# Regulation of Gene Expression and Development in Eukaryotes


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An amoeba is not likely to be confused with an *E. coli* cell. The phenotype of a corn plant is very different from the phenotype of an oak tree. Different human beings, excluding identical twins, can usually be readily distinguished. These different phenotypes, we know, result from different genes and different forms of genes (different alleles) in the genomes of various organisms and individuals.

Similarly, if we microscopically examine the nerve cells, kidney cells, liver cells, bone cells, blood cells, hair cells, skin cells, and so forth of any one organism or any one individual, we find vast differences in the phenotypes of these cells (see Chapter 1, Fig. 1.6). Some are short and fat; some are long and thin; some have appendages; and others are roughly spherical. Yet all of these cells (with a few notable exceptions) contain the same set of genes. All of them are produced from a single cell, the zygote in sexually reproducing species, by purely equational mitotic divisions. How, then, can their highly diverse phenotypes be explained?

At present, this question cannot be answered in detail. The superficial answer is that not all the genes present in a nucleus are ever expressed in a given cell at the same time. Furthermore, in differentiated cells of higher eukaryotes, only a small proportion (less than 10 percent) of the genes are ever expressed in any one tissue at any given point in time.

Nevertheless, detailed answers to several aspects of this question are emerging rapidly in the case of the fruit fly *Drosophila melanogaster* and the tiny worm *Caenorhabditis elegans*. Many of the *key genes that regulate specific patterns of morphogenesis, both spatial and temporal, have been identified* in these two model systems, and an elegant picture of the genetic control of development is beginning to emerge. This picture already shows that *development of a fly or a worm unfolds under the control of a cascade of regulatory genes* each in turn adding new pattern information to the blueprints that were in place at the time of the expression of that particular factor. By sequential rounds of regulatory gene fine-tuning, each group of cells produces the appropriate tissue or organ of each individual. In the end, each fly has the appropriate organs in the proper places: antennae and eyes on the head, legs and wings on the thorax, and genitals on the abdomen. However, mutations in key regulatory genes can destroy the normal pattern of development and produce flies with leglike structures where antennae should occur and so on. By studying the function of such mutant genes and their wild-type alleles, geneticists have discovered that many of these genes encode *trans*-acting regulatory proteins that bind to *cis*-acting regulatory regions of sets of genes and control their expression—in some cases turning them on and in other cases turning them off. Frequently, one or more of the genes that are activated by the original regulatory gene-product will in turn produce a *trans*-acting regulatory protein that turns on another set of genes. Development and cell differentiation thus result from the action of a cascade of key regulatory genes. Recently, the products of many of these key regulatory genes have been shown to be transcriptional activators that bind to the promoters or other regulatory sequences of the structural genes that they regulate. A molecular map of the process of development in higher animal model systems is thus rapidly emerging. Indeed, it seems likely that the important features of this map will be clearly established within the next few years.

As was discussed in Chapter 14, certain "housekeeping" genes such as those encoding ribosomal RNAs, ribosomal proteins, and transfer RNAs are necessarily expressed at some time in all cells. However, many other genes are apparently expressed for only a short period of time in one or a few cell types at a specific stage in development. In other words, the *expression of genes is regulated, and the coordinated regulation of sequential pathways of gene expression is primarily responsible for the diversity of cell phenotypes that unfold during the development of a higher plant or animal.*

## CELLULAR DIFFERENTIATION IN HIGHER EUKARYOTES

During the development of a higher eukaryote, a single cell—the zygote—gives rise by mitotic cell divisions to a vast array of cell types (in animals, skin cells, nerve cells, bone cells, blood cells, etc.) with highly divergent morphologies (Fig. 15.1) and macromolecular compositions. These different cell types are often highly specialized, carrying out only a few specific metabolic functions. For example, red blood cells are highly specialized for the synthesis and storage of hemoglobin. Over 90 percent of the protein molecules synthesized in red blood cells during their period of maximal biosynthetic activity are hemoglobin chains. Nerve cells are apparently the only cells capable of synthesizing neurotransmitters. What, then, is the mechanism by which these various cells differentiate

(Photo at left) Expression of the *Escherichia coli* β-glucuronidase coding sequence in flowers of transgenic *Arabidopsis* plants when regulated by (left) no promoter, (center) the 35S promoter of cauliflower mosaic virus, and (right) the promoter of the α1-tubulin gene of *Arabidopsis*. In the histochemical assay employed, β-glucuronidase converts the colorless substrate 5-bromo-4-chloro-3-indolyl glucuronide (X gluc) to a product that undergoes oxidative dimerization to form an insoluble blue indigo dye or pigment. (Photographs courtesy of Jeffrey L. Carpenter, D. Peter Snustad, and Carolyn D. Silflow, Department of Genetics and Cell Biology, Univ. of Minnesota, St. Paul.)

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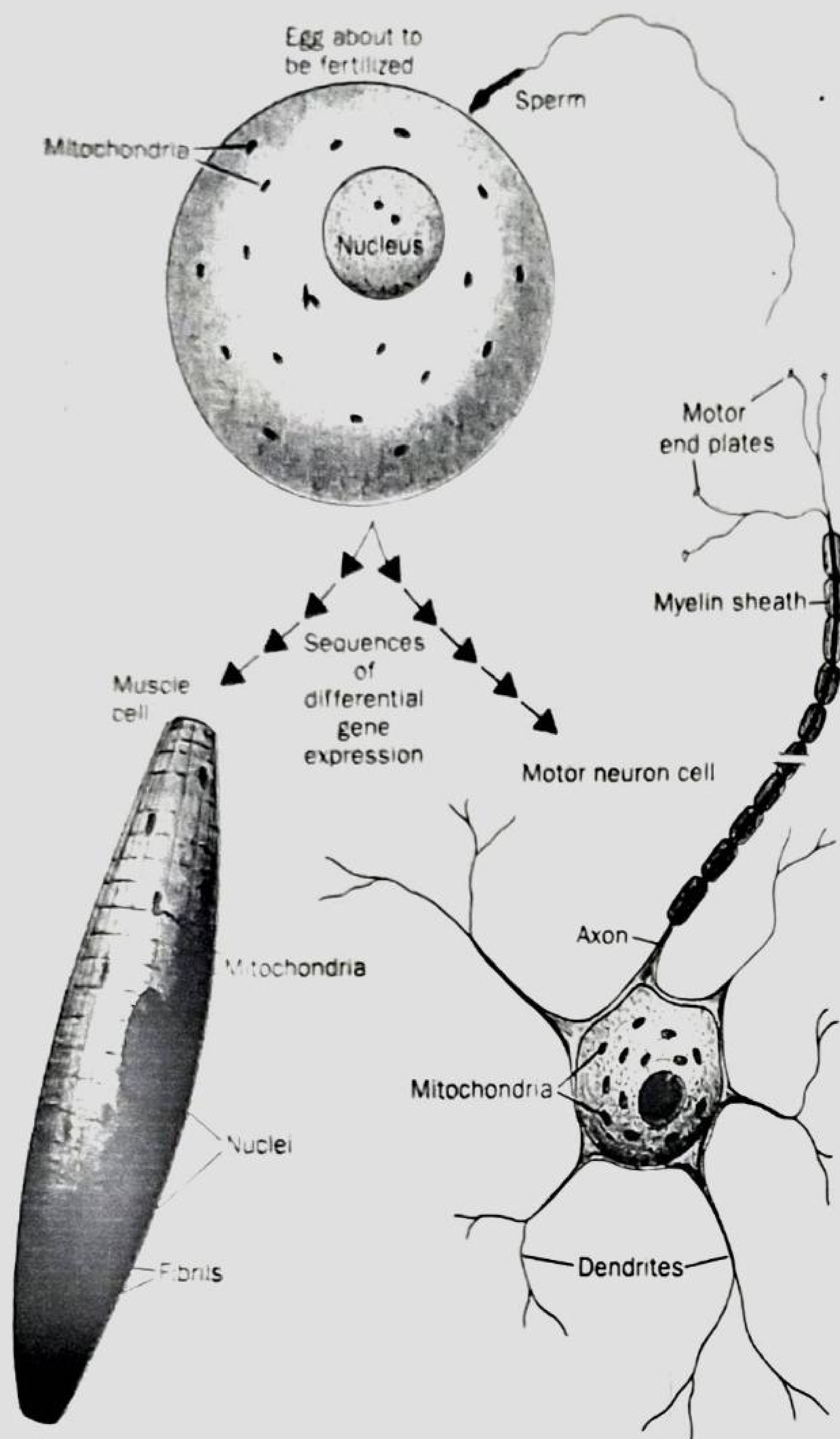


