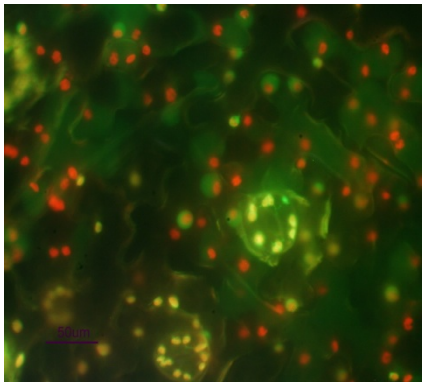
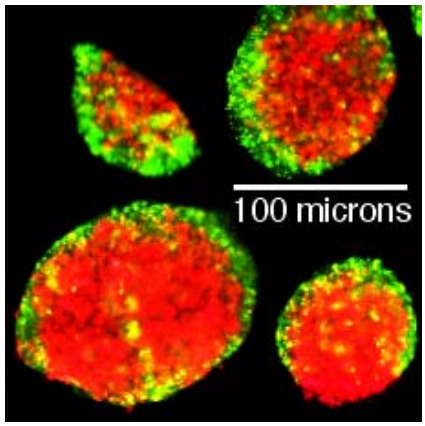
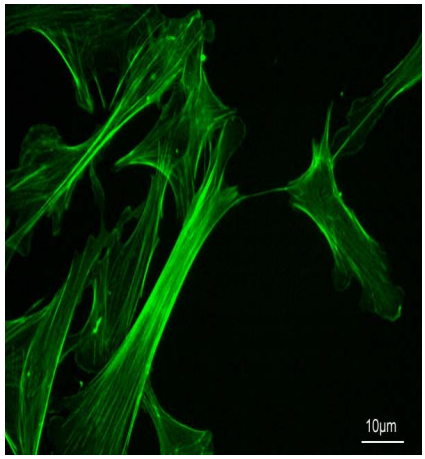






EMHGBN (EMGEN)* Newsletter

Vol. 2, Issue 10, October 20th, 2008

INSIDE THIS ISSUE:

Eastern Mediterranean Health Genomics and Biotechnology Network (EMHGBN) was created in 2004 with collaboration of representatives of selected centre of excellence in (health related) molecular biology, biotechnology & genomics in the Eastern Mediterranean region by recommendations and efforts of WHO/EMRO.

Address:
Biotechnology building, #69, Pasteur Ave., Pasteur Institute of Iran
Tehran, Iran, 13164
Tel: +98-21-66954324
Fax: +98-21-66465132
E-mail: emhgbn@gmail.com, secretariat@emhgbn.net
Website: www.emhgbn.net

Prepared by:
Milad Adibi
Page design: Milad Adibi
Editor: Dr. S. Sardari

* EMGEN is a shortened form of EMHGBN that was approved for the ease of use by the steering committee members of the network.

Detection of Plasmids in Heavy Metal Resistant Bacteria Isolated From the Persian Gulf and Nearby Industrial Areas

The article entitled "Detection of plasmids in heavy metal resistant bacteria isolated from thePersian Gulf and nearby industrial areas" focuses on identification and characterization of plasmids present in bacteria isolated from Persian Gulf and nearby industrial areas. The study was carried out by Hossein Zolgharnein, Mohd Lila Mohd Azmi Mohd Zamri Saad, Abdul Rahim Mutalib and Che Abd Rahim Mohamed. The corresponding author of this paper, Dr. Hossein Zolgharnein, is currently working in Department of Marine Biology, Faculty of Marine Science at the Khurramshahr Marine Science and Technology University. This paper was published in Iranian Journal of Biotechnology, Vol. 5, No. 4, 2007.

Dr. Hossein Zolgharneyn

Plasmid encoded genes play a significant role in adaptation of microbial communities to environmental factors by acting as a mobile gene pool. It has been shown that the frequency of catabolic plasmids increases 2 to 20-fold in polluted marine and freshwater ecosystems. A number of recent studies have focused on identification and characterization of plasmids and their role in adaptation of microbial communities in contaminated environments. Such information can provide insights into the potential of using bacteria as an effective and efficient means of detoxification of metal ions in polluted marine and freshwater environments, and therefore can play an essential role in decontamination and preservation of natural habitats.

