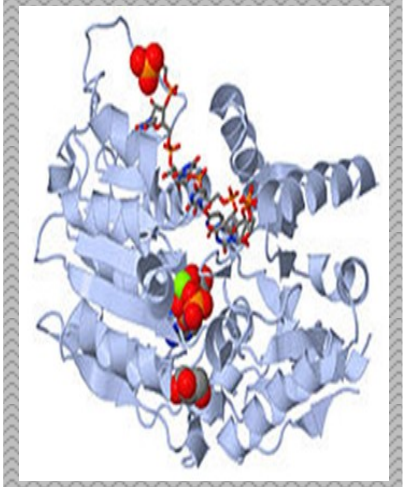

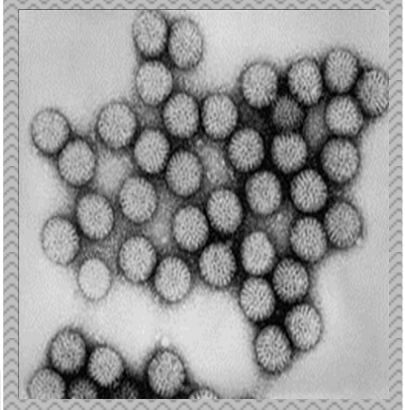

EMGEN Newsletter

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IN THIS ISSUE:

Eastern Mediterranean Health Genomics and Biotechnology Network (EMGEN) was created in 2004 with collaboration of representatives of selected centres 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, emgen@pasteur.ac.ir
Websites: www.emgen.net
www.emhgbn.net

Prepared by: Z. Mazinani
Page design: Z. Mazinani
Assistant editor: Mrs. A.B. Marschall, M.A
Editor: Dr. S. Sardari

Genetic analysis of peste des petits ruminants virus from Pakistan

The paper entitled: "Genetic analysis of peste des petits ruminants virus from Pakistan" which is published in BMC Veterinary Research (2013, 28 (3), 1-5) provides the information on the genetic nature of different PPRV populations circulating in small ruminants. Such information is essential to understand the genetic nature of PPRV strains throughout the country. Proper understanding of these viruses will help to devise control strategies in PPRV endemic countries such as Pakistan. The study was carried out by Dr. Muhammad Munir from the Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences.

Dr. Muhammad Munir

Peste des petits ruminants (PPR) is a highly contagious viral disease of domestic and wild small ruminants and camels. Despite continuous use of live attenuated vaccine, the disease is repeatedly being reported in small ruminants in Pakistan. However, very limited information is available on the genetic diversity and economic impact of the peste des petits ruminants virus (PPRV) in small ruminants in Pakistan.

Based on the molecular characterization, strains of PPRV can be categorized into four lineages, which are genetically different from each other. Lineage I contains strains from Western Africa, lineage II includes strains from West African countries, the Ivory Coast, Guinea and Burkina Faso, and lineage III comprises isolates from Eastern Africa, the Sudan, Yemen and Oman. The lineage IV represents PPRV isolates from the Arabian Peninsula, the Middle East and South Asia. Classification of PPRV is being investigated based on the sequence study of both F and N genes; however, parallel comparison of PPRV isolates has shown that N gene is most divergent and consequently most proper for molecular characterization of closely related strains. The virus has been identified to occur as only one serotype among four lineages.

Several studies were performed to provide information on the molecular characteristics, genetic diversity and the phylogenetic relationship of PPRV strains causing continuous outbreaks in Punjab, Pakistan. Thirty-five samples from 6 distinct outbreaks, suspected for PPR based on clinical picture and history, were confirmed to be PPRV positive using real-time PCR against the nucleoprotein (N) gene of the virus. Sequence analysis of the N gene of PPRV indicated that representative isolates were monophyletic and related to previously characterized Pakistani strains of PPRV.

However, these PPRV strains were significantly distinct from previously characterized PPRV strains from Pakistan. The results indicate that there are at least two different genetic variants of PPRV circulating in small ruminants in Pakistan and PPR has been established as an endemic infection in the country.

Moreover, the cost-benefit analysis of investment in small ruminants farming and production revealed that infectious diseases (PPRV is at the top) are the main hurdle in the growth of small ruminants sector especially in developing countries. This loss in farming is primarily the result of the high case fatality but also indirect losses are immense. These indirect losses include decreased milk production, overall debilitating conditions of the animal and cost on treatment.

