Review Article

ANTISENSE TECHNOLOGY

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ABSTRACT

Diseases are often connected to the insufficient or excess production of certain "Proteins". If the production of these proteins is disputed many diseases can be treated or cured. Antisense technology is a method that can dispute protein production. It may be used to design new therapeutics for diseases in whose pathology the production of a specific protein plays a circle role. Antisense technology is a tool that is used for the Inhibition of gene expression. The principle behind it is that an antisense nucleic acid sequence base pairs with its complementary sense RNA strand and prevents it from being translated into a protein. The complimentary nucleic acid sequence can be either a synthetic oligonucleotide, often oligodeoxy ribonucleotides (ODN) of less than 30 nucleotides, or longer antisense RNA (aRNA) sequences. An example of sense and antisense RNA is: 5'ACGU3'mRNA and 3' UGCA5' Antisense RNA. This article gives brief introduction regarding the antisense technology.

Keywords: Antisense technology, protein production, gene expression.

INTRODUCTION

Diseases are often connected to the insufficient or excess production of certain "Proteins". If the production of these proteins is disputed many diseases can be treated or cured. Antisense technology is a method that can dispute protein production. It may be used to design new therapeutics for diseases in whose pathology the production of a specific protein plays a circle role.[1]

Antisense technology is a tool that is used for the Inhibition of gene expression. The principle behind it is that an antisense nucleic acid sequence base pairs with its complementary sense RNA strand and prevents it from being translated into a protein. The complimentary nucleic acid sequence can be either a synthetic oligonucleotide, often oligodeoxy ribonucleotides (ODN) of less than 30 nucleotides, or longer antisense RNA (aRNA) sequences. An example of sense and antisense RNA is: - 5'ACGU3' mRNA, and 3'UGCA5' Antisense RNA.[2]

ANTISENSE TECHNOLOGY

In Antisense technology, synthetically – produced complementary molecules seek out and bind to messenger RNA (mRNA), blocking the final step of protein production. mRNA is the nucleic acid molecule that carries genetic information from the DNA to the other cellular machinery involved in the protein production. By Binding to mRNA, the antisense drugs interrupt and inhibit the production of specific disease-related proteins[1].

"Sense" refers to the original sequence of the DNA or RNA molecule. "Antisense" refers to the complementary sequence of the DNA or RNA molecules[1].

The basic idea is that if an oligonucleotide (a short) RNA or DNA molecule complementary to a mRNA produced by a gene) can be introduced into a cell, it will specifically bind to its target mRNA through the exquisite specificity of complementary based pairing the same mechanism which guarantees the fidelity of DNA replication and of RNA transcription from the gene. This binding forms an RNA dimmer in the cytoplasm and halts protein synthesis. This occurs because the mRNA no longer has access to the ribosome and cytoplasm by ribonucleotide.H. Therefore, the introduction of short chains of DNA complementary to m RNA will lead to a specific diminution, or blockage, of protein synthesis by a particular gave. In effect, the gene will be turned off[3].

THE BASICS OF ANTISENSE

A Sense strand is a 5' to 3' mRNA molecule or DNA molecule. The complementary strands or mirror strand to the sense is called an antisense.

Antisense technology is the process in which the antisense strand hydrogen bonds with the targeted sense strand. When an antisense strand binds to a mRNA sense strand, a cell will recognize the double helix as foreign to the cell and proceed to degrade the faculty mRNA molecule thus preventing the production of undesired protein. Although DNA is already a double stranded molecule, antisense technology can be applied to it building a triplex formation.

A DNA antisense molecule must be approximately seventeen bags in order to unction, and approximately thirteen bases for an RNA molecule RNA antisense strands can be either catalytic, or non catalytic. The catalytic antisense strands, also called ribozymes, which will cleave the RNA molecule at specific sequences. A Non catalytic RNA antisense strand blocks further RNA processing, i.e. modifying the mRNA strand or transcription.

The exact mechanism of an antisense strand has not been determined. The current hypotheses include. "Blocking RNA splicing, accelerating degradation of the RNA molecule, and preventing introns from being spliced out of the mRNA, impeding the exportation of mRNA into the cytoplasm, hindering translation, and the triplex formation in DNA". The two figures below illustrate the fact that no consensus has been reached concerning how antisense accomplishes the reduction of protein synthesis[4].

