

EMGEN Newsletter

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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.

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Epidemiological and clinical survey of scorpionism in Khuzestan province, Iran

The paper entitled: Epidemiological and clinical survey of scorpionism in Khuzestan province, Iran, which is published in *Toxicon*, Vol. 53 (2009) 454-459, described the demographic and clinical features of scorpion envenomation and the amount of antivenom required for treatments. The current study is the first epidemiological survey that covers the whole province. The study was carried out by Delavar Shahbazzadeh, Aref Amirkhani, Navid Dinparast Djadid, Shahla Bigdeli, Abolfazl Akbari, Hamed Ahari, Hossein Amini and Rouhullah Dehghani. Corresponding author of this paper, Dr. Delavar Shahbazzadeh is assistant professor of Medical Biotechnology Research Center, Pasteur Institute of Iran.



Dr. Delavar Shahbazzadeh

Khuzestan is a southwestern province of Iran, and it is tropic area nearby the Persian Gulf region (see figure 1). Envenomation in this tropical area by scorpion stings is a major public health problem, especially in children (Mirdehghan and Motlagh, 2001). There are more than 23 scorpion species in Iran; six species are dangerous and responsible for envenomings (Vachon, 1966).

Except for one species of *Hemiscorpius lepturus*, all species belong to the Buthidae family and include *Androctonus crassicauda*, *Hottentotta saulcyi*, *Hottentotta schach*, *Odontobuthus odonturus*, and *Mesobuthus eupeus* (Radmanesh, 1990a,b; Akbari et al., 1997; Fet et al., 2000; Bucherl, 1971).

Envenomations have many symptoms such as pain, sweating, fever and hypertension. The venom of Buthidae family has neurotoxic effects (Radmanesh, 1990b; Mirdehghan and Motlagh, 2001), and *H. lepturus* with cutaneous reaction. In most severe cases, hematuria due to hemolytic effect of *H. lepturus* venom was reported (Radmanesh, 1998; Pipelzadeh et al., 2007).

In Iran, serotherapy is the major therapeutic treatment of scorpion envenoming for the last 30 years (Akbari et al., 1997; Latifi and Tabatabai, 1979). The dose of antivenom and the administration protocol are based on the instructions recommended by the manufacturer or by the National Expert Committee, which mainly includes physicians. Very little is known about the envenomation and rate of scorpion stings in different areas of Khuzestan (Mirdehghan and Motlagh, 2001; Radmanesh, 1990a,b, 1998; Pipelzadeh et al., 2007).

Because of its high envenomation reports in among 30 provinces in Iran, Khuzestan, a southern province with the population of 3,972,309 consisting 6.6% of the whole country population was selected for investigation.

The study was investigated the different envenomation cases from the six major cities in the province which were selected by stratified cluster random sampling method. The selected cities consist of Shush (183,409 inhabitants), Ramhormoz (146,364), Masjed-Soleiman (237,418), Izeh (179,337), Baghmalek (105,504), and Behbahan (170,694).

The questionnaire contained information about the city where the incident was recorded, the place of the accident, the site of scorpion sting, the age and sex of the patients, the symptoms recorded at the time when patients came to the Emergency Units; possible known species of the scorpion; and measures taken by the Emergency Units for every envenomed individuals. The time between the sting and antivenom injection and the dosage of antivenom given to each patient was also recorded. In 2003, 12,150 scorpions stung patients, were studied. 7782 out of 12,150 patients in this study received scorpion polyvalent antivenom. The antivenom is manufactured by Razi Vaccine and Serum Research Institute in Karaj, Iran, through injecting the horses with a pool of six different scorpions venoms including: *A. crassicauda*, *H. saulcyi*, *H. schach*, *O. doriae*, *M. eupeus* and *H. lepturus*. This antivenom is usually presented in the vials of 5 ml.

The percentage of reported cases was as follows: Masjed-Soleiman (Bistodo-e-Bahman hospital) 27.1%, Ram-hormoz (Imam Khomeini hospital) 26.6%, Izeh (Shohada and Hefdah- e-Shahrivar hospitals) 15.4%, Shush (Nezamafi hospital) 12.0%, and Baghmalek (Tabatabaee hospital) 11.7%. It is estimated that the prevalence rate of scorpion envenomation in Khuzestan province is 3.1 cases/1000 inhabitants per year. It is notable that the accidents were occurred year-round. However, about 90% of the accidents took place in the warmest months of the year (April–October). While, although scarce, there were cases (2.5%) that detected even during the seasons in which scorpion activity is lower (December–March).



Figure 1: Map of Khuzestan and its major cities.

There were no significant differences between sexes of envenomated individuals (52.3% female; 47.7% male). The highest incidence rate of the scorpion stings fell in the group aged 6–45 years, which was significantly different ($P < 0.05$) from those recorded for the other age groups. Housewives and students were under high risk for envenomation (63.3%) than those of farmers and workers (9.4%).

