, which is however slowed down by the fact that very little information is known about the massive jute genome (1350Mb). Moreover, most of the small number of jute sequences deposited in GenBank is uncharacterized. In this context, we sought to carry out structural and functional analysis of an uncharacterized gene (LDLP) from jute encoding a putative low density lipo protein like protein that is over expressed under low temperature stress. This gene was identified through a RAPD primer OPG 05, which gave a 1200 bp polymorphic band present in all low temperature tolerant jute accessions, but absent in the sensitive varieties. A 153 bp terminal exon was found within this sequence which showed strong homology with hypothesized low density lipoprotein  $\beta$  -like protein of Arabidopsis and rice. Up to now we have explored  $\frac{3}{4}$  of the complete coding sequence by degenerate primer based gene walking and 5' RACE. We have also deduced most of the (90%) intronic sequence of the gene. Stress perception leading to tolerance involves a complex interplay of different gene products. To study the role of LDLP gene in low temperature tolerance, semi quantitative RT PCR, real time PCR and dot blot analysis were carried out. Findings from these results indicate that the expression level of LDLP is increased under low temperature and dehydration stress. The expression pattern of the gene is now being studied under salt stress and diseased conditions. Bioinformatics analysis indicates that LDLP gene may play a role as lipid binding transporter protein in cell membranes.

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**HEREGULIN INDUCTION OF HSP EXPRESSION, ANCHORAGE  
INDEPENDENT GROWTH AND PROTECTION FROM  
APOPTOSIS REQUIRE *HSF1***

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**Abstract**

Elevated levels of heat shock proteins (HSP) in cancer signal a poor prognosis and are frequently associated with resistance to therapy. Heregulin (HRG) is a trophic factor that binds to the cell surface of adjacent cells in a paracrine manner through the receptors ErbB3 and ErbB4, mediating the growth and survival cells of the developing mesenchyme. In addition, HRG is a potent inducer of malignant progression when overexpressed particularly in breast tissues. We demonstrate a role for the highly tumorigenic HRG/ErbB2 cascade in HSP induction. HSP induction by HRG requires an intact heat shock factor 1 (*hsf1*) gene. HRG mediates *hsf1*-dependent HSP transcription through a pathway involving ErbB2 tyrosine phosphorylation and phosphatidylinositol-3-kinase, but not ERK pathway. A role for HSF1 in HRG / HSP mediated tumorigenesis and treatment resistance is supported by the finding that *hsf1* disruption inhibits HRG-induced anchorage independent growth in MEF. A potential place for this pathway in treatment resistance is indicated by the finding that HRG protection of cells from chemotherapeutic drugs is inhibited by *hsf1* disruption and inhibitors of ErbB2 and PI-3 kinase activity.

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## **SALT TOLERANT BR11 AND BR28 THROUGH MARKER ASSISTED BACKCROSSING**

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### **Abstract**

Marker Assisted Backcrossing (MAB) has been adopted to improve the power and efficiency of breeding programmes. MAB approach is the most successful breeding technique for getting desirable genetic gains even over the more complex traits in the shortest possible time, e.g. Swarna *Sub1*. MAB can reduce the number of generations during crossing, thus decreasing the time and labour compared to conventional breeding. '*Saltol*', a major QTL at the short arm of chromosome 1 of rice was mapped in our lab from 11.2 to 12.79 Mb. One of the simplest forms of MAB is the use of molecular markers to improve the conventional backcross conversion method, where the desired trait is transferred to another line through crossing followed by repeated backcrossing to the recurrent parent to reconstitute the original variety.

MAB strategy was therefore undertaken to introgress the '*Saltol*' QTL into the widely accepted two mega rice varieties BR11 (T. Aman, monsoon) and BR28 (Boro, dry, winter). For '*Saltol*' QTL introgression crossing was done with the donor parent FL378, a near isogenic line (NIL) which was derived from Pokkali (a salt tolerant donor variety) and repeated backcrossing was done with high yielding varieties BR11 or BR28. Three step selections i.e. Foreground, Recombinant and Background were performed in each backcross population. Four markers were found significantly associated with the trait by fine mapping of the '*Saltol*' QTL region. These markers were used to locate the QTL in the backcross population. Similarly two flanking markers were used to delineate the QTL and reduce negative linkage drag. For the BR11 program, two double recombinants and 136 single recombinants were selected by genotyping of 342 BC<sub>2</sub>F<sub>1</sub> population using 3 foreground and 2 recombinant markers. Background screening was done with 75 SSR markers and the percentage of recurrent parent genotype (RPG) was found from the range of 65.33% to 80%. For the improvement of BR28 variety, a total population of >13000 BC<sub>1</sub>F<sub>1</sub> were developed for the introgression of '*Saltol*'. The BR28 introgression program will be also progressed as above and the final plants will be selected after field trial. It is expected that farmer popular salt-tolerant BR11 and salt-tolerant BR28 will be ready in our hands to give farmers by late 2009 and early 2010 respectively. After releasing of these two salt tolerant mega varieties, it will be easier for farmers to produce salt tolerant-high yielding rice which will contribute to food security of the country.