This situation is even more complicated due to unavailability of the vaccines. Contrary to hypothetical possibilities for easy eradication of the disease, owing to vaccine-induced long-term protective immunity, it remains one of the main problems in small ruminant farming.

In conclusion, these findings are essential in expanding the data on the genetics of local PPRV strains and their subsequent diagnostics and control. Moreover, such findings may lead to not only increase awareness for the farmer, but will also facilitate to estimating the economical impact the of disease and its subsequent consequences. However, what remains to determine the true epidemiology of the disease in the country and the high risk areas for vaccination on a priority basis.

Interview

In this issue, we present the following interesting interview with ***Dr. Firdos Alam Khan*** *from Manipal University Dubai* (Any views or opinions expressed are solely those of the author and do not necessarily represent those of EMGEN Newsletter).

Dr. Firdos Alam Khan

1. Dear Dr. Firdos Alam Khan could you please briefly introduce yourself and explain your educational status?

I have been working as a Full Professor, and Chairperson, Department of Biotechnology, Manipal University Dubai, (www.manipaldubai.com) UAE. I have received my Doctoral degree in Neuroscience from Nagpur University, India. I have more than 20 years of research and teaching experience in various domains of biotechnology. I did my first postdoctoral research at the National Institute of Biological Science, Tata Institute of Fundamental Research, Bangalore, India (www.ncbs.res.in) and second postdoctoral fellowship at the Massachusetts Institute of Technology, Cambridge, USA (www.mit.edu). I was associated with Long Beach Medical Center, California, USA as an Associate Research Director for a neurological disorder project. I worked with Reliance Life Sciences a multimillion dollar Biotech Company based in Mumbai, India, (www.rellife.com) as a research scientist and worked in adult and embryonic stem cell projects. My area of expertise in biotechnology includes stem cell technology, neuropharmacology, and neuroscience. I have written numerous articles in various national and international journals in the areas of neuroscience, neuropharmacology and stem cell biology. I have been awarded two US patents on stem cells. Recently I have been nominated as an International Advisor Board member of World Biotechnology Congress, UAE where four distinguished Noble Laureates are also members (http://www.biotechworldcongress.com/advisoryboard.php). I have been nominated for membership of the scientific advisory committee for Pharmaceutical & Biotechnology Middle East, (www.pabme.com). I have been associated with various international scientific organizations

like the International Brain Research Organization, France, and Society for Neuroscience, USA. I have work experience in multi-cultural environment with different positions such as researcher, teacher, academic administrator and educationist. I have contributed to more than 20 different national and international conferences in India, China, Singapore, UAE, Thailand, and USA.

2. Could you please tell us what your main research area is?

I have been involved in neuroscience research with projects based on neuronal mapping of neurotransmitters, neuro regeneration, neural stem cell and neural cell transplantation in mammalian and human subjects

3. Why did you choose this field of research?

I have always found the brain is very fascinating subject for research as a lot of its mysteries are still not fully understood. I especially like to explore further the neuronal regeneration fields and try to find out how damaged human neurons can be fully regenerated and be made functional.

4. Do you apply any biotechnology or genomics tools in your researches and please explain how and where?

With the advancement of molecular biology tools , it becomes necessary to utilize both cellular, molecular and genetic tools to understand the physiology and function of the neurons derived from stem cells and I have extensively used molecular techniques such as PCR, Rt-PCR and other techniques in my research projects.

5. What kinds of biotechnology facilities do you have in your laboratory?

At the Department of Biotechnology Manipal University Dubai we have approximately 1,200 square meters of laboratory space, well- equipped with state of the art scientific instruments. We have separate laboratories for BSc Biotechnology, BSc Bioinformatics, MSc Medical Biotechnology, and MSc Human Genetics & Forensic Science. We also have labs for conducting research in Molecular Biology, Cytogenetic-Microscopy and Imaging, Cell and Tissue Engineering. The state of the art laboratory facilities are

equipped with an autoclave, bio safety hood, CO2 incubator, compound microscope, conductivity meter, digital imaging system, ELISA reader, fluorescent microscope, freezers (minus 40 and 80), gel documentation system, gel electrophoresis, high speed refrigerated centrifuge, ice maker, inverted microscope, laminar hood, light microscope, microbial incubator, micro-centrifuge, PCR (gradient), PH meter, UV spectrophotometer, UV– trans illuminator, water bath and shaker and water distillation unit.