On admission to hospital, identification of scorpion species (color) by the information provided via the patients was obtained in 6209 (53.3%) cases as yellow and 2025 (17.4%) cases as black. About 91.5% of the individuals stung were occurred indoor, which may be in accordance with the synanthropic behavior of these scorpions in the region. The frequency of stings in feet and legs (37.3%), hands and arms (39.3%) was clearly different from those in bodies (16.8%) and head and necks (6.5%). Most stings were happened during the night (6 p.m.–6 a.m.) which is in accordance with the nocturnal behaviors of scorpions involved. Clinical signs and/or symptoms were reported in 44.8% of the accidents. Local symptoms were more frequent than systemic. All patients were received medical cares several hours after the envenomation. "997" (9.0%) cases treated with antivenom. Most patients (64.0%) received the antivenom within 3 h after sting, reaching 77.5% up to 6 h, and the remaining 2647 (22.5%) patients were treated with antivenom after 6 h. About 97.6% of total patients were administered antivenom via intramuscular injection. However, a total of 4074 cases did not receive antivenom. Allergic reactions to antivenom, in the form of skin rash, were observed in 0.4% of cases and anaphylaxis shock (due to the horse origin antibodies in serum) occurred in just three cases. Most patients received 1–5 ml of antivenom; however, the dose was increased 2–3 folds according to the clinical presentation in 339 (4.4%) cases. Hemoglobinuria was observed in 1763 (14.5%), in which 145 (8.2%) cases showed positive result even after 24 h. The severe hemoglobinuria was reported in 1193 envenomed patients; among those 15 cases have high degree of hemoglobinuria. During our study, three patients reported dead after being envenomated. Two patients in Izeh city; a 13-year-old girl stung by *H. lepturus* at 2 a.m., with local necrosis in hand, and high degree of hemoglobinuria. She received scorpion antivenom and blood transfusion, then for further treatment dispatched to the capital hospital and died at 2 p.m. Due to the heart failure occurred in Masjed-Soleiman which was a 3-year-old boy, who stung by unknown black scorpion at 2:30 a.m., in the neck, transferred to the hospital and in spite of receiving medical cares and scorpion antivenom died due to the respiratory failure at 6 a.m.

This study shows that scorpion envenomation in Khuzestan province of Iran is a major public health problem especially in summer times. Combination of symptomatic conventional protocols and antivenom therapy are recommended for treatment. Due to the involving of more than one scorpion species in the stings in each study in cluster areas of Khuzestan province, the quantification of venom antigens in body fluids of stung patients, by the development of immunoassay methods, is a crucial matter to evaluate the severity of envenomation by scorpions. According to the previous studies, it is possible to identify the type of scorpion venom in the sera of stung patients in each area by making a specific immunoenzyme assay kit (Krifi et al., 1998; Ghalim et al., 2000). The obtained results of this study could be useful for other similar tropical areas within Iran and neighboring countries. Furthermore, this data may be applied in joint projects on envenomation by scorpions of Eastern Mediterranean regions.

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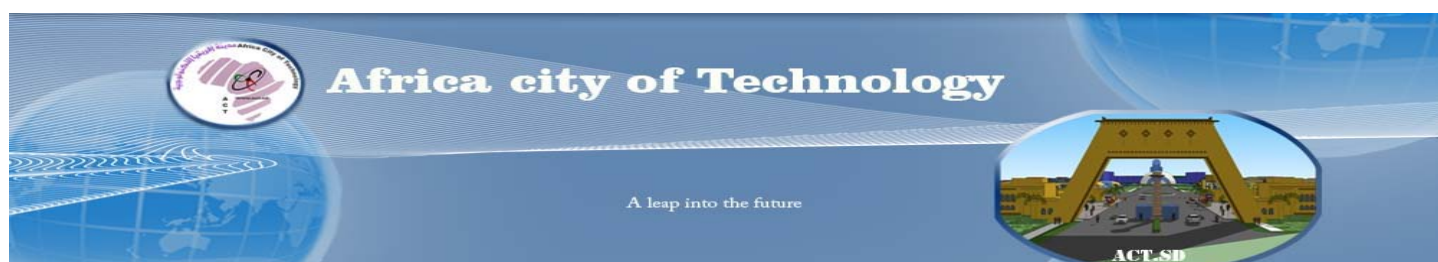


Article



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ANNOUNCEMENT



2nd International Conference on Applied Biotechnology “ICAB-2010”

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PLASMID DNA VACCINES

DNA vaccination is a technique that can protect an organism against disease by inserting bacterial plasmids into the cells for expressing an immunological response. The inner machinery of the host cells reads the DNA and translates it to pathogenic proteins, so these proteins are identified as foreign and when they appear on the surface of host cells, the immune system is alerted and starts a series of immune responses. (Figure 1)

Molecular Aspects and Preparation

DNA vaccine has a reporter gene. This gene codes a protein that could be between the other proteins like: an antigen of a disease organism, a tumor antigen, an allergen, a cytokine or a co-stimulatory molecule.

The suitable plasmids for this aim have been genetically engineered. Here we describe a plasmid which is more used and derived from *E. coli*. It includes:

1- A eukaryotic promotor. Most studies use viral promoters. 2- A LacZ gene, 3- A cloning site inside the LacZ gene to insert the reporter gene, 4- A 3' polyA tail, 5- A prokaryotic beginning of replication for plasmid vector amplification in bacteria and 6- A selectable marker as an antibiotic resistant gene (commonly kanamycin or ampicillin is used) to select the transformed *E. coli*.