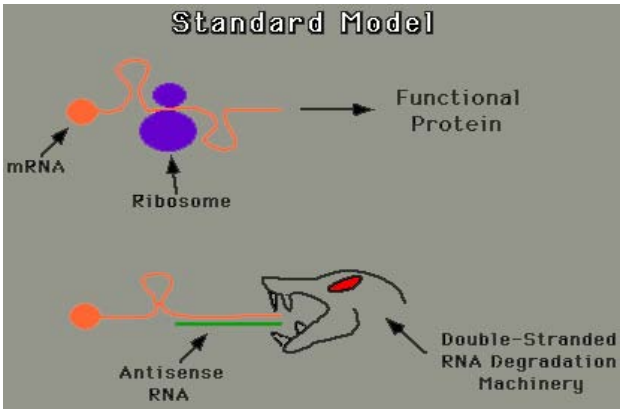

Figure 1, (by CMello Labs)

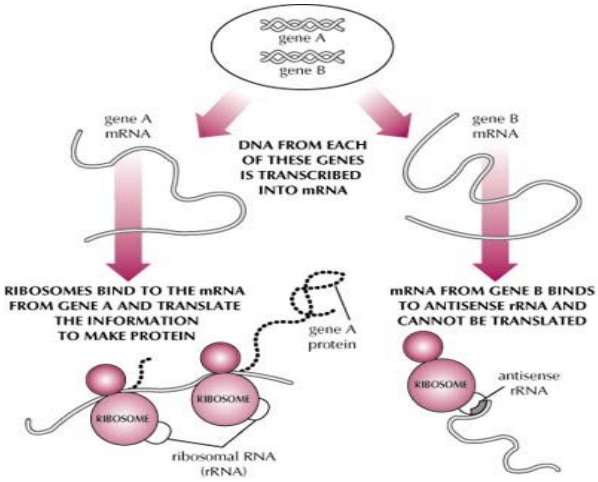

Figure 2, by Teri Platt, courtesy of Fred Hutchinson Cancer Research Center

PROTEIN FORMATION

Proteins are constantly being produced in the body. Protein production is triggered by various stimuli, such as hormones.

The formation of the proteins occurs in the following two steps

Step 1: Transcription

Step 2: Translation

Protein molecules are the expression of a gene. However, to get to a protein the cell must undergo two complex processes transcription and translation. Transcription is the process in which an RNA copy is made of the DNA. In order to get the copy, many enzymes such as polymerase, helicase, exonuclease, ligase and single stranded binding protein work together to unwind the double helix and match the base pairs of RNA (adenine, guanine, cytosine and uracil) to the DNA, once the copy is made, the RNA molecule, which is now in the heterogeneous nuclear RNA (hnRNA) made, is still not ready to go express the gene by making a protein. The hnRNA must be spliced to remove non coding sequence, and protected from the cellular environment with a 5'cap and poly A tail. Finally, the hnRNA is transported out of the nuclear membrane and into the cytoplasm where it achieves the status of mRNA.

In the cytoplasm, the mRNA molecule hooks up with the ribosomes where the protein production can start. Every three nucleotides in the mRNA molecule codes for a specific amino acid and are appropriately called a codon. The codon pair with an anticodon of t-RNA that has attached to an amino acid. In this manner a polypeptide chain is formed. It will eventually twist and contort itself into a unique configuration which aids in the function of the protein.

Occasionally, a bad mRNA molecule is synthesized so that the resulting protein cannot function properly. Abnormalities of protein cause many diseases that afflict humans. Therefore, it seems logical to conclude that if the expression of these malfunctioned proteins could be stopped, the sources of disease would be obliterated and the disease will be treated. This idea is the basis for the antisense technology[4].

HOW DOES ANTISENSE TECHNOLOGY WORK?