***Figure 15.1*** Schematic drawing illustrating the dramatic effects of differential gene expression during development in higher animals. A single cell, the fertilized egg or zygote, gives rise to the vast array of distinct cell phenotypes in the adult organism. The striated muscle and motor neuron cells shown are only two of the many cell types that exhibit highly divergent phenotypes in a higher animal such as a human being.

from one another during the growth and development of an organism? By what mechanism is the expression of the hemoglobin genes in red blood cells or the neurotransmitter genes in neurons, and the lack of expression of these genes in other cell types, brought about?

The only definitive answer that can be given to these questions at present is that the expression of these genes is controlled, at least in part, at the levels of transcription and transcript processing. Hemoglobin mRNA molecules are present in red blood cells, but are absent in other types of cells not synthesizing hemoglobin.

Differentiation occurs by the regulation of gene expression, rather than by changes in genome composition. This has been demonstrated by various techniques in many different organisms. In amphibians, for example, nuclei from differentiated cells can be transplanted into enucleated eggs (eggs from which the original nuclei have been removed) and shown to direct the development of normal embryos. Thus none of the genetic information required for the normal development of entire embryos is lost during the differentiation of the amphibian nuclei-donor cells. In addition, biochemical analyses of the DNA from the nuclei of various differentiated cells have in almost all cases shown that the genomes contain the same set of nucleotide-pair sequences. Rare exceptions are known. Mammalian red blood cells, for example, extrude their nuclei during the last stages of differentiation. By this time, large intracellular pools of stable hemoglobin mRNA have already been synthesized, so that the nuclei are no longer needed.

During the development of a complex plant or animal, gene expression has been shown to be regulated in different instances at essentially all possible levels—transcription, pre-mRNA processing, mRNA transport, mRNA stability, translation, posttranslational protein processing, protein stability, and enzyme function. However, extensive data now indicate that gene expression is regulated primarily at the levels of transcription and pre-mRNA processing. Clearly, regulation does occur at other levels as well. Regulatory fine-tuning at translational levels is obviously important in the overall control of metabolic processes in living organisms. However, the regulatory mechanisms with the largest effects on phenotype have been shown to act at the levels of transcription and RNA processing.

Many of the developmental processes in higher eukaryotes seem to be controlled, at least in part, by *preprogrammed circuits of gene expression.* In these cases, some event (such as release of a hormone in the bloodstream or fertilization of an egg) triggers the expression of one set of genes. The product (or products) of one (or more) of these genes functions by turning off the transcription of the first set of genes and/or turning on the transcription of a second set of genes. In turn, one or more of the products of the second set acts by turning on a third set, and so on. In these cases, the sequential expression of genes is genetically preprogrammed, and the genes *cannot* usually be turned on out of sequence.

In eukaryotes, we know that hormones can trigger the sequential expression of sets of genes. In addition, we know that regulatory genes are involved in the control of patterns of differentiation. In some cases, we know that regulatory elements called *enhancers* and *silencers* modulate levels of gene expression from nearby promoters. However, the question of how

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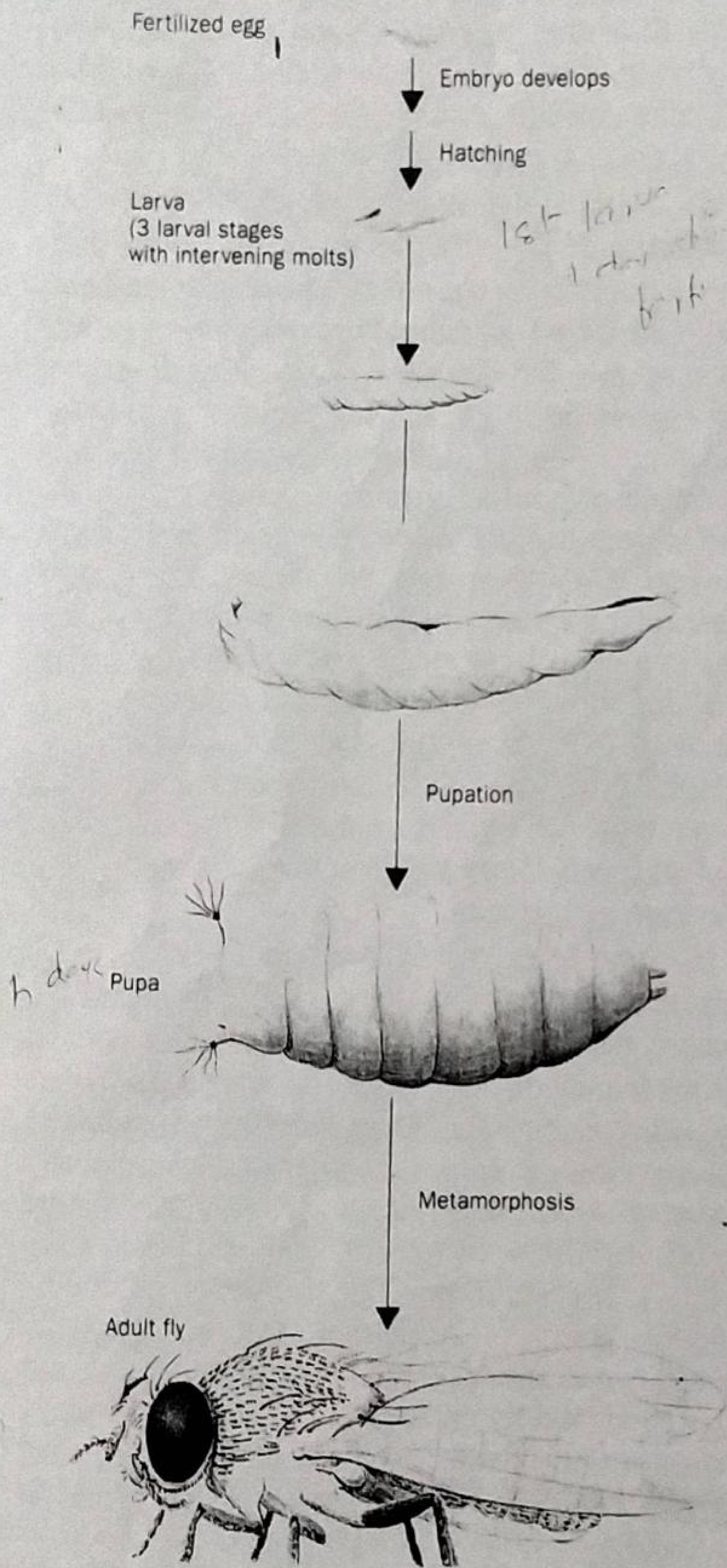


**Figure 15.2** Stages of development in *Drosophila*. Embryonic development (see Fig. 15.3) of the fertilized egg occurs very rapidly. The first larval stage hatches within about one day after fertilization. Pupation occurs at about five days postfertilization, and metamorphosis of the adult fly occurs at about nine days postfertilization.

