DNA Microarrays

Major refinements of the technology underlying DNA libraries, PCR, and hybridization have come together in the development of DNA microarrays (sometimes called DNA chips), which allow the rapid and simultaneous screening of many thousands of genes.

DNA segments from known genes, a few dozen to hundreds of nucleotides long, are amplified by PCR and placed on a solid surface, using robotic devices that accurately deposit nanoliter quantities of DNA solution.

Many thousands of such spots are deposited in a predesigned array on a surface area of just a few square centimeters.

Once the chip is constructed, it can be probed with mRNAs or cDNAs from a particular cell type or cell culture to identify the genes being expressed in those cells.

A microarray can answer such questions as which genes are expressed at a given stage in the development of an organism.

The total complement of mRNA is isolated from cells at two different stages of development and converted to cDNA, using reverse transcriptase and fluorescently labeled deoxynucleotides.

The fluorescent cDNAs are then mixed and used as probes, each hybridizing to complementary sequences on the microarray.

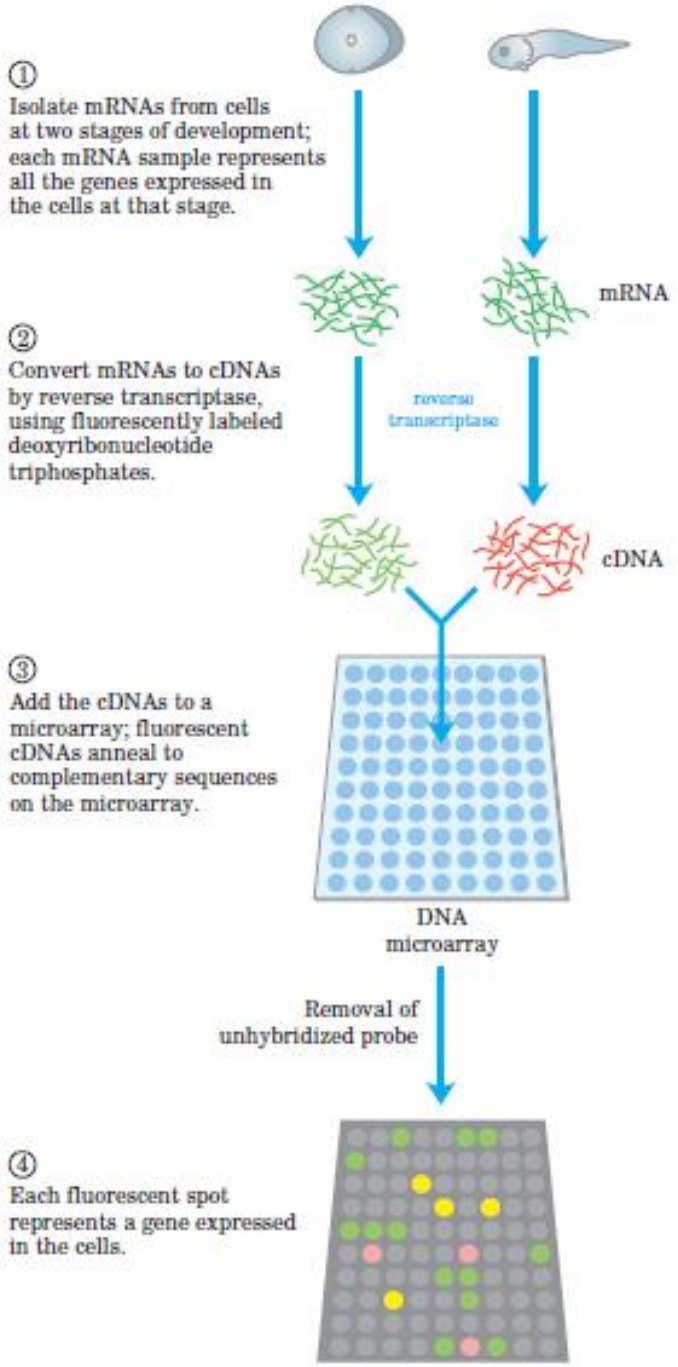
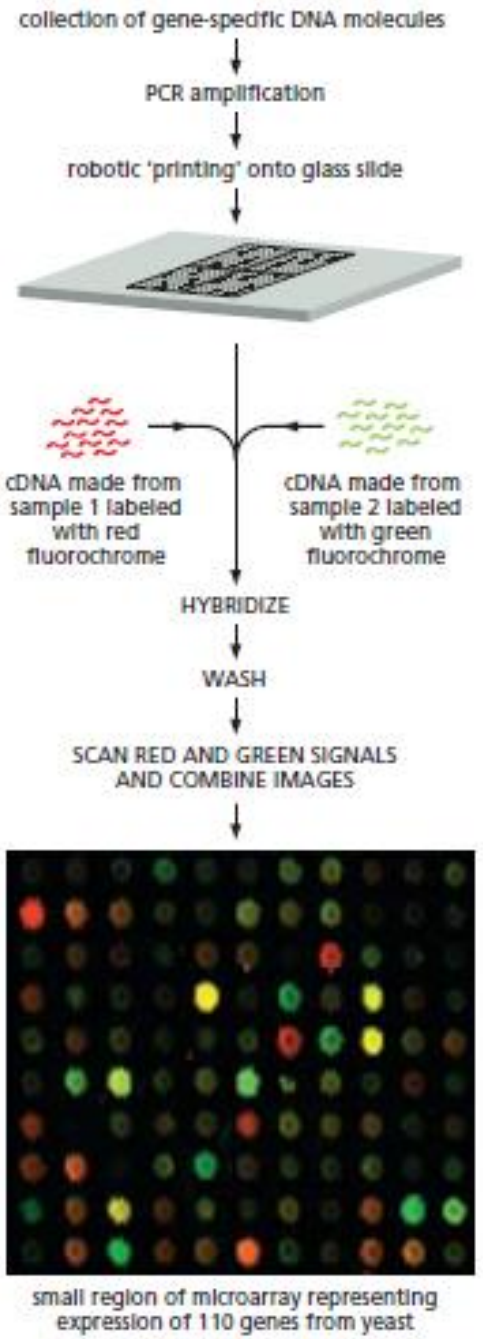


