14: The Eukaryotic Genome and Its Expression

Introduction

- Although DNA is used as genetic material by both eukaryotes and prokaryotes, content and organization is different.
- Prokaryotes have a smaller genome and few repeat DNA sequences.
- Eukaryotes have repeat DNA sequences, many of which do not code for proteins.

The Eukaryotic Genome

- Table 14.1 lists the differences between prokaryotic and eukaryotic genomes.

The eukaryotic genome is larger and more complex than the prokaryotic genome

- Viral genomes are smaller than bacterial genomes and bacterial genomes are smaller than eukaryotic genomes.
- Among eukaryotes, there is not always a direct relationship between complexity and genome size. (See Figure 14.1.)
  - Humans have 6 billion DNA base pairs (bp) in each cell's nucleus.
  - The lily, which produces fewer different proteins than a human, has 18 times more DNA.
- Most eukaryotic DNA codes for nothing.
  - Interspersed throughout the genome are various repeated sequences that are not transcribed.
  - Even within genes there are sequences that are not translated.
  - Some of these nontranscribed regions are structural, such as the telomeres; some regulate gene expression; and some have no known use.
  - The presence of so much noncoding DNA is an enigma.
- Eukaryotes have several separate, linear chromosomes.
  - Each eukaryotic chromosome has a single, continuous, double helix of DNA.
  - In humans, each chromosome is from 20 million to 100 million bp in length.
  - Each chromosome must have recognition sequences, a centromeric sequence, and, on the ends, telomeric sequences.
- In eukaryotes, chromosomes are separated from the cytoplasm by the nuclear envelope.
  - DNA duplication and RNA synthesis occur in the nucleus.
  - RNA translation occurs exclusively outside the nucleus.
- The DNA is packaged into nucleosomes, chromatin fibers, and ultimately chromosomes. (See Figure 9.7.)
- This compaction restricts the access of RNA synthesis machinery to the DNA.
- It also segregates replicated DNA during mitosis and meiosis.
- Genes have regulatory sequences that are not transcribed.
  - Promoter regions are where RNA polymerase binds to begin transcription.
  - A second set of regulatory DNA sequences, the enhancers and silencers, are common in eukaryotes, but rare in prokaryotes.
    - They can be located quite far away from the promoter.
    - They act by binding proteins that stimulate or inhibit transcription.
- Noncoding DNA sequences, scattered within protein coding portion of genes, exist. These get transcribed, but are spliced out of the pre-mRNA prior to transport out of the nucleus. (See Figure 14.2.)

- The entire genomes of a yeast (*Saccharomyces cerevisiae*), a roundworm (*C. elegans*), a fruit fly (*Drosophila melanogaster*), and a flowering plant (*Arabidopsis thaliana*) have been sequenced.
- In the year 2000, a "rough" sequence of the entire human genome was completed.

The yeast genome adds some eukaryotic functions onto a prokaryotic model

- The yeast *Saccharomyces cerevisiae* has 16 chromosomes and a haploid content of more than 12,068,000 bp.
- The sequencing has found 6,200 genes. Around 70% now have been assigned probable roles.
- The remaining 30% are being studied by gene inactivation techniques.
- The proportions of the yeast coding for specific metabolic roles are as follows:
  - About 11% of yeast proteins are for general metabolism, 3% are for energy production and storage, 3% are for DNA replication and repair, 12% are for protein synthesis, and 6% are for protein targeting and secretion.
- The most striking difference between the *E. coli* and yeast is the number of genes for protein targeting. (See Table 14.2.)
- Both of these organisms use about the same number of genes for cell survival.
- Eukaryotes require a greater number of genes because of the compartmentalization found in yeast and other eukaryotic organisms.
- This confirms the long-held idea that eukaryote cells are structurally more complex than prokaryote cells.