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## **RICE TRANSFORMATION WITH GENES REPORTED TO GIVE SALT TOLERANCE**

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### **Abstract**

Salinity is one of the major problems encountered during crop production in Bangladesh. Different transgenic approaches to solve this problem have been reported, but success with rice has been limited. Several genes, shown to confer improved salt tolerance were used for producing transgenic rice. We have transformed the tissue culture-responsive landrace, *Binnatoa* (BA), with the *Oryza sativa* vacuola sodium hydrogen antiporter (OsNHX1) constructs containing the 5' UTR and ORF. OsNHX1 transformed plants showed improved tolerance during seedling salinity screening. The transgenic *Binnatoa* (BA) has been crossed with farmer-popular dry-season cultivars, BR28 and BR45. The F<sub>1</sub> plantlets were selected by RT-PCR, F<sub>2</sub> progenies were screened for agronomic properties and F<sub>3</sub> plants were taken for salt screening. Half of the F<sub>3</sub> plants showed better tolerance than their parents and agronomic properties close to their farmer popular parents. Another gene, the Pea DNA helicase (*PDH45*), obtained from ICGEB, New Delhi, was also used for transformation. *PDH45*-transformed T<sub>1</sub> BA rice having a single gene insertion showed very good morphology as well as dramatically improved salt stress tolerance at the seedling stage. The Glyoxalase pathway was also reported to be important in improving salt tolerance. Therefore, genes for the two enzymes of this pathway obtained from ICGEB, New Delhi, are also being used for transformation of BA rice. To express all these genes, a good promoter is essential. Commercial binary vectors contain the CaMV 35S promoter which is weak promoter for gene expression in rice. Therefore, we are isolating and characterizing promoters reported to be salt stress-inducible.

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**SOLVING THE STRUCTURE - FUNCTION ANOMALY OF A REPORTED  
AMINOPEPTIDASE FROM JAPANESE EDIBLE MUSHROOM,  
*GRIFOLA FRONDOSA***

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**Abstract**

The N-terminal amino acid sequence of an aminopeptidase from Japanese edible mushroom, *Grifola frondosa*, was reported to have high similarity with that of a serine proteinase from basidiomycete, *Agaricus bisporous* (Nishiwaki and Hayashi, 2001, *Biosc.. Biotechnol. Biochem.* 65: 424-427). The full-length cDNA and the corresponding genomic DNA of the enzyme were cloned, based on the reported N-terminal amino acid sequence. The predicted open reading frame (ORF) of the cloned cDNA, encoding a product of 379 amino acids, was expressed in *E.coli* using pET expression vector. The expressed pro-enzyme (40 kDa) underwent autolysis to produce the mature protein (30 kDa) and a pro-peptide (10 kDa). The mature protein and the pro-peptide remained tightly bound to each other and could not be separated by Ni-NTA metal affinity chromatography or Q-Sepharose ion-exchange chromatography. The enzyme was inactive in the bound form. Upon treatment with subtilisin, the bound pro-peptide was further hydrolyzed and a high serine proteinase activity was recovered. No aminopeptidase activity was detected at any stage of the protein processing. These results clearly indicate that the N-terminal amino acid sequence and the function of the reported aminopeptidase were not derived from the same protein entity and hence caused the structure-function anomaly.

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***S. pombe* KINASE, OCA2 IS REQUIRED FOR STIMULATING CTD  
PHOSPHORYLATION AND RELEASE OF RNA POLYMERASE II FROM  
TRANSCRIPTION TERMINATION SITES**

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**Abstract**

Many features of transcriptional termination by RNA Polymerase II (PolII) are only poorly understood. To elucidate the mechanism more clearly, we employed a reverse genetic screen in *S.pombe* and identified mutants defective in Pol II termination based on TRO (transcription run-on) analysis on *ura4+* reporter gene. One such mutant (*m47*), which has a strong defect in termination was complemented by overexpression of a novel kinase gene (SPCC1020.10) called *oca2+*. In TRO analysis, *oca2* deletion mutant cells display an aberrant profile of transcription termination with an accumulation of RNA Pol II over the poly(A) site and the termination region.

To isolate proteins interacting with Oca2, immunoprecipitation was performed with HA-tagged Oca2 strain. Mass spectrometry analysis found Ctk2 (*S.pombe* homologue of mammalian cyclin T) to be associated with Oca2. To identify substrates of Oca2 kinase activity, total cell extract was fractionated by size exclusion chromatography and fractions were subject to phosphorylation by purified Oca2-HA. Mass spectrometry revealed one positive substrate to be Ctk2, the regulatory subunit of another kinase Ctk1. Ctk1 promotes serine 2 phosphorylation of the CTD heptad repeat of RNA polymerase II (Pol II), a dynamic modification associated with transcriptional elongation, termination and co-transcriptional pre-mRNA processing. TRO analysis of both fission and budding yeast deleted *ctk2* indicates a modest defect in Pol II termination.

Since the Ctk1/Ctk2 complex (P-TEFb) is known to phosphorylate Ser2 of the CTD of RNA Pol II, we investigated the distribution of Pol II phospho CTD across several *S.pombe* genes in WT and  $\Delta oca2$  cells. Chromatin immunoprecipitation (ChIP) analysis with antibodies against Ser2P and Ser5P show that both CTD modifications are reduced in *oca2* mutant cells, suggesting that Oca2 might regulate activity of one or several CTD-kinases. Both, Oca2 and Ctk2 localize predominantly to the terminator regions and in *oca2 $\Delta$  cells Ctk2 localization to the termination regions is lost. We further show that Oca2 stimulates CTD phosphorylation and consequently may be a prerequisite for correct Pol II release from termination sites. Moreover, microarray analysis revealed that subset of genes are affected by *oca2* deletion. Therefore an attractive possibility is that different pathways are involved in regulating CTD phosphorylation and hence transcription.*

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## SYNTHESIS AND STRUCTURE-FUNCTION STUDY OF RELAXIN HORMONES

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### Abstract

The chemical assembly of insulin-like peptides allows the detailed study of their structure and function. However, the two-chain, three-disulfide bond structure of this family of peptides, which includes relaxin, has long represented a significant challenge with respect to their chemical synthesis. We have developed highly efficient solid phase peptide synthesis methodology together with selective S-thiol protecting groups that allows the acquisition of individual chains that can be combined by effective sequential chemically-directed formation of each of the three disulfide bonds.