FIGURE 9–22 DNA microarray. A microarray can be prepared from any known DNA sequence, from any source, generated by chemical synthesis or by PCR. The DNA is positioned on a solid surface (usually specially treated glass slides) with the aid of a robotic device capable of depositing very small (nanoliter) drops in precise patterns. UV light cross-links the DNA to the glass slides. Once the DNA is attached to the surface, the microarray can be probed with other fluorescently labeled nucleic acids. Here, mRNA samples are collected from cells at two different stages in the development of a frog. The cDNA probes are made with nucleotides that fluoresce in different colors for each sample; a mixture of the cDNAs is used to probe the microarray. Green spots represent mRNAs more abundant at the single-cell stage; red spots, sequences more abundant later in development. The yellow spots indicate approximately equal abundance at both stages. Synthesizing an Oligonucleotide Array



New methods using **DNA microarrays** can simultaneously detect all the mRNAs present in a cell, thereby indicating which genes are being transcribed.

Such global patterns of gene expression clearly show that liver cells transcribe a quite different set of genes than do white blood cells or skin cells.

Changes in gene expression also can be monitored during a disease process, in response to drugs or other external signals, and during development

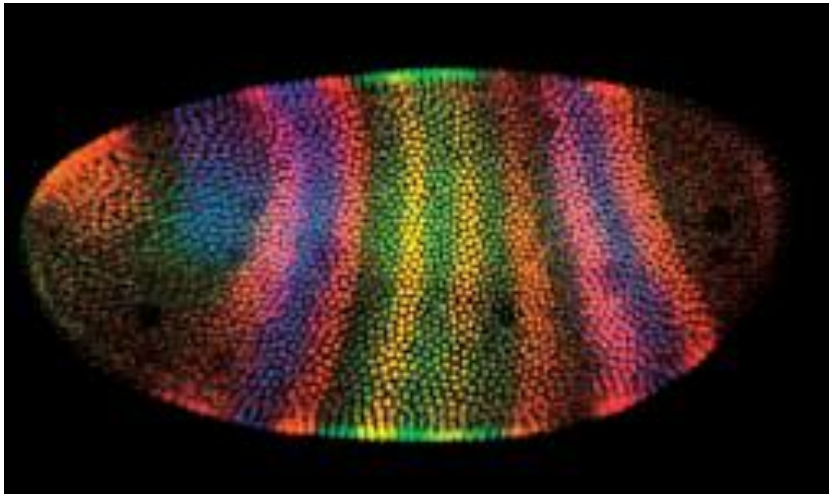
The microarray consists of tiny spots of DNA attached to a microscope slide.
Each spot contains many copies of a DNA sequence from a single human gene.

