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A REVIEW ON GENE SILENCING

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ABSTRACT

Gene silencing is a epigenetic term which means the expression of a particular gene is reduced by genetic engineering techniques using mechanism like DNA methylation and RNA interference. This technique if used perfectly and encouraged, can answer many mutational disorders which are incurable at present. These also give scope for us to explore into a new world of many unknown genes which have vital functions in the body. Even botanical silencing can be done to increase their resistance towards the attack of virus and many other harmful agents. It was found to be productive in combating against many cancers, viral diseases,bacterial infections and several mutational disorders. Research works are being done in institutes like the Scripps institute, California is undertaking studies on silencers which are helpful in treatment of cancer, rheumatoid arthritis, hemophilia etc. The national institute of health, Maryland, America are trying to implement silencers in Parkinsonism. Gene silencing in plants is being done in Howard Hughes medical institute.

Keywords: America, DNA methylation, California.

INTRODUCTION

Genetics

Genetics is the study of genes, heredity, and variation in living organisms. It is generally considered a field of biology, but it intersects frequently with many of the life sciences and is strongly linked with the study of information systems.

Genes

A **gene** is the molecular unit of heredity of a living organism. It is used extensively by the scientific community as a name given to some stretches of deoxyribonucleic acids (DNA) and ribonucleic acids (RNA) that code for a polypeptide or for an RNA chain that has a function in the organism.

DNA (Deoxy Ribonucliec Acid)

A molecule that encodes the genetic instructions used in the development and functioning of all known living organisms.

RNA (Ribonucliec Acid)

It is a family of large biological molecules that perform multiple vital roles in the coding, decoding, regulation, and expression of genes [1].

Genome

The genome is the genetic material of an organism which includes genes, sequences of both DNA & RNA.

Replication

Formation of DNA from DNA.

Transcription

Formation of RNA from DNA.

Translation

Formation of proteins from RNA.

GENE EXPRESSION

Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product. These products are often proteins, but in non-protein coding genes such as ribosomal RNA (rRNA), transfer RNA (tRNA) or small nuclear RNA (snRNA) genes, the product is a functional RNA. Gene expression is the most fundamental level at which the genotype gives rise to the phenotype.Generally gene expression is achieved by transcription, translation etc.

The actual mechanism can be understood by **"THE LAC-OPERON MODEL"**, which is familiar [2].

By taking the example of a gene PrP^C expression is explained.

- PrP^C Is a gene present on the p arm of chromosome 20 in human beings.

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- It encodes for a protein called PRNP, which is formed by the translation of this gene.
- The translation results in the insertion of amino acid aspartic acid (D) at the codon 178.
- The presence of aspartic acid at codon 178 helps in the normal functioning of the gene.
- But if a point mutation occurs at that position, it results in the formation of a prion protein which consists of asparagines (N) instead of aspartic acid at codon 178.
- This prion protein is characterized by heavy coiling which makes the protein PRNP.
- They form clumps which destroy the nerve cells.
- This degeneration leads to a rare genetic brain disorder called **"FATAL FAMILIAL INSOMNIA".**
- It involves progressively worsening insomnia, which leads to hallucinations, delirium, and confusional states like that of dementia.

GENETIC ENGINEERING

Genetic engineering, also called genetic modification, is the direct manipulation of an organism's gene using biotechnology.

WHY GENETIC ENGINEERING?

- New DNA may be inserted in the host genome by first isolating and copying the genetic material of interest using molecular cloning methods to generate a DNA sequence, or by synthesizing the DNA, and then inserting this construct into the host organism.
- Genes may be removed, or "knocked out", using a nuclease.
- Gene targeting is a different technique that uses homologous recombination to change an endogenous gene, and can be used to delete a gene, remove exons, add a gene, or introduce point mutations.

Applications of Genetic Engineering

Genetic engineering techniques have been applied in numerous fields including research, agriculture, industrial biotechnology, and medicine.

- In medicine, genetic engineering has been used to mass-produce insulin, human growth hormones, follistim (for treating infertility), human albumin, monoclonal antibodies, anti-hemophilic factors, vaccines and many other drugs.
- One of the best-known and controversial applications of genetic engineering is the creation and use of genetically modified crops or genetically modified organisms, such as genetically modified fish, which are used to produce genetically modified food and materials with diverse uses.
- Genetic engineering is used to create animal models of human diseases. Genetically modified mice are the most common genetically engineered animal model.
- Using genetic engineering techniques one can transform microorganisms such as bacteria or yeast, or transform cells from multicellular organisms such as insects or mammals, with a gene coding for a useful protein, such as an enzyme, so that the transformed organism will over express the desired protein.
- It is used to treat most of the mutational disorders.