The relaxin peptides, which are members of the insulin family, signal through two different classes of G-protein-coupled receptors (GPCRs), now referred to as the relaxin family peptide (RXFP) receptors 1–4, respectively. Although key binding residues have been identified in the B-chain of the relaxin peptides, the role of the A-chain in their activity is currently unknown. A recent study showed that INSL3, another insulin-like peptide, can be truncated at the N-terminus of its A-chain by up to 9 residues without affecting the binding affinity to its receptor RXFP2 while becoming a high affinity antagonist. This suggests that the N-terminus of the INSL3 A-chain contains residues essential for RXFP2 activation. We have now synthesized A-chain truncated human relaxin-2 and -3 (H2 and H3) relaxin peptides, characterized their structure by both CD and NMR spectroscopy, and tested their binding and cAMP activities on RXFP1, RXFP2, and RXFP3. In stark contrast to INSL3, A-chain-truncated H2 relaxin peptides lost RXFP1 and RXFP2 binding affinity and concurrently cAMP-stimulatory activity. H3 relaxin A-chain-truncated peptides displayed similar properties on RXFP1, highlighting a similar binding mechanism for H2 and H3 relaxin. In contrast, A-chain-truncated H3 relaxin peptides showed identical activity on RXFP3, highlighting that the B-chain is the sole determinant of the H3 relaxin-RXFP3 interaction. Our results provide new insights into the action of relaxins and demonstrate that the role of the A-chain for relaxin activity is both peptide- and receptor-dependent.<sup>1</sup>

### Reference

<sup>1</sup>Hossain MA, K. Rosengren, Haugaard-Jönsson LM, Zhang S, Layfield S, Ferraro T, Daly NL, Tregear GWT, Wade JD and Ross A. D. Bathgate RAD. *J. Biol. Chem.* **2008**, 283:17287-17297.

**THE RELATIONSHIP OF SMOKING AND *H. PYLORI* AND THEIR ROLE IN THE DEVELOPMENT OF GASTROINTESTINAL DISORDERS IN A COMMUNITY BASED STUDY IN BANGLADESH**

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**Abstract**

Gastric disorders are common in the community and several risk factors are reported including *Helicobacter pylori* and smoking. Studies on the association between *H. pylori* infection and smoking habit have given inconsistent results. In the present study, this relationship was investigated in Bangladesh. Stool and biopsies were obtained from subjects of Nandipara community, 7 miles northeast of Dhaka city. Two hundred and eighty seven subjects were investigated for *H. pylori* by stool antigen test (ELISA). Biopsy samples were collected from 259 stool antigen positive subjects and culture for *H. pylori* was done from biopsy samples. Information about their dyspeptic symptoms and smoking habit was collected in a standard questionnaire as well as gastrointestinal symptoms were recorded following upper gastrointestinal endoscopy. Our data shows that *H. pylori* infection was significantly associated with smoking habit ( $P < 0.05$ ). *H. pylori* was highly prevalent in smokers (82.5%) as compared to nonsmokers (54.8%). There were 111 subjects (38.7%) out of 287 with dyspepsia, 107 (96.4%) of them were stool antigen positive. Out of 104 of the stool antigen positive subjects 79 (76.0%) were *H. pylori* positive by culture. Out of 44 smokers, 31 (70.5%) had dyspepsia. The dyspeptic symptoms and gastrointestinal symptoms were strongly associated with *H. pylori* and smoking ( $P < 0.05$ ). Dyspepsia and gastrointestinal abnormalities are common in the Nandipara community of Bangladesh. Both *H. pylori* and smoking are the risk factors for dyspepsia and gastrointestinal abnormalities. Our data suggest that there is a high association between smoking habit and *H. pylori* infection.

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## FLAVOR CHARACTERIZATION OF BEEF MARROW BONE STOCKS

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Beef bone marrow has been part of the human diet since prehistoric times. Marrow bone stock is an important culinary base used by gourmet chefs. It is well known for its distinct savory character in foods. While there has been a great deal published on flavor active components in cooked meats, the flavor composition of beef bone marrow is still relatively unstudied and unreported.

For this study, commercial chopped fresh beef marrow bones were simmered in water for seven hours at 90°C. Three batches of cooked marrow bone mixtures were prepared. First batch was not enzyme treated. The second and third batches were treated with enzymes papain or umamizyme, and then heated for one hour at 65°C and 50°C respectively. All three batches (untreated and enzyme treated) were defatted by microfiltration.

A trained sensory panel evaluated the three stock samples. The umamizyme digested stock received higher organoleptic acceptance than the other two samples. GC-MS SPME analysis finds the stocks to possess lipid oxidation products including diacetyl, alcohols, aldehydes and ketones as principal volatile components. Nonvolatile analysis with LC-MS TOFS and LC-MS ESI showed numerous non-volatile components. We identified 20 novel peptides (di- and tri) in the papain and umamizyme digested stock samples. Free amino acids in these samples were analyzed by *o*-Pthaldehyde (OPA) and 9-Fluorenylmethyl chloroformate (FMOC-Cl) derivatization. These amino acids and small peptides participate in Maillard reaction to generate flavor active volatile components.

