Applications of Recombinant DNA Technology

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Analysis of Biological Processes

1. Developments in DNA technology have allowed advances in research. Useful new techniques include:
    a. Site-specific mutagenesis.
    b. Restriction mapping.
    c. Southern blotting to identify genomic regions.
    d. Northern blotting to study RNA.
    e. DNA sequencing.
2. Applications of these technologies have been applied to the study of:
    a. Functional organization of genes and regulation of gene expression.
    b. Key regulatory and target genes in development.
    c. Genetic influences on cancer and aging.
    d. Evolutionary relationships between organisms.
3. This chapter deals with selected examples of applied recombinant DNA and PCR techniques.

Regulation of Transcription: Glucose Repression of Transcription of the Yeast *GAL1* Gene

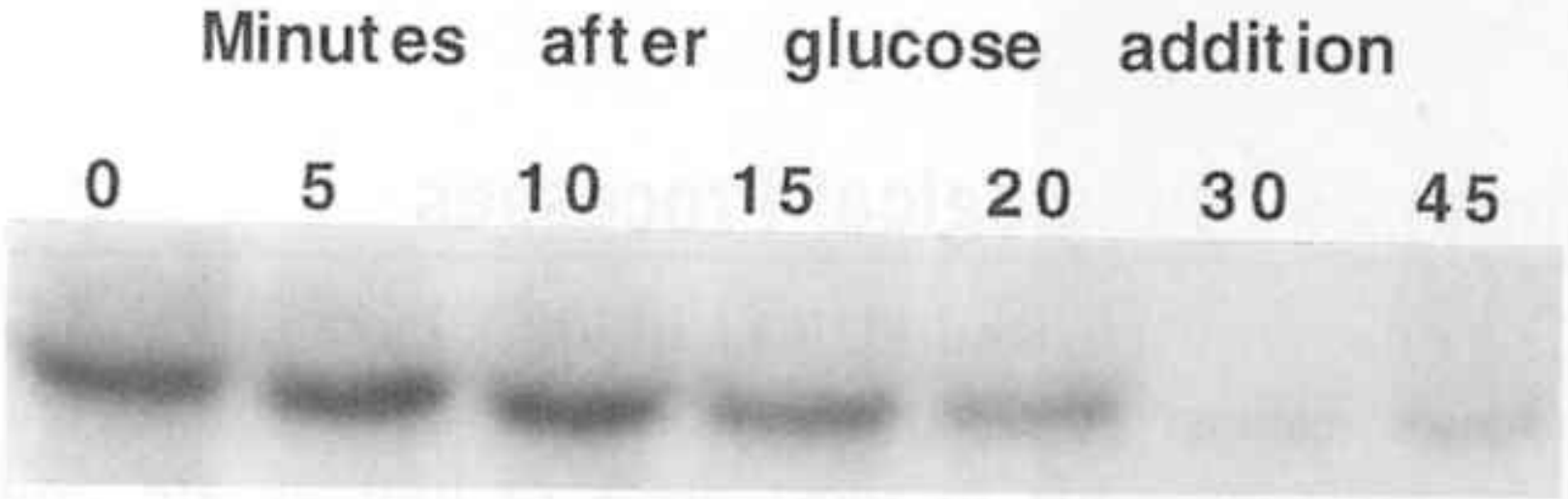
1. The GAL (galactose) genes in the yeast *Saccharomyces cerevisiae* serve as an example of eukaryotic transcriptional control of gene expression, and of recombinant DNA methods used to study transcription. Some details:

    a. GAL genes encode enzymes for catabolism of galactose, which serves as their inducer.

    b. Glucose represses GAL gene expression, and existing mRNAs are degraded, as shown by Northern blot analysis of yeast samples taken at time points and probed with the GAL genes (Figure 8.1).

Figure 8.1

Regulation of transcription of the yeast *GAL1* gene by glucose. Glucose was added at time zero, and the amount of *GAL1* transcripts was analyzed at various times thereafter by blotting and probing, as described in the text. (From Figure 5, Johnston, M., Flick, J. S., and Pexton, T., 1994. Multiple mechanisms provide rapid and stringent glucose repression of *GAL* gene expression in *Saccharomyces cerevisiae*. *Mol Cell Biol* 14:3834–3841.)



Alternative PremRNA Splicing: A Role in Sexual Behavior in *Drosophila*

1. Alternative splicing is one level of regulation of gene expression in eukaryotes. An example is control of male sexual behavior in *Drosophila melanogaster* (fruit fly).

   a. In *Drosophila* courtship, the male behaviors include:

      i. Orienting toward, following and tapping the female.

      ii. Singing a specific courtship song.

      iii. Curling the abdomen into copulation position.

   b. Regulatory genes in the sex determination pathways control these behaviors.

   c. Physiologically, the CNS (central nervous system) is responsible for key steps in male courtship behavior.

2. Genetic screening experiments have identified genes controlling aspects of *Drosophila* sexual behavior. An example is *fruitless (fru):*

a. In homozygotes, later steps of courtship (singing to copulation) are abnormal or absent.

b. Sexual organs and sperm are normal in these males, but they are functionally sterile due to defective courtship behavior.

c. Cloning and analysis of the *fru* gene has yielded this information:

i. The gene spans at least 140 kb.

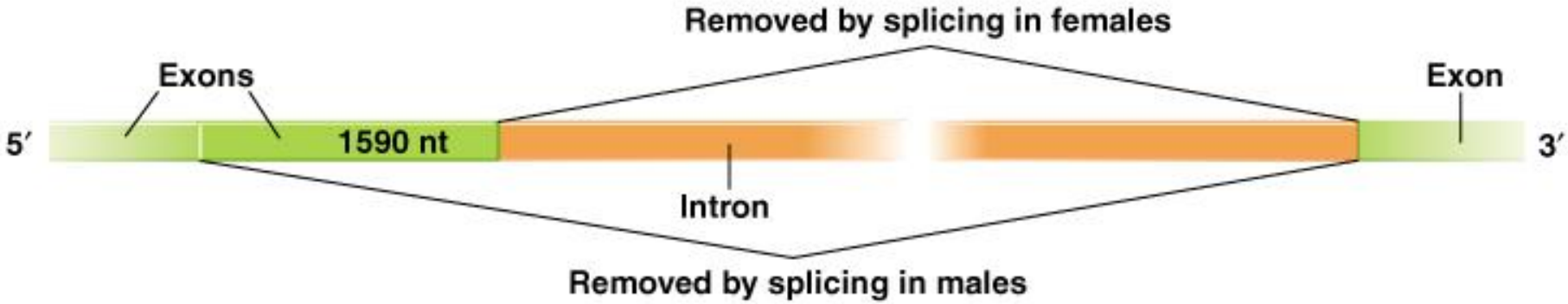
ii. Northern blots with *fru* as probe show a complex set of transcripts, some sex-specific.

iii. Sequencing of cDNAs shows some sex-specific transcripts result from alternative splicing at an intron 5' splice site, creating a shorter, male-specific version of the *fru* mRNA.

iv. The RT-PCR technique uses reverse transcriptase to make a cDNA from mRNAs, then amplifies the cDNA by PCR. Comparison of male and female mRNAs by this technique shows sex-specific transcripts of different sizes.

d. The sex-specific *fru* mRNAs are synthesized in only a few neurons in the CNS (500/100,000). The proteins encoded by these mRNAs regulate transcription of a set of specific genes, showing that *fru* is a regulatory gene. Its expression seems to be confined to neurons involved in male courtship.

Fig. 8.2 Alternative, sex-specific pre-mRNA in the *fru (fruitless)* gene of *Drosophila melanogaster*



Protein-Protein Interaction in the Cell: The Yeast Two-Hybrid System

Animation: The Yeast Two-Hybrid System

1. Understanding the quaternary structure of proteins (interaction of polypeptide subunits) requires study of the proteins themselves, and cannot be determined by the coding sequences alone.