By treating the plasmid and DNA, the plasmid-reporter gene is prepared. The DNA includes the reporter gene (one or more genes can be inserted) with the similar restriction endonuclease. After integrating two preparations, Ligase. *E. coli* gene is added. This codes a part of beta-galactosidase for culturing on the recombinant preparation, isopropylthiogalactoside (IPTG induces the transcription of the gene coding for beta-galactosidase), 5-bromo-4-chloroindolyl-beta-galactoside (X-gal) and the antibiotic (ampicillin or kanamycin) medium. Non-transformed cells can not grow in the presence of the antibiotic. To differentiate between *E. coli* containing plasmid-reporter gene and those including reporter gene free plasmid (both of which can cultivate in the presence of the antibiotic because now they possess the antibiotic resistant gene), a blue/white screening method was prepared, based on the activity of beta-galactosidase enzyme. It described that the LacZ gene codes the first 146 amino acids of the beta-galactosidase enzyme; Another part of this enzyme is encoded by a chromosomal gene of *E. coli*. The existence of IPTG in the medium will induce to form the LacZ gene and the colonies by the recombinant *E. coli* which contain the reporter gene-free plasmids and became blue because of X-Gal (a chromogenic substrate for the active beta-galactosidase) hydrolysis. In contrast, when the reporter gene is successfully inserted into the cloning site, which is located in LacZ gene, inactive beta-galactosidase is produced. Therefore X-Gal is not hydrolyzed and the *E. coli* colonies will appear in white.

PTargeT is an example of commercial mammalian expression method that is on hand nowadays and makes the preparation of the plasmid DNA possible. The plasmid can be extracted by commercial kits such as FlexiPrep[®] (Pharmacia Biotech, Uppsala, Sweden). This procedure includes alkaline cell lysis, RNase treatment and isopropanol precipitation. Purification of plasmid DNA is done by Sephaglas[™] FP, a commercial glass matrix, which selectively attaches to DNA. It has to be treated with a chaotropic salt so the remnants of proteins will be eliminated. The plasmid DNA is eluted in a buffer containing low down ionic strength in end.

Training



Routes of Administration and Dose

There are several procedures for plasmid DNA inoculation in animal models like: intra-muscular, subcutaneous, intra-peritoneal, intra-dermal, intravenous, oral, rectal, intra-bursal, intra-orbital, intra-tracheal, intra-nasal, and vaginal routes. In the case of a tumor, plasmid DNA vaccine can be injected into the tumor place. The most general methods of administration are done by injecting the dissolved plasmid DNA in saline via a hypodermic needle or bombarding plasmid DNA onto colloidal gold micro-particles, and inject this combination into the dermis or muscle by a gene gun. The gene gun speeds up entry of the particles into the aim tissue with a shock wave that is produced by a chemical propellant, expansion of a compressed gas or an electrical flash.

The dose of vaccine depends on the technique of administration. When 10-100ug of plasmid DNA vaccine is inserted, immune responses produce and increase when 1 or 2 boosters are given. On the other hand, time intervals among boosters become apparent to be critical. In these studies it has been shown that amplifying in the time interval among immunizing doses caused to an amplified immune response.

The production of an Immune Response to Reporter Gene Encoded Product

When a plasmid DNA vaccine encodes an antigenic section of tumor cell or a pathogen, the *in situ* expression of the antigen and the induction of an antigen- specific immune reaction are resulted. This vaccine goes into the cell and then enters the nucleus to be transcribed. The plasmid DNA is maintained in the host cell nucleus in an extra-chromosomal site for several time. When the transcribed mRNA of vaccine enters the cytoplasm, translates on ribosome and produces the antigens. Therefore a humoral and cell mediated immune response is caused.

Plasmid DNA processes the product of the reporter gene by the endogenous pathway and finally activates cytotoxic T-lymphocyte (CTL). Firstly it was thought that myocytes and keratinocytes were APC's, studies implied that myocytes would act such as APC's when the plasmid DNA vaccine is administered with the intra-muscular route. The myocytes express MHC class I molecules, but do not express co-stimulatory molecules. Also, deletion of the muscle injection spot within 10 minutes after immunization does not modify the production of the immune reaction; This shows that the plasmid DNA can rapidly leave muscle tissue and gained access to immunocompetent cell rich places. In contrast, when the spot of administration plasmid DNA to skin was eliminated in minutes following immunization, no immune response produce. These results implied that keratinocytes and Langerhans cells might be transfected APC.

Further results showed that the bone marrow derived from dendritic cells is the principal APC in the process of plasmid DNA immunization. Also, the plasmid DNA could transfect in somatic cells (myocytes, keratinocytes) or bone marrow, derived from dendritic cells, that penetrate to muscle or skin as a part of the inflammatory response to vaccination. In the previous case, it is thought that somatic cells like myocytes and keratinocytes are tanks of the antigen. They are transfected and the protein of the reporter gene is produced and dendritic cells take them up (cross priming). Bone marrow which is derived from dendritic cells, is transfected directly, this describes that plasmid DNA could be isolated from lymph node and skin after intra-muscular or intra-dermal administration, respectively.



Training



Adjuvanticity of Plasmid DNA

An immunologic adjuvant is an agent that can enhance the rate, level and duration of an immune reaction to an antigen. The adjuvant which is inserted to several vaccines to be used for human is alum (aluminum hydroxide and/or aluminum phosphate) that generates a biased Th2 response.