6. Are there any diagnosis products that have been made in your country? (i.e. your native researchers involved in the project)

Not aware of and I don't think we manufacture any diagnosis product here.

7. Are there any late stage biological products to be commercialized in your center? Could you please explain more? Not yet.

8. Are there significant biotechnology centers in your country?

We also have Dubai Biotechnology and Research Park, a cluster of life science companies whit about 100 companies currently based on the cluster.

9. Are there any academic training courses in biotechnology in your country? If yes, to above question, at which level and how many students are trained annually?

We have 4 Universities which offer bachelor degrees in biotechnology courses in the UAE and we also have two universities which offer master and doctoral programs in Dubai.

10. Are you familiar with EMRO countries and EMGEN (Eastern Mediterranean Health Genomics and Biotechnology Network)? Would you please tell us how you know the EMGEN?

EMGEN could be a vital link between regional countries and Iran in the development of biotechnology based research and products.

11. Do you have any suggestions for establishing/extending collaborations with EMRO countries?

We would like to associate with EMRO in promoting and developing the biotechnology education,

training, research as well as biotechnology product development.

12. Are there any possibilities for young researchers from EMRO countries to participate in training courses in your biotech centers?

We would like to associate with EMGEN in promoting and developing the biotechnology education, training, research as well as biotechnology product development.

13. What kinds of difficulties do you face, in research and commercialization of medical biotechnology in your country and the region?

There is a lack of research funding opportunities for biotechnology research here, especially for private Universities and research centers.

14. Do you have any training courses or workshops in your research center?

The Department of Biotechnology at Manipal University Dubai campus is the only program of its kind in the entire U.A.E. It was launched in 2004 with the vision of empowering students with the knowledge, skills, and latest developments in this upcoming field of biotechnology. The Department is committed to the development of this field by creating professionals

Currently, we are offering

B.Sc degree in Biotechnology

B.Sc degree in Bioinformatics

B.Sc Honors degree in Biotechnology

M.Sc degree in Medical Biotechnology

M.Sc degree in Human Genetics and Forensic Science

PhD in Biotechnology

15. What is your idea about genomics and its applications in improving public health?

The future goal is to launch a doctoral research program where students can work at the cutting edge areas of genomics, proteomics, drug discovery, and stem cell technology to fulfill their research dreams.

We see tremendous growth in this industry and career opportunities in Biopharmaceutical Industries, Research and Development Labs, Agriculture, Horticulture and Dairy farming, Biotech Industry (Food, Beverages, Chemicals and Solvents), Tissue Culture Labs, Health care data management and Molecular and Genetics data analysis

16. What is your idea about commercialization of researches in the field of bioscience?

I believe any research needs to be finally translated into a useful product and I trust that with development of the right environment for biotechnology education and research it would be possible to create a product development entity and considering the strategic position of the UAE and trading and business opportunities over here, it would be possible to start manufacturing units for biotechnology products and diagnosis kits.

17. What is your opinion about the development of the biotechnology and genomics in your place?

The entire Middle East is mainly very important for genetic screening and research in view of the presence of a high degree of genetic disorders and any company which makes any diagnosis and/or therapeutic products would be highly recommended.

18. Would you please tell us about the differences of genomics and its applications between developed and developing countries? What should we do in this regard?

There is a great deal of difference between how research is done here in developing countries and developed countries. Developing countries face a lot of challenges due to lack of research policies, and lack of research funding at both private and government levels. Other challenges are the lack of trained scientists and researchers in the developing countries, most of them migrate to developed countries for better salaries and a better life. The developing countries need to frame research policies, and try to fund research projects as much as they can, also retain trained scientists and researchers, pay the researchers and scientists excellent salaries and give them better career prospects.

Thank you Dr. Firdos Alam Khan for sharing information and your opinion with us. Also, we are grateful for your kind and useful cooperation.