The therapeutic objective of antisense technology is to block the production of disease technology is to block the production of disease causing proteins. This is achieved by creating a synthetic "antisense" or complementary nucleotide sequence of DNA or RNA that interacts with, and binds to the "sense" or original mRNA sequence. This "mRNA"- antisense complex" can no longer be translated and the disease causing protein cannot be produced[2]. The antisense polygalactouronase cDNA sequence is cut with restriction enzymes and fused in the inverted orientation to a plasmid (i.e. double-stranded, closed DNA molecule) containing an upstream promoter and a downstream terminator sequence. The cauliflower mosaic virus (CaMV) promoter is chosen because it produced the right amount of antisense RNA to provide an adequate delayed softening time. The degree of production of antisense RNA in the plant cells is dependent on a number of factors and the type of promoter sequence chosen is one of them. This plasmid is then transferred to Escherichia Coli (E-Coli) bacteria. E-Coil serves as the plasmid host.

Afterwards the hybrid gene is transferred to the Agro bacterium Tumefaciens by triparental mating with E-Coil. This type of mating is a recombinant DNA method which allows genetic information to exchange from one parent to the other. The gene of interest is now present in Agro bacterium Tumefaciens plasmid (Ti plasmid) and is situated beside the T-DNA[14].

Accomplishment of Antisense technology

A complimentary DNA sequence is cloned and inserted in front of the sequence of interest. This means that the last base of the inserted DNA is complimentary to the first base of the original DNA; the second last base is complimentary to the second base and so forth. When transcription takes place in this modified strand of DNA, the mRNA becomes double stranded. Depending on the size of the inserted DNA, the mRNA could be double stranded along all its length or could be partially double stranded. As a result of the double strand mRNA, ribosome's fined it difficult to process mRNA and very little protein is produced[15].

THEORIES ON HOW INHIBITION WORKS

When the aRNA binds to the complementary mRNA. It forms a double stranded RNA (ds-RNA) complex that is similar to double-stranded DNA. The ds-RNA complex does not allow normal translation to occur.

The exact mechanism by which translation is blocked is unknown[1].

Several theories include:

- That the dsRNA prevents ribosome's from binding to the sense RNA and translating [25].
- The ds-RNA cannot be transported from within the nucleus to the cytosol. This is where translation occurs[25].
- That ds-RNA is susceptible to endoribinucleases that would otherwise not affect single stranded RNA, but degrade the ds-RNA[25].

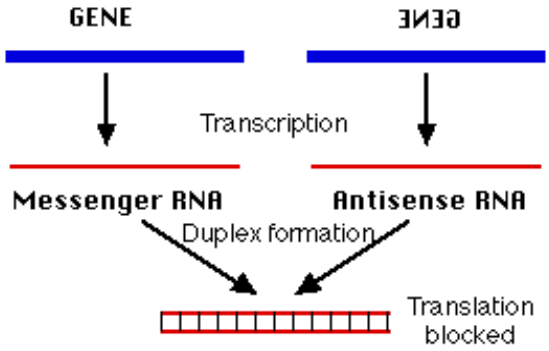

Figure 3: Cartoon of how sense mRNA and antisense RNA are transcribed and then anneal to form double-stranded RNA, which blocks translation of the protein coded for by the sense mRNA (Kimball, 2002).

Cloning of aRNA

In order for aRNA to black translation, it has to be inserted into the proper cells so it can bind to its complementary sense strand. There are several ways to incorporate aRNA into a cell. One method is to create a plasmid that codes for the aRNA. In essence, this is a plasmid that has a promoter that initiatives transcription in the direction of the 3'ends of the sense stand to the 5' end. Which corresponds to 5' to 3' transcription of the antisense strand? Next, this liposome's can be used to transfect target cells with the newly formed plasmids. Liposomes are cheap and efficient for this task[5].

Adenoviruses can also be used to infects cells and delivers aRNA sequences. This method has higher transduction efficiency than liposome (Tritton, 1998)

A northern blat can be used to detect whether the aRNA is produced within the cells, and a western blat can be used to measure amount of the mRNA gene that is expressed in wild type and mutant cells with. If the aRNA is properly expressed in the cells, then less of the m-RNA gene product should be produced.