The nematode genome adds developmental complexity

- *Caenorhabditis elegans* is a 1 mm long nematode (roundworm) that lives in soil.
- In 3 days it develops from an egg to an adult.
- Its transparent body makes it possible to observe its development.
- The adult has 1,000 cells. It has a nervous system and digestive system, it reproduces sexually, and it ages.
- *C. elegans* has been intensely studied, especially in regard to its development.
  - The genome, consisting of around 97 million bp and about 19,000 protein-coding genes, has now been completely sequenced.
  - Before sequencing, scientists guessed it would have about 6,000 genes.
- About 3,000 genes in the worm have counterparts in yeast.
  - These genes are the ones considered essential to all eukaryotes.
- Many of the remaining 16,000 genes perform roles related to multicellularity:
  - Holding cells together to form tissues
  - Cell differentiation and intercellular communication
- Table 14.3 identifies some of the genes in *C. elegans* that are essential to multicellularity.

The fruit fly genome has surprisingly few genes

- *Drosophila melanogaster* is much larger than *C. elegans*, having 10 times more cells.
- The *Drosophila* genome is also larger, containing about 180,000,000 bp.
- Surprisingly, *Drosophila* has fewer protein-coding genes (13,600) than *C. elegans* (19,000).
- One reason is that *C. elegans* has more copies of related genes than *Drosophila*.
- Many of the genes found in the worm have genes homologous to those of the fly.
- About half of the fly genes have mammalian homologs.
  - The fly genome contains 177 genes whose sequences are known to be directly involved in human diseases, such as cancer.
  - The roles of such genes are often more easily studied in the fly than in humans.

Gene sequences for other organisms are rapidly becoming known

- A rough human genome sequence has recently been completed.
- The sequencing of a genome is only the first step in the process of identifying genes and determining their functions and interactions.
- Over the next decades, tremendous progress should occur.

Repetitive Sequences in the Eukaryotic Genome

Highly repetitive sequences are present in large numbers of copies

- Three types of highly repetitive sequences are found in eukaryotes:
  - Satellites are 5 to 50 bp long, repeated side by side up to a million times.
  - Minisatellites are 12 to 100 bp long and repeated several thousand times. Individuals in a population can vary in the number of copies.
  - Microsatellites are very short (1 to 5 bp), and present in 10 to 50 copies per cluster. They are scattered all over the genome.
- The purpose for these repetitive sequences, if any exists, is currently unknown.

Telomeres are repetitive sequences at the ends of chromosomes

- Telomeres are moderately repetitive sequences at the end of the chromosomes.
- Telomeres are specialized sequences, generated by an enzyme called *telomerase*.
- A problem exists for the replication of linear DNA molecules, such as those of eukaryotic chromosomes.
  - There is nothing beyond the primer in the 5′ direction to replace the RNA that makes up the primer. (DNA polymerase can only add to an existing molecule.)
  - As a result, new chromosomes formed after DNA replication lack a bit of double-stranded DNA at each end.
  - These ends get trimmed, so chromosomes get shorter with each cell division.
- The human telomere repeated sequence is TTAGGG.
  - It is repeated around 2,500 times at each end.
  - These repeats bind special proteins that protect the ends. Without them, the DNA breaks down.
  - Telomeres can be elongated by an enzyme called *telomerase*. (See Figure 14.3.)
  - This enzyme contains not only proteins, but an RNA template sequence.
  - Telomeres shorten between 50 and 200 bp per cell division.
  - Telomerase activity occurs only during early embryonic periods of development in many human cell types.
  - As cells divide, their telomeres get shorter each time. After 20 to 30 divisions, the cells senesce (die).
  - Aging cells in culture have been demonstrated to have finite life spans in terms of the number of divisions.
  - Cells in culture have been experimentally altered to produce telomerase, and such cells become immortal.
  - Human cancer cells also divide continuously. Telomerase is expressed in more than 90 percent of human cancers.
  - Drugs targeting telomerase may help in attacking specific tumors.

Some moderately repetitive sequences are transcribed

- Some moderately repetitive DNA sequences code for tRNA's and rRNA's.
- Having copies of coding regions makes it possible to produce these RNA's more quickly.
- In mammals there are four different rRNA molecules that make up the ribosome: 18S, 5.8S, 28S, and 5S.