Samples were collected from 72 stations across the Persian Gulf, covering the whole area of the Gulf. After the Isolation of bacteria total DNA was exacted from all isolated strains. The 16S rRNA sequences were amplified through PCR by using two universal oligonucleotide primers designed for amplification of 16s rRNA. After sequencing, the resulting nucleotide sequences were compared against the published sequences in NCBI GeneBank in order to identify the isolated bacteria. A total of 35 isolated bacterial strains were identified and tested for the presence of plasmids using the modified alkaline lysate method. The frequencies of plasmid-carrying isolates from heavy metal resistant bacteria were as 32.25% with sizes of 4- 2 kb, 3.25% with sizes of 10 and 16 kb and 32.25% with sizes of 38-62 kb. Differences were evident between the marine and industrial samples both in terms of plasmid frequency and the strains carrying the plasmids. Some of the strains with high plasmid frequencies demonstrated the ability to take up heavy metals at high concentration. Furthermore, it was shown that some of these strains lost their capacity for accumulation of heavy metals, following the loss of their plasmids. Strains from the genus *Pseudomonas* were of particular Interest. It was previously shown that the incidence of plasmids in *Pseudomonas* strains was significantly higher in polluted water strains than in the same bacteria isolated from unpolluted water. The study also showed the higher plasmid levels in *Pseudomonas*

bacteria that other isolated bacteria, suggesting that bacteria from this genus might have a greater potential for adaptation to heavy metal contamination. The results demonstrate that at least some of the strains obtain their increased resistance to heavy metals via the plasmid encoded genes, which increase in frequency in response to selective pressure applied by contamination in their environment. Contamination in such environments has persisted for several decades, thus explaining the high level of adaptation observed in endogenous bacteria.

An Improved Experimental Model for Studying Vertical Transmission of Hepatitis B Virus via Human Spermatozoa

The article entitled "An improved experimental model for studying vertical transmission of hepatitis B virus via human spermatozoa" introduces an enhanced method for studying the vertical transmission of Hepatitis B virus through human spermatozoa. The study was carried out by Mohammad Morsi M. Ahmed, Tian-Hua Huang, and Qing-Dong Xie. The corresponding author of the article, prof Tian-Hua Huan, works at works at Research Center for Reproductive Medicine, Shantou University Medical College, Shantou, China. The coauthor of this article Dr. Mohammad Morsi M. Ahmed is Associate Professor and head of Department of Nucleic Acids Research (NAR) at Nucleic Acid Research Department, Genetic Engineering and Biotechnology Research Institute, Mubarak City for Scientific Research and Technology Applications, New Borg El-Arab City 21934, Alexandria, Egypt. This paper was published at the Journal of Virological Methods, Vol 151, issue 1, 2008.

Dr Mohammad M. Ahmed

Prof. Huang Tian-Hua Huang

Study of the mechanism of transmission of hepatitis B virus is important for public health. An improved experimental model is described for studying vertical transmission of hepatitis B virus (HBV) via human spermatozoa. Recombinant plasmid pIRES2-EGFP-HBx which would express enhanced green fluorescent protein (EGFP) used as a marker for the expression of hepatitis B virus X (HBx) gene was constructed successfully and confirmed by PCR, EcoR I and Sal I digestion, and DNA sequencing.

After exposure to the plasmid, human spermatozoa were used to fertilize zona-free hamster ova *in vitro*. Two-cell embryos were collected and classified into group A with green fluorescence and group B without green fluorescence under fluorescence microscope. The results showed that HBx DNA positive bands were detected in the embryos with green fluorescence (PCR and RT-PCR) and positive controls (PCR) indicating presence and expression of HBx gene. In contrast, HBx gene expression was not detected in the embryos without green fluorescence and negative controls (PCR and RT-PCR). This improved experimental model is more efficient, accurate and reliable for

studying further vertical transmission of HBV or other viruses causing chronic human disease possibly via the male germ line.