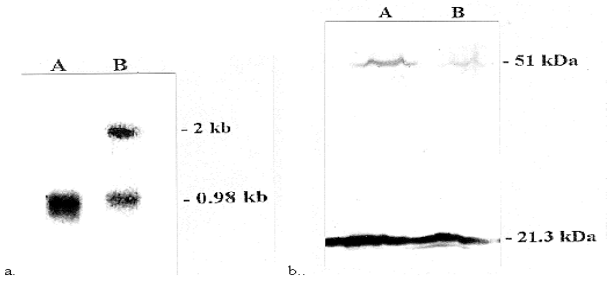

Figure 4: (A) Northern blot analysis of antisense RNA expression in mutant (lane B) and wild type (lane A) cells. The RNA was hybridized with in vitro labeled transcripts from the 21.3 KDa genes which encodes a subunit of the membrane arm of complex I, was included in the hybridization. A single with the expected 2kb length of the antisense RNA for the S1KDa protein could be seen only in the transformant RNA (lane B). While both wild type and transformant RNAs have a 0.98 Kb transcript which hybridized with the central probes for the 21.3 KDa proteins. This shows that the antisense RNA was only expressed in the wild type cells and that the sense RNA was expressed in both.

(B) Western blot analysis of one mutant (lane B) compared with the wild type (lane A). The same amount of mitochondrial protein was incubated with antisera against the 51 KDa subunit and as a control, against the 21.3 KDa submits of the membrane arm of complex I. The level of 51 KDa in lane B is significantly smaller than the band in lane A, showing that less of the gene product was made in the cells that expressed the aRNA (fecke, et al., 2003)[5]

RNA Inhibition

It has recently been shown that double stranded RNA in the cytoplasm triggers an as get poorly understood cascade of events leading to the suppression of the transcription of the gene producing the specific mRNA involved in the cytoplasmic RNA duplex. This could potentially lead to the development of new pharmacological agents[25].

Inserting Antisense into cells

(a) **Endocytosis:** One of the simplest methods to get nucleotide in the cell, it relies on the cells natural

process of receptor mediated endocytosis. The drawbacks to this method are the long amount of time for any accumulation to occur, the unreliable result, and the inefficiency[23].

(b) **Micro-Infection:** As the name implies, the antisense molecule would be injected into the cell. The yield of this method is very high, but because of the precision needed to inject a very small cell with smaller molecules only about 100 cells can be injected per day[23].

(c) **Liposome–Encapsulation:** This is the most effective method, but also a very expensive one[23]. Liposome encapsulation can be achieved by using products such as lipofect ACE™ to create a cationic phospholipids bilayer that will surround the nucleotide sequence. The resulting liposome can merge with the cell membrane allowing the antisense to enter the cell[23].

(d) **Electroporation:** The conventional method of adding a nucleotide sequence to a cell can also be used. The antisense molecule should transverse the cell membrane offer a shock is applied to the cells[23].

(e) Antisense PG gene: The PG enzyme is responsible for the breakdown pectin. Pectin is a building block in cell walls, and is what gives tomatoes their firmness. In an attempt to slow the softening process, the Flavr Savr employs antisense technology to block PG enzyme production. The use of antisense PG RNA is because the mRNA it generates is complementary to the mRNA produced by regular PG genes, it will actively inhibit PG enzymes by disabling their mRNA. This disabling is accomplished by having the small antisense fragment mRNA bind to the regular PG mRNA. This partial double-stranded complex will not for PG protein, and the complex is quickly degraded[18].

The CaMV35s promoter is used because of its constitutive effects. The CaMV35s is always expressed inside the tomato plants. This means the cell has readily large supply of antisense mRNA present. This means, that when tomato cells reach the ripening stage where PG enzymes are to be made, the normal PG mRNA is easily bound by the abundant supply of antisense mRNA. This is the key to disabling the PG gene/future enzyme.

The final stage plasmid is in agro bacteria. The bacteria infect the plant, thereby passing the genes between the LB & RB restriction site in to the plants genome. This genetic material is then expressed with subsequent cell divisions.

The use of a kinamycin resistant gene, is to create a selective marker. If the plant has the antisense gene which would mean it also has KanR, then it will grow on this selective medium. If the plant does not have the KanR gene (this means the antisense sequence was not incorporated into the genome of the plant), then it will not grow on the growth medium. Antisense technology in the Flavr Savr tomato does work. Levels of PG mRNA are 6% of those experienced in normal tomatoes[19].