- The 18S, 5.8S, and 28S rRNA's are transcribed as a single precursor RNA, which is twice the size of all three ultimate products. (See Figure 14.4.)
- There are 280 copies of sequences coding for the transcript located in clusters on five different chromosomes.
- Another moderately repeated sequence, which is not known to be transcribed, is the *Alu* family.
  - These are 300 bp long and scattered throughout the genome.
  - There are 300,000 copies of the *Alu* family, and these may act as multiple origins for DNA replication.

Transposable elements move about the genome

- Some moderately repetitive DNA sequences are transposons.
- There are four main types:
  - SINEs are short interspersed elements and are up to 500 bp long. They are transcribed but not translated.
  - LINEs are long interspersed elements. They are up to 7,000 bp long, and some are transcribed and translated into proteins. They constitute about 15% of the human genome.
- Both of these elements are present in more than 100,000 copies.
  - They move about the genome by making an RNA copy, which acts as a template for the new DNA.
  - The new DNA then inserts itself at a new location in the genome.
- Retrotransposons also make an RNA copy when they move.
  - These are rare in mammals but common in other animals and in yeast.
  - They resemble retroviruses, such as HIV, but code for no protein coat.
- DNA transposons do not use an RNA intermediate, but actually move to a new spot without replicating.
- Beneficial roles for these sequences are unknown.
- They seem to be cellular parasites that replicate themselves.
- Detrimental effects have been found; insertion of a transposon into a functional gene can disable it. (See Figure 14.5.)
  - *In some cases, insertions near a gene can alter its transcription rate.*
- When an insertion occurs in a germ cell line, a gamete carrying the new mutation might form.
- If it occurs in a somatic cell, cancer might result.
- Transposition certainly increases genetic variation by shuffling genetic material and creating new genes.
- Transposons may have played a role in the evolution of cell organelles such as chloroplasts and mitochondria.

The Structures of Protein-Coding Genes

- Many protein-coding genes in eukaryotes are single-copy DNA sequences.
- Unlike most prokaryotes, however, eukaryotes have genes with noncoding internal sequences.
- Eukaryotes also form gene families with structurally and functionally related cousins in the genome.

Protein-coding genes contain noncoding internal and flanking sequences

- Promoters, terminators, and introns are noncoding sequences in genes. (See Figure 14.6.)
- A gene's promoter occurs at the beginning of the gene and provides the site where RNA polymerase begins the transcription process.

- A gene's terminator occurs at the end of the gene and signals the end of transcription.
- The entire sequence between the promoter and the terminator is transcribed, but some parts are removed before translation occurs.
- The noncoding sequences are called *introns*.
- Introns are interspersed with the coding regions, called *exons*.
  - First the entire sequence, including introns, is transcribed. The resulting RNA is the primary RNA transcript, also known as *pre-mRNA*.
  - Pre-mRNA is processed into mRNA by removing the transcripts of the introns and splicing together the transcripts of the exons.
- Nucleic acid hybridization was useful for the initial discovery of introns.
  - Hybridization between globin-coding DNA and the mature globin mRNA showed a loop of DNA not present on the mRNA. (See Figures 14.7 and 14.8.)
- Most (but not all) genes of vertebrates contain introns. Other eukaryotes also have introns.
- Only a few prokaryotes have introns.
- In some cases, each exon in a gene codes for a distinct functional region (domain) in the final protein.

Many eukaryotic genes are members of gene families

- About half of all eukaryotic protein-coding genes have a single copy in the haploid genome.
- The rest have multiple copies.
- Pseudogenes are inexact, nonfunctional copies of genes, often found near the functional copy.
- Sometimes copies of genes are functional, but slightly different.
- A set of duplicated or related genes is called a *gene family*. (See Figure 14.9.)
  - Immunoglobulins have hundreds of members.
  - β-globins that are part of hemoglobin have a few members.
  - Immunoglobulins that make up antibodies have hundreds of members.
- Copies change over time, providing an opportunity to make useful new versions.
- As long as one member retains the original DNA sequence, the other members can mutate without negative effects.
- If the mutated gene is useful, it will be selected for in succeeding generations.
- During development, different members of the β-globin gene family are expressed at different times and in different tissues. (See Figure 14.10.)
  - Each member of the gene family is best suited for the time when it is expressed.
  - The globin gene family also includes nonfunctional pseudogenes.
  - These "black sheep" family members are evolutionary experiments gone wrong.
  - As long as some members of a gene family are functional and pseudogenes are not actively detrimental, there appears to be little selective pressure in evolution to eliminate the pseudogenes.