Bacterial DNA contains immunostimulatory sequences (ISS) that cause innate immunity for the host. ISS is composed of unmethylated CpG dinucleotides flanked with two 5' purines and two 3' pyrimidines. Analysis of the ampicillin resistant gene of plasmid DNA vaccines illustrated that there are two similar parts of a palindromic CpG hexamer 5'AACpGTT3'. This 6 base motif proved to be a potent adjuvant in mice. CpG motifs are about 20 times more usual in the bacterial than in the mammalian genome. In addition, less than 5% of the cytosine remains in the CpG dinucleotides of bacterial DNA are methylated as compared to 70-90% of the cytosine remainder in eukaryotic DNA.

Antigen Th1 and Th2 Lymphocyte Responses

When injected intramuscularly or intradermally, plasmid DNA vaccines would stimulate a Th1 response because the CpG motifs encourage the creation of IL-12 that favors the activation of Th1 lymphocytes. On the other hand, if the plasmid DNA is inserted by a gene gun, Th2 reaction is created. Reasons for a biased the response based on the method of vaccine administration are yet unknown, but there is a number of reasons that further investigation.

In some animal infection models, dominant Th1 responses have been related to protection. BALB/c mice are susceptible to *Leishmania major* because they are unable to generate a Th1 response unless they are given IL-12. In mice which immunized by an inactivated vaccine and infected with Respiratory Syncytial Virus (RSV), an increased pulmonary stimulating reaction with a predominant Th2 model and an unfavorable result was observed. A major Th1 protective reaction against RSV was persuaded in mice that were injected a plasmid DNA vaccine.

In autoimmune diseases, a Th1 response becomes apparent to associate with progression of the disease. Researchers have used a DNA vaccine coding for a T cell receptor to keep mice against experimental autoimmune encephalitis. Protection was related with a decline in the Th1, and an increase in the Th2 reaction.

Antibody Generation

Antibodies contribute to immunity in 3 main methods. Pathogen or toxin antibodies may deactivate a pathogen or toxin, in that way, some kinds of antibodies act like opsonins enhancing phagocytosis and some activate the Complement System. All these three mechanisms, stop or minimize the chance of contracting disease. Studies that have compared the humoral immune reactions obtained in animals which immunized with a DNA vaccine and a conventional vaccine or a sublethal dose of an infectious factor, dominated that higher levels of antibody attained by using the latter immunizing agents.

Influenza virus antibody that titers in mice that immunized with a DNA vaccine were lower than that in mice which immunized with live influenza virus. Similar results were gained when mice immunized with DNA encoding a malaria surface protein and the mice immunized with malaria protein alone. These results might not be considered as a shortcoming of DNA vaccines since memory was set up and lift up antibody levels would be expected on exposure to the infectious age.

In as much as antibody classes and sub-classes generated in an immune response to a DNA vaccine is concerned, it appears reasonable to imagine that this would rely on the system of vaccine administration.

Using a gene gun, a biased Th2 response would be obtained and the production of IgG1 and IgE antibodies