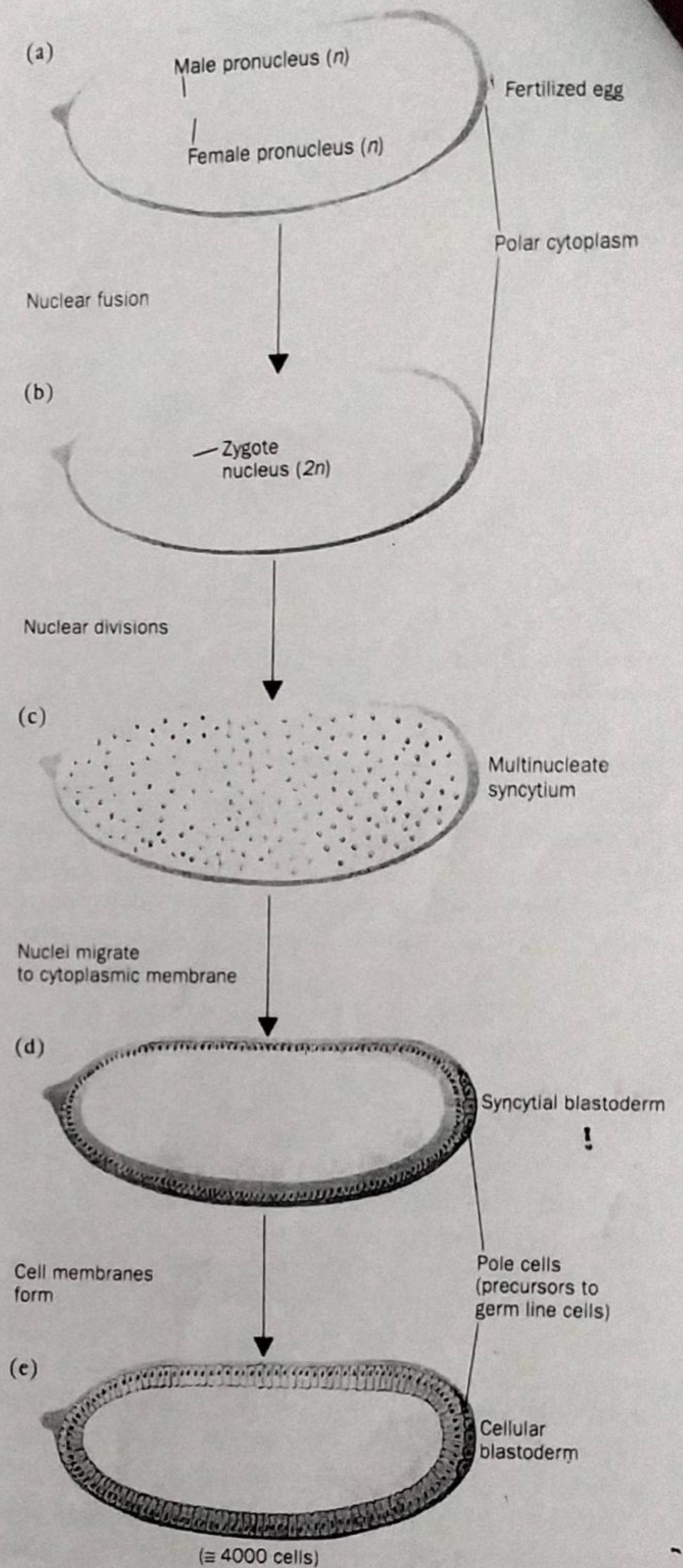


**Figure 15.3** Embryonic development in *Drosophila*. (a) The fertilized egg initially contains two haploid ($n$) nuclei, one from the egg and one from the sperm. (b) The two haploid nuclei then fuse to form the diploid nucleus ($2n$) of the zygote. (c) The zygotic nucleus divides very rapidly to produce a single cell (syncytium) with many nuclei. (d) After about 9 nuclear divisions have produced about 512 nuclei, the nuclei migrate to the periphery of the syncytium and continue to divide (about 4 more times each). A few nuclei migrate into the polar cytoplasm and form pole cells, which are the progenitors of all the germ line cells of the fly. (e) Cell membranes then form around the nuclei to produce the cellular blastoderm, composed of about 4000 cells. (After V. E. Foe and B. M. Alberts, *J. Cell Sci.* 61: 31–70, 1983.)

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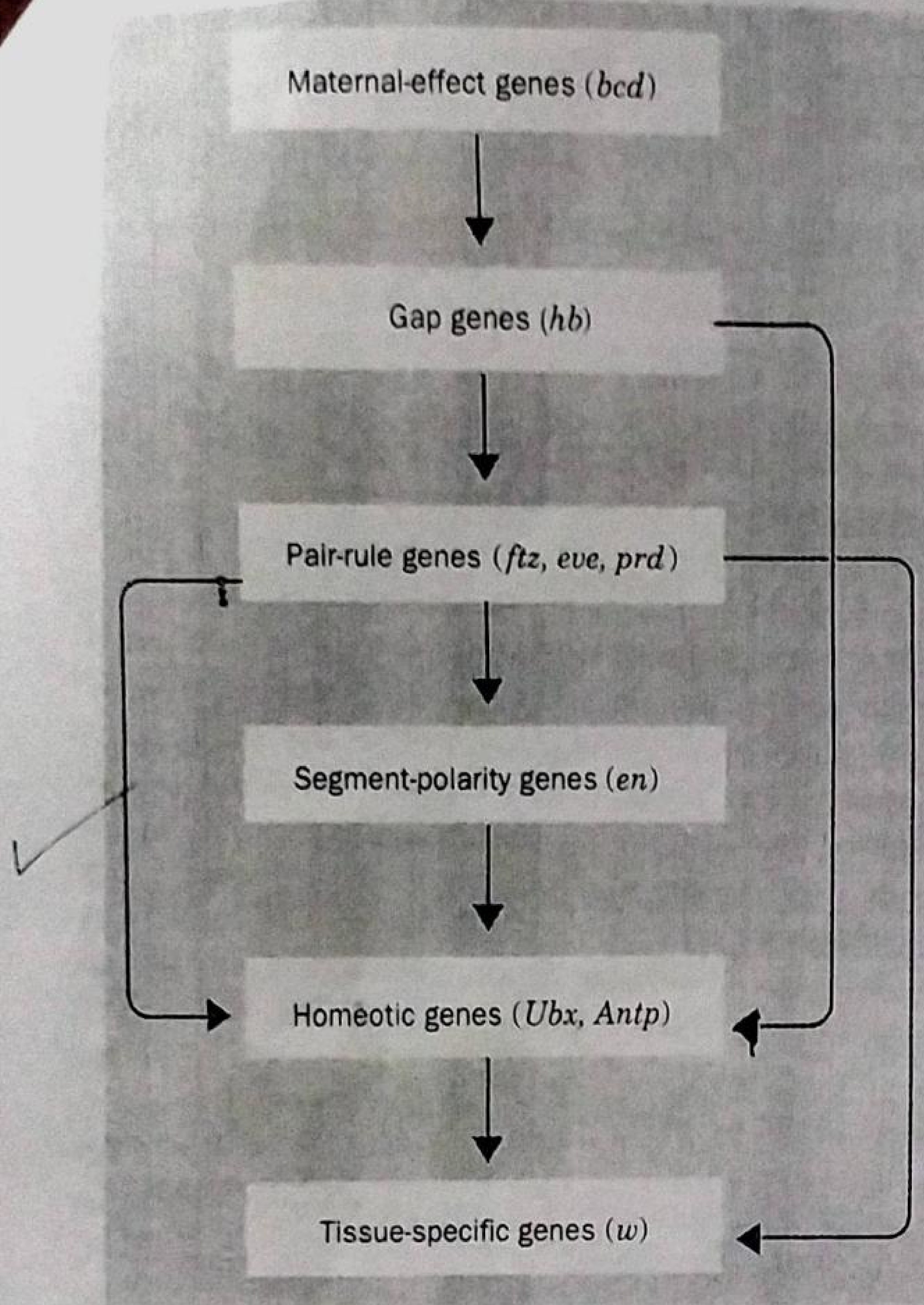


***Figure 15.14*** Regulatory cascade in the control of development in *Drosophila*. See Fig. 15.11 for the roles of the indicated classes of genes. Each arrow indicates a known pathway of gene regulation in which the product of one or more genes in one class affects the expression of one or more genes in another class. (After M. D. Biggin and R. Tjian, *Trends in Genetics* 5: 377–383, 1989.)

binds to the *hb* promoter in a sequence-specific manner (binding site TCCTAATCC). Other *Drosophila* pattern-determining genes that have been shown to encode proteins with sequence-specific binding properties are *Antennapedia (Antp), engrailed (en), Abdominal-B (Abd-B*$^r$*), even-skipped (eve), fushi tarazu (ftz), paired (prd), Ultrabithorax (Ubx),* and *zerknullt (zen).* In some cases, the gene-products bind to the same promoter sequences. For example, the proteins encoded by *Abd-B*$^r$*, en, eve, ftz, prd,* and *zen* all bind to the sequence TCAATTAAAT. Interestingly, some of these proteins also bind to other related sequences. In most cases, they stimulate transcription; however, in other cases, they repress transcription.