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**CHARACTERIZATION OF A NOVEL GROUP OF *SHIGELLA FLEXNERI* 1C STRAINS ISOLATED FROM PATIENTS IN BANGLADESH**

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**Abstract**

Infection of *Shigella* can often lead to severe complications especially in young children. Invasiveness is one of the common key characteristics of *Shigella* that attributed to the development of infection. Recently a large number of non-invasive *S. flexneri* 1c strains were isolated from patients having complications typical of *Shigella*. We are interested to study the molecular basis of pathogenicity of these *Shigella* strains. The aim of the study was to characterize the toxin produced by this group of noninvasive *Shigella* strains and their role in disease progression. Seventy two *S. flexneri* 1c strains isolated between 1997-2003 from patients at Dhaka treatment centre of ICDDR,B were studied. All these strains were characterized by plasmid profiling and tested for *set*, *sen* and *stx* by PCR. Cytotoxic (using HeLa cells) and neurotoxic (using cerebellar granule neurons with neurite length as indicator) activity of the toxin was determined. The toxin was tested for fluid accumulation in rabbit ileum. DNA fragmentation and chromatin condensation assay were used as markers of apoptosis. Of the 72 strains, 17 *S. flexneri* 1c did not contain 140 MDa invasive plasmid and any of *set*, *sen* or *stx* genes. However these strains exhibited very strong cytotoxic activity and induced chromatin condensation and chromosomal DNA fragmentation in HeLa cells. Culture filtrate (toxin) of these strains were unable to accumulate fluid in the rabbit ileal loops, but the histo-pathological report of the loop segments showed less maintained structure of the villi with Grade-3 inflammation. In our developed primary rat brain (cerebellar) neurons culture model we found that these strains showed strong neurotoxic activity by reducing the neurite length (tested in at least 100 neurons from random fields). We conclude from these results that the toxin produced by these non-invasive *S. flexneri* 1c causes apoptosis to HeLa cell and possess an inhibitory factor(s) for cerebellar granule neurons.

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**MUTATION IN PATERNALLY TRANSMITTED ALLELES AT FGA  
MICROSATELITE LOCI: A CASE OF ALLELE MISMATCH IN THE CHILD**

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**Abstract**

We investigated a case of paternity dispute with 10 autosomal STR loci and found a mismatch in one of the alleles of the FGA locus in the child. The composition of alleles of this locus in the mother, child and alleged father were, 19/24, 23/24 and 22/24 respectively. The combined paternity index ( $2.3 \times 10^3$ ) and probability of paternity (0.9998) suggest that the alleged father is the biological father of the child. Further analysis using 16 Y-chromosome STR loci, revealed matching of all Y-chromosome alleles of the child with that of the alleged father. Since there was a perfect match of all the paternal alleles inherited (10 autosomal and 16 Y-chromosomal STR) in the child with those of the alleged father except the allele at FGA, it might therefore be a case of mutation. The results suggest that either there was expansion of a complete repeat of the paternal allele 22 or a deletion of a complete repeat of paternal allele 24 which was transmitted as allele 23 in the child.

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## DISTRIBUTION OF ABO BLOOD GROUP IN MI PATIENTS AND ITS RELATIONSHIP WITH LIPID PROFILE AND OTHER RISK FACTORS

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### Abstract

A case control study was conducted with an aim to find out the relationship between ABO blood groups and myocardial infarction (MI) during August 2003 to July 2004. The study was conducted among 104 MI patients who were receiving treatments in the Department of Cardiology at NICVD, Dhaka. Ninety six age-matched subjects visiting the NICVD with normal ECG served as controls. The mean age of the respondents was  $54.1 \pm 10.1$  years. Among them 88.5% were male and 11.5% were female. The most prevalent age group for MI was between 50-59 years. The mean average monthly family income was between Tk.12723  $\pm$  Tk.1029. Among the 104 cases, 34 (33.3%) belonged to blood group A, 32 (31.4%) from B, 27 (26.5%) from O and 9 (4.5%) were from blood group AB. Total serum cholesterol (TC), serum triglyceride (TG), and HDL- and LDL-cholesterol were determined by standard methods. The mean TC in blood group A patients was  $234.8 \pm 0.8$  mg/dl in MI group and the same was  $187.1 \pm 8.0$  mg/dl in non-MI group ( $t = 9.856$ ,  $p = 0.0001$ ). In blood group B, the mean TC was  $223.8 \pm 10.3$  mg/dl in MI group, compared to  $169.6 \pm 10.3$  mg/dl in non- MI group ( $t = 8.955$ ,  $p = 0.00103$ ). Similarly, the mean TC in MI and non-MI patients of blood group O were  $219.8 \pm 18.6$  mg/dl and  $200.3 \pm 13.9$  mg/dl, respectively ( $t = 1.441$ ,  $p = 0.0001$ ) and in blood group AB the levels were  $216.6 \pm 22.2$  mg/dl and  $191.4 \pm 13.8$  mg/dl, respectively ( $t = 2.1001$ ,  $p = 0.1065$ ). Thus, a significant association of ABO blood groups with MI was noted. Association of several personal characteristics, both modifiable (such as smoking habit, alcohol consumption, BMI, physical activity, pattern of food intake) and fixed such as social class) with CVD was also examined in the study. The present study concluded that primary precaution should be the mainstay to prevent the premature occurrence of cardiovascular disease among population at risk possessing certain behavioral risk habits.

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## SERUM MINERAL LEVELS IN ESSENTIAL HYPERTENSION

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### Abstract

Serum ionized levels of sodium, potassium, calcium and magnesium were measured (by direct ISEs) in a selected group of 86 Bangladeshi subjects. Of these, 40 were normotensive subjects and 46 were newly diagnosed essential hypertensive patients. The aim of the study was to measure and compare serum levels of ionized Na, K, Ca and Mg between normotensive and hypertensive groups and also to correlate the serum levels of Na, K, Mg, Ca with the degree of hypertension. No significant differences were found in the level of serum sodium and potassium between hypertensive and normotensive groups. Serum ionized calcium and magnesium concentrations were significantly lower in the hypertensive group than the normotensive group (Ca:  $0.9097 \pm 0.1092$  mmol/L vs  $1.018 \pm 0.0497$  mmol/L,  $P = 0.000$ ; and Mg:  $0.4626 \pm 0.0530$  mmol/L vs  $0.4968 \pm 0.0391$  mmol/L,  $P = 0.001$ ). Though many studies showed a positive or a negative or no correlation between serum ionized calcium, ionized magnesium and blood pressure in essential hypertensive patients, in our study serum ionized calcium was found inversely correlated with blood pressure (sBP:  $r = -0.4304$ ,  $P = 0.000$ ; dBP:  $r = -0.4672$ ,  $P = 0.000$ ). A weak negative correlation was found between serum ionized magnesium and blood pressure (sBP:  $r = -0.2610$ ,  $P = 0.015$ ; dBP:  $r = -0.2997$ ,  $P = 0.005$ ). The study revealed that serum ionized calcium and serum ionized magnesium levels are significantly lower in hypertensive subjects than normotensive subjects in our study population, which both were inversely correlated with blood pressure.