2. The yeast two-hybrid system (interaction trap assay) is a technique to find genes for proteins that interact with a known protein (Figure 8.3):

   a. A regulatory protein, Gal4, binds a promoter element called UAS_G (upstream activator sequence G) that controls transcription of the *GAL1* gene.

   b. The Ga14 protein has two domains:

      i. A DNA binding domain (BD) that binds directly to UAS_G.

      ii. An activation domain (AD) that helps RNA polymerase to bind the promoter and initiate transcription.

   c. The two-hybrid system uses two types of yeast expression plasmids:

      i. The sequence for Gal4 BD fused to a known protein sequence (X).

      ii. The GAL4 AD fused to protein-coding sequences from a library of cDNAs (Y).

d. Reporter yeast strain is cotransformed with plasmid X (BD) and one of the Y plasmids (AD library).

e. A comnon yeast reporter gene is *E. coli lacZ* (β-galactosidase) inserted into the chromosome under control of UAS_G. β-galactosidase causes blue colonies when there is X-gal in the medium.

f. The reporter *lacZ* gene will only be expressed when the unknown protein, fused with AD interacts with the known protein fused with BD. The protein-protein interaction brings the AD and BD domains together, allowing them to bind the DNA region and activate the *lacZ gene.*

g. The cDNA from the successful AD fusion plasmid can be isolated and used for further study.

3. The two-hybrid system is widely used to find interactions between proteins. An example is human peroxin proteins:

a. Peroxins are encoded by *PEX* genes and used for peroxisome biogenesis.

b. Experiments using .the two-hybrid system show that normally PEXI and PEX6 proteins interact. Disrupting the protein-protein interaction results in neurological disorders like Zellweger syndrome.

c. Individuals with Zellweger syndrome:

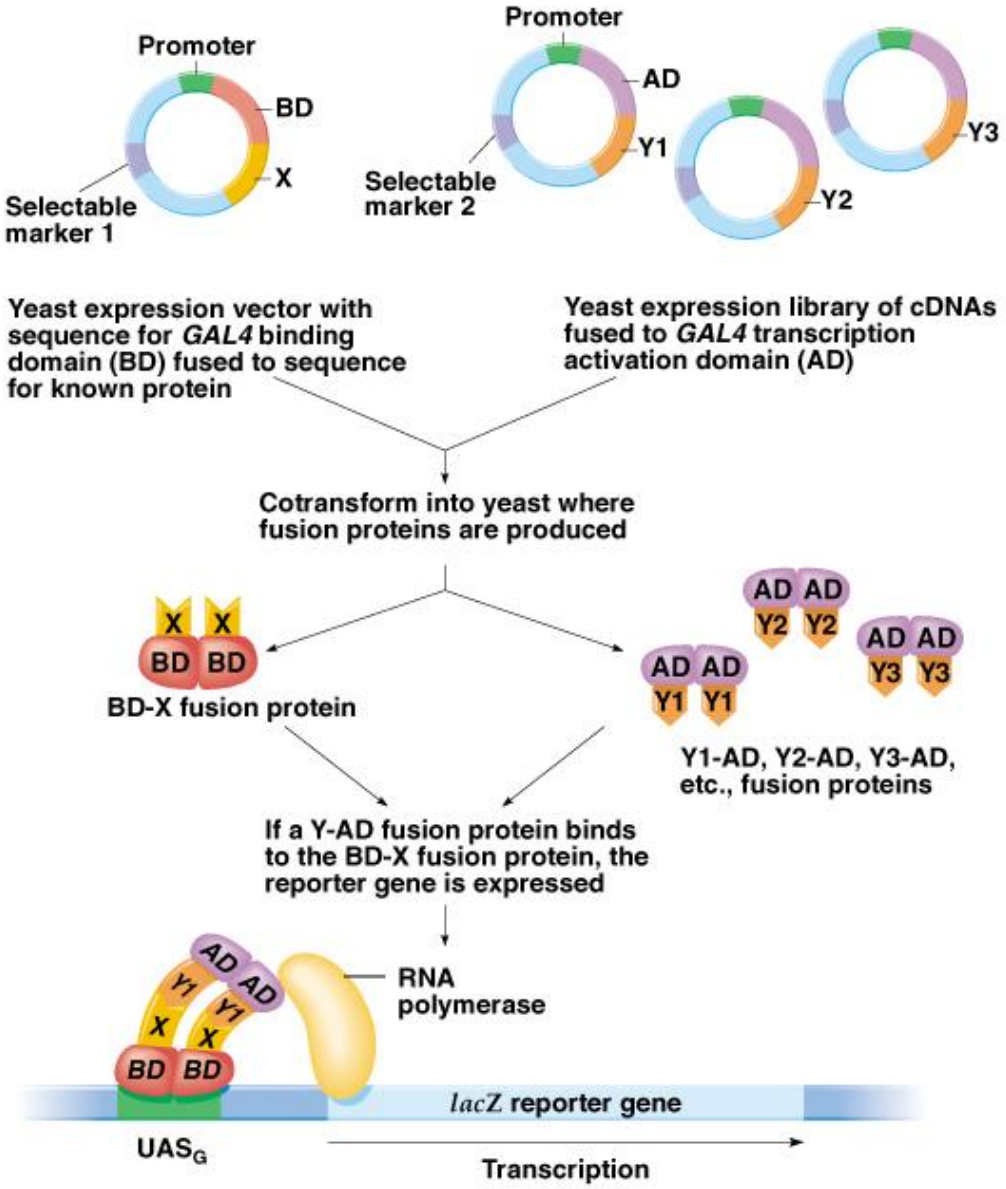
i. Lack many peroxisome enzyme functions.

ii. Have severe neurological, liver and renal abnormalities.

iii. Have mental retardation.

iv. Die in early infancy

Fig. 8.3 The yeast two-hybrid system for detecting protein-protein interactions



DNA Molecular Testing for Genetic Disease Mutations

1. Many human diseases result from protein defects caused by DNA mutations. DNA testing is increasingly available for genetic diseases, including:
    a. Huntington disease.
    b. Hemophilia.
    c. Cystic fibrosis.
    d. Tay-Sachs disease.
    e. Sickle-cell anemia.
2. Practical issues of DNA molecular testing are discussed in this section.

Concept of DNA Molecular Testing

1. Designing DNA molecular tests requires knowledge of gene mutations that cause a disease, derived from sequencing the gene involved.
2. Often a disease is caused by a variety of mutations, complicating its study. The breast cancer genes BRCA1 and BRCA2 are examples:
    a. Normal BRCA1 and BRCA2 genes control cell growth in breast and ovarian tissue.
    b. Mutations in the BRCA1 and BRCA2 genes can lead to cancer. Hundreds of mutations in these genes have been identified.
    c. Each BRCA1 or BRCA2 mutation confers a different risk of developing cancer, ruling out a single DNA molecular test to assess an individual's breast cancer risk associated with these genes.
3. Genetic testing reveals the presence of a mutation associated with a genetic disease. Genetic testing is usually done on a targeted population of people with symptoms or a family history of the disease.
4. Genetic testing is distinct from genetic screening, which involves the population at large, and it is also distinct from diagnostic testing to determine whether or not a disease is present, or the extent of its development.

Purposes of Human Genetic Testing

1. Human genetic testing serves three main purposes:
    a. Prenatal diagnosis.
    b. Newborn screening.
    c. Carrier (heterozygote) detection.
2. Prenatal diagnosis uses amniocentesis or chorionic villus sampling to assess risk to the fetus of a genetic disorder by analyzing for a specific mutation, or biochemical or chromosomal abnormalities.
    a. If both parents are carriers (heterozygotes) for the mutant allele, the probability is 1⁄4 that the fetus is an affected homozygote, 1/2 that it is a carrier, and 1⁄4 that it is homozygous for the normal allele. Genetic testing can determine the result of a particular conception.
    b. Genetic testing may be used during in vitro fertilization to eliminate before implantation embryos with mutated genes that could result in serious disease.

3. Examples of tests for specific mutations using blood from newborns include:
    a. Phenylketonuria (PKU).
    b. Sickle-cell anemia.
    c. Tay-Sachs disease.
4. Carrier detection tests using blood samples are available for many genetic diseases, including:
    a. Tay-Sach disease.
    b. Duchenne muscular dystrophy (a disease of progressive muscle atrophy and disfunction).
    c. Cystic fibrosis.

Examples of DNA Molecular Testing

1. Testing by restriction fragment length polymorphism (RFLP) analysis detects loss or addition of a restriction site in the region of a gene.

    a. The restriction map is independent of gene function, so RFLPs may occur without changing the phenotype.

    b. RFLP marker information is used in the same way as "conventional" DNA markers, and is assayed directly in the form of a restriction map.