Restriction enzymes

Restriction enzymes and their use

Restriction enzymes are the most powerful tools in molecular biology. Cutting DNA by using restriction endonucleases is one of the most current molecular biology methods and the availability of restriction endonucleases was one of the first main advances in the new science of molecular biology. These endonucleases exist naturally in bacteria and are used to maintain the bacteria free from invading foreign DNA such as bacteriophage.

Restriction endonucleases identify characteristic nucleotide sequences in a DNA molecule and then cut the phosphodiester bonds between the nucleotide sequences at that part. Any time this sequence becomes visible in DNA it will be cut by the enzyme, whether it is viral or animal or human DNA. This is why restriction endonucleases are so critical to molecular biology; they are most specific, cleaving only at their unique recognition sequence, however, they are also general because they will cleave any DNA having this sequence. They work by cleaving the DNA at a specific sequence, the recognition sequence of the enzyme. The result is the generation of DNA pieces from a DNA molecule including these recognition sequences. Thus, restriction of a population of similar DNA molecules with a determined enzyme always results in similar restriction pieces. This is recognized as an enzyme restriction and has made amplifying and other manipulations possible. Restriction endonucleases (abbreviated RE) usually identify palindromic sequences in a DNA molecule. For example, the widely used restriction enzyme *Bam*HI identifies the sequence GAATTC. This is presumed a palindrome because the complementary strand will have the similar sequence in the opposite direction.

Blunt and sticky ends

A determined enzyme will always cleave between the similar two nucleotides. As characterized in Table 1, some enzymes cleave in the center of the recognition sequence, which results in the generation

of "blunt ends" in a DNA molecule. Most restriction endonucleases make cuts two bases away from the axis of symmetry, resulting in the generation of single-stranded ends four base pairs long on the end of each piece. Because these ends are complementary, they are referred to as "sticky ends". Since a determined restriction enzyme produces similar ends in any DNA molecule, restriction pieces from one source can be recombined with DNA from other parts if they both cleave with the similar enzyme. This specification allows the generation of recombinant DNA molecules and is the basis of most DNA cloning protocols. Generally, sticky ends are used for recombinant DNA work although blunt ends can be adhered to other blunt ends when necessary (it is harder to ligate them together later).

Table 1. Some of restriction enzymes

Enzyme	Source	Recognition Sequence	Cut
Hae*III	*Haemophilus aegyptius*	5'GGCC 3'CCGG	5'---GG CC---3' 3'---CC GG---5'
Sma*I	*Serratia marcescens*	5'CCCGGG 3'GGGCCC	5'---CCC GGG---3' 3'---GGG CCC---5'
Pvu*II	*Proteus vulgaris*	5'CAGCTG 3'GTCGAC	5'---CAG CTG---3' 3'---GTC GAC---5'
Eco*RI*	*Escherichia coli*	5'GAATTC 3'CTTAAG	5'---G AATTC---3' 3'---CTTAA G---5'
Bam*HI*	*Bacillus amyloliquefaciens*	'GGATCC 3'CCTAGG	5'---G GATCC---3' 3'---CCTAG G---5'
Taq*I*	*Thermus aquaticus*	5'TCGA 3'AGCT	5'---T CGA---3' 3'---AGC T---5'

* = Blunt end
** =Sticky end

Mechanism of action of restriction enzymes

The action of restriction endonucleases is in some cases as varied as the enzymes themselves. In general, however, the process is one of recognition of the binding region, binding of the enzyme dimer to the DNA, division of the DNA, and enzyme release. To begin with, all restriction enzymes will bind DNA specifically and, with much less strength, non-specifically. This is a characteristic of proteins that interact with DNA. It is possible that even non-specific DNA joining will produce a conformational change in the restriction endonucleases dimer that will result in the protein adapting to the DNA strands. These changes are not similar to those that occur when the dimer joins to the recognition region though. As the dimer slides along the DNA strands, it probes for recognition regions and, when these are encountered, a reaction between the DNA and the protein ensues. This requires important conformational alterations in both the protein and the DNA as well as exclusion of water molecules from the protein/DNA interface so that more intimate contacts can be created. In general, intimate contact is held by 20 hydrogen bonds created between the protein and the DNA bases in the recognition region. These bonds are shown to be mediated through specific amino acids, primarily Asparagine and Glutamine, held in an appropriate three-dimensional structure. There are differences among restriction endonucleases with respect to how much water is expelled, but in all cases, it is a considerably higher amount than is expelled during non-specific binding.