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## DETERMINATION OF EXPRESSIONAL HIERARCHY OF *ESCHERICHIA COLI* GENOMIC GENES

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### Abstract

*Escherichia coli* is a most extensively characterized microorganism. The complete genome sequence has revealed that *E. coli* has more than 4,000 genes. These genes are transcribed by only 1000 promoters of which functions of about 2000 genes are still unknown. In order to understand the overall structural and functional analysis of *E. coli* genomic genes under various growth conditions, a promoter-protein fusion library was constructed previously, which represents about 10% promoters from *E. coli* genome (Talukder et al. 1994). Library clones were analyzed in respect to their reading frames, protein productivity as well as expressional regulations under normal and various environmental stress conditions. Thirteen of 77 genes exhibited significant changes in expression in response to at least one of the six treatments, and six of them appeared to be controlled by more than one  $\sigma$  (sigma) factor of RNA polymerase (Talukder et al. 1996; 1999; 2005; Yamada et al. 2002). DNA database searching revealed that out of 77 genes, 75 genes were found to known genes. The remaining 2 genes showed no homology indicating them as unknown or new genes. Moreover, three genes have interesting structural organizations were recorded. All together the results clearly indicated that about 5-10% *E. coli* genes are expressed under the control of complex regulatory circuits. These findings are important to determination of global gene expressional hierarchy of *E. coli* genome.

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## **OXIDATIVE STRESS AND DNA DAMAGE FROM ARSENIC IN DRINKING WATER: ASSESSMENT OF HEALTH RISK**

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### **Abstract**

In this study we explore the relationship between oxidative stress and genotoxicity due to chronic arsenic exposure from drinking water in human. Sixty three inhabitants who lived in four villages (Vaumik para, Charkamaldi, Sheikhkaadi and Bishnadi) located within 10 km radius in Narayanganj had been drinking tube-well water with high concentrations of inorganic arsenic (mean value = 237.12 µg/L) for about 10 years constituted the arsenic-patient group, and thirty three residents who lived in Dhaka city but were exposed to much lower concentrations of arsenic in their drinking water (mean value = 11.3 µg/L) were selected as the control group. Here we investigated the possible genotoxic activity of arsenic compounds in the human lymphocyte using the alkaline Comet assay. The results were evaluated principally by computer analysis of comets. The arsenic patients showed significantly higher DNA damage in their lymphocytes than the control participants. The results indicate that arsenic species were able to induce significant increase in the comet tail DNA (%), total comet length and Olive tail moment; the parameters used to determine genotoxicity. Moreover, elevated plasma lipid peroxidation and protein carbonyl group levels with decreased activity of superoxide dismutase, glutathione peroxidase and catalase indicated the production of reactive oxygen species in arsenic patients. These results provide evidence that chronic exposure to arsenic from drinking water in humans results in induction of oxidative stress. Therefore our results suggest that the arsenic induced oxidative stress and resulted oxidative DNA damage could be the possible mechanism of clinical manifestations in Bangladeshi arsenic patients.

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**EFFECT OF MOBILE PHONE RADIATION ON MEMORY AND  
OXIDATIVE STRESS: EFFECT OF *CENTELLA ASIATICA***

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**Abstract**

Exponential increase of mobile phone use within the last few decades has raised the public concern about the health risk associated to long term mobile phone use. Recent studies suggest that low frequency electromagnetic radiation of mobile phone causes several types physiological abnormalities which possibly are linked with the formation of reactive oxygen species (ROS), injury to the nervous system and behavioral abnormalities. However, the correlation between low frequency radiation induced oxidative stress and memory dysfunction is obscure. Therefore, in the present study we examined the effect of 21 days of low frequency radiation exposure (3 hours per day) on DNA, protein, lipid and anti-oxidative enzymes in both plasma and hippocampal brain tissue. Spatial memory performance was assessed by classical T-maze in this experimental paradigm. We further used *Centella asiatica* extract (200 mg/kg of body weight) to examine whether radiation evoked changes are prevented or not. Radiation exposure significantly impaired the working memory as measured by T-maze. Furthermore, mobile phone radiation increased the level of DNA damage, lipid peroxidation, protein carbonyl content and decreased protein sulfhydryl content along with a down regulated level of superoxide dismutase in both plasma and hippocampus. Pre-treatment with *Centella asiatica* extract blocked the mobile phone-induced cognitive decline, oxidative DNA damage and protein and lipid peroxidation. In conclusion, the present study gives an indication of an association between oxidative stress induced changes in the levels of anti-oxidative enzyme, oxidative DNA damage, lipid and protein peroxidation and memory impairment in low frequency radiation exposed animal model.