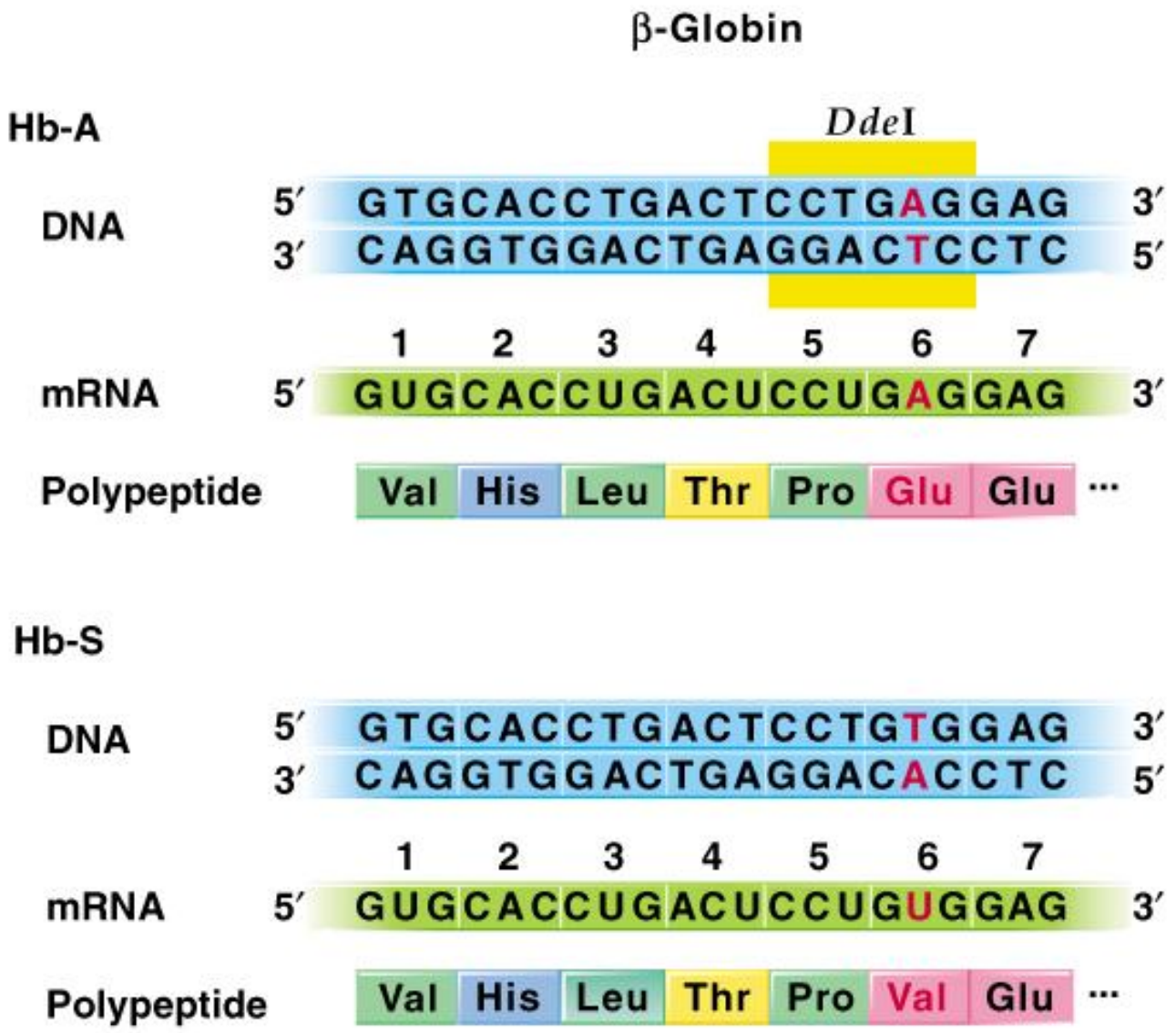
    c. In heterozygotes both parental types are seen, allowing easy detection of carriers.

2. RFLPs are associated with many genetic disorders. Sickle-cell anemia is an example:

    a. A single base-pair change in the b-globin gene results in abnormal hemoglobin, Hb-S, rather than the normal Hb-A. Hb-S molecules cause sickling of red blood cells.

    b. The Hb-S mutation is an AT-to-TA base pair change in the 6th codon of b-globin, resulting in a valine rather than a glutamic acid, and also eliminating a DdeI restriction enzyme site (Figure 8.4).

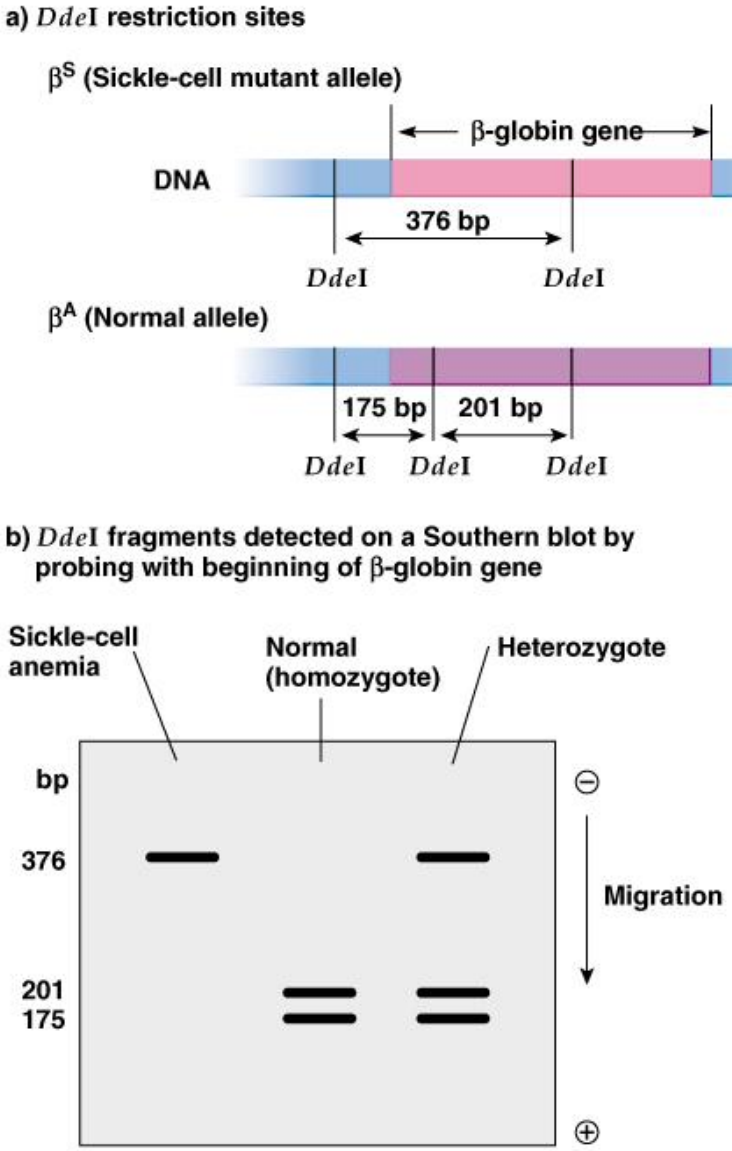
Fig. 8.4 The beginning of the β-globin gene, mRNA, and polypeptide showing the normal Hb-A sequences and the mutant Hb-S sequences



c. In the normal b-globin (Hb-A) gene there are three D*deI* sites, while the sickling form, Hb-S, has only two D*deI* sites. This difference can be detected using Southern hybridization of genomic DNA with a b-globin gene probe.

3. RFLPs associated with genetic disorders may also result from changes in flanking sequences. PKU is an example:
   a. PKU results from defective phenylalanine hydroxylase enzyme.
   b. Genomic DNA digested with H*paI*, Southern blotted and probed with cDNA probe from phenylalanine hydroxylase mRNA shows different restriction fragments for PKU and normal individuals.
   c. The RFLP results from DNA sequences located 3' to the gene that usually segregate with it. Recombination events that occur between the site of the RFLP and the gene mutation can complicate this test.