Main groups of restriction enzymes

There are three main groups of restriction enzymes. Their grouping is related to the nature of the cut made in the DNA molecule, the enzyme structure, and the types of sequences identified. Type I and III enzymes are not appropriate for gene cloning because they cut DNA at regions other than the recognition regions and thus cause random cleavage patterns. In contrast, type II enzymes are most frequently used for mapping DNA *in vitro* because they identify characteristic sites and cut just at these sites. In addition, the type II enzyme and methylase activities are commonly segregate, single subunit enzymes. Although both of the enzymes identify the similar target sequence, they can be purified separately from each other.

Several type II restriction enzymes do not conform to this narrow definition, making it essential to define further subdivisions.

Naming of restriction enzymes (REs)

Restriction enzymes are named based on the bacterial genus (the first letter), species (the first two letters) and strain. Thus the first three letters of a restriction enzyme′s name are abbreviations of the bacterial species designation and the fourth letter represents the particular bacterial strain. They must therefore be underlined or italicized. The restriction enzymes name may have additional numbers or letters and these may determine a particular strain, plasmid, or isolate. These numbers or letters should not be italicized or underlined. For example, *Hae*III enzyme was isolated from *Haemophilus* aegyptius, and was the third restriction enzyme isolated from this species. *Eco*R1 takes from *Escherichia coli* RY13 (this is the thirteenth strain of *E. coli*).

To date, many REs are commercially available, offering a great variety of options for cleaving DNA molecules at different parts. Restriction enzymes are activated by adding the DNA to be digested and a restriction enzyme to a buffer that is optimal for that specific enzyme.

Sometimes it is necessary to use several restriction enzymes in the same digestion, called a “double digest”. This can commonly be accomplished with an appropriate buffer that provides sufficient activity from each enzyme.

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DNA Vectors

In order to investigate a DNA fragment (e.g., a gene), it needs to be stored, amplified and finally purified. These tasks are carried out by cloning the DNA into a vector. A vector usually is a small, circular DNA molecule (a plasmid) used as a carrier to artificially carry foreign genetic material into another cell. It can replicate inside a bacterium such as *Escherichia coli*. A vector consisting foreign DNA is called recombinant DNA. The vector is a DNA sequence that contains an insert fragment and a larger sequence that serves as the "backbone" of the vector. The goal of a vector which transfers genetic materials to another cell is generally to isolate, replicate, or express the insert in the target cell. Vectors called expression vectors are specially are for the expression of the insert in the target cell, and usually have a promoter sequence that drives expression of out the insert. Simpler vectors only capable of being transcribed, but not translated, are called transcription vectors: they can be duplicated in a target cell unlike expression vectors. Transcription vectors are used to amplify their insert.

Vector DNA

Cloning vectors act as carrier DNA molecules. Four main properties of all cloning vectors are: (i) they can autonomously duplicate themselves and the other DNA fragments they carry; (ii) they contain a number of unique restriction enzyme cleavage regions that are present only once in the vector; (iii) consist a selectable marker (generally in the form of genes for enzymes missing in the host cell or antibiotic resistance genes) to determine host cells that transport vectors from host cells that do not contain a vector; and (iv) they are relatively easy to recover from the host cell. There are many possible selections of vectors depending on the goal of cloning. The major type of cloning vectors has been designed for use in *Escherichia coli*. Thus, the first practical skill commonly required by an investigator is the capability to grow pure cultures of bacteria. The major types of vectors are plasmids, phages, and artificial chromosomes.

