



Sense vs antisense rna

Messenger RNA (mRNA) is single-stranded. Its sequence of nucleotides is called sense because it results in a gene product (protein). Normally, its unpaired nucleotides are read by transmitting RNA anticodons as the ribosome continues to translate the message. Figure 11.9.1 Sense Strand Dock, RNA can form duplexes just like DNA does. All that is needed is a second part of RNA whose sequence of bases is a complement to the first string. Example 5' C A U G 3' mRNA 3' G U A C 5' Antisense RNA The ribosome cannot access nucleotides in mRNA or because duplex with an additional antisense RNA sequence, the translation is blocked. This can happen becauses in a cannot access nucleotides in mRNA or because duplex with an additional antisense RNA molecules can be introduced into the organism. Most tomatoes that need to be delivered to the market are harvested before they are ripe. Otherwise, eten synthesized by the tomato causes them to mature and destroy before they reach the customer. Transgenic tomatoes have been designed which in its genome carries an artificial gene (DNA) that is transcribed into an antisense RNA for an enzyme involved in ethean production. These tomatoes make only 10% of the normal amount of the enzyme. The goal of this work was to provide supermarket tomatoes with slightly closer appearance and flavor of tomatoes harvested where ripe. However, these tomatoes were often damaged during transport and handling and have been removed from the market. Fig. 11.9.3 Tobacco Flower Flower of a tobacco plant carrying a transgenic plant tark has not had its normal pigmentation altered. There are several methods for inserting genes into plants, including infecting plant cells with plasmid vectors that carry the desired gene that shoots microscopic pellets containing the gene directly into the cell Unlike animals, there is no real difference between somatic cells and settcells. Somatic tissues of plants, e.g. If all goes well, the transgenic of other sequence in the cell? The answeris yes, and these seem to repres hybrids: emotion and antisense strands that form a double spiral of double-stranded RNA (dsRNA). Double-stranded RNA corresponding to a particular gene is a powerful suppressant of that gene. In fact, the suppressive effect of antisense RNA probably also depends on its ability to form dsRNA (with the corresponding mRNA as a template). The ability of dsRNA to suppress the expression of a gene corresponding to its own sequence is called RNA interference (RNAi). It is also called post-transcriptional gene silent or PTGS. The only RNA molecules normally found in the cytoplasm in a cell are single-stranded RNA molecules. If the cell finds molecules of double-stranded RNA (dsRNA), it uses an enzyme called Dicer to cut them into fragments containing ~21 base pairs (~2 turns of a double helix). The two strands of each fragment then separate – release the antisense string. With the help of a protein, it binds to a complementary sense sequence on a molecule of mRNA. If the bass-pairing is exact, the mRNA is destroyed. Because of their action, these fragments of RNA have been named small (or short) disturbing RNA (siRNA). The complex of siRNA and protein is called RNA-induced silent complex (RISC). There is growing evidence that siRNAs may also inhibit the transcription of genes perhaps by binding to complementary sequences on DNA or perhaps by binding to the incipient RNA transcript it is being formed. In fission veast, at least, siRNA is complex with a molecule of each of three different proteins. The entire complex is called the RITS complex (RNA-induced initiation of transcription gene silencing) How these siRNAs — synthesized in the cytosun — gain access to the DNA in the nucleus is unknown. Synthetic siRNA molecules that bind to gene promoters can — in the laboratory — overpressure transcription of that gene. The oppression is conveyed by the methylation of DNA in the promoter and, perhaps, methylation of histones nearby. There is a strain of rice (LGC-1) that produces abnormally low levels of proteins called glutelins. It turns out that of several glutelin genes found in rice two closely similar glutelin genes lie back to back the same chromosome. In LGC-1, there has been a deletion between the two genes, which removes the signal that would normally stop the transcription after the first gene. Thus RNA polymerase II transscribes right the first gene and on into the second. The result is a messenger RNA with nearly-identical sequences running in opposite directions. This causes mRNA to fold up to a molecule of double-stranded RNA (dsRNA). A Dicer-like enzyme cuts up dsRNA into small disruptive RNAs (siRNAs) that suppress further transcription of these genes as well as other glutelin genes. RNAi has been shown to work in organisms as diverse as plants, fungi and animals as Drosophila melanogaster, Caenorhabditis elegans, and also mice and zebrafish. Such a universal cell response must have an important function. What could it be? Some possibilities: Some viruses of both plants and animals have a genome of dsRNA. And many other viruses of both plants and animals have an RNA genome that in the host cell is briefly converted into dsRNA. So RNAi can be a weapon to counteract infections of these viruses by destroying their mRNAs and thus blocking the synthesis of essential viral proteins. Transpoons can be transcribed into RNA molecules with regions that are double-stranded. RNAi can then destroy these. RNA interference may be the unexpected distribution of another basic process of controlling gene expression. In any case, the discovery of RNAi adds a promising tool to the molecular biologist's toolbox. Introducing dsRNA corresponding to a particular gene will knock out the cell's own expression of that gene. (Feeding C. elegans on E. coli manufacturing of dsRNA will also do the trick.) Heroic example In