The advantages of the improved model are as follows: (1) it is possible to determine which embryo contains human sperm-mediated virus genes by observation of green fluorescence and avoid the uncertainty which occurred in previous studies (Huang *et al*., 2002; Ali *et al*., 2006, 2005; Xiong et al., 2005). (2) The results can be obtained by performing only one experiment with a single embryo with the new model while many experiments with many embryos were needed with the previous model. This was not only less time consuming and less expensive but also the results were more accurate and reliable. (3) The improved model would make it easy to explore vertical transmission of other viruses (X) via human spermatozoa if recombinant plasmid pIRES2-EGFP-HBx was replaced by pIRES2-EGFP-X.

Overall, the improved experimental model will be more efficient and reliable for studying further vertical transmission of sperm-mediated virus genes without moral and ethical complications.

Hype in Biotechnology

In recent years biotechnology has been put forward as the solution to many of major problems in the modern world. The scope of benefits from biotechnology industry has expanded from just curing diseases to providing food for developing countries through genetically modified (GM) food products and development of clean renewable energy sources. But how realistic are such approaches? A detailed examination of the current biotech reveals that such an idea is far-fetched.

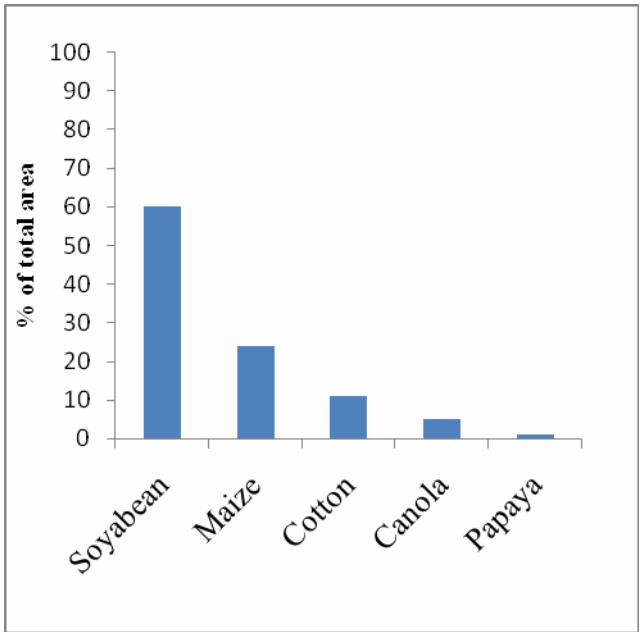
The use of GM crops is common in many countries around the world. However current state of GM crops is far away from solving the main agricultural issues of developing countries. At the present time only a few GM food staples are available that have the potential to contribute significantly towards the food sources in developing countries. In the case of biofuels such as ethanol, biotech is only one of the required technologies to establish biofuels as a reliable and renewable energy source.

Biotech has been successful in improving the treatment methods for some diseases. Thanks to biotech new techniques are available for treatment of cancer, autoimmune disorders etc. However the notion of cures based on gene therapy and molecularly targeted medicine are far from being realized. Furthermore biotech has done little toward addressing the problem of diseases prevalent among under-privileged populations.

An Alternative view-point

It should be emphasized that the aim here is not to show biotech as a futile field with poor outcomes, which is definitely not the case. Since the establishment of the biotech industry, every new trend, such as microarrays, has been hyped as the 'solution'. 'Omics', Protein drugs and other ideas failed to deliver on the overstated promises.

Such a forceful and unrealistic approach can be successful in gathering attention and investment at the early stages. However in the long-run it results in strong criticism and opposition from various fractions of the society. A more efficient and practical approach would be to let pubic and critics assess the ability of biotech to tackle global problems for themselves. By informing the general public and politicians of capabilities of biotech, they can draw their own conclusions about the potential of biotech to solve the problems that society faces.



Percentage of global area under GM plant cultivation for each GM crop. Only 24% (maize) belongs to food staples.

In other words biotech supporters need to try less hard. They need to be more careful about overstating the current abilities of biotech and its future prospects. The general public would inevitably realize the prospects of biotech as one of the major technologies for solving the world's most critical problems.

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SNP Genotyping as a Diagnostic tool

The sequencing of whole human genome revealed millions of sequence variations in human DNA. Most of these variations are short nucleotide polymorphisms (SNPs). SNPs are precursor of many human diseases and as results are drawing increasing attention in pharmacogenetics. The availability of a dense set of SNP markers brings about the opportunity to study the genetic basis of human diseases, as well as determining the basis of variable responses to drugs. SNP genotyping comprises a variety of methods for detection of SNPs among human populations. Sequencing of the human genome paved the way for SNPs to become promising mean for research.