Training

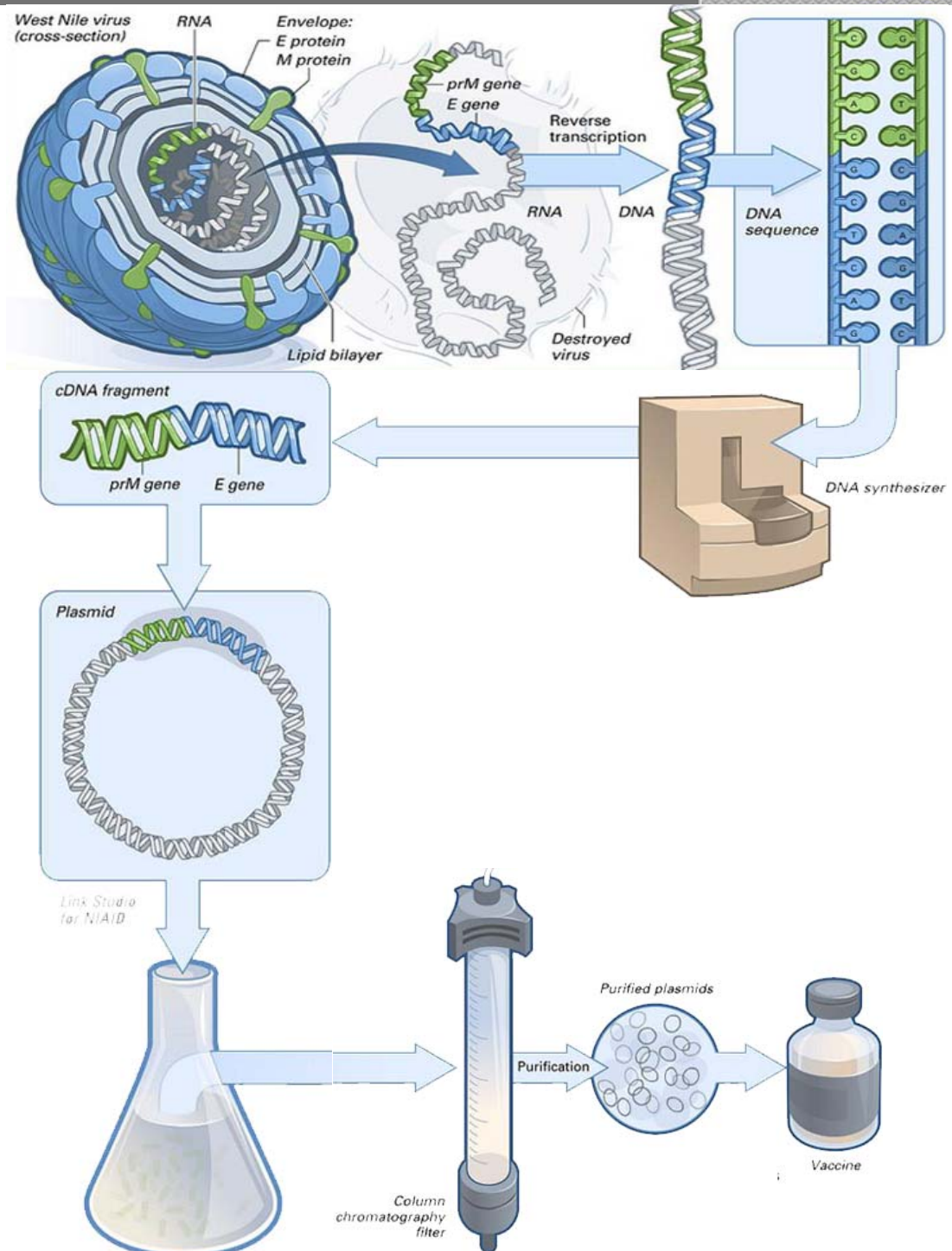


Figure 1: The making of a DNA vaccine.



Training



would predominated. Alternatively, if the DNA vaccine were administered with injection, a biased Th1 reaction and the creation of IgG2a would predominate the duration and strength of the Immune Response. End results of studies have shown that long-term immunity is achieved when a DNA vaccine is used. Researchers have reported that antigen-specific CD4⁺ T-lymphocytes stayed superior up for about 10 months following immunization with a DNA vaccine. they have also reported long lasting antigen- specific Th1 activity in mice immunized with a DNA vaccine including a gene that encoded for a *Leishmania* antigen.

Advantages, Clinical Trials of Plasmid DNA Vaccines

Plasmid DNA is non-infectious and cannot duplicate. It encodes just the antigen of interest. It does not contain heterologous protein components to which the host may respond. It induces both cell mediated (Th1 and CTL) and humoral immunity that are long-term. DNA vaccines persuade *in vivo* expression of immunogens so protecting the native conformation of epitopes. Conserving an appropriate tertiary structure of proteins is essential for the induction of conformational specific antibodies and cellular reactions. They may be constructed to contain more than one immunogenic gene, so potentially decrease the number of vaccinations needed in children. They suggest the possibility of creating effective immune reactions against diseases like malaria and HIV where other types of vaccines have failed. Furthermore, they may be safer to utilize than live attenuated vaccines particularly in immune-compromised hosts. They are steady, easy to freeze dry and reconstitute, and can be manufactured inexpensively in great quantities on high levels of purity.

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NEWS

Broccoli Compound Slows Growth of Breast Cancer Cells

A compound in vegetables of the genus *Brassica*, like broccoli and Brussels sprouts can inhibit development of breast cancer. The compound, indole-3-carbinol (I3C) degrades an oncogene that is over expressed in more than 50% of breast tumors.

Investigators revealed that treating cultures of breast cancer cells with I3C caused the destruction of Cdc25A and reduced the growth of the tumors.

Oral treatment with I3C reduced breast tumor size by up to 65% in mouse. The I3C and Cdc25A had an interaction at the serine-124 (Ser124) site on the Cdc25A molecule. Growth of breast cancer cells containing a Cdc25A mutant with alanine instead of serine at the amino acid 124 site was not inhibited by I3C.

Cdc25A is present at abnormally high levels in about half of breast cancer cases also occurs at abnormally high levels in cancers of the breast, prostate, liver, esophagus, endometrium and colon, and in non-Hodgkin



lymphoma, and in other diseases such as Alzheimer's disease. Therefore, by eating some steamed fresh broccoli, can get the benefits of I3C.

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http://www.biotechdaily.com/genomics_proteomics/articles/294730205/broccoli_compound_slows_growth_of_breast_cancer_cells.html

In Pursuit of the Energy of Life

An essential role of mitochondria, has been discovered by the scientists from the institute of Biochemistry and Molecular Biology University of Freiburg.

Mitochondria make the energy a cell needs to divide, move and produce secretory products. The generators in the cellular power plants are biological membranes located inside the mitochondria. An error in the composition of the inner mitochondrial membrane can lead to severe metabolic disorder.

In order to function, there are many highly specialized membrane proteins in the inner mitochondrial membrane. Almost always, these proteins are imported to the mitochondria with the help of translocases. The Scientist used baker's yeast as a model for their study and now has been published in *Current Biology*.

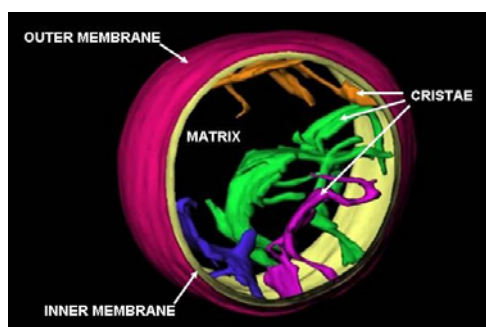


Figure 1. Diagram of a mitochondria

During the insertion of ABC transporters, a family of membrane proteins, scientists surprised that some segments of the transporters are apparently initially skipped by the insertion machinery and transported completely over the membrane.