the March 24, 2005 issue of Nature, Sönnichsen et al reported that they have injected dsRNAs corresponding to 20,326 of C. elegans's genes (98% of the total!) and monitored the effect of each on embryonic development from the completion of meiosis (after fertilization) through the second mitotic breakdown producing the 4-cell embryo. They found that at least 661 different genes changed any process during this period: about half of those involved in cell division and half in general cell metabolism. (Another thousand genes produced phenotypic effects that were seen in later stages of development.) Since RNAi can be done in particular tissues at a selected time, it often gives an advantage over conventional gene knockouts where the missing gene is carried in terms of and thus whose absence can kill the embryo before it can be studied. Another example: screening genes for their effect on drug sensitivity Distribute your cells in thousands of wells and add — from a library of thousands of siRNAs representing the entire genome — siRNA molecules that target the expression of a gene to each well Add the drug to all wells See which wells Cells Responding Some Other Promising Applications of RNAi In Mammalian Cells In Mammalian Cells, the introduction of dSRNA fragments only temporarily reduces gene expression. However, mammalian cells can be infected with a DNA vector that encodes an RNA molecule of 50-80 nucleotides called a small hairpin RNA (shRNA) that contains a sequence corresponding to the gene that one wishes to suppress. Because shRNA is synthesized, dicer converts it into a typical siRNA molecule. Since the cell can continuously synthesize shRNA, the disturbance is prolonged. In fact, with vectors that become integrated into the host genome, the RNA ican be passed on to the descendants. In Plants On June 19, 2003 the issue of Nature reported on coffee plants that were designed to express a transgen that makes siRNA that interferes — of RNAi — with the expression of a gene needed to make caffeine. So, decaffeinated coffee may one day no longer require the chemical removal of caffeine from coffee beans. Monsanto develops a transgenic corn (corn) that expresses a dsRNA corresponding to the sequence of an important gene in the western corn rotor, a devastating pest of the crop. After the procedure of this dsRNA, the insect's own cells process it into a siRNA that targets the mRNA of the gene for destruction and kills the worm in a few days. In C. elegans, plants, and neurospora, the introduction of some molecules of dsRNA has a potent and long-lasting effect. In plants, the sound attenuation of the gene spreads to adjacent cells (by plasmodesmata) and even to other parts of the plant (through phloem). RNAi within a cell can continue after mitosis in the offspring of that cell. Ejaculation of RNAi in C. elegans can also pass through the setten into its descendants. Such amplification of an initial trigger signal suggests a catalytic effect. It turns out that these organisms have RNA-dependent RNA polymerases (RdRPs) that use mRNA that is targeted by the initial antisense siRNA as a template for the synthesis of more siRNAs. Synthesis of these secondary siRNAs also occurs in adjacent regions of mRNA. So not only can these secondary siRNAs target additional areas of the original mRNA, but they are potentially able to silence mRNAs of other genes that may carry the same sequence of nucleotides. This phenomenon, called transitive RNAi, can complicate the interpretation of geneinactive experiments as expressions of other genes can be suppressed in addition to the target gene; raises a warning flag for the use of RNAi to suppress single genes in human therapy (although RdRPs and amplification have not been observed in mammalian cells). Because its target is so specific, the ability to use RNAi to turn off the expression of a single gene has created great excitement that a new class of therapeutic agents is on the horizon. Many clinical trials are underway to explore siRNA molecules in the treatment of a wide variety of diseases. To date, the most promising results have used RNAi to target an inherited disease in which the liver secretes a mutant form of transthyretin that leads to the accumulation of amyloid deposits in neurons and elsewhere. In C. elegans requires successful development through its larval stages and on to the adult presence of at least two microRNAs (miRNAs) — single-stranded RNA molecules containing approximately 22 nucleotides and thus approximately the size of siRNAs. These small single-stranded transcripts are generated by the cleavage of larger precursors with the C. elegans version of Dicer. They operate by either destroying or inhibiting the translation of multiple messenger-RNAs in the mask (usually by binding to a region of complementary sequence in the 3' untranslated region [3'-UTR] of mRNA). MicroRNA (miRNAs) in C. elegans (first called small temporal RNAs) turns out to be representatives of a large class of RNA encoded by the organism's own genes. The initial product of gene transcription is a large molecule called pri-miRNA. While still within the nucleus an enzyme called Drosher cuts pri-miRNA into a shorter molecule (~70 nucleotides). called pre-miRNA. Pre-miRNA is exported into the cytosol where it is keyboarded (by Dicer in animals) into the miRNA. MicroRNAs are found in all animals (humans generate some 1000 miRNAs) and plants but not in fungi. contain 19–25 nucleotides; are encoded in the genome some of the independent genes (which can encode multiple miRNAs) some of the elements of an intron of the gene whose mRNA they will regulate. may be expressed in only certain cell types and at only certain times in differentiation of a particular cell type. While direct evidence of the function of many of these newly discovered gene products remains to be discovered, they regulate gene expression by regulating