Testing the Expression of the Antisense Construct

After the plants have been selected on the kinomyosin medium, further tests are performed to examine if the endogenous gene is expressed appropriately in the organism. To ensure that the antisense gene is still intact, a Southern Blot is performed to locate the newly incorporated genomic sequence. The DNA is digested with a restriction enzyme and broken into smaller fragments which are subjected to gel electrophoresis and separated according to their size. The fragments are then transferred onto a nitrocellulose filter or nylon membrane and incubated with a radio labeled cDNA probe that is complementary to the endogenous gene. This probe binds to the fragment of the digested DNA corresponding to the newly inserted sequence and can be detected by autoradiography. This demonstrates that the DNA has incorporated the endogenous gene into its genome.

The next step that is essential in determining if the gene is being expressed is to test the activity of the endogenous sequence. First, a Northern Blot analysis is performed to determine if the RNA complement to the new gene is present. A Northern Blot is performed like the Southern Blot, except the probe used is an RNA complement to the transcribed gene. Although this is an effective test to determine if the RNA is present in the cell, it does not reveal the amount of RNA expressed. To test if transcription is occurring at a sufficient level, Nuclear Run-on experiments are performed. The nuclei are isolated from cells and allowed to incorporate ^{32}P into the growing RNA chains. The resulting labeled RNA is allowed to hybridize with the same probe as in the Northern Blot, and by autoradiography, the amount of RNA can be determined[20].

The expression of the endogenous gene can also be examined at the level of the translated protein. This is done by Western Blotting, or Immunoblotting. A mixture of the cellular protein is separated on a SDS-polyacrylamide gel, and then transferred to a nitrocellulose membrane. The membrane is soaked in a solution of antibody that is specific only for the protein from the endogenous gene. The membrane is then soaked in a second antibody that is labeled, and specific for the first antibody. This reveals the antibody: protein of interest complex, and is used as an indicator of gene expression[21].

The final test in assessing the activity of the endogenous gene is to ensure that integration of the antisense gene has not affected the expression of adjacent genes. This is accomplished using Nuclear Run-on experiments. As in the tests for the level of RNA transcription, ^{32}P is incorporated into growing RNA chains. The resulting RNA fragments can be separated according to size, and probed with an RNA probe. If any of the resulting fragments are longer than the expected length compared to the gene and/or the probe, it is indication that transcription run-

through has occurred, and the gene has affected the expression of adjacent genes. This is an undesired result that must be carefully monitored during antisense experiments.

Triplex Antisense Technology

In the face of all this progress, still newer technologies are being developed based on concepts related to antisense biology. For example, it is known that oligonucleotides can, in certain instances, bind to duplex DNA molecules through an unusual kind of base pairing. In this triplex binding mode, oligonucleotides insert themselves into the major groove of the DNA double helix on a reasonably specific basis determined by the nucleotide sequence f the target DNA[21].

This triplex technology provides the opportunity to reduce gene transcription itself rather than to destroy mRNA once it is produced. Because the triplex oligonucleotides can be made to permanently after the DNA after localizing to specific target sites, the technology actually has the potential to permanently silence genes[21].

In the laboratory of "Alton ochesner Medical foundation, New Orleans, L.A. (U.S.A); this technology is used in an effort to produce triplex binding to one of the consensus binding sites of the anti-tumor protein P^{53}. The protein P^{53} is in normal cells and is turned on when cells became damaged or malignant, implanting their growth and preventing the reproduction of faulty cells. Certain cancer cells lack P^{53} function, which allows continued growth and reproduction. The P^{53} protein binds to specific DNA sequences, and we speculated that triplex oligonucleotides designed to bind to those sequences might mim ic P^{53} effects. We subsequently demonstrated that the use of triplex oligonucleotides will suppress cell growth in certain cancer cells lacking normal P^{53} function. Thus raising the possibility of that triplex biology may lead to the development of effective anticancer agents[21].

Biotechnology and antisense technology

The impact of Biotechnology an antisense technology is expected to increase dramatically as the links between genetics, protein production and disease are better understood[1].