- Many of the differences among differentiated cells are due to production of specific sets of proteins needed to carry out the unique functions of each cell type.

- That is, only a subset of an organism's genes is transcribed at any given time or in any given cell.

- Such **differential gene expression at different** times or in different cell types occurs in bacteria, fungi, plants, animals, and even viruses.

- Differential gene expression is readily apparent in an early fly embryo in which all the cells look alike until they are stained to detect the proteins encoded by particular genes



RNA Interference Provides an Additional Tool for Disrupting Gene Expression

An extremely powerful tool for disrupting gene expression was that required the introduction of RNA into a cell.

The introduction of a specific double-stranded RNA molecule into a cell was found to suppress the transcription of genes that contained sequences present in the double-stranded RNA molecule.

Thus, the introduction of a specific RNA molecule can interfere with the expression of a specific gene.

When a double-stranded RNA molecule is introduced into an appropriate cell, the RNA is cleaved by an enzyme referred to as ***Dicer*** *into fragments* approximately 21 nucleotides in length.

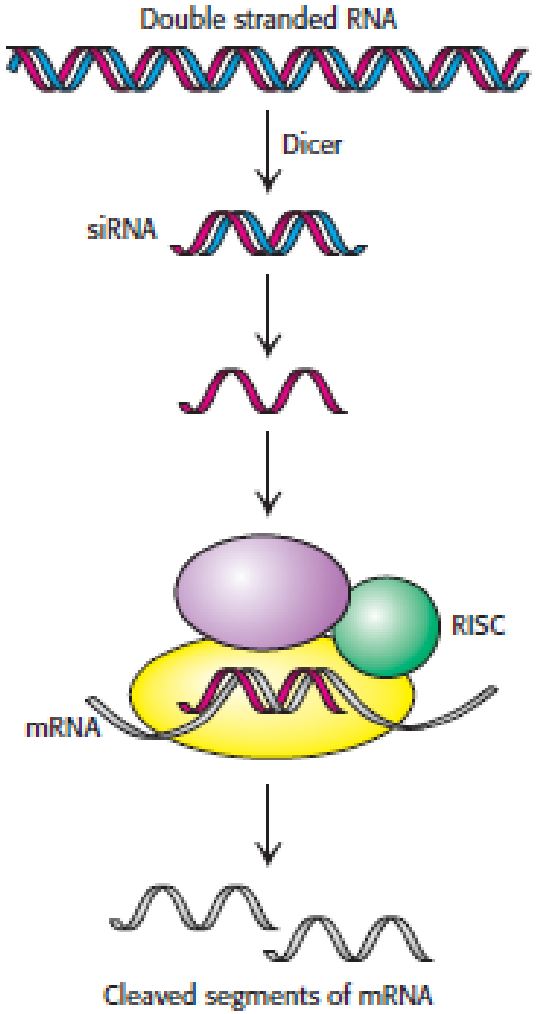


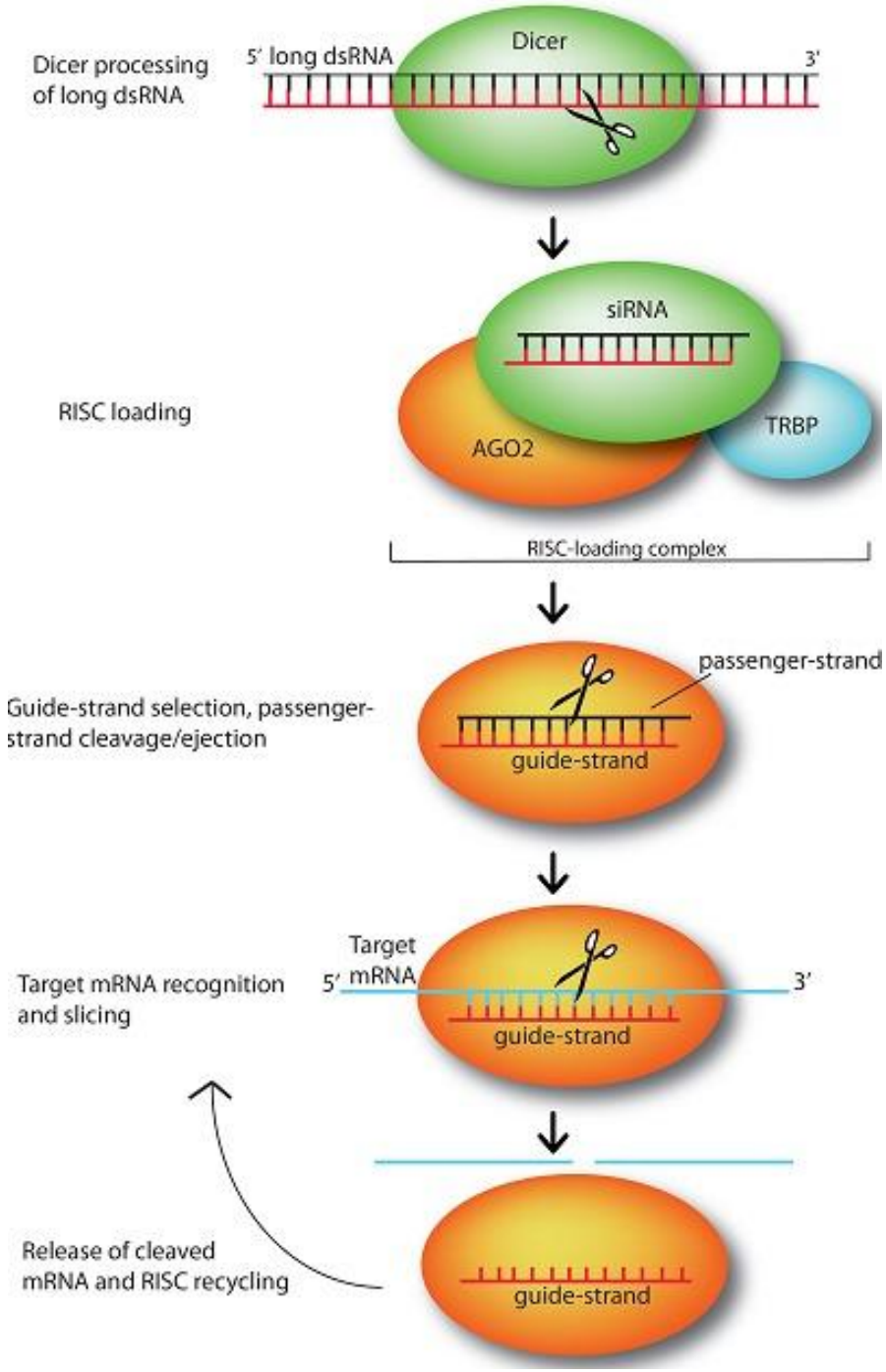
Figure 5.36 RNA interference mechanism. A double-stranded RNA molecule is cleaved into 21-bp fragments by the enzyme Dicer to produce siRNAs. These siRNAs are incorporated into the RNA-induced silencing complex (RISC), where the single-stranded RNAs guide the cleavage of mRNAs that contain complementary sequences.

Each fragment consists of 19 bp of double-stranded RNA and 2 bases of unpaired RNA on each 5 end.

After separation, the two single strands of the RNA molecule, termed small interfering RNAs (siRNAs), are each incorporated into a different enzyme referred to as ***RNA-induced silencing complex (RISC).***

The single stranded RNA segment incorporated into the enzyme acts as a guide that allows RISC to cleave mRNA molecules that include segments that are exact complements of the sequence.