MUTATIONAL DISORDERS

It is a genetically related disorder which is caused due to changes in the activity or function of a gene. This change is called as Mutation [3].

Example

Tay-Sachs disease

It is a rare autosomal recessive genetic disorder. It causes a progressive deterioration of nerve cells and causes disturbances in mental and physical abilities that begins around six months of age and usually results in death by the age of four.

Cause

- Mutations in the HEXA gene cause Tay-Sachs disease.
- The HEXA gene provides instructions for making part of an enzyme called beta-hexosaminidase A.
- Hexosaminidase A is a vital hydrolytic enzyme, found in the lysosomes, that breaks down glycolipids.
- Hexosaminidase A specifically breaks down fatty acid derivatives called gangliosides; these are made and biodegraded rapidly in early life as the brain develops.
- When hexosaminidase A is no longer functioning properly, the lipids accumulate in the brain and interfere with normal biological processes.

Signs & Symptoms

There are several forms of this disease

- **Infantile Tay–Sachs disease.** Infants with Tay–Sachs disease appear to develop normally for the first six months after birth. Then, as neurons become distended with gangliosides, a relentless deterioration of mental and physical abilities begins. The child may become blind, deaf, unable to swallow, atrophied, and paralytic. Death usually occurs before the age of four.
- **Juvenile Tay–Sachs** disease is rarer than other forms of Tay–Sachs, and usually is initially seen in children between two and ten years old. People with Tay–Sachs disease develop cognitive and motor skill deterioration, dysarthria, dysphagia, ataxia, and spasticity. Death usually occurs between the ages of five to fifteen years.

Adult/Late-Onset Tay–Sachs disease. A rare form of this disease, known as Adult-Onset or Late-Onset Tay–Sachs disease, usually has its first symptoms during the 30s or 40s. In contrast to the other forms, late-onset Tay–Sachs disease is usually not fatal as the effects can stop progressing.

BACKGROUND

Gene Silencing is an epigenetic term which involves preventing the extent of expression of a particular gene in a cell. SILENCING only involves in the reduction of expression of a particular gene in a sequence.

Advantages

Silencing techniques were successful in combating many mutational and inherited disorders like Diabetes, Neurodegenerative Disorders etc.

If one could silence the gene that is over reacting in something like the production of white blood cells (lymphoma) that person could control possible damaging effects, or symptoms like Leukemia [4].

Disadvantages

This technique though useful, is very expensive. It can also show some abnormalities like undesirable extent of silencing which cannot be corrected or re-engineered.

Application

Silencing techniques are applied in studying the genetic field in studying the mechanism of disease production in genes and also the gene expression of a particular gene under study.

TECHNIQUE

Generally to carry out silencing the gene must be isolated or targeted.

Isolation

Finding the right gene to insert usually draws on years of scientific research into the identity and function of useful genes.

- To determine if a useful gene is present on a particular fragment the bacterial library are screened for the desired phenotype.
- If the gene bears close homology to a know gene in another species then it could be isolated by searching for genes in the library that closely match the known gene.
- If the DNA sequence of the gene and the organism is known then it can be isolated relatively easily.
- Restriction enzymes can cut the DNA either side of the gene and gel electrophoresis can be used to separate the fragments according to length.
- The DNA band at the correct size should be the one containing the gene, and it can then be excised from the gel.
- After the restriction enzymes cut the DNA, gel electrophoresis can be used to separate the fragments according to length [5].

Gel electrophoresis

- **Gel electrophoresis** is a method for separation and analysis of macromolecules (DNA, RNA and proteins) and their fragments, based on their size and charge.
- Gel electrophoresis uses a gel as an anticonvective medium and/or sieving medium during electrophoresis, the movement of a charged particle in an electrical field.
- The types of gel most typically used are agarose and polyacrylamide gels.

Targeting

- The most common form of genetic engineering involves inserting new genetic material randomly within the host genome.
- Gene targeting uses homologous recombination to target desired changes to a specific endogenous gene.
- This tends to occur at a relatively low frequency in plants and animals and generally requires the use of selectable markers.
- The frequency of gene targeting can be greatly enhanced with the use of engineered nucleases.
- In addition to enhancing gene targeting, engineered nucleases can also be used to introduce mutations at endogenous genes that generate a gene knockout [6].