Currently, antisense technology is used to design therapeutic compounds which target specific mRNA sequences to obstruct the production of certain disease causing proteins. Traditional drug therapies focus on a drug's interaction with the disease causing proteins. However, antisense drug therapies inhibit the production of the disease – causing proteins altogether[9].

Antisense in AIDS

(1) The expression of anti – HIV – 1 hammerhead ribozymes in the context of retroviral vectors. To determine optimal vector designs for ribozyme expression, we compared three vectors. Each of which contained the same pair of anti-HIV-1 hammer head ribozymes in tandem. Despite the presence of vastly different amaints of vector-derived flanking sequences, the ribozymes produced by each vector had similar cleavage activity when assayed in vitro. The ribozyme vectors were packaged into amphotropic virion and used to transduce human CEMT-lymphatic. Analysis by Northern blot and RNAse protection assays demonstrated that the highest steady state levels of ribozyme-containing transcripts were produced by a vector in which the ribozyme were expressed under transcriptional control of the vector MoMuLV LTR[4].

Despite these differences in the levels of ribozyme transcripts achieved by the vectors, their ability to confer resistance to HIV-1 replication was similar. Therefore, other factors then the absolute levels of ribozyme play a role in determining the effectiveness of ribozyme vectors to inhibit HIV-1. These may include structural features of the transcripts that affect the antisense effects of the ribozyme constructs, the actual catalytic activity of the ribozyme, their RNA folding, the binding of proteins, and the intracellular localization, greater understanding of these factors may permit more effective application of ribozyme to inhibit gene expression[4].

(2) Antitat is an auto regulated gene expressing an inhibitory RNA with dual function: It sequesters the Tat protein by polymeric – TAR and blocks the translation of the Tat messenger RNA by antisense Tat using human T cell lines and peripheral blood lymphocytes as the in vitro target, we have previously shown that anti-tat is an effective long term suppressor of HIV-1, including "field" isolated[5]. To assess the efficacy or this inhibitory gene better in the setting of an infected individual with late – stage AIDS, we examined its antiviral activity in an in vivo established infection. Peripheral blood mononuclear cells isolated from AIDS patients were transduced with replication defective retroviral vectors carrying the anti-tat gene. In the absence of cell selection. The anti-tat gene blocked virus replication and allowed infected CD4+T cells to expand in culture[5].

These results suggest that anti-Tat gene therapy may be beneficial to black HIV-1 replication and reconstitute the immune system of late-phase AIDS patients. We introduced a new parameter, CRF, which defines the effectiveness of the ex-vivo gene therapy treatment of AIDS patients. Anti-Tat treatment was efficient in cells of all patients regardless of viral quos species; however, it was most potent in severely immunocompromised individuals[5].

(3) The Human immunodeficiency virus type-1 (HIV-1) Tat activation response (TAR) region is essential for Tat-mediated trans-activation of the HIV-1 transcripts and is relatively consumed among different HIV-1 isolates. These properties make it an attractive target for anti

HIV-1 gene therapy strategies. We have constructed moloney nurine leukemia (MoMuLu). Based retroviral vector that expressed a chimeric tRNA (iMet) antisense TAR fusion transcript complementary to the HIV-1 TAR Region[6]. The potential of this anti-TAR retroviral vector to inhibit HIV-1 was initially tested by transient transfections with an HIV-1-LTR-Tat expression plasmid into Hela-CAT cells. Anti-TAR inhibited Tat-mediated HIV-1 LTR-driven CAT reporter gene expression in a dose dependent fashion. The antisense-TAR vector was then used to transducer the human supT1 T –cell line. Co transfection of these SupT1cells with a Tat expression Plasmid plus an HIV-1 LTR-CAT reporter plasmid resulted in decreased CAT gene expression in comparison to control transducer sup T-1 cells. The antisense-TAR engineered sup T1 cells line was then challenged with HIV-1 MN. HIV-1 viral production was inhibited in sup T1 cells transducer with the antisense –TAR retroviral vector. Greater inhibition of HIV-1 was observed with antisense –TAR or compared to antisense – Tat expressing retroviral vector. These observations suggest that antisense –TAR retroviral vectors are potentially useful for clinical anti-HIV-1 gene therapy[6].