In the sequence of events in anterior–posterior segmentation in *Drosophila* embryos (Fig. 15.11), there are many examples where a gene of a class expressed early produces a sequence-specific transcription factor that controls the expression of a regulator gene that acts later in the pathway. Fig. 15.14 shows some of the known transcriptional regulators that are part of this regulatory cascade. It seems likely that other genes that control pattern formation in *Drosophila* will be shown to be part of this regulatory cascade in the future. If the transcription of regulatory genes in a morphogenetic pathway requires the concerted action of two or more sequence-specific, *trans*-acting regulators, one can easily visualize how complex patterns of gene expression could result (Fig. 15.15).

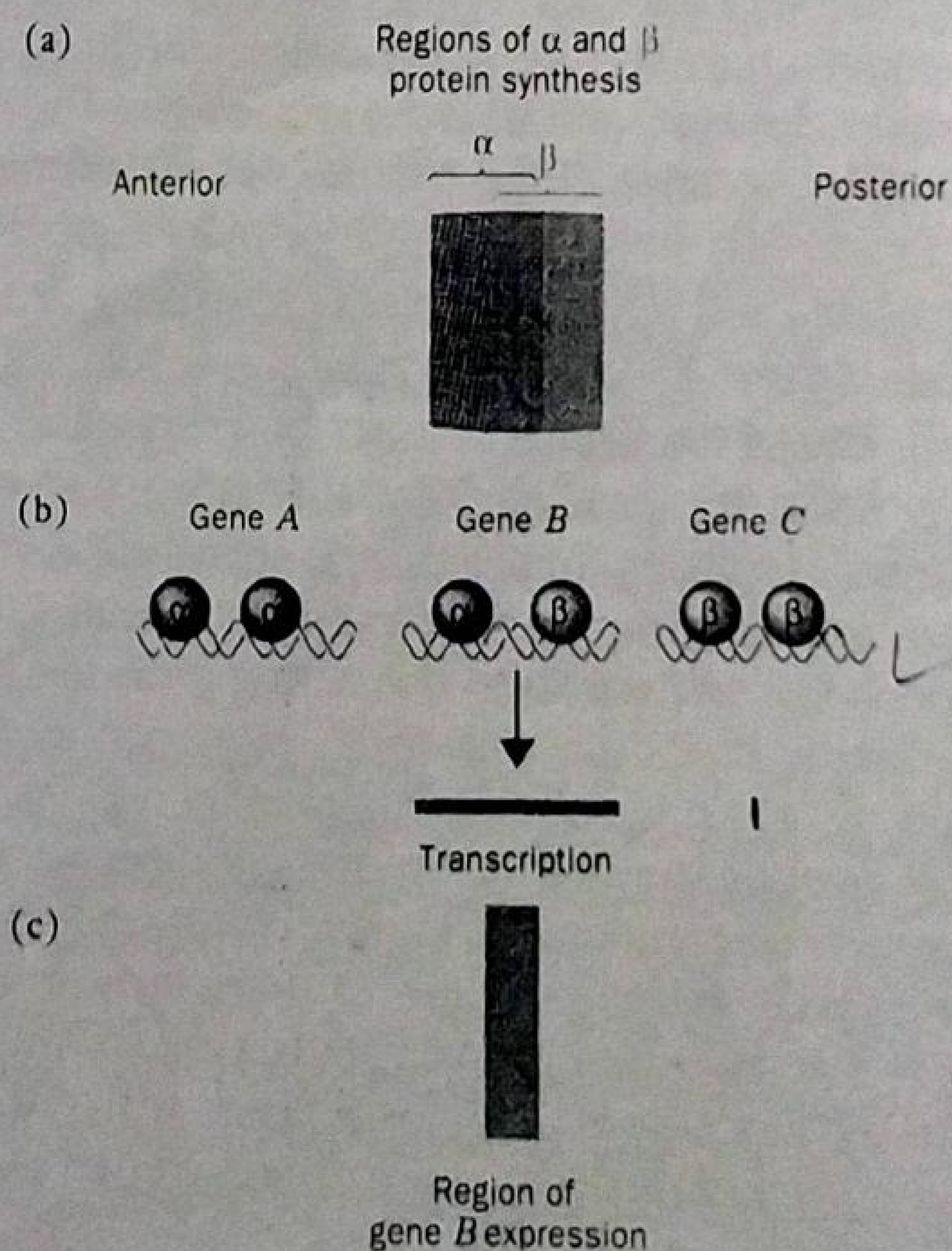


***Figure 15.15*** Pattern formation based on the effects of two different sequence-specific transcription activators synthesized in overlapping segments of an embryo. The region in which the α activator is synthesized is shown shaded in red. The region containing the β activator is shown shaded in purple. The gene *B* promoter requires both activators, α and β. By adding additional transcription regulators, both activators and repressors of transcription, and additional promoter binding sites, one can produce complex patterns of gene expression. (After M. D. Biggin and R. Tjian, *Trends in Genet.* 5: 377–383, 1989.)