RNA Processing

- To produce mRNA from pre-mRNA, introns are removed, exons are joined, and the ends of the transcript are modified.

The primary transcript of a protein-coding gene is modified at both ends

- The pre-mRNA gets modified at the 5′ and 3′ ends. (See Figure 14.11.)
  - The G cap, a modified guanosine triphosphate (GTP), is added to the 5′ end.
  - A poly A tail is added to the 3′ end. It is 100 to 300 residues in length.
  - This tail may assist the export of mRNA from the nucleus.

Splicing removes introns from the primary transcript

- If introns are not deleted, a nonfunctional protein would result.
- Figure 14.12 illustrates the removal of introns, a process called *splicing*.
- At the boundaries between introns and exons are consensus sequences.
- A small ribonucleoprotein particle (snRNP) binds by complementary base-pairing to the consensus sequence at the 5′ exon–intron boundary.
- Another snRNP binds near the 3′ exon–intron boundary.
- Next, other proteins bind, forming a large RNA–protein complex called a *spliceosome*.
- The spliceosomes require energy acquired from ATP to cut out the introns and join the exons together to make mature mRNA.
  - Beta thalassemia is a human disease that is caused by a mutation at the consensus sequence of the β-globin gene.
  - It causes an inadequate supply of β-globin, which in turn results in an inadequate supply of red blood cells.
- After processing, the mature mRNA exits the nucleus; unprocessed or incompletely processed pre-mRNA's stay in the nucleus.

Transcriptional Control

- Regulation of gene expression can occur at many points during development. (See Figure 14.13.)

Specific genes can be selectively transcribed

- Different cells in multicellular organisms produce some proteins found in all cell types, but also some that are unique to each cell type.
- With few exceptions, all cells in an organism have the same genes or DNA sequences, but they express genes differently.
  - For example, both brain and liver cells transcribe "housekeeping" genes.
  - Housekeeping genes code for enzymes and other molecules essential to the survival of all cells, such as the enzymes needed for glycolysis and ribosomal RNA's.
  - But liver cells transcribe some genes for liver-specific proteins, and brain cells transcribe some genes for brain-specific proteins.
  - The difference in the production of proteins is due to differential transcription.
- Contrasting eukaryotes and prokaryotes:
  - Unlike prokaryotes, in which related genes are transcribed in units called *operons*, eukaryotes tend to have solitary genes.
  - Regulating several genes at once requires common control elements in each of the genes. These allow the genes to respond to the same signal.
  - Eukaryotes have three different RNA polymerases, each catalyzing transcription of a specific type of gene.
    - RNA polymerase II transcribes protein-coding genes to mRNA.
    - RNA polymerase I transcribes rRNA coding sequences.
    - RNA polymerase III transcribes tRNA and small nuclear RNA's.
  - Most eukaryotic genes have other DNA sequences that regulate transcription: regulators, enhancers, and silencers.
  - Unlike prokaryotes, in eukaryotes many different proteins are involved in initiating transcription.
- Transcription factors:

