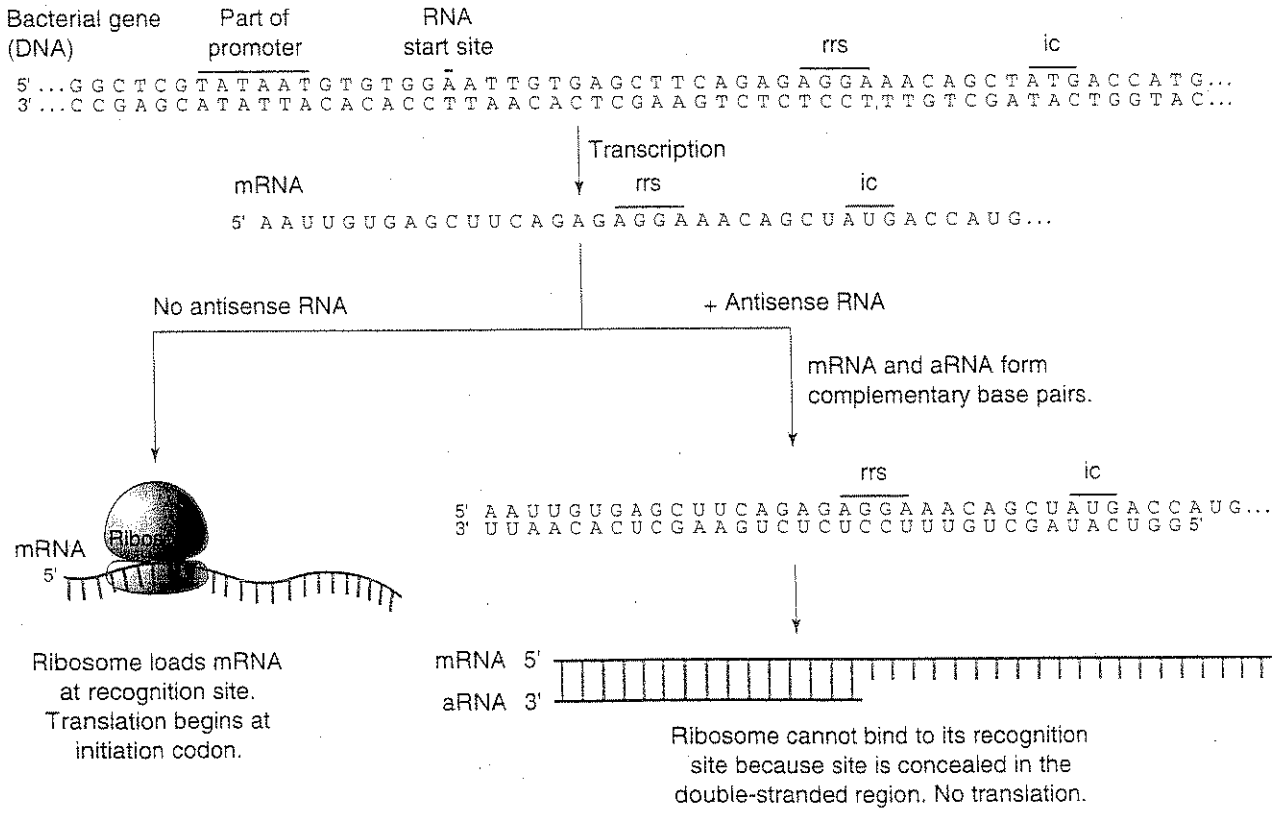
Does Antisense Make Sense?

Imagine what you might be able to do if you could prevent or decrease the expression of any gene. Prevent cancer from developing? Prevent viral diseases? Control certain genetic diseases?

Biotechnologists are pondering these and other questions largely because of a new technology for gene regulation: antisense. What is antisense technology, and how does it work? Antisense technology, like the rest of biotechnology, is based on a natural phenomenon. In antisense gene regulation, a cell synthesizes a very short piece of RNA that is exactly complementary to the ribosome recognition region (usually the 5′ or "front" end) of the messenger RNA (mRNA) of the gene to be regulated. Because of its complementary base sequence, the antisense RNA can base pair with the mRNA. When it does this, the 5′ end of the mRNA becomes double stranded. The ribosome can no longer recognize and translate it. By preventing translation of mRNA, antisense RNA can decrease gene expression (see figure).

How antisense regulation works. The gene to be regulated is transcribed normally, producing an mRNA molecule. The antisense oligonucleotide is exactly complementary in sequence to the area of the message where the ribosome normally binds. When the antisense molecule base pairs to the mRNA, the ribosome cannot bind to its recognition site, and translation does not occur. (The bacterial gene sequence shown is taken from the *Escherichia coli lacZ* gene.) RRS, ribosome recognition site; ic, initiation codon; aRNA, antisense RNA.



Antisense technology copies this natural approach except that the antisense molecule can be RNA, DNA, or even a chemically modified version of either one, just as long as it will base pair with the target molecule. A general term used to refer to these short molecules is "antisense oligonucleotides" (the prefix "oligo" means "several"; "nucleotide" can refer to the building blocks of DNA or RNA). It is theoretically possible to reduce the expression of any gene by introducing an appropriate antisense oligonucleotide into the cell.

Regulation by antisense is very precise because of the specificity of base pairing. For any oligonucleotide, the chance that its complementary sequence will occur randomly is 1 in 4^n, where n is the number of bases in the oligonucleotide. So the chance of a 15-nucleotide antisense molecule accidentally pairing with an unintended target is 1 in 4^{15}, which is less than 1 in 1 billion (multiply it out for yourself). For longer antisense molecules, the odds are even more remote.

How is it possible to introduce antisense oligonucleotides into a cell or an organism? There are basically two choices: give the molecule as if it were a drug, or genetically engineer a gene encoding the antisense molecule into the organism. To genetically engineer an antisense molecule, a biotechnologist must synthesize a piece of DNA that encodes the proper sequence, then attach appropriate genetic traffic signals such as a promoter and a terminator (to tell RNA polymerase to start and stop making RNA), and then put the new gene into the organism. Once the artificial gene is introduced, the new host cells will synthesize the antisense RNA.

Is antisense technology being used for any practical applications? Yes. One genetically engineered food that will soon be available is the Flavr Savr tomato. The Flavr Savr is an "antisense tomato." It will be tastier than regular grocery store tomatoes because scientists have designed it so that large-scale growers can let the Flavr Savr ripen on the vine before harvesting it. Normal grocery store tomatoes are picked when they are green and hard. They are then shipped all over the country and turned red by exposure to ethylene gas. The tomatoes never develop ripe tomato flavor. The reason growers do not let the tomatoes ripen before picking them is that as they ripen, tomatoes become too soft for mechanical handling and a long shelf life.

Scientists at the biotechnology company Calgene, Inc., reasoned that if a tomato could ripen without softening, growers could let them ripen and develop flavor before picking them. They developed an antisense molecule for a gene encoding a softening enzyme. An artificial gene for the antisense RNA was constructed and introduced into tomato plants, where it prevents softening during ripening. Calgene claims the result is a much tastier grocery store tomato. You will soon be able to test this claim for yourself.

Classroom Activities

(#5) * Answer the 3 Review Questions

1) Explain the concept of **Antisense** technology?

2) Explain HOW and WHY the **Flavr Savr** tomato was designed using **Antisense**?

3) If an Antisense-based therapy were given to a human patient, how large (# of nucleotides) should the **Antisense** molecule be to avoid "hitting" any unintended targets ... EXPLAIN?