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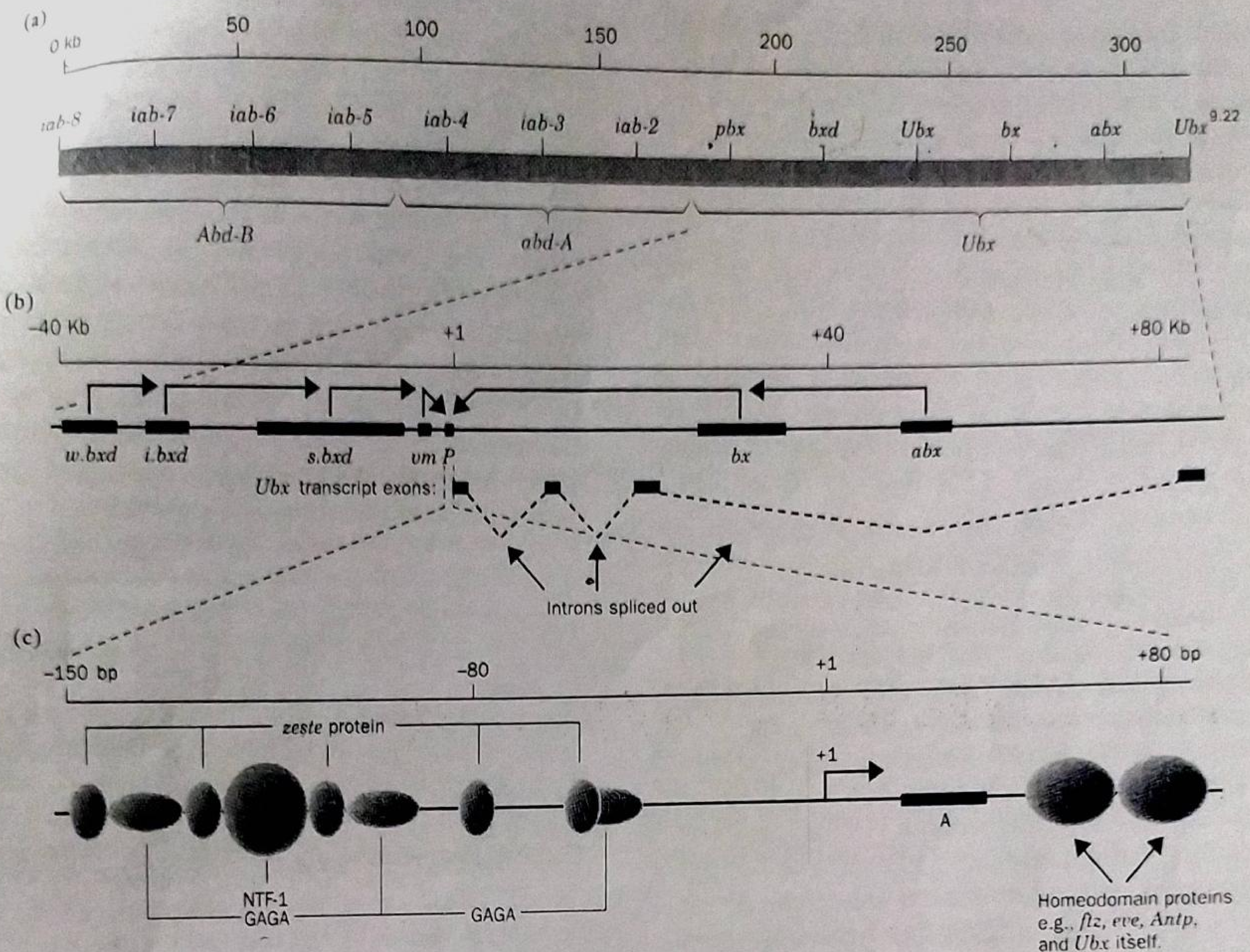


***Figure 15.16*** Structure of the *bithorax* complex (BX-C) of homeotic mutations. The locus includes three complementation groups and spans 300,000 nucleotide-pairs (300 kb) on chromosome 3 of *D. melanogaster*. Mutations within BX-C affect thorax segments T2 and T3 and abdomen segments A1 through A8 (see Fig. 15.6). (a) Map of BX-C showing the locations of some of the classical homeotic mutations and the positions of three complementation groups: *Ubx, abd-A,* and *Abd-B*. The map is oriented such that transcription of the major *Ubx* transcription unit occurs from left to right. (After E. B. Lewis, *Cold Spring Habor Sympos. Quant. Biol.* 50: 155–164, 1985.) (b) Enlarged map of the *Ubx* major transcription unit showing the positions of *cis*-acting regulatory sequences that have been identified by correlated genetic and *in vitro* transcription studies. Sites *w.bxd, i.bxd, s.bxd,* and *vm* are four distinct *cis*-acting sequences within the *bxd* region; *P* is the *Ubx* major promoter. The exon and intron regions of the primary transcript and the splicing pattern are indicated below the map (in green). (c) Map of known protein binding sites within the *Ubx* major promoter. ***Note*** that the ***scale in (c) is in base-pairs,*** whereas the ***scales in (a) and (b) are in kilobase-pairs.*** Again, +1 represents the 5′ base of the primary transcript. The *zeste* gene has been known to interact with other *Drosophila* genes for several decades. NTF-1 and GAGA are known transcription factors in *Drosophila;* NTF-1 = neurogenic transcription factor 1; GAGA = protein transcription factor that binds to promoter sequence CGAGAGAG. The morphogenic genes *ftz, eve,* and *Antp* are described in the text. The box labeled A is known to be an important *cis*-acting regulatory sequence, but the protein(s) that binds to it is not yet known. [(b, c) After M. D. Biggin and R. Tjian, *Trends in Genet.* 5: 377–383, 1989.]

more divergent homeobox with only 45 percent sequence conservation when compared to the *Antennapedia (Antp)* group of homeoboxes. In all cases, the homeobox lies within the coding region of the gene and specifies a 60-amino acid segment, called the *homeodomain,* of the polypeptide product.

Once it became clear that many of the homeodomain-containing proteins were transcription regulators that functioned via sequence-specific binding to DNA *cis*-control elements, attention was directed at the role of the conserved homeodomain regions of the proteins. When *in vitro* studies showed that several different homeobox proteins would bind specifically to the same consensus sequence, namely, TCAGCACCG, researchers began to look for evidence that the homeodomains of these proteins were directly in-

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genesis of *C. elegans* has been extremely rapid since that time. Only *D. melanogaster* rivals *C. elegans* as a model system for the study of animal development, with each system having some advantages. *Caenorhabditis elegans,* called "the worm" by the large group of *C. elegans* researchers, is particularly suited to correlated genetic and ultrastructural dissection. Its major advantages as a system are given in the following list.

1. *Small size*— the adult worms are about 1 mm in length.
2. *Short generation time*—reproduces with a 3-day life cycle under optimal growth conditions.
3. *Simple growth conditions*—grows on agar plates with *E. coli* as food.
4. *Two sexes: hermaphrodites and males*—the existence of hermaphrodites (male and female sex organs in the same worm) allows geneticists to produce worms homozygous for newly induced mutations very easily by simply allowing self-fertilization, just as Mendel allowed his pea plants to self-pollinate.
5. *Large numbers of progeny*—a single hermaphrodite will produce about 300 eggs during its life.
6. *Small genome*—about $8 \times 10^7$ nucleotide-pairs, which greatly facilitates molecular analyses such as cloning, sequencing, and physical mapping of the genome.
7. *No exoskeleton, transparent bodies*—all cells of the body can be observed directly with the light microscope (Nomarski optics) at all stages of development.
8. *Precise and invariant developmental program*—each adult hermaphrodite is composed of exactly 945 cells and these are produced from the zygote by precise pathways of cell division, cell migration, cell growth, and cell death; these are called *invariant cell lineages.*

The transparent body of *C. elegans* has permitted J. E. Sulston and coworkers to establish the entire cell lineage for the adult worm from the single-celled zygote, an accomplishment that would be impossible in almost any other higher animal. Now, a small army of developmental geneticists are dissecting this pathway at the molecular, cellular, and morphological levels. The dominant approach is induce mutations, select those of the desired type, and use biochemical and microscopic tools to define the resulting blocks in the pathway. The final result of their efforts will be a molecular map of the morphogenesis of "the worm" just like that worked out for phage T4 in earlier studies (see Chapter 11, Fig. 11.33).

## NEOCLASSICAL EXAMPLES OF DEVELOPMENTALLY REGULATED GENE EXPRESSION

The recent explosion of new information about the regulation of gene expression in eukaryotes that has resulted from the application of recombinant DNA and gene cloning technologies should not cause us to overlook some of the elegant neoclassical studies of developmentally regulated gene expression. The striking pictures of transcription on lampbrush chromosomes and of the amplification of ribosomal RNA genes in amphibian oöcytes are two important examples of developmentally regulated gene expression that predated the recombinant DNA epoch of biology.

### Transcription on Lampbrush Chromosomes in Amphibian Oöcytes

In all higher organisms studied so far, the fertilization of a mature egg by a sperm triggers a dramatic increase in protein synthesis, followed by the rapid nuclear and cell divisions of early cleavage stages of embryogenesis. In most eukaryotes, this protein synthesis is not accompanied by RNA synthesis. Instead, all the components required for protein synthesis during early embryogenesis are present in the egg prior to fertilization. Gene transcripts, in the form of mRNA or pre-mRNA molecules, must therefore be stored in the egg in a dormant state. Translation of these preformed mRNA molecules must somehow be triggered by events associated with fertilization.