“These errors in membrane insertion are then repaired by another translocase which is very old from an evolutionary perspective,” says Maria Bohnert, doctoral student and Boehringer-Ingelheim Scholarship recipient. The scientists were able to illustrate for the first time that at least two different protein translocases cooperate closely to insert proteins with complex structures into the inner mitochondrial membrane.

In clarifying this coupled mechanism of membrane insertion, project head Dr. Martin van der Laan and his team have solved a hotly debated scientific problem and made a major contribution to our understanding of the composition and functioning of cellular power plants.

Citation: 'Cooperation of Stop-Transfer and Conservative Sorting Mechanisms in Mitochondrial Protein Transport', Maria Bohnert, Peter Rehling, Bernard Guiard, Johannes M. Herrmann, Nikolaus Pfanner und Martin van der Laan, *Current Biology*. Published online: June 17, 2010. 10.1016/j.cub.2010.05.058

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<http://www.sciencedaily.com/releases/2010/06/100618082215.htm>

Application



The Polymerase Chain Reaction: a Magic Reaction in the Test Tube

"Sometimes a good idea comes to you when you are not looking for it." It is the first sentence of Kary B. Mullis inventor of PCR in 1983 in Scientific American. A new discovery which was changed basic researches in world so much that today PCR is one of the most applicable methods in any laboratories. The first idea of polymerase of a specific region of DNA is belonged to 1971 by Khorana and his colleagues. They introduced a new method for replication of special sequence of DNA by oligonucleotide primers which their 3' end were free But the idea of using this reaction in a polymerase chain reaction pathway was untouched tile 12 years later when new idea was inspired to Mullis during a moonlight drive through the mountains of California.

PCR mechanism:

The polymerase chain reaction is a system for DNA replication that allows a certain DNA sequence to be amplified several million-fold in just a few hours. In a cell, DNA replication includes a series of enzymatic reactions, whose result is an exact copy of the whole genome. Within a test tube, PCR uses just one enzyme-DNA polymerase- to amplify a specific region of the genome.

During cellular DNA replication, firstly enzymes unwind the double strand DNA into single strands. Then, RNA polymerase synthesizes a short oligonucleotide primer of RNA which is consummating to one of the DNA strands at the start point of replication (replication fork). This DNA/RNA heterodimer acts as a marked place for attaching of the DNA polymerase, which then produces the complementary DNA strand. In PCR different temperature performed enzymatic roles, high temperature is used to separate the DNA molecules into single strands, and synthetic sequences of single-stranded DNA (containing about two dozen nucleotides) take part as primers. Two different primers are used to close the target region to be amplified. One primer is complementary to sense DNA strand at the beginning of the target region (forward); a second primer is complementary to a sequence on the antisense of DNA strand at the end of the target region (reverse).

To direct a PCR reaction, a small amount of the target DNA which you know its exact sequence, is added to a test tube with a buffered that is contained DNA polymerase (Taq polymerase), oligonucleotide primers, the four deoxynucleotides triphosphate and the cofactor $MgCl_2$ which is essential for enzyme activity. The PCR mixture is used through replication cycles contain of:

1. 94-96 degrees C, one to several minutes which the DNA is denatured into single strands and mixed with primers (denaturing degree)
2. 50-65 degrees C, one to several minutes which the primers anneal to their complementary sequences on both side of the target sequence and bind to them (annealing degree)
3. 72 degrees C, one to several minutes which the polymerase binds and extends a complementary DNA strand from each primer 5' to 3' end (elongation degree)



Application

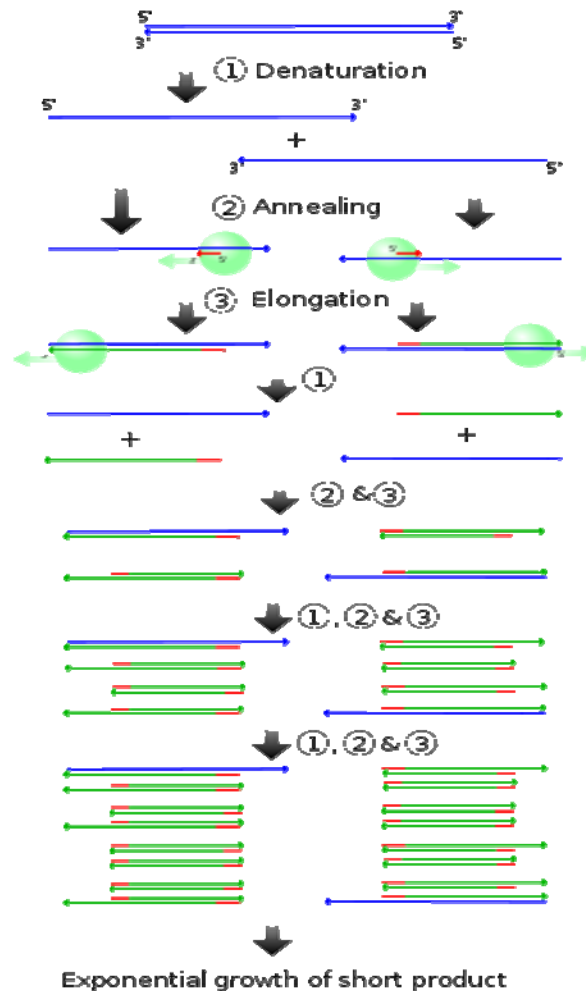


Figure 1. Schematic drawing of the PCR cycle. (1) Denaturing at 94–96 °C. (2) Annealing at 50–65 °C (3) Elongation at 72 °C. Four cycles are shown here. The blue lines represent the DNA template to which primers (red arrows) anneal that are extended by the DNA polymerase (light green circles), to give shorter DNA products (green lines), which themselves are used as templates as PCR progresses.