Initially it was suggested that SNPs, evenly distributed along the genome, can be used in Genetic association studies, where two populations (usually diseased subjects and healthy controls) are screened to determine whether single-locus alleles or genotype frequencies differ between the two. SNPs are useful determinant of such differences. Close association of certain SNPs with the diseased group would associate the SNPs or the adjacent DNA with the disease state. The ability of SNP genotyping to recognize genetic variation patterns among different populations can be utilized in human population studies. This can prove useful studying the origins of human populations, as well as revealing patterns of genetic variation causing prevalence of certain diseases among human subpopulations

In the early days of SNP genotyping, inefficiency and expensiveness of techniques were a major problem towards development of SNP genotyping into a useful and reliable research and medical tool. Recent developments have significantly lowered the costs and increased the number of SNPs analyzed, thus making whole genome analysis more feasible. In recent years, several algorithms have been developed for identification of Tag SNPs. Tag SNPs are representatives SNPs in the regions of genome with high linkage disequilibrium. Such SNPs can be used to identify genetic variation in a given chromosomal region, without the need to genotype every SNP In addition, as more SNPs are firmly linked to specific disorders and responses to drugs, focused SNP genotyping techniques, which use fewer SNP markers, are becoming more reliable.

Genetic Risk Factors:

To this date the success of SNP genotyping technique has not reach the initial high expectations. The association between SNPs and any particular disease state were often shown to be small. Currently the major prospect of SNP technology is in the field of personalized medicine. SNPs can be used in determining the genetic variations in drug-metabolizing enzymes and drug metabolism in order to prescribe medicine according to genetic profile of the individual. The role of SNPs in identification of genetic risk factors is evident. However recent studies have shown the existence of non DNA-based hereditary mechanism, the most well-known case being the regulation of expression by DNA methylation. Thus SNPs cannot be solely relied on as indicators of hereditary variation in relation to a

disease state. Therefore, in most cases, a link between risk factors and SNPs cannot be definitely established. Identification of known risk factors does not guarantee a particular response to a therapeutic method in a patient; the association can only be presented in terms of probability. This can prove to be a problem when it comes to choosing between therapeutic options.

In order for SNP-based technologies to be more than just research tools in the lab, SNP-based technology must deliver results in a reliable, comprehensible, and interpretable fashion. In addition medical practitioners must have the knowledge and skill to communicate the implications to the patient.

Approach	Processing steps		
	DNA purification	Amplification	Postamplification modification
Allele-specific PCR	Yes	PCR	None
Flow cytometry	Yes	PCR	Allele-specific pimer extension, hybridization, washing and flow cytometry analysis
Microarray or DNA cheap	Yes	PCR	Purification, loading, hybridyzation and detection
Rolling circle amplification (RCA)	Yes	Ligation/RCA	None
Standard displacement amplification	No	SDA	None
Mass spectrometry	Yes	PCR	Primer extension, purification and purification and mass spectrometry analysis
Invader assay	Yes	NO PCR	Cleavage reaction, detection
Single-strand conformational polymorphysm (SSCP) analysis	Yes	PCR	Gel electrophoresis

Comparison of some technologies used for detection of SNPs in relation to processing steps required for obtaining results

Challenges Ahead:

Large-scale SNP genotyping methods usually use small amounts of samples and reagents, making them particularly vulnerable to contaminations, which can lead to unreliable results. This situation presents a major hurdle in establishment of SNP analysis as a useful medical technique and needs to be addressed before SNP genotyping can gain widespread acceptance among the medical community. Other challenges ahead of SNP genotyping technology in near future include, further reduction of costs, increasing the speed of essay development and performing multiple essays in parallel. However considering the high speed development of new methods, it is reasonable to expect an ideal SNP genotyping method to emerge soon.

Another potential problem can arise from the issues concerning the confidentiality of DNA sequence data produced by SNP genotyping techniques. Public would likely be concerned about how such information can be used by insurance companies, employers and governments. Introduction of reasonable regulations and protocols regarding the privacy of such data should significantly help towards satisfying these concerns.