Fig. 8.5 Detection of sickle-cell gene by the *Dde*I restriction fragment length polymorphism



Animation:Polymerase Chain Reaction

4. PCR is another approach to DNA molecular testing. It requires sequence information so that specific primers can be designed. An example is allele-specific oligonucleotide (ASO) hybridization used to detect mutations in *GLC1A*, a gene involved in maintaining normal eye pressure.

a. Abnormal pressure in the eye results in glaucoma, which can cause blindness. Mutations in *GLC1A* can be responsible for this condition.

b. Sequence of *GLC1A* is known, and glaucoma-inducing mutations identified.

i. PCR primers were designed to amplify a region of the gene where glaucoma-inducing mutations occur.

ii.PCR products undergo agarose gel electrophoresis, are extracted from the gel and dotted onto duplicate membrane filters, and denatured to single strands.

iii. One blot is probed with a labeled oligonucleotide specific to the wild-type allele, while the other receives labeled oligo specific for a particular mutation.

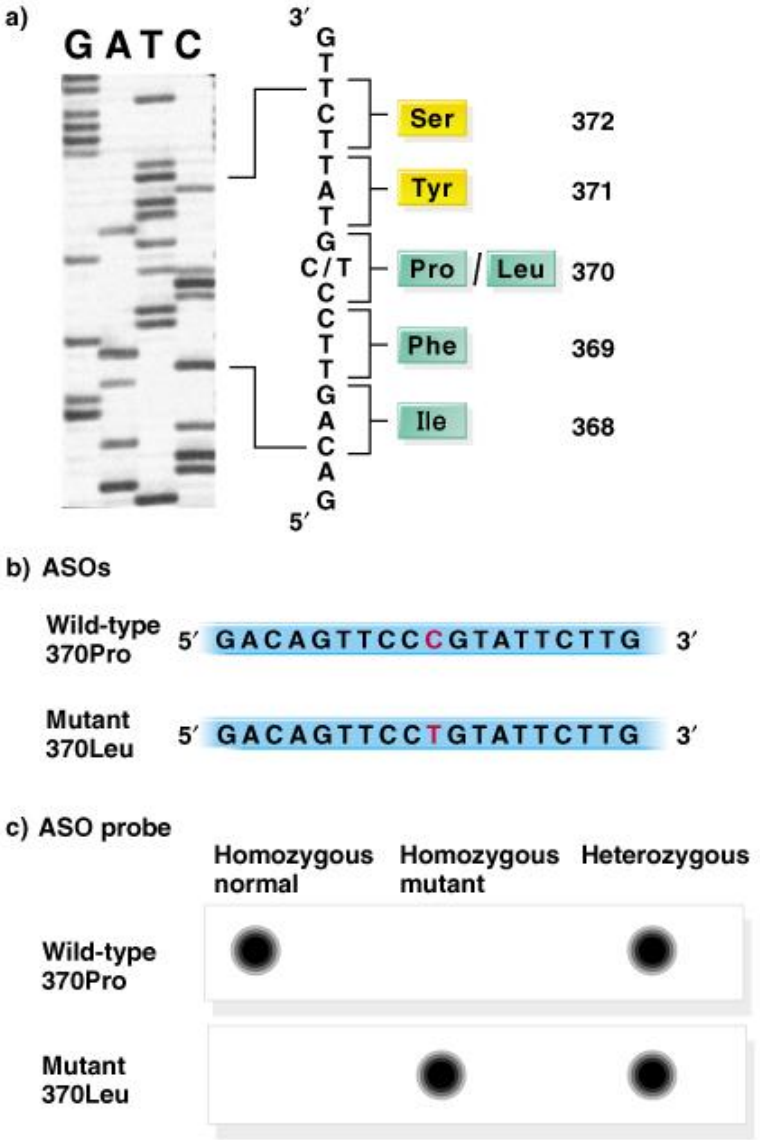
(1) Signal only with the wild-type oligo indicates an individual homozogous for the normal allele.

(2) Signal only with the mutant oligo shows an individual homozygous for the mutant *GLC1A* allele, and at risk of developing glaucoma.

(3) Signal on both filters indicates a heterozygous (carrier) individual.

5. A related PCR procedure uses labeled PCR product as a probe against a filter blotted with an array of normal and mutant alleles of the gene. The dot(s) to which the probe binds indicate the genotype of the individual.

Fig. 8.6 DNA molecular testing for mutations of the open-angle glaucoma gene, *GLC1A*, using PCR and allele-specific oligonucleotide (ASO) hybridization



Availability of DNA Molecular Testing

- 1. Genetic testing is not available for every disorder. Reasons include:
  - a. Lack of information about the gene. Its location may be unknown, or it may not yet be sequenced.
  - b. Many different mutations in the gene result in disease, so a single molecular test is of little use. An individual maybe tested for the presence of a known subset of mutations, but a negative test does not guarantee the absence of a deleterious allele.
  - c. Not every individual carrying a disease-associated mutation develops that disease, and so genetic testing is of limited use, and is usually performed only in high-risk families.

Isolation of Human Genes

1. Locating a gene is relatively easy when its gene product is known.
2. If the gene product is not detectable using antibodies, an RFLP marker associated with the disease phenotype can identify the gene's approximate position on the chromosome, and its DNA can be isolated by positional cloning.
3. Because positional cloning involves identifying a gene without knowing its product, it is sometimes called reverse genetics.

Cloning the Cystic Fibrosis Gene

1. Cystic fibrosis (CF), the most common lethal genetic disease in the U.S., is inherited as an autosomal recessive. Its gene was the first to be isolated solely by positional cloning.

2. Vast screening of individuals in families with CF found a single RFLP with a weak linkage to the CF locus.

3. Using ^{3}H-labeled RFLP probe for in situ hybridization, the CF gene was localized to chromosome 7.

4. Other known chromosome 7 RFLPs were investigated to find those most closely linked with the CF gene. Two were found that flank the CF gene, localizing it to region 7q31-q32 (7 is the chromosome, q is the long arm, 31-32 indicates subregions).

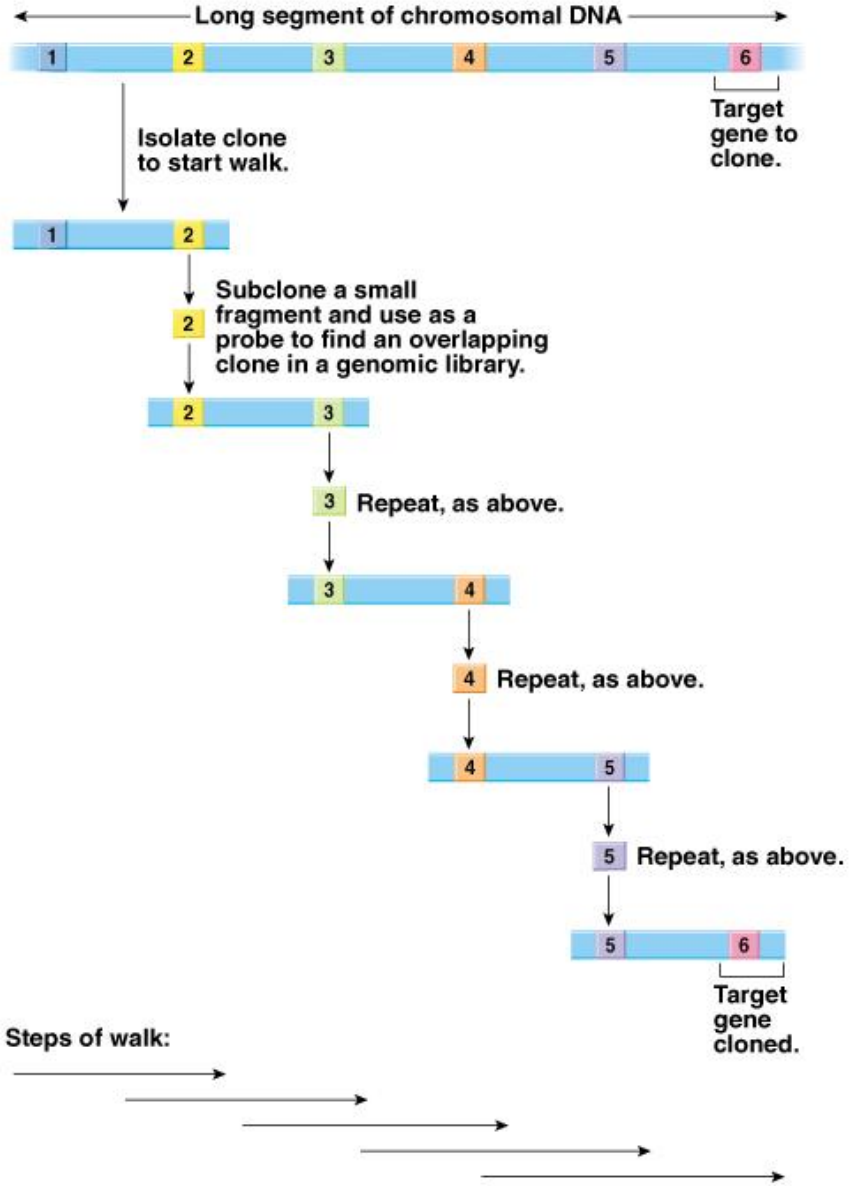
5. The DNA region of the CF gene was cloned using chromosome walking to identify adjacent chromosomal fragments from clones in a genomic library. (Figure 8.7)

    a. The end of a known sequence is used as a probe to find an adjacent fragment. The end of that DNA is then used to find the next fragment, and so on.

    b. Repetitive DNA sequences can complicate chromosome walking.

    c. The length of each step in the walk is limited to the size of the inserts in the library, minus the size of the overlap.