(4) The Tat gene produce (Tat) of HIV-1 is an early regulatory protein necessary for viral gene expression and replication. Tat may also play a role as an extra cellular protein in both HIV-1 replication and AIDS associated disorders such as Kaposi's sarcoma[6].

Thus, Tat represents a good target for gene therapy against AIDS. Here we show that when vectors expressing antisense tat RNA are transiently transected into CD4+ cells, they block about 70% of HIV-1 replication and inhibit the rescue of Tat-defective HIV-1 proviruses by inhibition of Tat protein expression and consequent lack of transcriptional activation of the HIV-promoter. However, antisense tat vectors cannot block the activity of extra cellular Tat protein. Another tat inhibitory construct (poly-Tat activation response; TAR) previously suggested to inhibit HIV-1 Tran activation by sequestering the Tat protein, inhibited the activity of extra cellular Tat protein[6].

Another Tat inhibitory construct (Poly-Tat Activation response; TAR) previously suggested to inhibit HIV-1 Tran activation by sequestering the Tat protein, inhibited the activity of extra cellular Tat, but like antisense tat RNA did not completely block viral gene expression and replication. These results suggested that one mode of inhibition is not sufficient to block Tat function. However, when the antisense tat poly-TAR constructs were combined HIV-1 gene expression was completely blocked (94-98%) suggesting that a combination of inhibitory genes blocking Tat by sequential steps may be better approach for AIDS gene therapy[6].

(5) A ribozyme was constructed that specifically cleaves RNA that contains the first coding axon of the tat – gene of HIV-1. This anti-tat ribozyme was incorporated into a moloney murineleukemia virus vector. A sequence containing only the 48 nucleotide antisense region of the ribozyme was also inserted into the retroviral vector[7].

Human T-cell lines constitutively producing the tat-antisense and the anti-tat ribozyme RNA were create by transduction into Jurkat Cells. When challenged with HIV-1 both the tat-antisense and anti tat ribozyme producing cells inhibited the replication of HIV-1. The antisense vector conferred a greater resistance to HIV-1 replication than did the anti-tat ribozyme vector[7].

(6) Replication of human immunodeficiency virus type (HIV-1) was inhibited by stable intracellular expression of antisense RNA in the human T-lymphoid cell line Junkat. When the viral sub region encoding the HIV-1 activator proteins was targeted, the extent of antisense RNA. Mediated inhibition was greater than 97% during the first 2 weeks post infection. Later HIV-1 infection broke through at high initial infective days,

However at initial multiplicities of infection equal to or smaller than 01, HIV -1 production was not detectable during the 5 weeks of observation. The results underline the effectiveness of stable intracellular antisense RNA expression in inhibiting HIV-1 replication[8].

Antisense therapies in development

Applications of antisense technology in therapeutics are expected to increase substantially as the link between diseases and genes are made. Antisense drug therapies are currently unavailable in Canada. However, there are numerous antisense therapies in development, some of which are in clinical trials and one of which has been approved for use in the United States[1].

Problems with antisense technology

Antisense technology has not been perfected. It is still difficult to express aRNA only in targeted tissues. Precision gene therapy using aRNA needs to be improved because as it is right now, aRNA sometimes binds to mRNA that is not its target. It is known to improperly bind to human growth factor. Furthermore, the uptake of aRNA is still imprecise [23].

APPLICATION

The possibilities that aRNA has to offer the scientific and medical fields seem endless. Projects are in the works already on how to use antisense technology for the deactivation of oncogenes[25], for the suppression of viral RNA expression[24], and to understand naturally occurring aRNA that can be used for genomic imprinting [25].

Antisense technology has already been successful in suppressing the gene for the protein that makes

tomatoes – Poil Flavor saur tomatoes were transgenic tomatoes constructed to have artificial DNA that coded for aRNA that was complementary to the RNA that coded for the protein that caused spoiling. The aRNA suppressed the expression of this spoilage gene by 10% which was enough to save the tomatoes from rotting while being shipped to grocery stores. The tomatoes are no longer on the market due to complications in the harvesting process[25].