Principal features and applications of different cloning vector systems

Vector	Basis	Size limits of insert	Major application
Plasmid	Naturally occurring multicopy plasmids	≤ 10 kb	Sub cloning and downstream manipulation, cDNA cloning and expression assays
Phage	Bacteriophage l	5–20 kb	Genomic DNA cloning, cDNA cloning, and expression libraries
BAC (Bacterial Artificial Chromosome)	*Escherichia coli* F factor plasmid	75–300 kb	Analysis of large genomes
YAC (Yeast Artificial Chromosome)	*Saccharomyces cerevisiae* centromere, telomere, and autonomously replicating sequence	100–1000 kb	Analysis of large genomes, YAC transgenic mice

Plasmid DNA as a vector

Plasmids are naturally occurring extra chromosomal double-stranded circular DNA molecules found in bacteria and carrying an origin of replication and replicating independently within host cells. One of the first genetically engineered plasmids to be used in recombinant DNA was the plasmid vector pBR322. Naming of plasmids is based on a system of uppercase letters and numbers, where the lowercase "p" stands for "plasmid." In the case of pBR322, the BR determines the primary manufacturers of the vector (Bolivar and Rodriquez), and 322 is the determination number of the specific plasmid. These early vectors were often of low copy number, meaning that they duplicate to yield only one copy in each bacterial cell.

Plasmid vectors are modified to possess a specific antibiotic resistance gene and a multiple cloning region (also called the polylinker region) which has a number of unique target regions for restriction enzymes (endonucleases).

Cutting the plasmid vector with one of these endonucleases results in a single cut, producing a linear plasmid. A foreign DNA molecule, referred to as the "insert", cut with the same enzyme, can then be adhered

to the vector in a ligation reaction. Ligations of the insert to plasmid vectors are not 100% productive, because the two ends of a vector can be readily ligated together (self-ligation). Treatment of the vector with the enzyme phosphatase may reduce the degree of self-ligation, which removes the terminal 5′-phosphate. When the 5′-phosphate is deleted from the plasmid vector it cannot be recircularized by ligase enzyme since there is nothing with which to create a phosphodiester bond. But, if the vector is adhered with a foreign insert, the 5′-phosphate is provided by the foreign DNA. Another strategy involves using two different restriction enzymes cutting regions with noncomplementary sticky ends. This inhibits self-ligation and promotes joining of the foreign DNA in the desired orientation within the vector.

Bacteriophage lambda (λ) as a vector

Since engineering of the first viral cloning vector in 1974, bacteriophage lambda (λ) has been widely used in DNA manipulation. Because phage λ vectors can transport a larger fragment of DNA than a plasmid vector, they are particularly useful for preparing genomic libraries. Today many variations of λ vectors exist. Insertion vectors have unique restriction enzymes regions that allow the cloning of small DNA pieces in addition to the phage λ genome. These are often used for preparing cDNA expression libraries. Replacement vectors have paired cloning regions on either side of a central gene cluster. This central cluster consists of genes for lysogeny and recombination, which are not essential for the lytic life cycle.

The central genes can be removed and foreign DNA adhered between the “arms.” All phage vectors have been unarmed for safety and can only function in special conditions. The recombinant viral fragment infects host cells, in a process called “transduction.” The bacterial host cells lyse after phage reproduction, releasing progeny virus fragments. The viral fragments appear as a clear part of lysed bacteria or “plaque” on an agar plate containing a layer of bacteria. Each plaque demonstrates progeny of a single recombinant phage and consists of millions of recombinant phage fragments.

Artificial chromosome vectors

The important tools for mapping and analysis of complex eukaryotic genomes are bacterial artificial chromosomes (BACs) and yeast artificial chromosomes (YACs). Because they can hold more than 300 kb of foreign DNA, much of the investigation on the Human Genome Project and other genome sequencing projects depends on the utilization of BACs and YACs. BACs are created using the fertility factor plasmid (F factor) of *E. coli* as a starting point. The plasmid is usually 90 kb in size and occurs at a low copy number in the host cell. The engineered BAC vector (7.2 kb) including a duplication origin, cloning regions, and selectable markers can accommodate a large insert of foreign DNA. The characteristics of YAC vectors are discussed below.

Immediately after the production of the first YAC in 1983, attempts were undertaken to design a mammalian artificial chromosome (MAC). From then on, it took 15 years until the first archetype MAC was published in 1997. Like YACs, MACs rely on the presence of centromeric sites, sites that can commence DNA duplication, and telomeric sites. Their advancement is considered an important progress in animal biotechnology and human gene therapy for two main reasons. First, they involve independent duplication and separation in mammalian cells, as opposed to random integration into chromosomes (as for other vectors). Second, they can be improved for their use as expression systems of large genes, containing not only the coding site, but all control elements. A major obstacle limiting application at this time, however, is that they are difficult to handle due to their large size and can be recovered only in small quantities. Two important methods exist for the production of MACs. In one method, telomere-directed fragmentation of natural chromosomes is used. For example, HAC or human artificial chromosome has been derived from chromosome 21 using this method. Another method involves *de novo* assembly of cloned telomeric, centromeric, and duplication origins *in vitro*.