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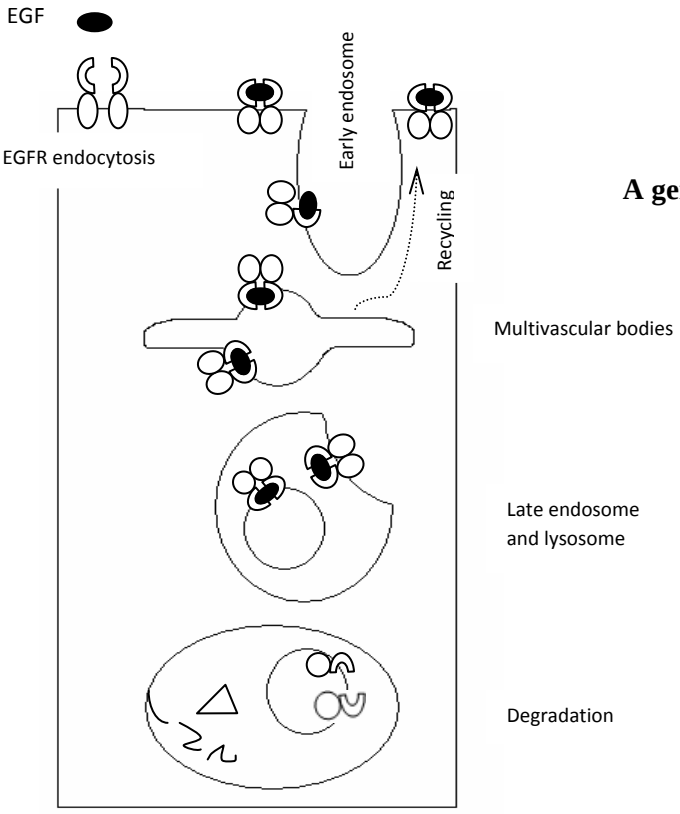
Engineering Protein-based Agonists

Engineering Agonists on the Basis of Ligand–Receptor Binding Affinity

An appealing approach to create enhanced agonists is to mutate the natural ligand in order to improve its ability to bind to receptors. However an increased receptor-ligand binding affinity does not always result in an increase of the target biological activity. It has been shown that application of mutant human growth hormone (hGH) proteins with 400-fold higher binding affinity, does not result in an increased rate of cell proliferation. It was later revealed that the increased internalization rate of receptor-ligand complexes limits the maximum agonistic activity of the ligand. Therefore changes to ligand binding affinity cannot always single-handedly alter the target biological activity.

Engineering Agonists on the Basis of Ligand–Receptor Trafficking

Receptor trafficking and internalization plays a significant role in controlling cellular responses. In general, the rate of internalization is induced as the activity of the receptor is increased. Many therapeutically important ligands are internalized along with their receptors. Such ligands might be released undamaged or can be sent to lysosomes for degradation. The ligand-receptor complexes with longer duration of activity are more readily degraded, while the short-lived complexes have a higher recycling rate. Logically this situation would be problematic when engineering ligands for high affinity.



A general overview of receptor down-regulation, degradation and recycling

Theoretically a ligand that binds strongly to the receptor on the cell surface but dissociates easily in the endosome, would have an increased activity due to reduction in degradation rate. Using this strategy granulocyte colony-stimulating factor (GCSF), a cytokine that increases white blood cell proliferation, has been successfully engineered for improved potency. This engineered molecule has greater degree of pH difference in extracellular space (ph ~ 7), compared to endosome (pH~ 5-6). Histidine side-chains are neutral in the extracellular medium but will be protonated in the endosome. Regions of the GCSF molecule which had high negative charge relative to their interaction site on the receptor were substituted with neutral histidine or positively charged histidine. Computer-based predictions identified two histidine-mutant GCSFs that showed ten-fold longer half-life and two-fold increase in cell proliferation rates in comparison to wildtype equivalent.