GENERAL MECHANISMS

Silencing can be achieved by many mechanisms, but most productive mechanisms are: DNA Methylation & RNA Interference.

DNA Methylation

✓ Methylation process is one among the key mechanisms for modification of DNA molecule, and hence the epigenetic control of gene expression in vertebrates.

✓ Such a regulatory mechanism allows a cell to stop transcription, ensures inactivity of the majority of genes of one X chromosome of the female organism, and enables the process of genomic imprinting, as well as protection of the endogenous genome from eventual intrusion of a parasitic genome.

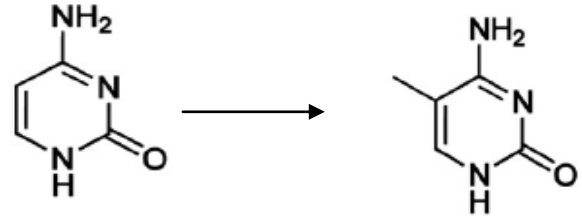
✓ Mechanism of methylation refers to the binding of a methyl group to the 5^{th} carbon atom of the cytosine ring, and is carried out with the help of the enzyme DNA methyltransferase (Dnmt).

✓ The result is the formation of a new base, 5-methylcytosine (5Mc).

✓ Addition of the methyl group takes place immediately after replication, and is completed within 1 minute after completion of replication.

✓ The result of such modification changes the affinity for particular transcription factors towards DNA molecule which prevents the formation of the transcriptional initiation complex, or elongation.

✓ Hence the expression of particular gene is silenced [7].



(cytosine) (5-MC)

RNA Interference

✓ RNA interference (RNAi) is a natural process used by cells to regulate gene expression.

✓ The process to silence genes first begins with the entrance of a double-stranded RNA (dsRNA) molecule into the cell, which triggers the RNAi pathway.

✓ The double-stranded molecule is then cut into small double-stranded fragments by an enzyme called Dicer.

✓ The fragments integrate into a multi-subunit protein called the RNAi induced silencing complex (RISC), which contains Argonaute proteins that are essential components of the RNAi pathway.

✓ One strand of the molecule, called the "guide" strand, binds to RISC, while the other strand, known as the "passenger" strand is degraded.

✓ The guide strand remains bound to RISC and directs it towards the silencing of target m RNA molecule.

✓ With the cleavage or translational repression of the mRNA molecules, the genes that form them are essentially inactive.

Examples

DNA Methylation

A classic example of mammalian gene silencing can be studied under methylation mechanism:

- Generally every offspring inherits two copies of chromosomes, one from mother and the other from father.
- The genes present in both the chromosomes are in a "turned on" condition.
- In some conditions only one of the chromosomes is present in on condition while other is switched off. This is called as

"Genomic imprinting".

- The main mechanism of such imprinting is silencing via methylation.

Mechanism

➢ In the "chromosome 11" inherited from the parents, two genes called "**Igf2**" and "**H19**" are present.

➢ Igf2 promotes growth during gestation and H19 has a role in some cancers.

➢ In general cell of an offspring materna H19 is on and paternal one is off

➢ In case of Igf2 reverse takes place, paternal is on and maternal is off.

➢ This silencing depends upon the activity of enhancer and insulator sies present on the chromosomes.

➢ The enhancer is present downstream to H19, where as insulator is present between Igf2 and H19 genes.

➢ In the maternal chromosome, the insulator site is bound with protien "**CTCF"** (or) **" ZINC FINGER PROTIEN".**

➢ Due to this the enhancer is prevented in making the Igf2 to express, and it makes H19 to be activated.

➢ Hence Igf2 gene is silenced in the offspring.

➢ In case of H19, in the paternal chromosome the insulator is methylated, which aids in the methylation of H19 promoter region.

➢ Due to methylation the enhancer is prevented from activating it, hence it is silenced.

➢ The Igf2 gene is activated by the enhancer.

RNA Interfernce

➢ This can be studied taking the example of *fission yeast* i.e; ***"Schizosaccharomyces pombe".***

➢ In this species some of the genes are down regulated pre-transcriptionally by modification of histones.

➢ This process is called **"RNA-INDUCED TRANSCRIPTIONAL SILENCING".**

➢ As studied earlier the enzyme dicer facilitates the binding of dsRNA to RITS complex.