In vitro application

Antisense technology has been applied successfully in two general areas. The first is in fundamental research where the introduction of antisense oligonucleotides can help determine the role of a specific gene in a specific physiological process[10-12]. For example, in the laboratory of Alton ochsmir medical foundation, New Orleans. L.A (U.S.A) was interested for same time in the idea that local components of the rennin angiotensin system can be produced by specific cells. They hypothesized that the production of angiotensin II by cell feeds back on those cells resulting in cell growth and other changes.

In their view this tissue (cellular rennin angiotensin system) could therefore potentially play a role in a wide variety of cardiovascular disorders, including atheroeclerosis and vascular hypertrophy. It is difficult to demonstrate that a cellular system is operative in any given process. However, because a circulatory rennin antiotensin system exists that produces angiotensin II in tissue culture medium as well as in tissues. To approach this problem they developed oligonucleotides to inhibit the synthesis of angiotensinogen, the substrate from which cells make angiotensin II[18].

Thus, through the application of antisense technology, they are able to demonstrate the biological principle that cells can make their own angiotensin II with growth promoting effects. Now they extended this antisense work to certain cancers and demonstrated, for the first time, that neuroblastoma cells[18].

Therapeutic Application

A second application of this technology, and one that is potentially of more immediate relevance to the practicing physician, in the use of this technology in therapy[2-3,14-19]. In principle, antisense oligonucleotides complementary to viral RNAs can suppress a wide variety of viral infections, a tremendous amount of research is ongoing in this area, similarly, antisense oligonucleotides directed towards the products of oncogenes can play a role in reducing the growth of cancer cells, and this leads in being hotly pursued[20].

Perhaps the most widely discussed application of antisense technology lies in its applications to gene therapy. In this case, a variety of vectors is used to introduce antisense encoding genes into a large number of cells in a patient or animal to produced long term inhibition of a protein. For example, in animal models the introduction of vectors encoding antisense angiotensin II receptor sequences results in long term normotension in spontaneously hypertensive animals[20].

These are but a few of the possible application of antisense technology. As familiarity with the relevant chemistry increases, it is likely that more effective oligonucleotides and gene vector will be developed thereby providing the ability to interfere at will with the translation of specific mRNA.

Obstacles of Antisense

The successful result of any of those methods is reduced protein production as long as the surrounding conditions are favorable (Driver). However, there are more problems that need to be overcome then just inserting the antisense molecule into the cell. If antisense is being used as a treatment in the human body it might be degraded before stopping any protein production if it is unmethylated because the body would recognize it as an invader[26]. When antisense is used is living organisms, there are also many complications that can arise such as high blood pressure and a low white blood cell count[26].

Antisense and how it applies to Flavr Savr

The first step is to create a cDNA of the PG gene. This involves generating an mRNA, then re-transcribing it back into a cDNA. This cDNA contains the entire PG gene. Next a 730 base pair (bp) region, including a 50bp non-coding region is excised called the Hinf1 fragment. This is cloned into a plasmid. Next the cloned fragment was excised and legated into a second plasmid. The insertion occurred just after the CaMV35s (Cauliflower Mosaic Virus Promoter) finally, this plasmid was inserted into an agrobacteria, where the complete fragment was inserted yet again, into another plasmid, this one containing the KanR gene[19].

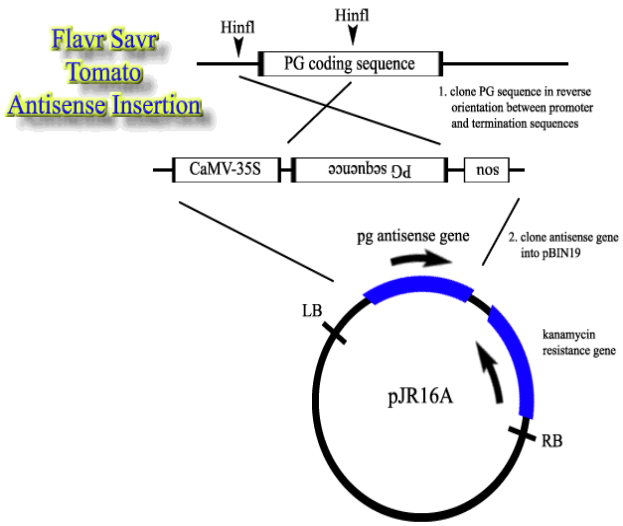

Figure 5

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