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## **BOILAM: A NOVEL SALT TOLERANT RICE VARIETY OF BANGLADESH**

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### **Abstract**

Boilam is a photoperiod insensitive and early maturing rice, unlike the common salt tolerant donor rice, Pokkali. Pokkali is also not very receptive to crossing. This investigation was designed to identify novel Quantitative Trait Loci (QTL) linked to salt tolerant traits of Boilam and introgress these into modern rice varieties. BC<sub>2</sub>F<sub>2</sub> progenies from a cross between salt tolerant rice Boilam and farmer popular rice variety BR27 was developed for this experiment. A total of 200 BC<sub>2</sub>F<sub>2</sub> plants genotyped by means of 108 SSR markers. QTLs were identified using single-point analysis and interval mapping performed using QGene-3.0. A total of 4 QTLs located at chromosome 1, 9, 12 were detected using LOD >3.0 threshold. The range of R<sup>2</sup> value of QTLs was 19.57% – 25.81%. Four QTLs linked to salinity are QTL1 (LOD 6.16, R<sup>2</sup> 25.81%), QTL2 (LOD 4.88, R<sup>2</sup> 21.67%), QTL3 (LOD 4.72, R<sup>2</sup> 20.26%) and QTL4 (LOD 4.54, R<sup>2</sup> 19.57%) were identified on chromosome 9 and 1 and two on chromosome 12 respectively. Salt tolerant BC<sub>2</sub>F<sub>3</sub> having the background genotype of BR27 will be developed further for release or use as parents in breeding programs with the help of the markers-linked to the salt tolerant QTLs. Mapping of salinity QTLs have been worked out in seedling stage in Backcross population of Boilam and BR27. A study at reproductive stage tolerant is also set up to observe where the salinity QTLs are sharing the same map location or different in respect of different plant growth stages. This added information will be helpful in a way to establish Boilam as an alternative salt tolerant donor rice variety.

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## INVESTIGATION OF SEEDLING-STAGE SALINITY TOLERANCE QTLs USING NEAR ISOGENIC LINES DERIVED FROM POKKALI

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### Abstract

The major quantitative trait locus (QTL), *Saltol*, for seedling-stage salinity tolerance and low Na<sup>+</sup>/K<sup>+</sup> ratio, has been previously identified on rice chromosome 1 using recombinant inbred lines (RILs) from the cross of tolerant rice landrace 'Pokkali' and sensitive 'IR29'. A backcross mapping population (BC<sub>3</sub>F<sub>3</sub>, BC<sub>3</sub>F<sub>4</sub> and BC<sub>3</sub>F<sub>5</sub> near isogenic lines, NIL) derived from a cross of the same parent lines Pokkali and IR29 has been used for the fine mapping of *Saltol* (continuation of the previous work) and for the investigation of additional QTLs. Salinity tolerance is a multi-trait system. For durable tolerance to salinity, it is necessary to find out other QTLs for salinity tolerance and introgress to farmer popular mega varieties along with the *Saltol*. This mapping population consisted of a different combination of genotypes i.e. tolerant genotypes (with SES 3-5) with and without the *Saltol* locus and sensitive genotypes (with SES 6-9) with and without the *Saltol* locus. All of these genotypes were subjected to salinity tolerance screening (in hydroponics) and many of those were genotyped with markers at the *Saltol* locus as well as throughout the 12 chromosomes. The polymorphic marker data was analyzed by QGene mapping software. The results showed 4 QTLs at markers RM26063, RM3867, RM20224 and RM222 on chromosome 11, 3, 6 and 10, respectively, having LOD >5. The next plan would be the characterization of the QTL with highest LOD score 6.25 and coefficient of determination (R<sup>2</sup>) of 15%. Major QTLs will be targeted for fine mapping by saturating identified regions with more polymorphic markers. Identification of markers flanking the additional QTLs will be important for introgression of multiple QTLs in farmer popular varieties.

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## **EXPRESSION OF TRANSGENE UNDER STRESS INDUCIBLE PROMOTER FOR PRODUCING SALT TOLERANT RICE**

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### **Abstract**

Among the various stresses that impair crop productivity, salinity is a major threat to agriculture. Salinity tolerance is a multigenic trait. Therefore it has been difficult to obtain salt tolerance by using single genes. Many genes are now reported to confer salt tolerance in plants, particularly in the case of dicots like tobacco. In most of these cases, the genes have been expressed by the constitutive cauliflower mosaic virus promoter (CaMV35S). The same genes with the CaMV35S promoter when transformed into rice do not perform as well because this promoter is not good for monocots such as rice. It can cause yield penalty. Therefore it is necessary to find efficient promoters for rice gene transformation and expression.

The promoters of the NHX1 (vacuolar Na<sup>+</sup>/H<sup>+</sup> antiporter) from the salt tolerant landrace Pokkali (PKN) and sensitive cultivar IR-64 (IRN), respectively, were isolated and characterized. Promoter efficiency is generally measured by promoter-reporter gene constructs and used to transform rice. In transgenic T<sub>2</sub> rice homozygous for the promoter-GUS gene, the PKN promoter was found to be salt-inducible with tissue-specific expression in leaf epidermal tissues whereas IRN shows all over expression except in the epidermal layer. The significance of this result is that, like salt tolerant barley, the landrace Pokkali is likely to partition Na<sup>+</sup> away from the photosynthesizing mesophyll layer and thus require high activity of the antiporter during salt stress. Antiporter constructs with the CaMV and PKN promoter were used to transform rice.

Other stress related promoters - HKT8, Asr1, CCOMT and APX have also been isolated from Pokkali and are being characterized as they are known to be expressed under salt stress conditions. By sequencing and comparing HKT8 promoter region with the sequence of database of Nipponbare rice sequences, 20 single nucleotide polymorphisms (SNPs) were observed. Further work with this construct can confirm the role, if any, of the SNPs in salt inducibility.