➢ This complex is guided towards the specific mRNA sequence where the gene to be silenced is present.

➢ Thus this causes disruption of histone methylation and stops the formation of centromere.

➢ This silenced gene causes the cell to have slow or stalled anaphase period.

SPECIAL MENTION

Ribozymes

These are specially used as gene silencing tools in medicinal and pathological researches.

- Catalytic RNA molecules used to inhibit gene expression.
- These catalytic RNA molecules bind to a specific site and attack the neighboring phosphate in the RNA backbone with their 2' oxygen, which acts as a nucleophile, resulting in the formation of cleaved products.This helped in silencing of disease causing gene.
- Many motifs of ribozymes are present which are listed as follows

Hammer head

- The hammerhead ribozyme is a RNA module that catalyzes reversible cleavage and joining reactions at a specific site within an RNA molecule.

Hairpin

- The **hairpin ribozyme** is an RNA motif that catalyzes RNA processing reactions essential for replication of the satellite RNA molecules in which it is embedded [8].

Hepatitis delta virus

- The **hepatitis delta virus (HDV) ribozyme** is a non-coding RNA found in the hepatitis delta virus that is necessary for viral replication.

Breakthroughs

Gene silencing was found to be extremely productive in diagnosis and treatment of several diseases and disorders.Some of them are explained below:

Cancer

➢ In chronic myelogenous leukemia (CML), the infected cells produce a fusion protien called **BCR-ABL**, which

prevents the drug **IMITINB** (anti cancer) binding to the cells, so that growth is not altered. By employincing silencing techniques, BCR-ABL expression is silence so that the cells are exposed to the drug.

- **CXCR4** (chemokine receptor type 4), involving in proliferation of breast cancer was silenced and the cance cell division was reduced.
- Targeted silencing of **B-CATENIN** helped in increasing the life span of mice infected with colon adenocarcinoma.

Viral Diseases

- RNAi has been used to target **CCR5** (chemokine receptor 5), primary HIV receptor. This prevents the entry of virus into the peripheral lymphocytes and haemopoietic stem cells.
- Targeting the surface antigen of HEPATITIS B & C virus decreased the amount of virus by 98% in the cell.
- Replication of WEST NILE VIRUS, which causes neuroinvasive disorders was achieved by silencing techniques.

Bacterial Diseases

- Bacteria are less susceptible for silencing because they replicate outside the host and also they do not have specific binding sites for targeting.
- But, the bacterial infections can be controlled by silencing the host cells which are prone to the attack of bacteria.
- siRNA was used to prevent the bacteria, *Psueomonas aeruginosa*, from invading murine lung epithelial cells by silencing caveolin-2 gene.

Respiratory Diseases

- RNAI techniques were found helpful in ASTHMA & COPD (chronic obstructive pulmonary disease).
- Targeted silencing of (TGF)-α (TRANSFORMING GROWTH FACTOR) reduced mucus secrection.
- TGF-β helped in the reduction of chronic inflammation.

Hungtington's Disease

- It is a neurodegenerative disease caused by mutation in huntingtin gene, which causes the production of huntingtun protien(orange spot in the figure).
- The mutant huntingtin protein has been targeted through gene silencing, using allele specific oligonucleotides [9].
- It has been found that approximately 85% of patients with HD can be covered and there was a 50% decrease in the mutant protein

Amyotrophic Lateral Sclerosis

- Also called **Lou Gehrig's disease** is a motor neuron disease that affects the brain and spinal cord.
- The disease causes motor neurons to degenerate, which eventually leads to their death.
- Hundreds of mutations in the Cu/Zn superoxide dismutase (SOD1) have been found to cause ALS.
- Gene silencing has been used to knock down the SOD1 mutant that is characteristic of ALS.
- siRNA molecules have been successfully used to target the SOD1 mutant gene and reduce its expression through allele-specific gene silencing.

RELATED METHODS

A closely related method of GENE SILENCING is "**GENE KNOCKOUT".** This involves complete inhibition of gene expression.The gene is "TURNED OFF" completely.

Method of Gene Knockout in mice

- ✓ It Is Similar To Silencing.
- ✓ The Gene To Be Knocked Out Is Selected(White Mouse), Isolated And Engineered To Form A DNA Sequence
- ✓ Embryonic Stem Cells From A Mouse Are Isolated And Grown In Vitro
- ✓ The Ko Sequence Is Introduced In The Culture Of Stem Cells By Electroporation
- ✓ The Cells incorporating the Ko sequence are taken and the others are killed
- ✓ They Are Injected Into A Female Uterus (Grey Mouse).
- ✓ They Form Chimeras Where White Area Is Of Ko Cells And Grey Is Of Mother.

