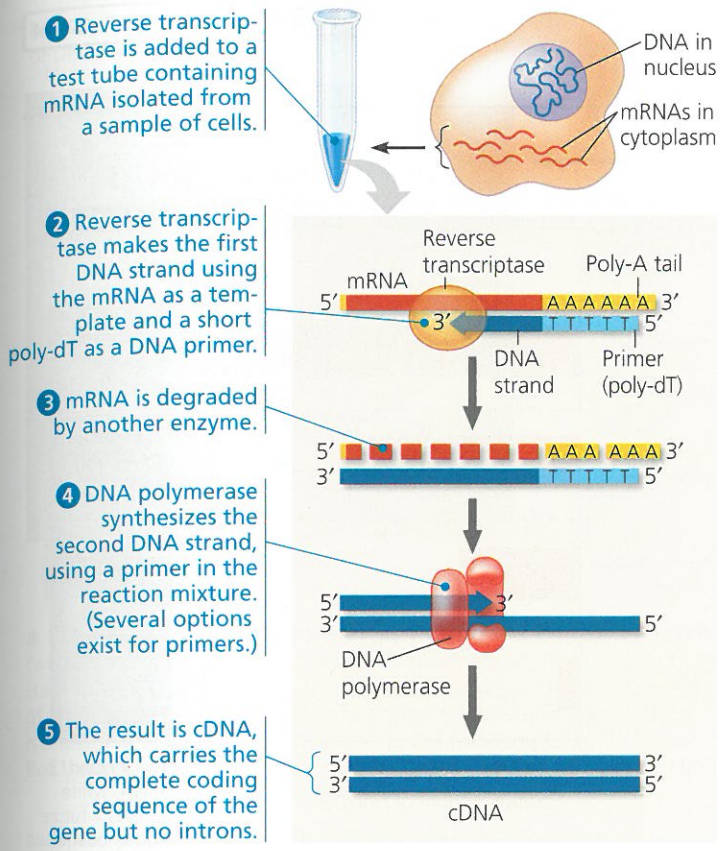


▲ Figure 20.11 Making complementary DNA (cDNA) from eukaryotic genes. Complementary DNA is made *in vitro* using mRNA as a template for the first strand. The mRNA contains only exons, so the resulting double-stranded cDNA carries the continuous coding sequence of the gene. Only one mRNA is shown here, but the final collection of cDNAs would reflect all the mRNAs present in the cell sample. Figure 20.12 shows how the cDNA of interest is identified.

providing quantitative data, a distinct advantage. RT-PCR can also be carried out with mRNAs collected from different tissues at one time to discover which tissue is producing a specific mRNA.

In the **Scientific Skills Exercise**, you can work with data from an experiment that analyzed expression of a gene involved in paw formation in the mouse. The study investigated mRNA expression using two techniques. One of these methods was qualitative (*in situ* hybridization), whereas the other approach was quantitative (PCR).

Studying the Expression of Interacting Groups of Genes

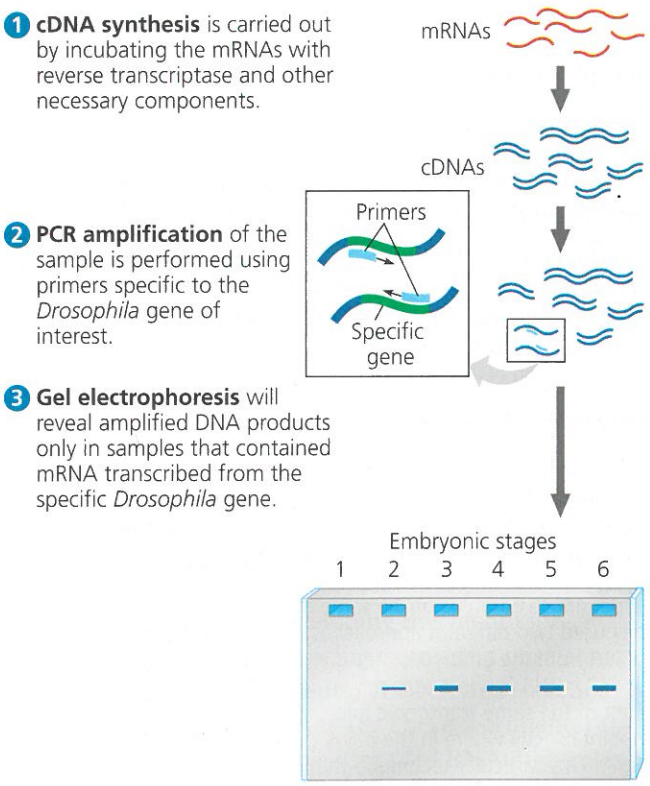
A major goal of biologists is to learn how genes act together to produce and maintain a functioning organism. Now that the genomes of a number of species have been sequenced, it is possible to study the expression of large groups of genes—the so-called *systems approach*. Researchers use what is known about the whole genome to investigate which genes are transcribed in different tissues or at different stages of development. One aim is to identify networks of gene expression across an entire genome.

▼ Figure 20.12 Research Method

RT-PCR Analysis of the Expression of Single Genes

Application RT-PCR uses the enzyme reverse transcriptase (RT) in combination with PCR and gel electrophoresis. RT-PCR can be used to compare gene expression between samples—for instance, in different embryonic stages, in different tissues, or in the same type of cell under different conditions.

Technique In this example, samples containing mRNAs from six embryonic stages of *Drosophila* were analyzed for a specific mRNA as shown below. (In steps 1 and 2, the mRNA from only one stage is shown.)



Results The mRNA for this gene first is expressed at stage 2 and continues to be expressed through stage 6. The size of the amplified fragment (shown by its position on the gel) depends on the distance between the primers that were used (not on the size of the mRNA).

Genome-wide expression studies can be carried out using **DNA microarray assays**. A DNA microarray consists of tiny amounts of a large number of single-stranded DNA fragments representing different genes fixed to a glass slide in a tightly spaced array, or grid. (The microarray is also called a *DNA chip* by analogy to a computer chip.) Ideally, these fragments represent all the genes of an organism.

The basic strategy in such studies is to isolate the mRNAs made in a cell of interest and use these molecules as templates for making the corresponding cDNAs by reverse transcription. In microarray assays, these cDNAs are labeled with fluorescent molecules and then allowed to hybridize to a DNA microarray. Most often, the cDNAs from two