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## MOLECULAR CLONING AS A TOOL TO IMPROVE CROP YIELD

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### Abstract

Molecular cloning is widely used as a method to transfer a particular trait to any variety of same or even completely unrelated species. It is also used to over express a gene or a group of genes to change its property like yield or content etc. We are interested to improve the salt tolerance of rice and are therefore cloning several genes in order to transform into farmer popular varieties to improve their acceptability to our local farmers in salt affected areas. The most promising genes reported to improve salt tolerance are for the Vacuolar Sodium/Hydrogen Antiporter, DNA helicase and Glyoxalase pathway. Traditional digestion-ligation methods were bypassed using Gateway technology where insert is transferred primarily to an entry vector. The linearized entry vector contains topoisomerase enzyme tagged at the end which transfers the bond to the insert and enables cloning within 5 minute incubation at room temperature. The entry vector contains recombination site flanking the insert site allowing transfer of the insert to the destination vector simply by incubating two vectors with recombinase enzyme. A wide choice of destination vectors allows us to characterize the insert in a different way. Targeted genes were cloned and transgenic plants were regenerated and are being characterized. They are showing promising results upon salinity screening. But commercial binary vectors contain the constitutive CaMV 35S promoter which has been shown to poorly express in monocots. Constitutive expression also causes yield penalty, an undesirable character for high yielding varieties. Therefore a salt inducible efficient promoter was needed for these destination vectors. We have isolated an upstream region and characterized to be an efficient promoter. The CaMV 35S promoter had to be replaced by traditional digestion-ligation method. Digestion with the same enzyme was not compatible and therefore we modified the traditional process. We filled the digested vector ends and added T- overhang. At the same time we added A-overhang to the amplified insert. This enabled the vector and inserts to be ligated as TA cloning method. We have now produced transgenic plants with the Na/H antiporter driven by the salt-inducible promoter and are comparing salt tolerance of these plants with those that have the same gene driven by the CaMV 35S promoter.

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## **RICE LANDRACES OF BANGLADESH: MUTATION IN THE WAXY AND Rc GENES.**

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### **ABSTRACT**

Most Bangladeshi rice varieties are Indica and nonglutinous. However, glutinous rice also exists, for example, Beruin varieties. Glutinous rice produces cohesive grains and are therefore of importance in festivals where the main food item is rice cake. Some of these glutinous rice varieties are red in color. The red pericarp color of rice is of much significance due to its nutritional, evolutionary and domestication history

Glutinous rice contains much higher level of amylopectin compared to nonglutinous rice and high amylopectin is easy for digestion, therefore it has a high glycemic index. This is due to lack of the synthesis of the starch amylose, because of a defect in the gene (*waxy*) for granule-bound starch synthase. This defect is due to G to T mutation, which causes defective splicing of the gene transcript. The amylose content in nonglutinous rice however varies considerably, apparently because of the suppression of the *waxy* gene mutation due to sequences elsewhere. The *waxy* gene from both glutinous and nonglutinous varieties was sequenced in an attempt to find the G to T mutation or suppression in Bangladeshi landrace. There were two SNPs found to be unique for Bangladeshi glutinous and nonglutinous landraces compared to database sequences in the promoter for the *waxy* gene. SNPs found in this study may therefore be indicative of the origin of Bangladeshi rice.

Some glutinous varieties included in the study were also red in color. Knowledge on genetics of red rice is important to avoid the transfer of herbicide resistance property of weedy red rice as well as to implement good features like disease resistance trait. Among them some red and white accessions were found with the same name and could not be distinguished visually unless dehulled. It was found in a study that either a 14 bp deletion or a C to A mutation in the red pericarp regulating gene *Rc* which encodes a bHLH transcription factor can turn a rice from red to white. For this purpose a portion of the *Rc* gene was sequenced and the 14 bp deletion was found in white Bangladeshi varieties under study. In addition, rice microsatellite fingerprinting of Non Glutinous and Glutinous rice varieties with both red and white pericarp were performed for characterization and dissimilarity analysis. The dendrogram from the study could differentiate the glutinous varieties from others and the heterozygosity analysis could differentiate the red and white varieties with the same name as different accessions.

In future more varieties can be collected from different parts of Bangladesh for analyzing sequence variation. The evolutionary linkage of these varieties with respect to global rice accessions can be made by comparison with database sequences.

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## **LYMPHOID ENHANCER FACTOR INTERACTS WITH GATA-3 AND CONTROLS ITS FUNCTION IN T HELPER TYPE 2 CELLS**

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### **Abstract**

GATA-3 is the master transcription factor for T helper 2 (Th2) cell differentiations and is critical for the expression of Th2 cytokines. Little is known, however, about the nature of the functional molecular complexes of GATA-3. We identified a high-mobility group (HMG)-box type transcription factor, lymphoid enhancer factor 1 (LEF-1), in the GATA-3 complex present in Th2 cells using a Flag-calmodulin-binding peptide (CBP)-tag based proteomics method. The interaction between GATA-3 and LEF-1 was confirmed by co-immunoprecipitation experiments using LEF-1-introduced T-cell lineage TG40 cells. The HMG-box domain of LEF-1 and two zinc finger domains of GATA-3 were found to be important for the physical association. The introduction of LEF-1 into developing Th2 cells resulted in the suppression of Th2 cytokine production. The suppression was significantly lower in the cells into which a HMG-box deleted LEF-1 mutant was introduced. Moreover, LEF-1 inhibited the binding activity of GATA-3 to the interleukin (IL)-5 promoter. These results suggest that LEF-1 is involved in the GATA-3 complex, while also regulating the GATA-3 function, such as the induction of Th2 cytokine expression via the inhibition of the DNA-binding activity of GATA-3.