DRAWBACKS

Even though the method of silencing led to many discoveries and research studies, it also showed some undesirable effects.

- A useful trait may not be exhibited. A person can suffer from the adverse effects of the other exhibited gene.
- Silencing of disease-associated genes using RNA interference (RNAi) has become a most powerful therapeutic option in the battle against cancer, infections and other human disorders.
- Yet, recent evidence suggests that over-dosing RNAi can have detrimental side effects on cells and tissues, and cause toxicities and lethalities in small animals.
- ACRE (Advisory Committee on Releases to the Environment) considered a report on the technique of RNA mediated gene silencing. RNA methods can be unstable in that gene silencing may be of variable efficacy in otherwise genetically identical plants and their offspring.
- The silencing mechanism is not transferable to other organisms; specifically bacteria and nematodes. Direct harm to humans would require deliberate and complex actions.
- Inclusion of GM silencing mechanism in a GM crop could encourage the evolution of some viruses [9].

Fig 1. A DNA Strand

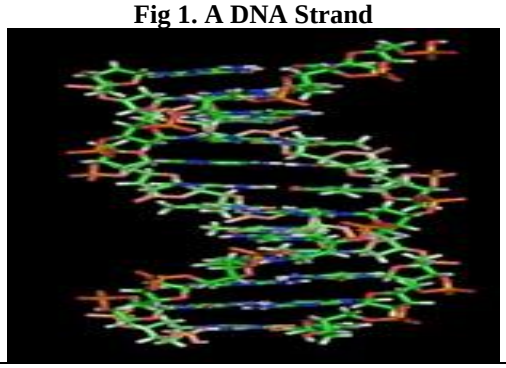


Fig 2. A RNA Strand

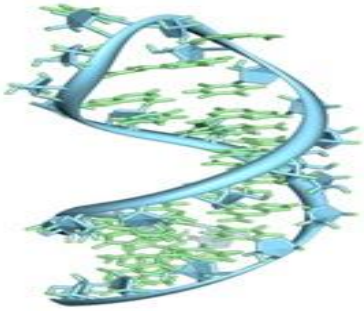


Fig 3. FFI Infected areas

Fig 4. Contrast Between a Normal Neuron And Infected Neuron

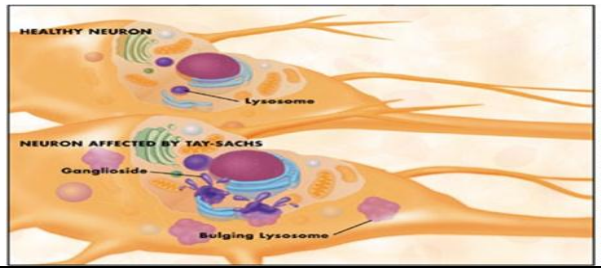


Fig 5. A gel electrophoresis apparatus