Yeast artificial chromosome (YAC) vectors

Yeast is a small single cell that can be manipulated and grown in the lab much like bacteria. YAC vectors are designed to work like chromosomes. Their design would not have been feasible without a detailed knowledge of the requirements for chromosome stability and duplication, and genetic study of yeast mutants and biochemical pathways. YAC vectors contain an origin of duplication (independently duplicating sequence, ARS), a centromere to ensure separation into daughter cells, telomeres to seal the ends of the chromosomes and ensure stability, and growth selectable markers in each arm. These markers permit the selection of molecules in which the arms are adhered and which include a foreign insert. For example, the yeast genes *URA3* and *TRP1* are usually used as markers. Positive selection is performed by auxotrophic complementation of an *ura3-trp1* mutant yeast strain, which requires supplementation with uracil and tryptophan to grow. Orotidine-5′-phosphate decarboxylase enzyme that is essential for the synthesis of the base uracil is encoded by *URA3*. Phosphoribosylanthranilate isomerase enzyme that is essential for synthesis of the amino acid tryptophan is encoded by *TRP1*. After digesting with restriction enzymes *Eco*RI and *Bam*HI, the left arm and right arm change to linear form, with the terminal sequences forming the telomeres. Foreign DNA is cut with *Eco*RI enzyme and the YAC arms and foreign DNA are adhered and then shifted into yeast cells. The yeast cells are maintained as spheroplasts (lacking yeast cell wall). Yeast cells are grown on selective nutrient regeneration media that lack tryptophan and uracil, to select for molecules in which the arms are adhered bringing together the *TRP1 and URA3* genes.

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Fatty Acids Could Aid Cancer Prevention and Treatment

In vitro experiments indicated that omega-3 fatty acids induced apoptosis (cell death) in virulent cells, meaning they have the potential to be employed in both the treatment and prevention of oral cancers and certain skin cansers. Omega-3 fatty acids cannot be made by humans in large amounts and so we must acquire those amounts from our diet. A particular type of cancer called squamous-cell carcinoma (SCC) was studied by scientists. Squamous cells are the major part of the outer layers of the skin, and SCC is one form of skin cancer. However, squamous cells also exist in the lining of lungs, the digestive tract, and other areas of the body. Oral squamous cell carcinomas (OSCC) are the sixth most common cancer worldwide and are hard and very expensive to treat. In the experiments, the scientists grew cell cultures from many different cell lines to which they added fatty acids. The cell lines contained both virulent oral and skin SCCs, along with pre-virulent cells and normal skin and oral cells. They found that the omega-3 fatty acid selectively prevented the growth of the virulent and pre-virulent cells at doses which did not affect the normal cells. Surprisingly, we noticed this was due almost to an over-stimulation of epidermal growth factor which activated apoptosis. This is a novel mechanism of action of omega-3 fatty acids.

Reference: http://www.sciencedaily.com/

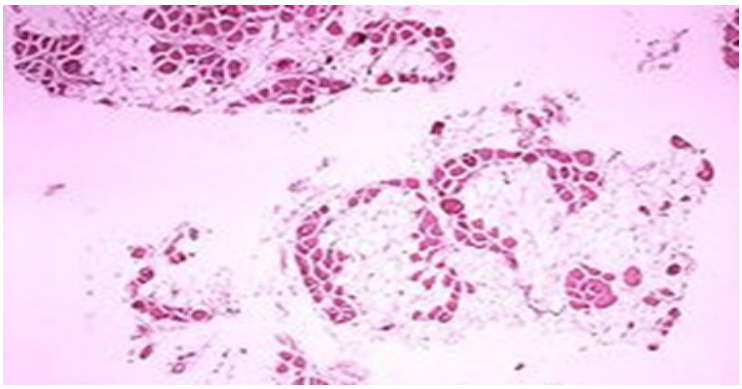
Skin Cell Defect Is Surprising Allergy Trigger

Desmoglein 1 is best understood as the “glue” that protects the outer layer of body skin by holding it together. Historically, the molecule was essentially believed to play a significant role: adhesion between cells is essential to the physical barrier that regulates water amounts and also acts as the body's major defense against environmental elements. Researchers studied clinical data from two families, combined with genetic analysis consisting next-generation DNA sequencing and light and electron microscopy, among other methods. They discovered that when desmoglein 1 does not properly function or does not exist, the resulting barrier disruption can affect the immune response, with severe consequences.