Fig. 8.7 Chromosome walking



6. Chromosome jumping was also used in isolating the CF gene. It is similar to chromosome walking, but eliminates the need to detect overlapping regions (Figure 8.8):

a. Partial restriction digestion produces large overlapping DNA fragments.

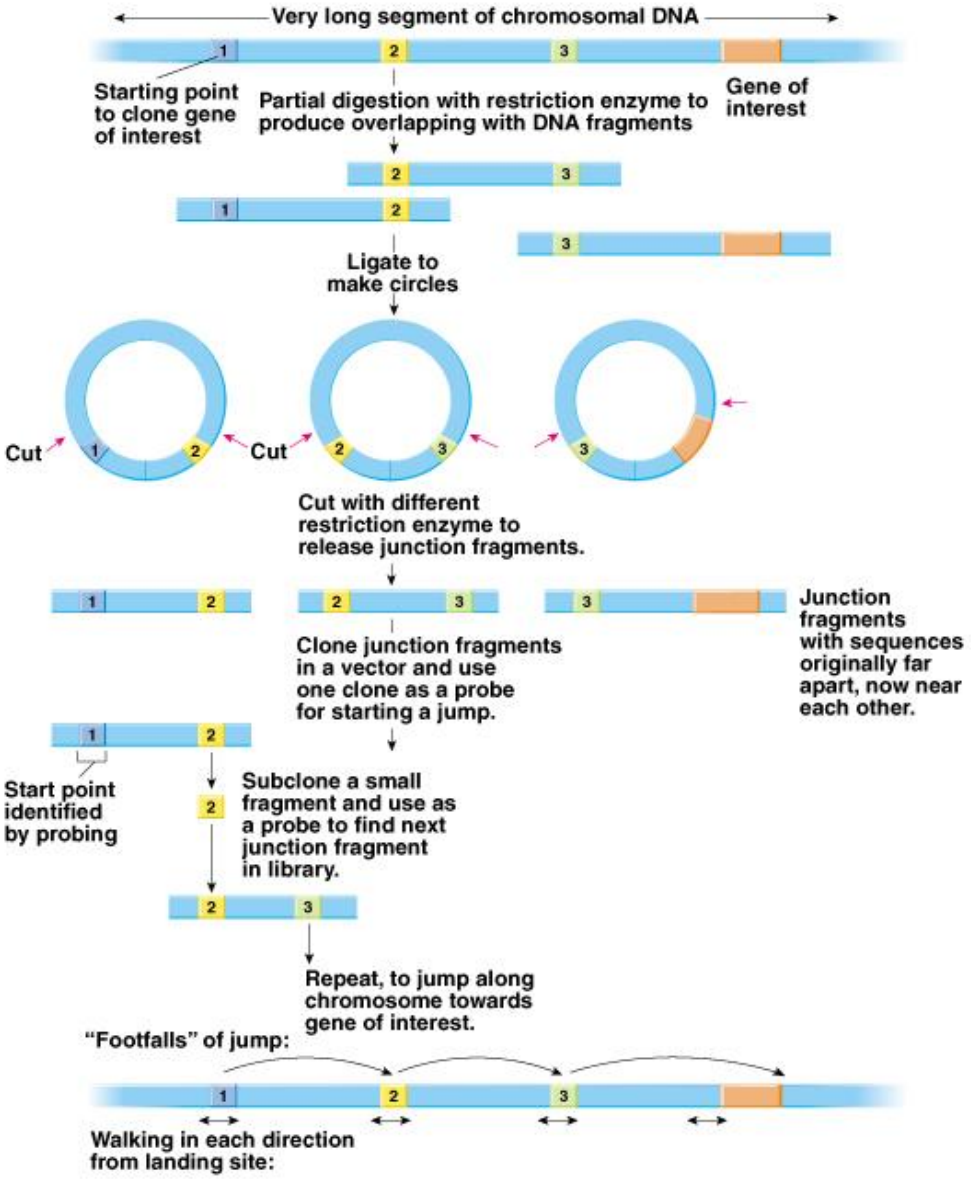
b. Fragments arc circularized with DNA ligase, bringing ends that were some distance apart together.

c. Resulting circles are cut to release the junction region, which is cloned to form a jumping library.

d. When a probe detects a homologous DNA in the library it will be associated with DNA from some distance away in the chromosome.

e. The associated DNA is then used as a probe to make another jump in the library, or as the starting point for chromosome walking.

Fig. 8.8 Chromosome jumping



7. Identifying a gene of interest in a set of clones is a challenge. Two techniques were used to determine whether specific regions of cloned DNA contain the CF gene.

    a. Cloned DNA was used as a probe against genomic DNA of other species, because genes are likely to be more conserved than nongene sequences. Genomic fragments from a variety of species were analyzed by Southern hybridization (a zoo blot).

    b. DNA probes from genes are expected to hybridize with mRNAs in a Northern, and this technique can test whether a sequence is transcribed.

8. The putative CF DNA was used to screen a cDNA library derived from mRNAs of normal sweat gland cells, which would be expected to express the normal allele. This probe identified a single cDNA clone by Northern blot, and that clone was then used to find the genomic CF sequences.

9. Isolation of the CF gene was confirmed when DNA sequences in this region proved to be different in a normal and a CF individual, showing a deletion of 3-bp in the CF patient.

10. Further investigation showed 68% of caucasian CF patients had the 3-bp deletion (resulting in the protein losing a phenylalanine), while the remaining patients had over 60 different mutations.

11. Computer analysis of the CF gene predicts that the CFTR (cystic fibrosis transmembrane conductance regulator) protein has two similar membrane-association motifs, and an ATP binding domain. The common CF mutation removes phenylalanine from the 1st predicted protein domain.

DNA Typing

1. DNA typing (DNA fingerprinting or DNA profiling) identifies DNA from particular individuals, using techniques similar to those described in for DNA molecular testing for genetic diseases. Relevant molecular markers include:

    a. RFLPs detected by restriction digestion and blotting.

    b. PCR product polymorphisms detected by agarose gel electrophoresis.

    c. Alleles detected by allele-specific oligonucleotides (ASOs).

2. Highly polymorphic DNA regions are preferred for typing, and great variation is shown in regions of DNA consisting of short tandem repeats:

    a. Microsatellites, also called single tandem repeats (STRs), have repeating units of 2–4 bp