Two major processes had effected on PCR progressing in laboratory and made it automatically. First, a heat-stable DNA polymerase was isolated from the bacterium *Thermus aquaticus*, which inhabits hot springs. The DNA polymerase originally used for the PCR was extracted from the bacterium *E. coli*. After each cycle of DNA synthesis, the reaction must be heated to denature the double stranded DNA product. Additionally, high temperature inactivated this polymerase, so new enzyme had to be added at the start of each cycle. The bacterium *T. aquaticus* produces a DNA polymerase which is not inactivated at high temperature. This enzyme called the Taq polymerase. Second, DNA thermal cycler machines that use a computer to control the repetitive temperature changes required for PCR were invented.



Application



Finally for ensuring of real products, the PCR products are usually loaded on an agarose gel and electrophoresed. PCR products can be visualized with a chemical stain such as ethidium bromide. The important point is that the amplification is selectively, it means that only the genome fraction which is located between the forward and reverse primers is amplified and the rest of the DNA in the genome is not amplified and is invisible in the gel.

Applications of PCR:

It is not surprisingly that the first applications of new method were detection of genetic mutations in laboratory. PCR has verified a quick as a trustful method for detection all manner of mutations related to genetic disease such as insertions, deletions and point mutations.

Additionally PCR enable scientist to early diagnosis of malignant diseases such as leukemia and lymphomas, which is recently the highest developed in cancer research and is already being used customarily. PCR can be done directly on genomic DNA samples to detect translocation at an accuracy which is at least 10,000 fold higher than others.

PCR also allows distinguish of slow-growing microorganisms such as mycobacterium, anaerobic bacteria, or viruses from tissue culture test and animal models. In this concept PCR diagnostic applications in microbiology is detection of infectious factors and the insight of non-pathogenic from pathogenic strains by virtue of specific genes.

Viral DNA can similarly be detected by PCR. The primers which are used must be specific for the targeted sequences in the DNA of a virus, and the PCR can be used for diagnostic analyses or DNA sequencing of the viral genome. The high sensitivity of PCR let virus detection soon after infection and before the beginning of clinical symptom of disease. Additionally the amount of virus (viral load) in a patient can also be quantified by PCR-based DNA quantitation techniques (real time PCR).

Also PCR is a strong instrument in forensic analysis, because PCR can amplified less amount of DNA and it is useful specially when there is just a little evidence of DNA. Additionally PCR can be used in ancient research in archeological sciences. This techniques have been successfully used on animals and human DNA.

Beside all these usage of PCR technique, scientists are able to measure the level of expression of specific gene in cell with Quantitative PCR methods. These methods permit to estimate certain sequences which are given in sample. Real-time PCR is one of the best tools for this goal.

Variations of PCR technique:

As scientists acquainted with PCR, they modified PCR on their own and put the technique to new uses. Very soon PCR became a standard research technique. Gradually based on research needs scientists had to innovate on the basic PCR which some of them are more common than others?

Some of these modified PCR techniques include Nested PCR, Real-Time qRT-PCR, PCR-RFLP, RT-PCR, RACE-PCR, AFLP, Asymmetric PCR, CAPS, dCAPS, ISH, MPSS, MIP, RAPD, SSO, STS, Alu PCR, , Colony PCR, DD-PCR, Degenerate PCR, Hot-start PCR, Inverse PCR, Long-PCR, Multiplex PCR, PCR-ELISA, PCR-SSCP, QC-PCR, Rep-PCR, TAIL-PCR, Touchdown PCR and Vectorette PCR.

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WEBLINK

www.Genengnews.com

This website is a very useful portal, which consist of five different information sections:

Biobusiness

Drug discovery

Omies (all omixes)

Bioprocessing

Translational medicine

In “Top Stories” section, you can read different news about these fields.

This site also have a section by the name of “Blogbiotech” that introduce the reliable blogs or web-pages of Scientifics and persons who write about science.

“Breaking News” is the pars where you can find daily updated top news. Visitors can also be alerted about novel industrial news at “Industry update” page of this site. The last, not the least, part of this useful informing site is named “Analysis and Insight” in which you can be informed of the news analysis and insights such as “The consequences of merging two international companies and its effect on their products.”

This site has been instructed since 1981 by “Coedi engineering and Biotechnology news” institute, New Rochelle city, New York state, USA and its papers are published 21 times a year by Mary Ann Liebert, Inc.

Teams



Bioinformatics and Drug Design Unit of Pasteur Institute of Iran



From left to right at this picture: Shiva Shajari, Horieh Rahimi, Hosna Gomari, Maryam Mehravar, Elham Jahangiri, Marzieh Zamani, Ghazaleh Ghavami, Dr. Soroush Sardari, Azim Dehghani Amirabad, Behrad Shaghaghi, Saeid Malekzadeh, Kaveh Mosavihzaheh, Mohammad Sani

This group is established by Dr. Soroush Sardari in 2003 as soon as he came to Pasteur Institute.