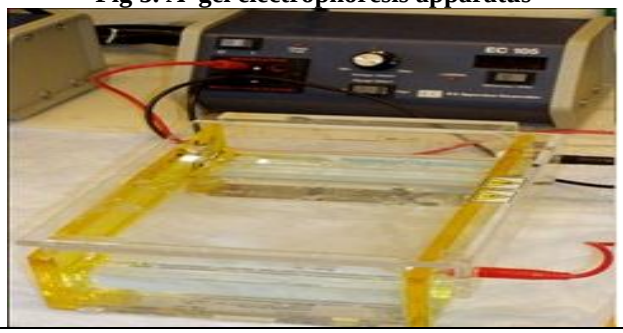


Fig 6. Gene Targeting Overview

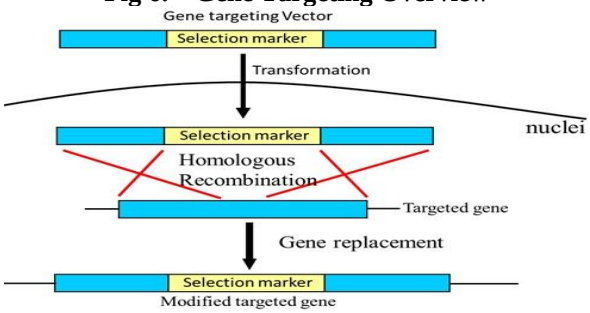


Fig 7. Overview of RNA Interference

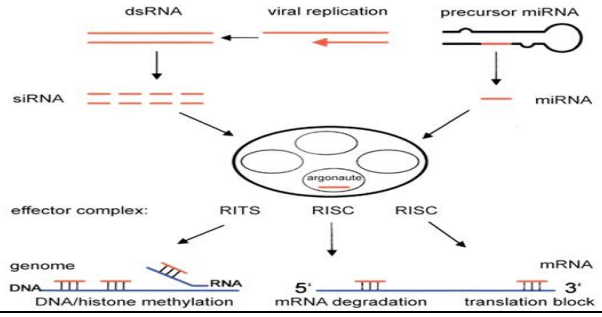


Fig 8. Schematic representation of silencing in chromosome 11

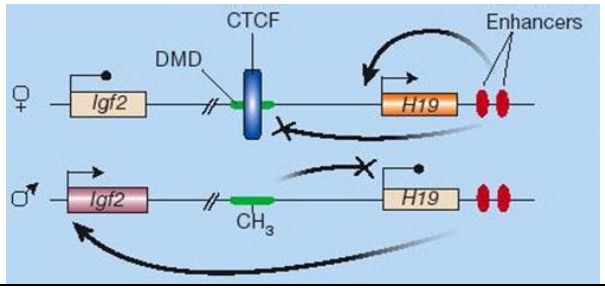


Fig 9. RNA Interfernece in Fission Yeast

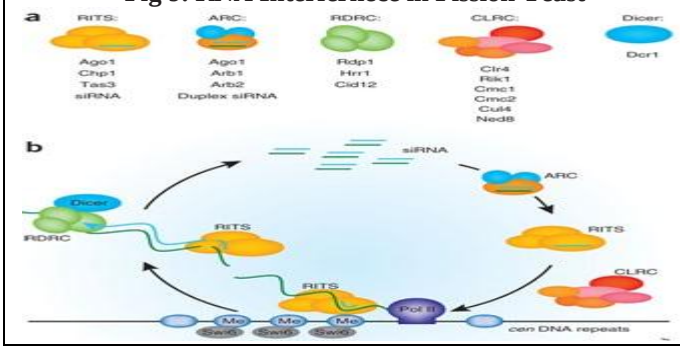


Fig 10. General mechanism of a ribozyme

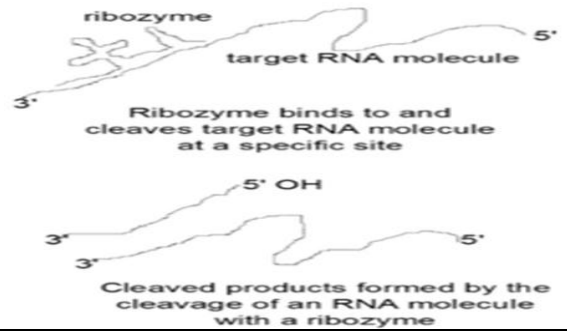


Fig 11. Hammerhead Ribozyme

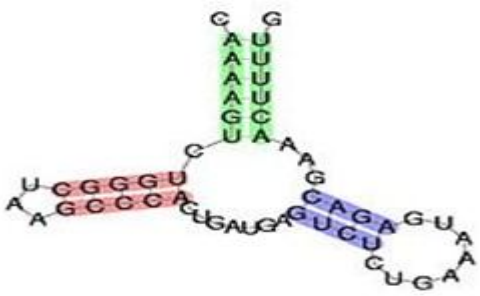


Fig 12. Hairpin Ribozyme

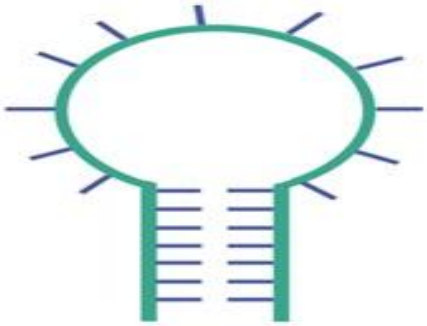


Fig 13. Hepatitis Delta Virus

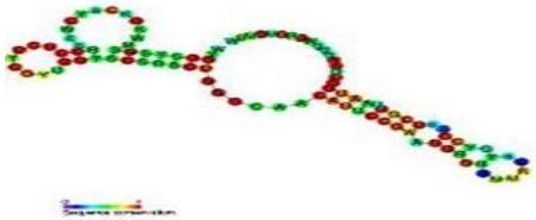


Fig 14. Orange Spot of HD

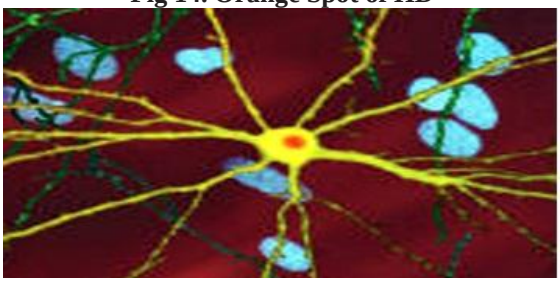


Fig 15. Normal Neuron and Atrophied Muscle

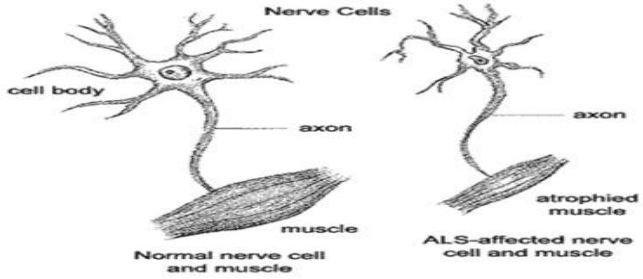
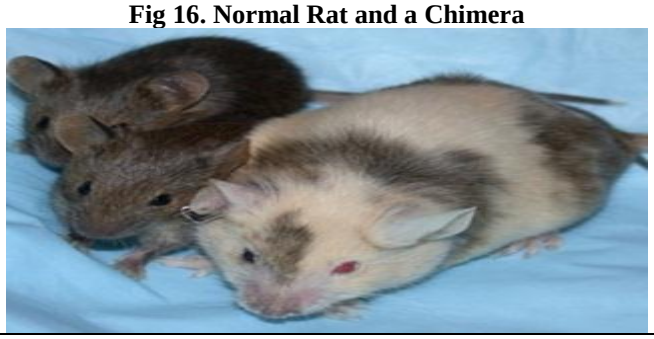


Fig 16. Normal Rat and a Chimera



CONCLUSION

Even though the technique is expensive, it may help in the treatment of many disorders caused due to mutation in single (or) multiple genes like:

Type of Disorder	Affect ratio
1. Familial hypercholesterolemia	1 in 500 people
2. Polycystic kidney disease	1 in 1250 people
3. Neurofibromatosis type 1	1 in 2500 people
4. Marfan syndrome	1 in 4000 people
5. Muco polysaccharidoses,	1 in 25000 people
6. Progeria	1 in 1000000 people

Research works are being done in institutes like