Reference: http://www.sciencedaily.com/

Genetic Editing Shows Promise in Duchenne Muscular Dystrophy

Duchenne muscular dystrophy is a genetic disorder affecting one in 3,600 newborn boys. The genetic mutation is discovered on the X chromosome. People with Duchenne muscular dystrophy cannot create the dystrophin protein, which is essential in maintaining the structural integrity of muscle fibers. Over time, patients with the disorder suffer gradual muscle degeneration, which leads to disablement and eventual death, usually by the age of 25. The scientific studies, which were carried out on Duchenne muscular dystrophy patients, were made feasible by using a new method for structural synthetic proteins named transcription activator-like effector nucleases (TALENs), which are enzymes that can be engineered to bind to and change approximately any gene sequence. These TALENs bind to the damaged gene, and can correct the mutation to produce a normally functioning gene. Patients currently are in a wheelchair by their teens and many die in their early twenties. This disorder has been extensively investigated by scientists, and it is believed that more than 60 percent of persons with this type of mutation can be treated by adding normal genes to compensate for the mutated genes. Previous studies indicate that reforming the production of dystrophin proteins will be functional to a large extent, and decrease disorder symptoms when expressed in skeletal muscle tissue.



Histopathology of gastrocnemius muscle from patient

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Genome Analysis: Current Procedures and Applications

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Editor: Maria S. Poptsova

Weill Cornell Medical College, New York, USA and Moscow State University , Russia

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Editor: Torben L. Skovhus, Sean M. Caffrey and Casey R.J

Hubert Danish Technological Institute, Aarhus, Denmark; Genome Alberta, Calgary, Canada; Newcastle University, Newcastle, UK

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Announcement

http://www.eubce.com/

http://www.ecb16.com/

http://www.biotherapeuticsanalyticalsummit.com/

http://www.pegsummitchina.com/

Title: Cytosol

The cytosol or cytoplasmic matrix is the fluid found inside cells. It is separated into parts by membranes. In the eukaryotes, the cytosol is a component of the cytoplasm, which also contains the plastids, cell nucleus, mitochondria, and other organelles. In prokaryotic cells, most of the biochemical reactions occur in the cytosol, whereas a few occur in membranes or in the periplasmic space. In eukaryotes, while several metabolic pathways still take place in the cytosol, others take place within organelles. The cytosol is a complex combination of substances dissolved in water.

Reference: http://en.wikipedia.org/wiki/Cytosol

Title: RNA helicases

RNA helicases are necessary for most processes of RNA metabolism such as translation initiation, pri-mRNA splicing and ribosome biogenesis. Besides being involved in all the prior processes RNA helicases also play a key role in sensing viral RNAs. RNA helicases are involved in the mediation of antiviral immune response because they are able to recognize foreign RNAs in vertebrates. About 75% of the viruses are RNA viruses and they have their own RNA helicases. Damaged RNA helicases have been attributed to cancer, infectious diseases and neuro-degenerative disorders.

Reference: http://en.wikipedia.org/wiki/Rna_helicases

Title: Reoviruses

Reoviruses are viruses that can affect the respiratory tract and gastrointestinal system. These viruses have genomes including fragmented, double-stranded RNA (dsRNA). Their name comes from *respiratory enteric orphan viruses.* The term "orphan virus" means that a virus is not related to any known disorder. Reoviruses are non-enveloped and have an icosahedral capsid (T-13) composed of an inner and outer protein shell. The genome of viruses in Reoviruses contains 10-12 compartments which are categorized into three groups corresponding to their size: L (large), M (medium) and S (small). Compartments range from ~ 3.9 kbp – 1kbp and each compartment encodes 1-3 proteins.

Reference: http://en.wikipedia.org/wiki/Reovirus