Therefore, the informational molecules that direct protein synthesis during the early cleavage states following fertilization must be synthesized during oögenesis. Studies of oögenesis in vertebrates, particularly amphibians, reveal that extensive transcription occurs during prophase I (specifically diplotene) of meiosis. During this stage, the chromosomes exist as large *lampbrush* structures. (The structure of these oöcyte *lampbrush chromosomes* is described in Chapter 6, pp. 134–135).

Most of the DNA in lampbrush chromosomes exists in a highly condensed, transcriptionally inactive state in the so-called axial regions of the chromosomes. Certain segments of the DNA in each lampbrush chromosome, however, exist in highly extended lateral loops (see Figs. 6.6 and 6.7). Each loop consists of a central molecule of DNA that is surrounded by a matrix of newly synthesized RNA and protein. By means of pulse-labeling with [$^3$H]uridine and autoradiography, the loops of lampbrush chromosomes have been shown to be regions of active transcription. The lampbrush chromosomes of oöcytes thus appear to be an excellent example of the correlation between structure and function—the lampbrush morphology being the structural correlate of the transcription of a specific set of chromosomal genes. The transcribed genes of the lampbrush chromosomes apparently are those whose products are required during the early stages of embryogenesis. The gene transcripts synthesized dur-

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ing oögenesis must be stored in an *inactive but stable form* (possible in RNA–protein complexes) until fertilization occurs. Clearly, regulatory mechanisms are involved that act at a posttranscriptional (mRNA processing?) or translational level.

In addition, particular gene transcripts and/or other gene-products must become *localized* in specific areas of the egg cytoplasm during oögenesis. This is evident from experiments that show that the destiny of a particular cell depends on the section of the egg cytoplasm that the cell receives in the early cleavage divisions.

In amphibians, then, and probably in most vertebrates, the genetic programs controlling early development (up to about the blastula stage) are established during oögenesis. Later stages of development, when cell differentiation begins (from about the gastrula stage on), require new programs of gene expression.

### rRNA Gene Amplification in Amphibian Oöcytes

Despite the rapid initiation of protein synthesis following fertilization, no rRNA is synthesized in amphibian embryos until the gastrula stage. This means that large amounts of rRNA must also be synthesized during oögenesis. In fact, the large eggs of amphibians contain vast quantities of ribosomes, on the order of $10^{12}$ per mature egg. The requirement for the synthesis of such enormous amounts of a particular gene transcript (the 40S amphibian rRNA precursor) in a single cell has resulted in the evolution of a novel mechanism of *specific gene amplification*. In amphibian oöcytes, the rRNA genes are selectively amplified about a thousandfold to facilitate the synthesis of the huge quantities of rRNA stored in mature eggs.

As discussed in Chapter 10, the rRNA genes are normally present as tandemly repeated copies located within the nucleolar organizer regions of the chromosomes. D. Brown, J. Gurdon, and colleagues have shown that there are about 500 copies of the rRNA gene in each of the two nucleolar organizer regions of diploid nuclei of *Xenopus laevis*. The rRNA precursors are synthesized and processed in the nucleoli.

Given that about 1000 rRNA genes exist per diploid nucleus, it has been estimated that over 450 years would be required to synthesize the large number of rRNA molecules present in mature *Xenopus* eggs, hardly a plausible situation given the average life expectancy of a toad. This potential dilemma has been resolved by the evolution of a mechanism by which the rRNA genes are selectively replicated in oöcytes.

D. Brown and I. Dawid have shown that the nuclei of oöcytes of *Xenopus laevis* contain hundreds of nucleoli (Fig. 15.18), each containing circular DNA molecules carrying tandemly repeated copies of the rRNA gene. Once formed, these extrachromosomal DNA molecules appear to replicate by the rolling circle mechanism (Chapter 5, pp. 122–124). However, how the nucleolar regions of the chromosomes are selectively replicated to produce the first extrachromosomal DNA molecules is not yet established.

*Figure 15.18* Photomicrograph of a nucleus from an oöcyte of the South African clawed toad *Xenopus laevis*, showing a large number of supernumerary nucleoli that contain circular extrachromosomal molecules of DNA carrying tandemly repeated rRNA genes. (From D. W. Brown and I. B. Dawid, *Science* 160: 272–280, 1968. Copyright © 1968 by the American Association for the Advancement of Science.)

The selective replication of the rRNA genes in oöcytes is the best-known example of this type of specific gene amplification. When large quantities of specific protein are required, as in the case of hemoglobin in red blood cells, extensive amplification can be accomplished at the translation level; each mRNA molecule can be translated many times. Of course, this is not possible when the required gene-product is an RNA molecule.

## GENE TRANSCRIPT POPULATIONS ARE DIVERGENT IN DIFFERENT CELL TYPES

In higher eukaryotes, only a small proportion of the genome is represented among mRNA molecules in any given cell type. This has been demonstrated by *RNA–DNA saturation hybridization experiments*. RNA is extracted from cells of a particular type and allowed to hybridize with total nuclear DNA (denatured). The

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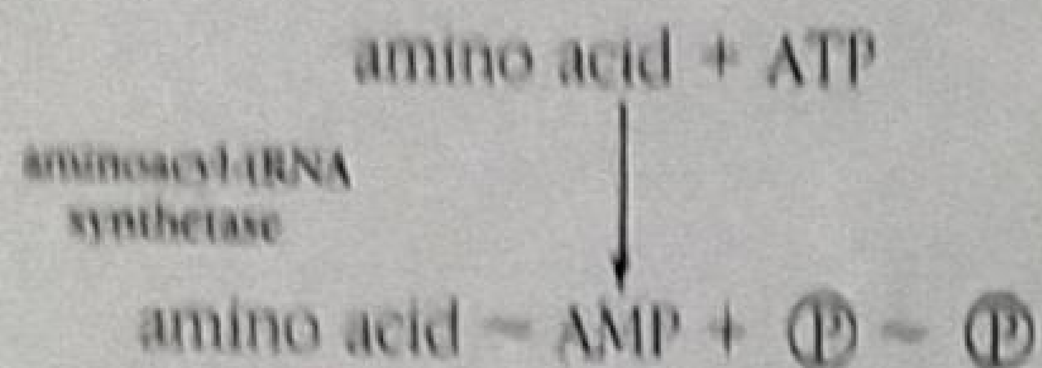
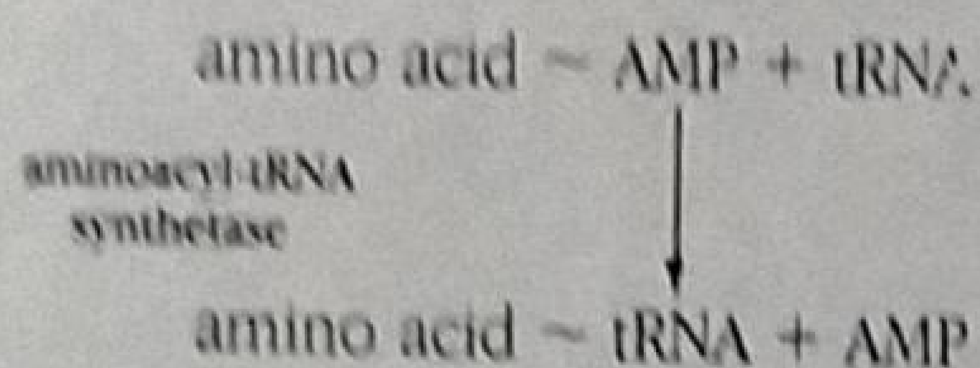
are formed in a two-step process, both steps being catalyzed by a specific "activating enzyme" or *aminoacyl-tRNA synthetase*. There is at least one aminoacyl-tRNA synthetase for each of the 20 amino acids. The first step in aminoacyl-tRNA synthesis involves the *activation* of the amino acid using energy from adenosine triphosphate (ATP):




The amino acid ~ AMP intermediate is not normally released from the enzyme before undergoing the second step in aminoacyl-tRNA synthesis, namely, the reaction with the appropriate tRNA:




The aminoacyl~ tRNAs (amino acid ~ tRNAs) are the immediate precursors of polypeptide synthesis on ribosomes, with each activated tRNA recognizing the correct mRNA codon and presenting the amino acid in a steric configuration (three-dimensional structure) that facilitates peptide bond formation.