Thus, levels of such mRNA molecules are dramatically reduced.


Dicer processing of long dsRNA
5′ long dsRNA
Dicer
3′
siRNA
RISC loading
AGO2
TRBP
RISC-loading complex
passenger-strand
Guide-strand selection, passenger-strand cleavage/ejection
guide-strand
Target mRNA
5′ mRNA
3′
Target mRNA recognition and slicing
guide-strand
Release of cleaved mRNA and RISC recycling
guide-strand

TRANSGENESIS IN *M. MUSCULUS*

Mice are the most important models for mammalian genetics.

Much of the technology developed in mice is potentially applicable to humans.

There are two strategies for transgenesis in mice, each of which has its advantages and disadvantages:

• ***Ectopic insertions****. Transgenes are inserted randomly in* the genome, usually as multicopy arrays.

• ***Gene targeting.*** *The transgene sequence is inserted into a* location occupied by a homologous sequence in the genome.

That is, the transgene replaces its normal homologous counterpart.

Recombinant DNA molecules can be introduced into animal cells in several ways.

DNA can be *microinjected into cells.*

A finetipped (0.1-mm-diameter) glass micropipet containing a solution of foreign DNA is inserted into a nucleus. A skilled investigator can inject hundreds of cells per hour.

About 2% of injected mouse cells are viable and contain the new gene.

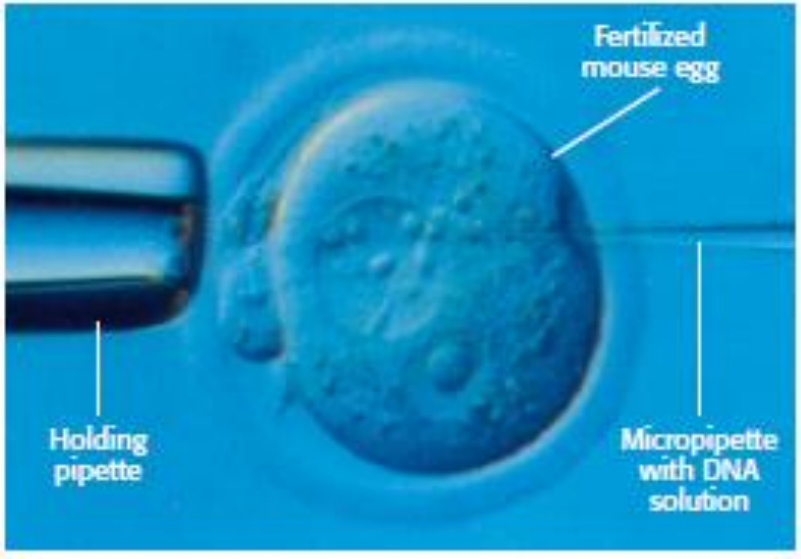


Figure 5.31 Microinjection of DNA. Cloned plasmid DNA is being microinjected into the male pronucleus of a fertilized mouse egg.

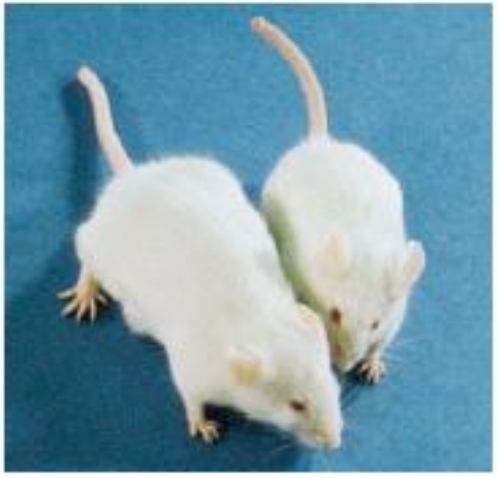


Figure 5.32 Transgenic mice. Injection of the gene for growth hormone into a fertilized mouse egg gave rise to a giant mouse (left), about twice the weight of his sibling (right). [Courtesy of Dr. Ralph Brinster.]