Engineering Agonists on the Basis of Sequence Variation

An alternative strategy in ligand engineering involves taking advantage of the natural variations present in protein families similar in structure or sequence. This is based on the notion that natural evolution has already created amino acid residues that are very efficient in binding interaction, folding etc. Assuming that residues present in majority of homologues of a protein contribute towards maximum stability, mutations can be introduced to create ligands with improved stability. This approach has been employed to create more stable variants of human fibroblast growth factor 1

(FGF1), Due to low thermodynamic and proteolytic stability, FGF1 ligand has a short *in vivo* half-life. The analysis of consensus residues for an FGF1 alignment of 140, and the subsequent stability analysis of proposed sites for substitution, identified point mutations that could potentially increase the half-life of the ligand. Mutations found in orthoulogues of a protein are used in a similar fashion.

Ligand Design Strategies

Approaches to ligand-design are divided into two groups based on their utilization of structural information of ligands and receptors.

In directed evolution strategies (also referred to as combinatorial protein engineering), a population of protein mutants are generated. The resulting genetically diverse population of candidate mutants is screened for improvement in a phenotype of interest. This method is applied even when the libraries of mutants are generated from random mutagenesis. This is commonly achieved by error prone PCR, where different polymerases, reaction conditions, or non-natural nucleotide analogs are used. Such strategies are particularly useful when engineering ligands for a receptor with unknown structure.

Another more refined method involves identification of specific sites on the protein of interest that are more important to functionality, and focus mutation efforts on those sites (referred to as rational protein design.). Such information can be obtained via experimental approaches. Alternatively computational approaches can be used. When information is available on receptor binding properties, computational approaches are also useful at the screening stage. This can be done by designing libraries to target specific coverage of the mutant proteins.

Computational approaches are ever more utilized in protein engineering. Such methods have been significantly improved as a result of the increase in computational power, the availability of high-resolution protein structures, the development of combinatorial chemistry and the advent of the genome era.

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Announcment

EMHGBN Grant 2008-2009 for Research in Health Genomics and Biotechnology for Member States of the Eastern Mediterranean Region

Call for Pre-proposals

Eligibility

Only collaborative proposals are considered eligible for this grant in which investigators from two or more different institutions of minimum two countries of the Eastern Mediterranean Region collaborate on an unified research topic pertaining to one of all health genomics and biotechnology areas. However, the presences of researchers and/or companies from non-EMRO countries are welcome. In cases that such researchers link to the proposal and can bring matching fund, they can be considered as one of the Principle Investigators as well. A condition for approval of the proposals would be matching fund from the researchers host institute.

Research Areas

All areas of HEALTH genomics and biotechnology, with focus on solving problems for eastern Mediterranean countries and preferably lead to production or establishing a new foundation, means of treating people or diagnosing conditions, new set ups of research, databases, patents, industrial partnership, etc.

Deadline

EMHGBN Grant has established 15 November as deadline for submission of ***pre-proposals*** to allow time for their consideration by EMHGBN Selection Committee.

For more comprehensive information on eligibility criteria, conditions, ***pre-proposal*** preparation and review process you can kindly find the attached guideline and application form and visit our website; www.emhgbn.net

Cover Pictures Description (From top to bottom)

Title: Epidermal cells from tomato leaf with green fluorescent protein (GFP) expression in chloroplasts
Description: GFP protein is expressed along with major plastid proteins. Red color represents chlorophyll which naturally omits red fluorescent light. In stomata were chlorophyll containing chloroplasts have already formed, overlapping red and green colors produce yellow
Source: Created by Dr. Kevin Pyke and Milad Adibi at Plant Science Department, biosciences school, University of Nottingham.

Title: Glofish (not Goldfish)
Description: Genetically modified fluorescent goldfish; the first GM animal to be sold as a pet
Source: http://en.wikipedia.org/wiki/Glofish

Title: Cell sorting out
Description: Illustration of "cell sorting-out" for w:Morphogenesis. Cultured P19 embryonal carcinoma cells. Live cells were stained with either DiI (red) or DiO (green). The red cells were genetically altered and express higher levels of E-cadherin than the green cells. The image was captured by scanning confocal microscopy.
Source: http://commons.wikimedia.org/wiki/Image:P19_cell_sorting_out.png

Title: Microfilament
Description: Microfilament (actin cytoskeleton) of mouse embryo fibroblasts, stained with FITC-phalloidin (100-fold magnification.).
Source: http://commons.wikimedia.org/wiki/Image:MEF_microfilaments.jpg