The tRNAs are transcribed from chromosomal genes. As in the case of rRNAs, the tRNAs are transcribed in the form of larger precursor molecules that undergo posttranscriptional processing (cleavage, trimming, methylation, etc.). The mature tRNA molecules contain several nucleosides not present in mRNA or in the primary tRNA gene transcripts. These unusual nucleosides, such as inosine, pseudouridine, dihydrouridine, 1-methylguanosine, and several others, are produced by posttranscriptional, enzyme-catalyzed modifications of the four nucleosides incorporated into RNA during transcription.

Because of their small size (70–80 nucleotides long), tRNAs have been more amenable to structural analysis than the other, larger molecules of RNA involved in protein synthesis. The complete nucleotide sequence and proposed "cloverleaf" structure of the alanine tRNA of yeast (Fig. 10.15) was published by R. W. Holley and colleagues in 1965; Holley shared the 1968 Nobel Prize in physiology and medicine for this work. Since then, many tRNAs have been sequenced, and the yeast alanine tRNA gene has even been synthesized *in vitro* from mononucleotides by H. G. Khorana (another 1968 Nobel Prize winner; in Khorana's case, for work on the nature of the genetic code) and coworkers. The three-dimensional structure of the

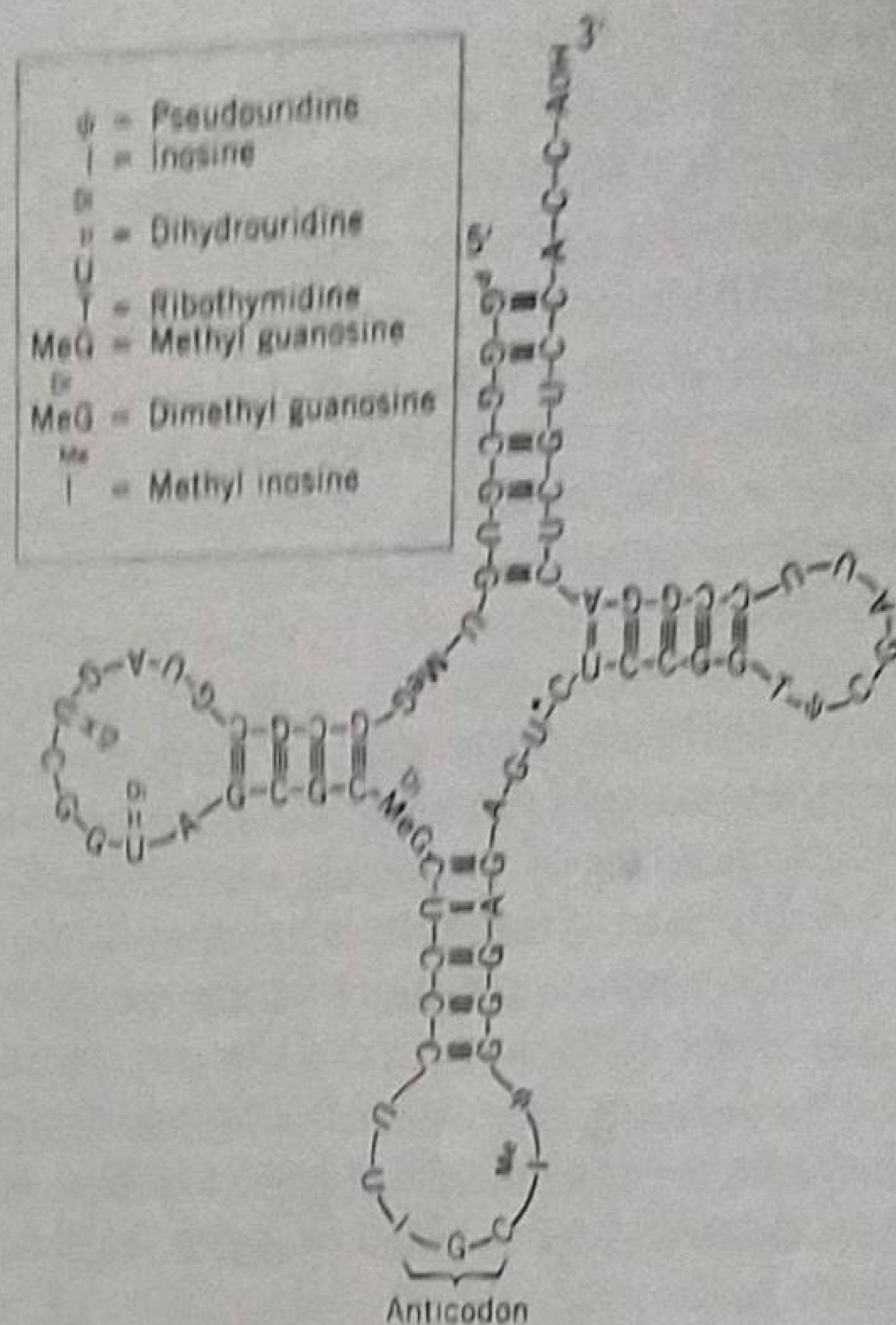



*Figure 10.15* Nucleotide sequence and cloverleaf configuration of the yeast alanine tRNA. The secondary structure results from hydrogen bonding between bases in different segments of the molecule as shown. The structure contains three loops, within which no base-pairing is possible. One of these loops contains the anticodon sequence; in the case of the yeast alanine tRNA, the sequence is 3′-CGI-5′. The names of the modified nucleosides present in the yeast alanine tRNA are shown in the inset. (Adapted from R. W. Holley *et al., Science* 147: 1462–1465, 1965. Copyright © 1965 by the American Association for the Advancement of Science.)

phenylalanine tRNA of yeast has been determined by X-ray diffraction studies (Fig. 10.16). The anticodons of the alanine (Fig. 10.15) and phenylalanine (Fig. 10.16) tRNAs of yeast occur within a loop (nonhydrogen-bonded region) near the center of the molecule. In fact, the anticodons of all the tRNAs sequenced to date (over 70 from all organisms) have been found within comparably located anticodon loops.

Each ribosome has two tRNA binding sites (Fig. 10.17). The A or *aminoacyl site* binds the incoming aminoacyl-tRNA, the tRNA carrying the amino acid that is next to be added to the growing polypeptide chain. The P or *peptidyl site* binds the tRNA to which the growing polypeptide is attached. The specificity for aminoacyl-tRNA binding in these sites is provided by the mRNA codons that make up part of the *A* and *P* binding sites. As the ribosome moves along an mRNA (or as the mRNA is shuttled across the ribosome), the specificity for the aminoacyl-tRNA binding in the *A* and *P* sites changes as different mRNA codons move into