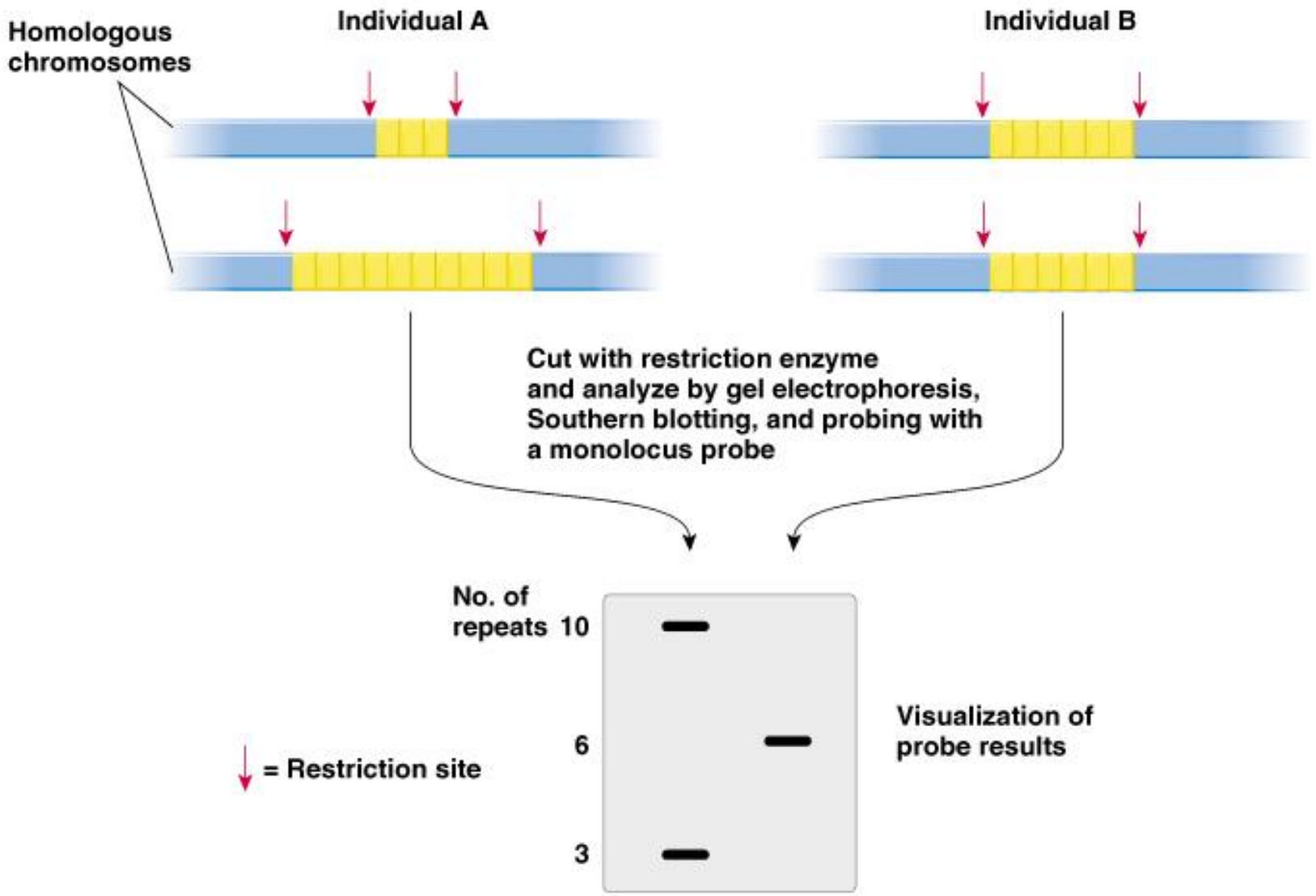
    b. Minisatellites, also called VNTRs (variable number of tandem repeats), have repeating units ranging in size from 5 to a few tens of base pairs.

3. STRs and VNTRs are used for DNA typing by DNA digestion with restriction enzyme, which releases different sized fragments depending on the length of the repeat sequence (Figure 8.9).

4. The size variation is detected by probing for the particular repeat sequence. The probe may be specific for STR or VNTR sequences at one genomic locus (a monolocus or single-locus probe) or at a number of loci (a multilocus probe).

5. If sequence data are available, PCR from flanking sequences may be used to amplify the STRs or VNTRs.

Fig. 8.9 The concept involved in using STRs (microsatellites) or VNTRs (minisatellites) as DNA markers

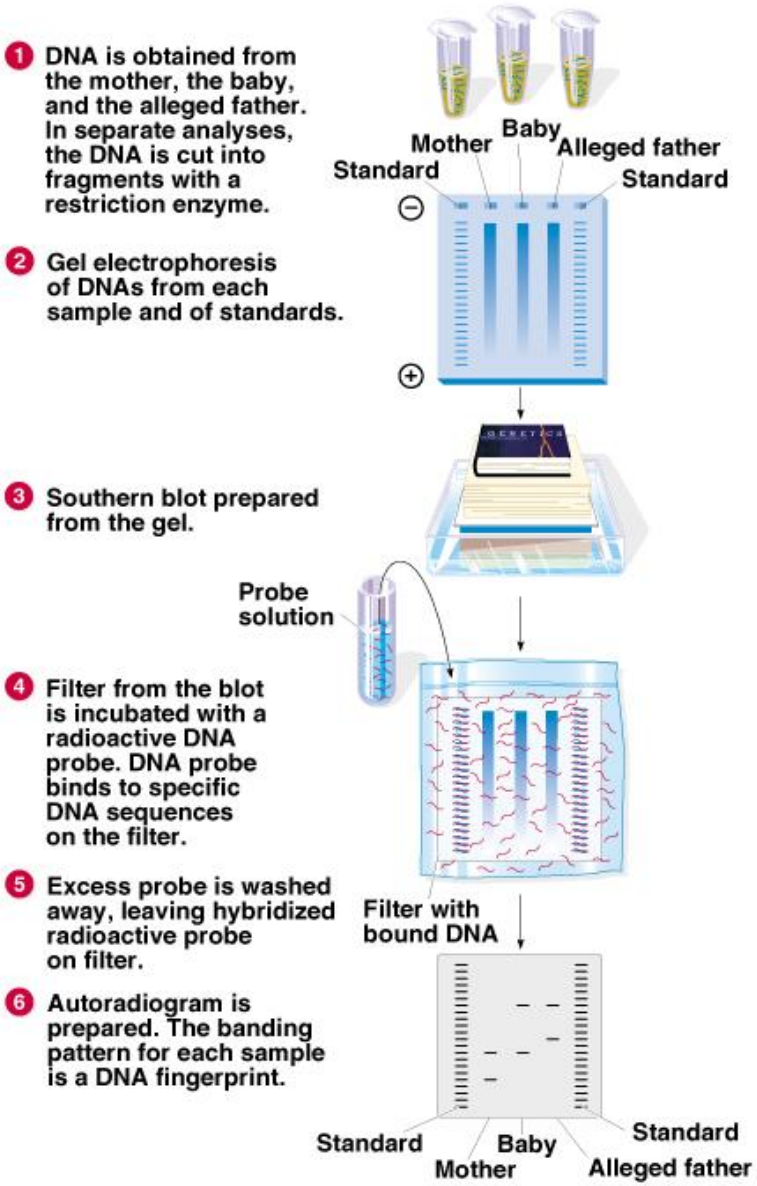


- iActivity: Combing Through "Fur"ensic Evidence

DNA Typing in a Paternity Case

1. DNA typing in a paternity case would proceed as follows (Figure 8.10):
    a. DNA samples (typically from blood) are obtained from mother, baby and putative father.
    b. DNA is cut with a particular restriction enzyme, electrophoresed and transferred to a membrane filter by Southern blotting.
    c. Hybridization is performed with a labeled monolocus STR or VNTR probe, and the banding pattern is analyzed.
    d. Baby and mother are expected to share one allele, while baby and father share the other allele.
    e. If the man and baby do not share a common allele, DNA typing has proved he is not the father. If they do share an allele, paternity is possible, but not proven, since other men also carry the allele at some frequency that can be calculated.
    f. Often five or more different polymorphisms are characterized. If all match with the putative father, the combined probabilities calculated for the array of polymorphisms can be convincing evidence in court.

Fig. 8.10 Procedure for DNA typing as used for a paternity case



2. The role of DNA typing in court cases is still being determined, and DNA typing is not generally accepted for proving parenthood or guilt, although it is widely used as evidence of innocence.
3. DNA evidence is most commonly rejected for procedural reasons, such as errors in evidence collection or processing, or due to lack of population statistics for the alleles in question.

Other Applications of DNA Typing

1. Examples of DNA typing used to analyze present-day samples include:
    a. Forensic analysis in criminal cases. DNA samples from victims and suspects are compared, sometimes using PCR to amplify the relevant DNA.
    b. Population studies to determine variability in groups of people.
    c. Proving horse pedigrees for registration purposes.
    d. Forensic analysis in wildlife crimes, allowing body parts of poached animals to be matched and used as evidence.
    e. Detection of pathogenic E. coli strains in foods such as ground meat.
    f. Detection of genetically modified organisms (GMOs) in bulk or processed foods, by the presence of inserted sequences.

