Each of our cells contains a complete copy of the genes required to create a whole organism. Thus, it was possible to clone Dolly from a single sheep epidermal cell, and we should be able to reconstruct a dinosaur from the genetic material buried in the mud of “Jurassic Park.” However, it is hard to believe that by adding water to the $5 worth of chemicals in a human, it is possible to create a human being. Although 30000 instructions, or genes, sounds like a lot, the creation of a hand or a heart or a mind is still a daunting task. Our current genetic code is a lot like the King James Bible—both have evolved. The travails of Moses or Noah were initially “transcribed” by clerics into huge books safely stored in cathedral vaults. With frightening frequency, these imaginative priests “improved” the story as they wrote. The testaments were then “translated” into many languages to enhance access by many peoples. These translations were found, and the data enjoyed further incremental evolution. In eukaryotes, the functional DNA rarely leaves the safety of the nuclear cathedral vault. Traditionally, we have envisioned the Gideon messengers as transcribing reliable molecular copies and distributing them out to motel rooms. While chromosomes are made of double-stranded DNA comprising millions of nucleotide pairs, single-strand messenger RNA (mRNA) carries only single verses, requiring a nucleotide code of only 50 to several thousand nucleotides. Ribosomal RNA synthesizes protein from the mRNA script, while transfer RNA serves an adaptor role in converting the message to protein.¹

Two decades ago, RNA assumed exalted status when catalytic RNAs, or ribozymes, were recognized as capable of promoting reactions similar to protein enzymes. The realization that RNA could also store information spawned the concept that RNA, as a simpler molecule than DNA, was a logical earlier step in the “origin of life.” As brighter light was shone on RNA, a rapidly expanding group of noncoding RNAs (ncRNAs) has surfaced. These short-segment, or micro-RNAs, contain only several dozen nucleotides. In creating functional cells and organs, it is clear that coordinating the expression of genomically orchestrated protein synthesis requires highly sophisticated temporal and spatial tuning. Micro-ncRNAs appear to exert considerable posttranscriptional control of molecular-level activities within a cell. It seems that this regulatory function of ncRNA also serves as antiviral protection in a fashion that intuitively might be harnessed for therapeutic benefit. The purposes of this article are (1) to delineate the rapidly expanding subtypes of RNA that appear critical to healthy cellular function; (2) to explore RNA silencing as a highly conserved method of cellular regulatory protection; (3) to examine mechanisms by which ncRNA protects the genome from external viral attack; and (4) to present posttranscriptional RNA interference or silencing as a therapy for medical and surgical disease.

CLASSICAL DNA-DRIVEN PROTEIN SYNTHESIS

RNA is formed from the same nucleotides as DNA (adenine, cytosine, guanine, and thymine) except, in RNA, uracil pairs with adenine as thymine does in DNA. Under the control of promotors and enhancers, segments of chromosomal DNA are isolated for copy into relatively short segments of

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WHAT DO ncRNAs DO?

Until recently, we could comfortably assume that any change in phenotype or cellular function was driven directly by the genome’s control of protein structure. It is now clear that ncRNAs exhibit both posttranscriptional and posttranslational regulation of protein function, structure, and location. Estimates of the number of ncRNAs in Escherichia coli range up to 200, and several thousand may be operative in man. Criteria used in searching for ncRNAs include (1) large gaps between identified protein coding genes, (2) extended stretches of introns conserved among species, and (3) orphan (unlinked) promoter or terminator sequences. It is also clearly possible that ncRNAs are produced by the opposite DNA strand of protein-coding genes.5

In its simplest fashion, ncRNAs work by direct base-pairing with RNA or DNA molecules. In this manner, the ncRNAs can bind to the target nucleotide sequence (similar to a blocking antibody) and clog transcription or translation. Some ncRNAs appear to masquerade as promoters or ribosomal start sequences and can fool the polypeptide synthesis machinery into producing the desired protein. Noncoding RNAs can also participate as signal recognition sites in large RNA-protein complexes.5

HOW DOES ncRNA PROTECT THE CELL?

The critical first step in any successful military coup is to take over the central government and the radio and television stations. A virus successfully incorporating its genetic material into the genome of a cell is analogous to a foreign agent infiltrating the politburo. Human cells are an attractive target for invasion by viruses and other transposable elements. Plasterk3 estimates that as much as 45% of the human genome consists of remnants of prior viral/transposon infiltration. In wading off a viral attack, the human cell faces the standard array of immunological problems apparent in the spectrum from transplantation (accepting “good” nonself) to surgical oncology (rejecting “bad” self). Thus, the cell must (1) recognize the viral/transposon invader as nonself and (2) amplify the defensive response specific to the invader. Eukaryotic cells (like ours) appear to have evolved a posttranscriptional gene silencing strategy that is analogous to “clonal selection” in the vertebrate immune system.

All functional flavors of RNA are relatively short single strands. Thus, the cell’s warning alarm of nuclear attack is sounded by double-stranded RNAs (dsRNAs). When an infiltrating transposon/virus provokes production of dsRNA, its cover is blown. There are several methods (all hypothetical) by which an exogenous nucleotide sequence might promote dsRNA constructs: (1) inserted transposons might contain a terminal inverted repeat that would trigger duplicate complementary RNA transcription; (2) identical virus/transposons might incorporate into multiple random locations on the genome with the resultant production of duplicate RNA strands; and (3) “good” self genes may share some ZIP code or Social Security number motif on their mRNA, identifying the mRNA message as self.7 Whatever the mechanism, cells recognize dsRNA as a death warrant. A successful cell must heed the dsRNA warning and use it as a code for self-catharsis.

mRNA. The transcribed mRNA is the complement of the gene to be expressed. After the mRNA is disengaged from the DNA, the coding regions (exons) are retained, and the apparent noncoding introns are discarded. The resulting mRNA travels out to the cytoplasmic ribosomes. The short-segment mRNA is translated into protein within the cytoplasmic ribosome. Typically, there is a 3-nucleotide code for each of the 20 amino acids. Thus, ribosomal polypeptide synthesis begins by reading the start nucleotide codon, proceeding through the triple nucleotide amino acid identifying codons, and finishing at the stop codon with a complete protein. Messenger RNA is quite unstable, and 80% of it is degraded before it is ever transcribed into protein.