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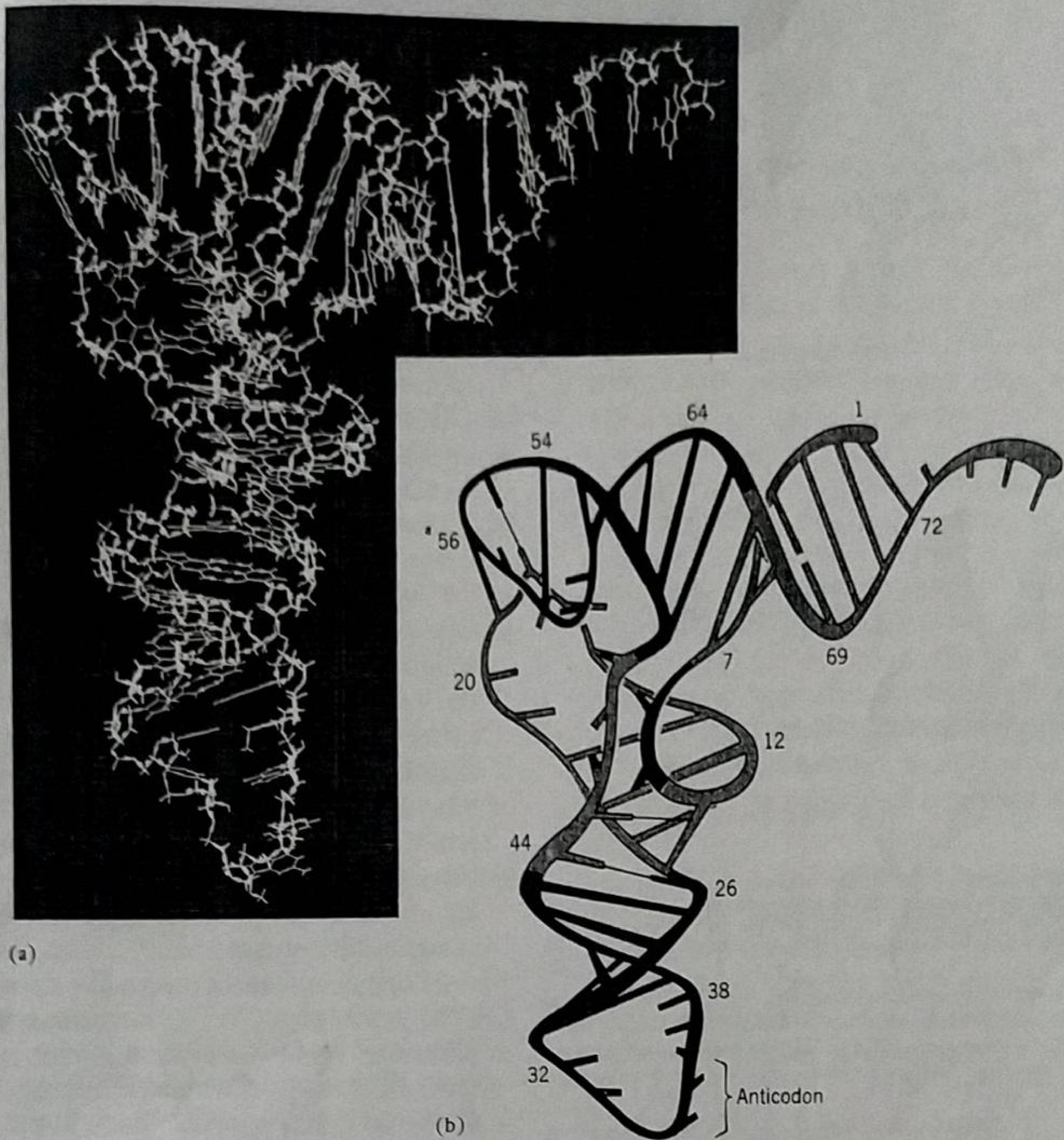


*Figure 10.16* Photograph (a) and interpretative drawing (b) of a molecular model of the yeast phenylalanine tRNA based on X-ray diffraction data. The anticodon loop is at the bottom. The ribose–phosphate backbone of the tRNA is drawn as a continuous cylinder. The crossbars indicate hydrogen-bonded base-pairs. Unpaired bases are indicated by shortened rods. (From S. H. Kim, F. L. Suddath, G. J. Quigley, A. McPherson, J. L. Sussman, A. H. J. Wang, N. C. Seeman, and A. Rich, *Science* 185: 435–440, 1974. Copyright © 1974 by the American Association for the Advancement of Science.)

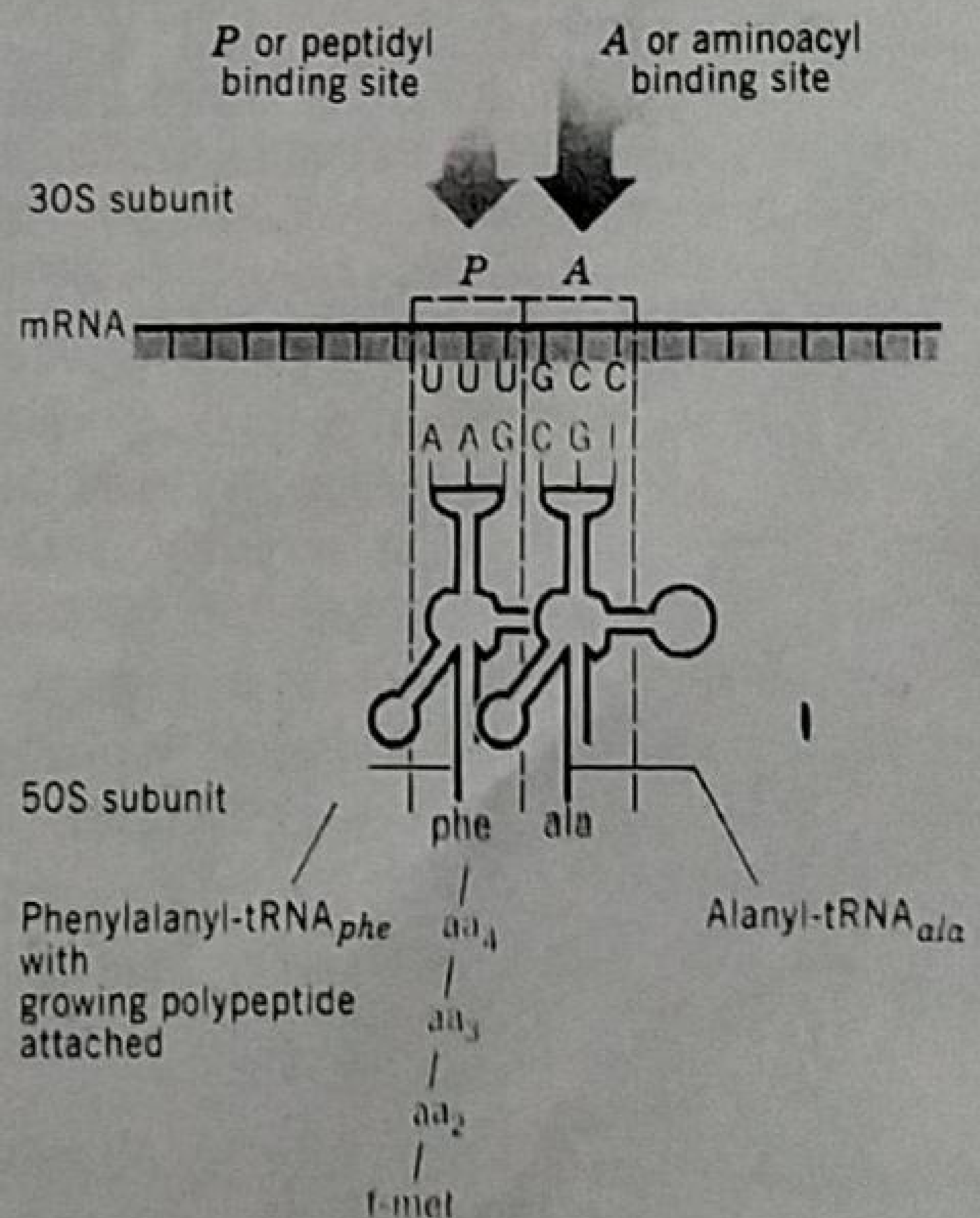


*Figure 10.17* The aminoacyl-tRNA binding sites formed by each ribosome–mRNA complex. The *A* or aminoacyl-tRNA site is shown occupied by an alanyl-tRNA$_{ala}$ complex. The *P* or peptidyl site is shown occupied by a phenylalanyl-tRNA$_{phe}$ complex, with the growing polypeptide chain covalently linked to the phenylalanine tRNA. The next step in protein synthesis will involve the formation of a peptide bond between phenylalanine and alanine, followed by the translocation of the alanine tRNA (with the growing polypeptide now attached) from the *A* site to the *P* site.

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