2. DNA typing is increasingly being used on much older samples, as well.

   a. DNA extracted from the remains of ancient organisms can be analyzed. Examples include an insect in amber (40 million yrs. old), a fossil leaf (17 million yrs.) and a mammoth (40,000 yrs.).

   b. Historic questions can be addressed by DNA typing. An example is the lingering doubt about whether a boy who perished in captivity in 1795 was the sole surviving son of Louis XVI and Marie Antoinette. Comparison of DNA from preserved tissue and hair, and from living descendants, shows that the dead boy was indeed the Dauphin.

Gene Therapy

1. Theoretically, two types of gene therapy are possible:
    a. Somatic cell therapy, modification of non-reproductive cells to prevent a genetic disorder. Treats disease in an individual, but mutant gene can be passed to progeny.
    b. Germ-line cell therapy, altering the reproductive cells. Replaces mutant allele with a normal one, changing what offspring will inherit.
2. Somatic cell therapy is used in humans, but due to ethical issues, germ-line cell therapy is not.
3. Somatic cell therapy is most likely to be successful in disorders resulting from a simple defect in a well-understood gene.
4. The general procedure for somatic cell therapy:
    a. An individual's mutant cells are sampled.
    b. Normal (wild-type) copies of the mutant gene are introduced into the cells.
    c. The modified cells are returned to the donor.
    d. If all goes well, cells produce a normal gene product, and disease symptoms decrease or disappear.

5. The cell type selected for modification varies with the disorder.

Examples:

a. Blood disorders (e.g., thalassemia, sickle-cell anemia) require modification of blood-line bone marrow cells.

b. Skin fibroblasts can be modified and implanted back into the dermis to produce proteins that circulate in blood.

6. Producing transgenic cells by introducing a transgene has several problems:

a. Transformation is inefficient, so many cells are needed for the attempt. Viral vectors are presently used to make delivery more efficient.

b. When "foreign" DNA enters a cell, only a fraction of it replaces a mutant gene. Integration often must be precise for normal gene expression to occur.

7. Successful somatic cell therapy is well documented in experiments with other mammals, and results of a few human gene therapy trials are now available. For example:

a. Deficiency in adenosine deaminase (ADA) enzyme causes severe combined immunodeficiency (SCID).

i. T cells from a young SCID patient were grown in vitro, receiving the normal ADA gene by a viral vector.

ii. When reintroduced, the cells expressed the ADA gene, and immune function became more normal.

iii. The therapy is repeated periodically to replace obsolescent T cells.

b. Sickle-cell anemia (SCA) has also been treated with a type of somatic gene therapy.

   i. A patient's bone marrow cells were replaced with stem cells from umbilical cord blood.

   ii. One year later, no sickle cells had been detected in the patient's blood.

8. Other genetic disorders that are good candidates for somatic gene therapy:

   a. Thalassemias.

   b. Phenylketonuria.

   c. Lesch-Nyhan syndrome.

   c. Cancers.

   d. Duchenne muscular dystrophy.

   e. Cystic fibrosis (human clinical trials are in progress).

9. Gene therapy raises scientific, ethical and legal questions.

Commercial Products

1. Biotechnology has become big business, and one aspect of it is manipulation of DNA to make commercial products. The general approach is to:

   a. Express a cloned gene or cDNA in a transformed organism using an expression vector appropriate to the host organism (which can range from bacterium to mammal).

   b. In mammals, proteins are relatively easy to isolate if secreted into milk (Figure 8.11):

      i. Gene of interest (GOI) is placed next to a promoter active only in mammary tissue (e.g., β-lactoglobin).

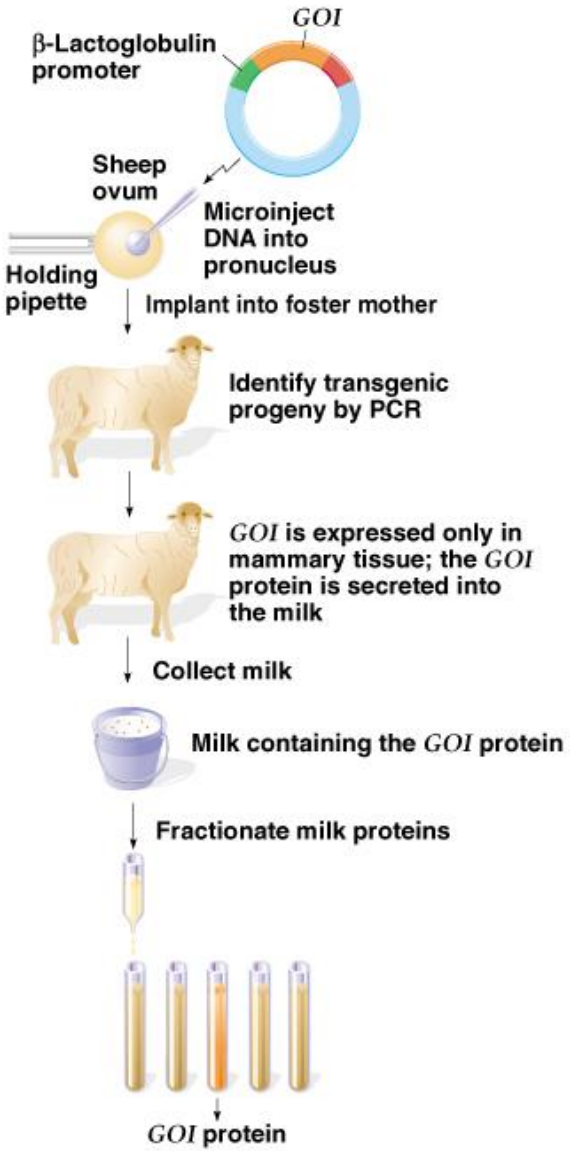
      ii. Recombinant DNA is microinjected into ova, and each ovum implanted into a foster mother.

      iii. Transgenic offspring are detected by PCR.

      iv. At maturity, the b-lactoglobin promoter is activated, and the GOI is expressed.

      v. Product of interest is obtained from milk.

Fig. 8.11 Production of a recombinant protein product (here the protein encoded by the gene of interest, *GOI*) in a transgenic mammal, in this case a sheep



2. Examples of products from biotechnology companies:

   a. Tissue plasminogen activator (TPA), used to prevent and dissolve blood clots.

   b. Human growth hormone, to treat pituitary dwarfism.

   c. Tissue growth factor-beta (TGF-β), to promote blood vessel and epidermal growth, and thus healing.

   d. Human blood clotting factor VIII, to treat hemophilia.

   e. Human insulin ("humulin"), to treat insulin-dependent diabetes.

   f. DNase, to treat cystic fibrosis.

   g. Recombinant vaccines, to prevent human and animal viral diseases.

   h. Bovine growth hormone, to increase meat and dairy yields.

   i. Platelet derived growth factor (PDGF), to treat skin ulcers in diabetics

   j. Genetically engineered bacteria to produce industrial enzymes, flavorings and ethanol.

   k. Genetically engineered bacteria specialized to degrade pollutants.

Genetic Engineering of Plants

1. Humans have selectively bred plants for centuries. Now, recombinant DNA technology has made possible the genetic engineering of agricultural plants.

Transformation of Plant Cells

1. Genetic engineering is relatively difficult in plants. Most plant transformation involves the bacterium *Agrobacterium tumefaciens*, a plant pathogen with a wide host range.

   a. Causative agent of crown gall disease, *Agrobacterium* can invade most dicots at a wound site and insert a defined segment of bacterial DNA into the plant genome, producing a tumor (gall).

   b. Plant cell transformation is mediated by the Ti (tumor-inducing) plasmid, a 200-kb circular DNA. A 30-kb region of the Ti plasmid is called T-DNA because it is excised from the plasmid and transforms the plant cell.

   c. T-DNA is excised by nicking the flanking pairs of 25-bp sequences called the borders. It is then transferred to the nudeus of a plant cell in a manner similar to bacterial conjugation.

   d. The T-DNA integrates into the plant genome, and the plant acquires its genes. No genes for DNA excision, transfer or integration are encoded by the T-DNA.

   e. The only signal required to excise, transfer and integrate the T-DNA is the flanking 25-bp repeat sequences at the borders. Any DNA placed between the border sequences will be integrated into the host genome

2. T-DNA transformation is not effective for monocots, and so won't work for most crop plants. In monocots, DNA is delivered into the cell by electroporation of plant cell protoplasts.