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## INTER-PROPHAGE INTERACTIONS AND CONTRIBUTION OF DEFECTIVE PROPHAGES OF A PROPHAGE POOL IN DISSEMINATING VIRULENCE DETERMINANTS

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### Abstract

Bacteriophages are well-known mediators of horizontal gene transfer and drivers of bacterial evolution. Until recently they have been ignored by most of the scientific community, however, they put forward a gigantic impact upon bacterial community that the encounter with such a bacterial species, harboring a phage genome in the chromosome called a prophage, may cause severe disease in humans. The enterohemorrhagic *Escherichia coli* O157: H7 Sakai strain, produce severe disease in humans, contains a pool of 18 such prophages with various anomalies that are generally considered as genetic defects. Among these prophages, two contain *stxAB* genetic region encoding a potent cytotoxin “Shiga toxin” and the rest contain many other virulence related genes, and morons. Interestingly, some prophages have identical morphogenetic regions. However, their contribution to the spread of virulence has long been neglected considering them simply as remnants. Thus, this prophage pool is definitely a striking biological model to study potential activities of the defective prophages to spread virulence determinants.

Our combinational approach of *in silico* prediction and experimental evaluation using DNA microarray, qualitative PCR and quantitative real-time PCR, and by field-inversion-gel-electrophoretic analysis, we analyze spontaneously as well as SOS response inducible phage genome replication and packaging. We also analyze transferability of those replication and packaging proficient prophages employing their antibiotic-marked derivatives. Thus, we inquire whether the defective or remnant prophages have the potential as mobile genetic elements and illustrate a complete picture on their biological activity. We observe that the prophages in the genome of *E. coli* O157 Sakai follow a proliferative pathway, unless lack a phage regulatory region, an intact site-specific recombination system, or phage DNA replication machinery forming a stable lysogeny otherwise. Notionally, a proliferative pathway should end up yielding replication intermediates for the lack of genes for morphogenesis. But, surprisingly, we observe, such defective prophages can proliferate and propagate into *E. coli* K-12 from Sakai strain. Thus, we speculate that a prophage pool in a genome with similarities among morphogenetic functions would have a positive influence on winning induction by complementation of missing function(s) and may further process the ended up pathway executing production of virions with genetically defective genomes. Another possible scenario would be, replication-intermediates may interact with each other to undergo recombination to generate chimera with infectivity. Consequently, we suggest for the first time that various inter-prophage interactions in a defective prophage pool can occur to generate and spread progeny carrying virulence determinants.

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**INACTIVATION OF THE GENE *SLR1923*: A POTENTIAL STEP TO  
DISCOVER DIVINYL REDUCTASE INVOLVED IN CHLOROPHYLL A  
BIOSYNTHESIS IN  
*SYNECHOCYSTIS* SP. PCC6803**

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**Abstract**

Chlorophyll is the most vital and inevitable photon-capturing pigment in photosynthesis. During biosynthesis of normal monovinyl-chlorophyll, reduction of the 8-vinyl group that is located on the B-ring of the macrocycle is a necessary step. The enzyme responsible for catalyzing this step, divinyl reductase (DVR), has recently been identified in higher plant, *Arabidopsis thaliana*. But the counterpart is yet to be discovered in most of the cyanobacteria. We have created a mutant of *Synechocystis* sp. PCC6803 by insertion of spectinomycin cassette into the gene *slr1923* (*slr1923<sup>M</sup>*) and have characterized it. The mutant showed a strong sensitivity to high-light illumination ( $300 \mu\text{E m}^{-2}\text{s}^{-1}$ ) and greatly reduced the cellular content of chlorophyll. By absorption spectra, HPLC and <sup>1</sup>H-NMR analysis, we found that the molecular identity of the chlorophyll of the mutant is 3,8-divinyl chlorophyll (DV-Chl) but not 3-monovinylchlorophyll (normal chlorophyll). We have concluded that *slr1923* is inevitable for conversion of 3,8-DV-(proto)chl(ide) *a* to 3-MV-(proto)chl(ide) *a* in *Synechocystis* 6803. We thus designated the gene as *cvrA* (a gene indispensable for cyanobacterial vinyl reduction). We have further characterized the photosystems (PSs) of the mutant. 77K fluorescence spectra of PS I and II particles are not different from those of wild type, indicating that the micro-environments of chlorophyll responsible for fluorescence emission are not much altered in the mutant. However, 77K absorption spectra indicated that chlorophyll forms were mainly blue-shifted by 1-2 nm in the mutant suggesting that the binding mode and/or stability of the modified chlorophyll molecule to the most of chlorophyll-protein complexes have been altered in the mutant. The difference spectrum of P700 showed that Soret peak is also shifted to a longer wavelength. This is the same characteristic of DV-Chl *a* absorption spectrum. These data confirmed that PSI primary electron donor (P700) is also composed of divinyl-Chl *a*.

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**NUCLEOPHOSMIN IS REQUIRED FOR CHROMOSOME CONGRESSION,  
PROPER MITOTIC SPINDLE FORMATION, AND KINETOCHORE-  
MICROTUBULE ATTACHMENT IN HELA CELLS**

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**Abstract**

Nucleophosmin (NPM) is an abundantly and ubiquitously expressed multifunctional nucleolar phosphoprotein, which plays a key role in the regulation of a number of processes including ribosome biosynthesis and maintenance of genomic integrity. Although several other functions of NPM such as centrosome duplication and chromatin regulation have been reported, its roles during the cell cycle are still unknown in HeLa cells. In this study, we show that NPM is dynamically localized throughout the cell cycle of HeLa cell. Using a combination of high-resolution microscopy and RNAi method, we show that depletion of NPM causes defects in cell division followed by an arrest of DNA synthesis due to activation of p53-dependent checkpoint response in HeLa cells. Depletion of NPM leads to mitotic arrest due to spindle checkpoint activation. The mitotic cells arrested by NPM depletion have defects in chromosome congression, proper mitotic spindle and centrosome formation, as well as defects in kinetochore-microtubule attachments. Loss of NPM thus causes severe mitotic defects and delayed mitotic progression. These findings indicate that NPM is essential for mitotic progression and cell proliferation.

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