messenger RNA (mRNA), either destroying mRNA when the sequences match exactly (the usual situation in plants) or suppressing its translation when the sequences are only a partial match. In this latter case, it probably requires several miRNAs to bind simultaneously in 3'-UTR. MicroRNAs have two properties ideal for this: Being so small, they can be quickly transcribed from their genes. They do not need to be translated into a protein product to act (as opposed, e.g. to transcription factors). MicroRNAs regulate (repress) expression of genes in mammals as well. Genome analysis has revealed thousands of human genes whose transcripts (mRNAs) contain sequences that one or more of our miRNAs can bind. Probably each miRNA can bind to as many as 200 different mRNA targets while each mRNA has binding locations for multiple miRNAs. Such a system provides many opportunities for coordinated mRNA translation. A study reported in Nature (Lim, al., 433: 769, Feb 17, 2005) used DNA chip analysis to show that when a particular miRNA was expressed in HeLa cells, a miRNA normally expressed in brain repressed mRNA production of 174 different genes while a miRNA normally expressed in the cardiovascular and skeletal muscles reprinted mRNA production of 96 genes —all but 8 of them are different from those pressed by the brain miRNA. As work progresses rapidly in this area, the pattern that is beginning to emerge is that: Many genes — especially those involved in such housekeeping activities (e.g. cellular respiration) common to all cells — do not have 3'-UTRs that can be blocked by any of the miRNAs encoded in the genome. The genes that need to be expressed in a certain type of differentiated cells and/or at a certain point in the life of that cell do not express any of the miRNA genes that could block their expression but do express miRNA genes that block the expression of other genes for specialized functions that would not be appropriate in that cell at that time. Rather than being simple switches that turn gene expression on or off, miRNAs seem to exert a more subtle effect — raising or lowering the level of gene expression (much as protein transcription factors do). Thus repression of gene expression by miRNAs appears to be a mechanism for ensuring regulated and coordinated gene expression, which cells differentiate along specific paths. For example, when zygote genes begin to be turned on in zebrafish blastula, one of them encodes a miRNA that triggers the destruction of maternal mRNAs that have run things up to then. So miRNAs can play as important a role as transcription factors in regulating and coordinating the expression of multiple genes in a particular type of cell at certain times. The ease with which miRNAs can be introduced into cells and their widespread effects on gene expression have given rise to hopes that they may be useful in controlling genetic diseases, such as the use of genetic diseases. So far, some laboratory studies have been quite promising. A miRNA that blocks the expression of G1 and S-phase cyclins — thus stopping the cell cycle in its tracks — protects mice from liver cancer. A miRNA that inhibits genes needed for metastasis suppresses the metastasis of treated human breast cancer cells. In addition to protein transcription factors, eukaryotes use small RNA molecules to regulate gene expression — almost always by suppressing it — so the phenomenon is called RNA silencing. There are two sources of small RNA molecules: small disturbing RNA (siRNAs) Plant cells make these from the double-stranded RNA (dsRNA) of invading viruses. Researchers and pharmaceutical companies do these as agents to turn off the expression of specific genes (called RNA interference or RNAi). micro RNAs (miRNAs) These are encoded in the genomes of all plants and animals. Both siRNA's and the cell's cytosol. Both are generated by Dicer. Both are incorporated into an RNA-induced sound attenuating complex (RISC). If the nucleotiden sequence of the small RNA exactly matches that of the mRNA, the mRNA is cut and destroyed. If there is only one partial match (usually in its 3' UTR), translation (i.e. protein synthesis) is repressed. Both of these activities take place in the cytosun-perhaps in P-bodies. For some small RNAs, however, the RISC complex enters the nucleus and turns off transcription of the corresponding gene(s) by binding to the unwound DNA sequence (or perhaps the RNA transcript it is being formed) converting the euchromatin to the heterochromation methylation of lysine-9-histon H3 in the nucleosomes around the gene(s) Apart from their use as a laboratory — and perhaps therapeutic tools —, small RNAs are clearly essential to the organisms that make them. Some examples: Plants and animals use them to defend themselves against viruses. For example, when human cells are infected by hepatitis C virus (HCV), they produce miRNAs that interfere with the gene expression of this RNA virus and thus its ability to replicate. Some herpes viruses use them to keep their host cell alive long enough to complete virus replication (by blunting a host's immune response to the infected cell and preventing its premature death by apoptosis). Of the 46 miRNAs expressed in the Drosophila embryo, 25 have been shown to be essential for normal development. Proper embryonic development in other animals (e.g.C. elegance, zebrafish, mice) also requires them. They protect against the danger of mutations caused by transpounions moving in the genome. They are also needed to regulate the size of the pool of at least certain types of stem cells. Transgenic mice with a single miRNA gene rash develop severe immunodeficiency affecting dendritic cells, help-T cells and B cells. Impaired, or no, expression of certain miRNAs is characteristic of several different cancers in humans. Contributors and Attributions Attributions

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