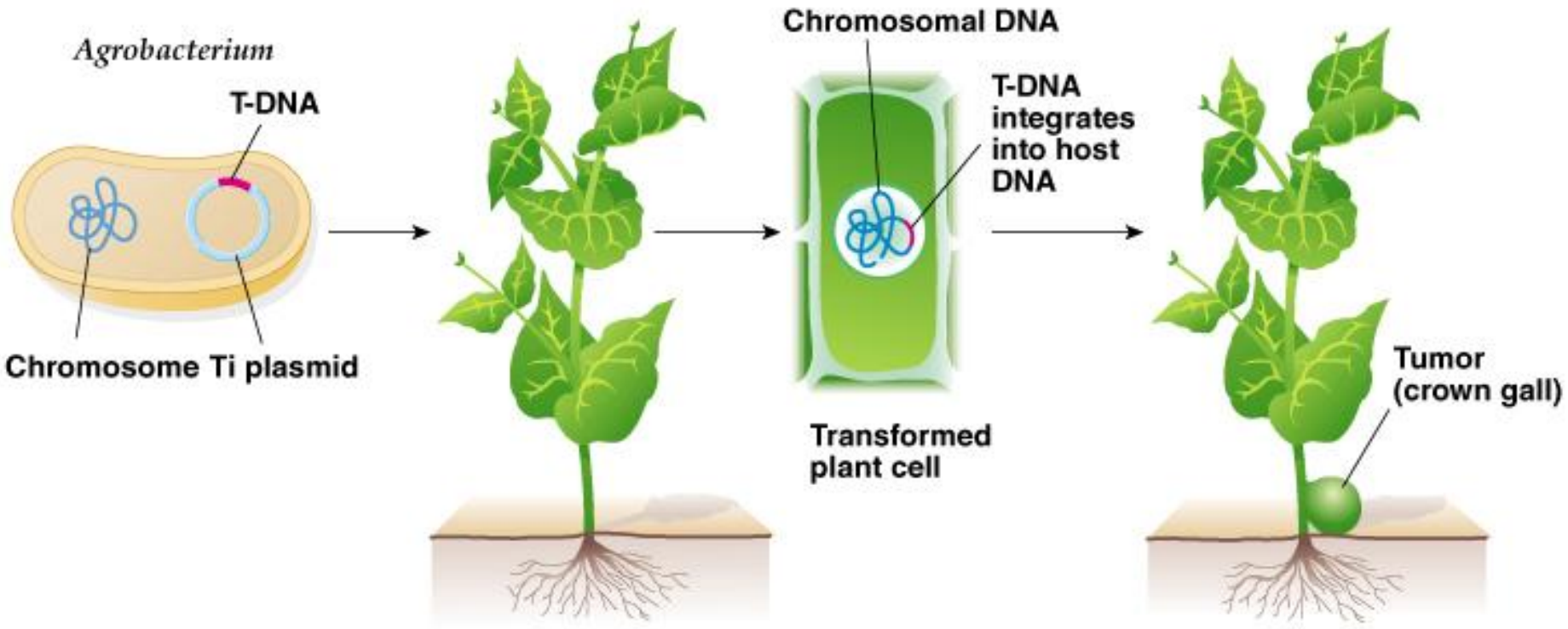
   a. DNA is added to plant cells, and high voltage is used to introduce it into the cells.

   b. Cells are tissue cultured until they recover and cell walls are. restored.

   c. Transformed cells are selected and propagated to produce whole plants.

3. DNA may also be introduced by a gene gun that fires a plastic bullet carrying tiny tungsten beads coated with DNA. At the end of the gene gun's barrel is a plate which stops the plastic bullet but allows the tungsten beads to move into target cells, delivering the DNA.

Fig. 8.12 Formation of tumors (crown galls) in plants by infection with certain species of *Agrobacterium*



Applications for Plant Genetic Engineering

1. Genetically modified crops are currently on the market and are part of our food supply. Roundup™ tolerant plants are an example.

   a. The active ingredient of Roundup™ is glyphosate, which inhibits EPSPS, a chloroplast enzyme required for aromatic amino acid synthesis.

   b. Roundup™ is active in relatively low doses, and is rapidly degraded by soil microbes.

   c. Roundup tolerant crop plants are desired because weeds can be killed by application of the herbicide, without harming the crop.

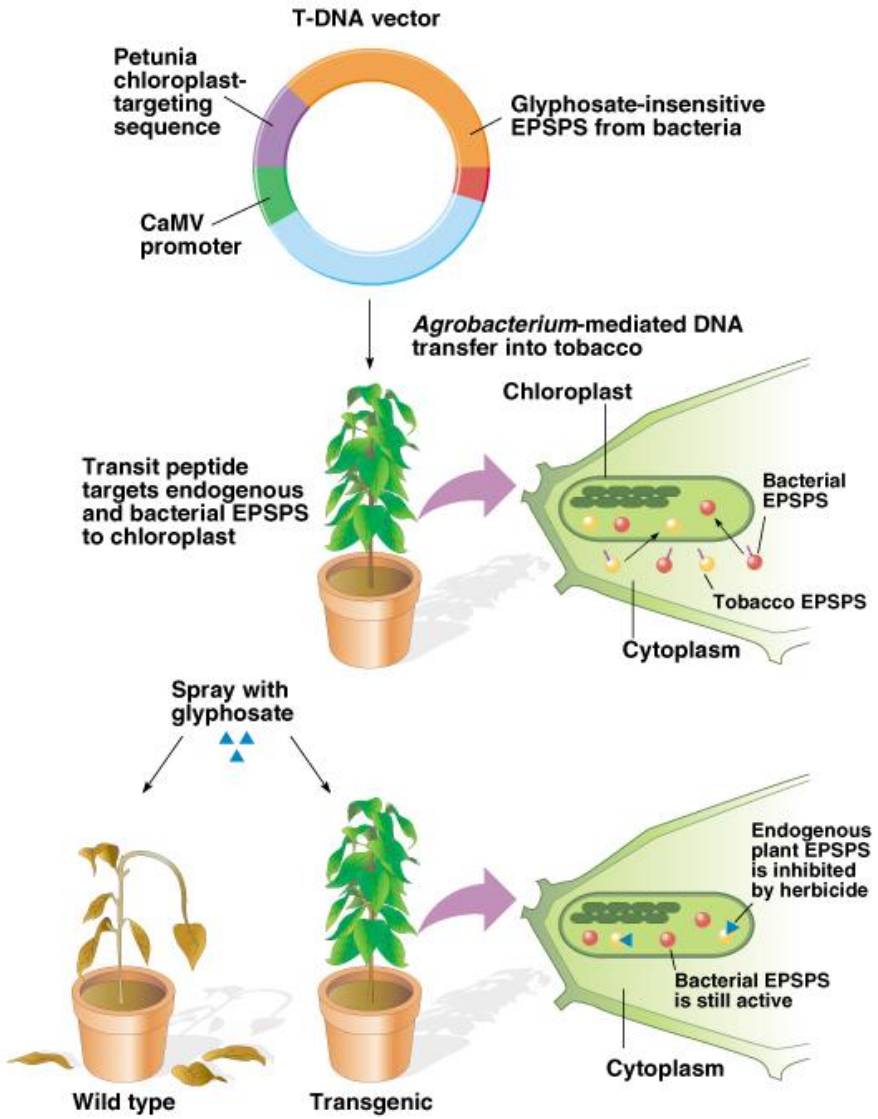
   d. Approaches to Roundup™ tolerant plants include:

      i. Introducing a modified bacterial form of EPSPS that synthesizes aromatic amino acids even in the presence of Roundup™ (Figure 8.13).

      ii. Introducing genes for enzymes that convert Roundup to an inactive form.

   e. Monsanto's Roundup™Ready soybeans have been on the market since 1996, despite controversy over the safety of genetically engineered crop plants.

Fig. 8.13 Making a transgenic, Roundup™-tolerant tobacco plant by introducing a modified form of the bacterial gene for the enzyme EPSPS that is resistant to the herbicide



2. Another example is the “Flavr Savr” tomato, in which production of the fruit-softening enzyme polygalactouronase (PG) is inhibited by an antisense mRNA, resulting in vine-ripened . tomatoes that are less prone to bruising and over-ripening.

3. Genetic engineering of plants is likely to increase. Particularly valuable traits might be:

   a. Increased yield.

   b. Insect pest resistance.

   c. Herbicide tolerance.

4. Developers of such plants promise advances in preventing world hunger, but significant public resistance to genetically modified plants exists in many countries, including a growing resistance in the U.S.