Research area and interest:

The main focus of this group is discovery and design of anticancer and antimicrobial agent through computer aided drug design protocols and natural products.



Teams



This group includes students from many disciplines such as biotechnology (pharmaceutical, microbial, industrial, agricultural), chemistry (analytical and polymers), biology, computers, at both graduate, and undergraduate levels.

In addition, the group uses many strategies in design and discovery of new drug-like compounds. In the *in-silico* part to design anticancer agent, first *in silico* modeling of signaling pathway in cancer is performed with various applications and the best targets are selected in regard to its function in the cell. In many cases, homology modeling and molecular dynamics simulations of target is performed to archive the best model of target. Finally suitable mimetic or inhibitor is designed with use of database assembly, cheminformatics analysis and mining, virtual screening, docking simulation, artificial intelligence, etc.

Active ingredient synthesis to test real bench top interaction analysis that is performed by UV and CD spectrometric facility.

Also another focus of this group is to identify pharmacologically active small molecules from natural sources, particularly plants and soil microorganism. Secondary metabolites of soil microorganisms screened to find antifungal agents. Toxicity measurement are performed by *Artemia* assay and/or cell line assay; other bioassay that is carried out routinely.

Other studies of this group are simulation nanostructure for nano-drug loading and delivery, metabolic pathways and metabolic fingerprinting that would be related to human health and genetically modified organisms.

Collaboration and Services:

Bioinformatics and drug design unites currently perform international project with European and Eastern Mediterranean countries related to drug design discovery such as those for *Mycobacterium tuberculosis*. Other services of this group includes:

Bioinformatics analysis of drugs.

Performing projects related to protein-protein and protein-drug interactions.

Evaluation of antifungal, and toxicity effect of drugs or potential products with standards methods.

Services and methods design related to quality control of drugs and biological products.

Holding Bioinformatics workshop.

Determination of secondary structure of proteins with Circular Dichroism.

In silico protein design through truncation, chimera and their stability analysis.



Announcement



Computational Biology September 27 – October 1, 2010, China

The abstract deadline for the 2010 Cold Spring Harbor Asia meeting on Computational Biology, September 27-October 1, 2010 taking place at Cold Spring Harbor Asia in Suzhou, China is quickly approaching on July 9th! As one of the key topics of the full-fledged programs of Cold Spring Harbor Asia, this computational biology meeting will cover a wide range of the most updated topics including Nextgen Sequencing and Microarray Bioinformatics, Protein Gene Expression and Regulation, RNA Gene Expression and Regulation, SNP/CNV, Genome Variation, WGAS, Systems Biology & Biological Networks -Omics, Stem Cells / iPSs, Development, Disease and Cancer, Evolution, Phylogeny, Comparative Genomics. For detailed information, please visit the webpage at:
<http://www.csh-asia.org/stipends.html>

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The pressure has never been so great to conduct successful global clinical trials on time and on budget and the pharmaceutical industry has continued to explore emerging markets in an effort to deliver sustained growth and reduce costs.

The role of China as a key player for conducting preclinical and clinical trials has strengthened as the regulatory authorities have sought to soften the regulatory climate and also due to an ever growing labour pool available to support clinical trials.

China's changing population is beginning to provide increased consumer demand as large numbers of Chinese seek to become more westernised. As the population ages and lifestyles change we are seeing a subsequent increase in western diseases such as diabetes and cardiovascular issues amongst others creating an increased demand for drugs to treat these diseases.

Inevitably China's huge population provides for faster patient recruitment and the large

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Cover Picture



Title: Molecular Docking

Description: Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. The first requirement is a structure of the protein of interest that is determined by X-ray crystallography or NMR spectroscopy. The success of a docking program depends on two components: the search algorithm and the scoring function. A binding interaction between a small molecule ligand and an enzyme protein may result in activation or inhibition of the enzyme. If the protein is a receptor, ligand binding may result in agonism or antagonism. Docking is most commonly used in the field of drug design.

Source: [http://en.wikipedia.org/wiki/Docking_\(molecular\)](http://en.wikipedia.org/wiki/Docking_(molecular))

Title: Biological Engineering, biotechnological engineering or bioengineering

Description: is the application of engineering principles to address challenges in the fields of biology and medicine. It is used to describe the use of vegetation in civil engineering construction and also be applied to environmental modifications such as surface soil protection, slope stabilization, watercourse and shoreline protection, windbreaks, vegetation barriers including noise barriers and visual screens, and the ecological enhancement of an area. Bioengineers are engineers who use the principles of biology and the tools of engineering to create usable, tangible products. The scheme shows modeling of the Spread of Disease Using Cellular Automata and Nearest Neighbor Interactions.

Source: http://en.wikipedia.org/wiki/Biological_Engineering

Title: Cisgenesis

Cisgenesis is the process by which genes can be artificially transferred between organisms that could be conventionally bred. Unlike in transgenesis, genes are only transferred between closely related organisms. Cisgenesis results in far less change to an organism's genes compared to mutagenesis which was widely used before genetic engineering was developed. It has the advantage over conventional breeding that it can produce new varieties of crops more quickly and cheaply and only selected beneficial genes are transferred and not other genes nearby on the chromosome. One application of cisgenesis is to create blight resistant potato plants by taking resistance from wild varieties and transferring them into high yielding varieties.

Source: <http://en.wikipedia.org/wiki/cisgenesis>