If the genetic message (mRNA) is so unstable, the opportunity for posttranscriptional regulation by modification and stabilization of the mRNA should be huge—and it is. Recently, a family of RNA that is not classified as mRNA, transfer RNA, or ribosomal RNA, has been identified.2 This was initially termed other RNA. It is now termed ncRNA. Some are short, consisting of 21- to 25-nucleotide segments, and are sometimes referred to as micro-RNA. Other larger ncRNA appear to influence protein structure and function by regulating transcription, RNA processing, RNA modification, and RNA stability.2 Following protein synthesis, ncRNAs also provide posttranslational regulation by influencing protein stability and translocation.3 Intuitively, ncRNA and, presumably, nonfunctioning RNA should not be conserved through evolution. In this instance, our intuition is correct. These small bits of ncRNA seem to do a lot.
As indicated, dsRNAs contain both sense and anti-sense fragments of the genetic material (viral/transposon) that needs to be silenced. Small bits of the dsRNA are sufficient to bind and clog the target mRNA. It is now clear that these warhead fragments, or small interfering RNAs (siRNAs), are still double stranded and are cleaved from longer dsRNAs by an adenosine triphosphate–dependent ribonuclease, appropriately named “dicer.” The cell performs a security check at this point. A 5′ phosphate is required on the target complementary strand of siRNA for it to perform its war on terrorism. After the siRNA is confirmed as targeting the appropriate transposon/gene, it is transferred to the RNA-induced silencing complex (RISC), which peels off the antisense strand of the siRNA. This antisense strand of siRNA is now armed and ready to attack and clog the mRNA to be silenced. Chemically synthetic siRNAs patterned after dicer products have triggered silencing of genes in animals and cultured human cells.

siRNA AMPLIFICATION

Clearly, the unique RNA-dependent RNA polymerase that amplifies the workhorse siRNA probe is the key to this ancient immune process. DNA transcription is not impeded, but as soon as the suspect mRNA emerges into the cytosol, the expanding number of siRNA copies generated there destroys it. Just as Guttenberg’s printing press mushroomed the availability of scriptural knowledge, the amplification of stabilized siRNA fragments (that have successfully found an existing mRNA in the RISC) ensures that soon, all copies of threatening mRNA will be quenched. Thus, only a few nanograms of siRNA (21 nucleotides long) will completely silence a gene in a day or 2. This amplification of a few RNA molecules harkens back several hundred million years, when RNA-based life preceded the arrival of DNA. Unfortunately—but predictably—several viruses have evolved strategies to counterattack the components of the silencing/surveillance system.

SURGICAL GENE KNOCKOUT OWNER’S MANUAL

1. Identify the target gene, such as the tumor necrosis factor gene (in a patient with an acute myocardial infarction) or the toll-like receptor-4 gene (in a patient with gram-negative bacteremia) or the inducible nitric oxide synthase gene (in a patient with sepsis).

2. Go to PubMed and find the gene nucleotide code corresponding mRNA sequence.

3. You will find a nucleotide sequence (which for human tumor necrosis factor is a 1581-item sequence), within which you must locate 21 sequential nucleotides that begin with AA. With this template, you can construct an antisense or short-segment, mRNA-“clogging” strand.

4. Copy this sequence and feed it into Ambion’s siRNA target finder and designer tool engine (www.ambion.com /techlib/misc/siRNA_finder.html).

5. Order your nucleotide sequence at $15 a pop for each nucleotide (21 X $15 = $315).

6. You may now inject/transpose your designer siRNA or ncRNA into a cell, tissue, or intact patient.

7. Do not try this at home.

RNA SILENCING AS THERAPY

Although it sounds fantastic, multiple groups now state that virtually any gene can be disrupted in cultured human cells. The problem—likely solvable—is to extrapolate this strategy to the intact patient. In that small ncRNAs are ubiquitous processors of gene expression, it is up to us to harness the cell’s regulatory machinery. Novina and colleagues have successfully used ncRNA to inhibit events in the life cycle of human immunodeficiency virus (HIV) both prior to and after HIV infection. Double-stranded RNA segments (ncRNA) were introduced into human cells, targeting mRNA for the HIV1 CD4 cellular receptor (prior to infection) or the viral structural Gag protein (postinfection). This group persuasively espoused the therapeutic potential of ncRNA for HIV and other viral infections. Korneev and coworkers seem to have overcome the animal ncRNA delivery problem in rodents. These investigators injected whole animals with small-segment, nc-dsRNAs, targeting neuronal nitric oxide synthase. By decreasing nitric oxide synthesis in the brain, feeding was reduced. Hamon has championed the concept of “RNA therapeutics.” This laboratory has successfully delivered ncRNA with high efficiency, using replication-deficient viruses and has harnessed the exquisite specificity of RNA silencing, making it possible to single out and silence the cancer-causing activated oncogene without affecting the healthy allele. Similarly, once the genetic mutation that provokes the risk of cardiac arrhythmias has been identified, RNA silencing of this potentially fatal gene will soon be feasible. Noncoding RNA can now control the biological spectrum from viral receptor docking to animal behavior. These observations are presented with the conviction that surgeons should be tuned into the information superhighway—we must drive the steamroller, and not be part of the road.

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