✓ **The Scripps institute, California** is undertaking studies on silencers which are helpful in treatment of cancer, rheumatoid arthritis, hemophilia etc.

✓ **The national institute of health, Maryland, America** are trying to implement silencers in Parkinsonism.

✓ Gene silencing in plants is being done in **Howard.**

✓ **Hughes medical institute**

If some disorders are difficult to cure or incurable, silencing techniques may help either in reducing the mortality rates or understanding the disorder.

By spreading awareness about the value and the consequences of this technique, there might be a day where world's fatal disorders doesn't matter anymore.

REFERENCES

1. http://www.sciencedirect.com/science/article/pii/S1740674910000260
2. https://www.promega.com/~/media/.../epigeneticwebinarsept2012.pdf
3. www.biochem.emory.edu/labs/xcheng/CH002.pdf
4. www.nature.com/.../the-role-of-methylation-in-gene-expression-1070
5. http://www.reliabilityoxford.co.uk/2006-gene-silencing-is-it-a-new-risk/
6. Handbook of Epigenetics, published by Elsevier.
7. DNA Methylation as a Regulatory Mechanism for Gene Expression in MammalsAlan [erman2, Maja Vlahovi}1, Ljiljana [erman1 and Floriana Buli}-Jaku, Department of Biology, School of Medicine, University of Zagreb, Zagreb, Croatia General Hospital »Sveti Duh», Zagreb, Croatia.
8. Molecular biology of gene, 5th edition by Watson, baker, bell, gann, Levine, losick.
9. http://perpetualsummer.co.uk/diseasemotifs/prion/ffi.html