    - In prokaryotes, the promoter is a DNA recognition sequence about 40 bp from the 5′ initiation point and a TATA box (area rich in AT base pairs) just upstream from the intersection point.
    - In contrast, in eukaryotes the TATA box is about 25 bp away from the initiation site, and one or two recognition sequences are about 50 to 70 bp 5′ from the TATA box.
    - Transcription in eukaryotes requires various regulatory proteins called *transcription factors*. (See Figure 14.4.)
        - RNA polymerase II does not bind until several other proteins, such as TFIID, have already bound the protein–DNA complex.
        - Some DNA sequences, such as the TATA box, are common to most promoters; others are unique to only a few genes.
        - Transcription factors play an important role in cell differentiation during development.
- Regulators, enhancers, and silencers in DNA:
    - In addition to those regions associated with the promoter, nearby regulator regions also affect transcription. (See Figure 14.15.)
    - Regulator proteins bind these regions.
    - Much farther away (up to 20,000 bp away) are enhancer regions.
        - Enhancers bind activator proteins, which strongly stimulate the transcription complex.
    - There also are negative regulatory regions of DNA called *silencers*.
        - They have the opposite effect of enhancers.
        - Silencers bind proteins called *repressors* and turn off transcription.
    - All genes in most tissues can transcribe a small amount of RNA.
    - However, the correct combination of factors is what determines the maximum rate of transcription.
        - Rates can be modulated by the varying combinations of factors.
- Coordinating the expression of genes:
    - In prokaryotes, a single regulatory system can regulate several genes in an operon.
    - In eukaryotes, genes on different chromosomes may required coordination.
    - Regulation of the various genes can be coordinated if all have the same regulatory sequences that bind to the same activators and regulators.
    - One example is the stress response element in plants. (See Figure 14.16.)
    - Stress response elements near each of the scattered genes stimulate RNA synthesis.
    - RNA then codes for proteins needed for water conservation.
- The binding of proteins to DNA:
    - Key to transcription regulation in eukaryotes is the binding of protein to specific DNA sequences.
    - Proteins need to recognize and bind appropriate sites.
    - There are four different structural themes or motifs for protein–DNA interactions.
    - The helix-turn-helix motif involves several α-helices, one of which makes contact with DNA. (See the in-text art on page 273 for this and other motifs.)
        - This motif is common in genes involved in embryonic development.
    - The zinc finger motif has a loop that forms when a zinc ion is held by the amino acids cysteine and histidine.
        - This motif is common for steroid hormone receptors.

- The leucine zipper motif has hydrophobic leucine residues on one side of a polypeptide.
  - Two polypeptide chains interact (zipper) hydrophobically.
  - This sets up positively charged residues just past the zipper, which interact with the DNA.
  - This is a common DNA-binding motif. One of these, AP-1, is involved in cell growth and division.
  - Overactivity of transcription factor AP-1 has been linked to several types of cancer.
- The helix-loop-helix motif is two helices separated by a loop. Two adjacent regions associate with the DNA.
  - This motif occurs in the activator proteins that bind to enhancers for immunoglobulin genes that make antibodies and in factors involved in muscle protein synthesis.

Genes can be inactivated by chromatin structure

- The packaging of DNA by the nuclear proteins in chromatin can make DNA physically inaccessible to RNA polymerase and associated components. (See Figure 14.17.)
- Local effects:
  - Nucleosomes inhibit both initiation and elongation of transcription.
  - These effects are countered by two protein complexes.
    - One binds upstream of the initiation site, disaggregating the nucleosomes and allowing the large initiation complex to form.
    - The other binds after transcription begins, allowing the transcription complex to move through these nucleosomes.
    - These processes are called *chromatin remodeling*.
- Global effects:
  - There are two kinds of chromatin; they can be distinguished by staining the interphase nucleus.
  - Euchromatin is diffuse and stains lightly. It contains DNA that is transcribed into mRNA.
  - Heterochromatin stains densely and is generally not transcribed.
    - Any genes in heterochromatin are thus inactivated.
    - X chromosome inactivation is an example.
    - In mammals, just one of the X chromosomes in each cell of a female is activated.
    - This creates a gene dosage equivalence between the cells of XX females and XY males.
    - Early during development, one of the X chromosomes in each of the cells of a female embryo is inactivated.
    - Which one is inactivated is usually chance. However, once inactivated, all descendants of the cell have the same X inactivated.
  - X chromosome inactivation was discovered independently in 1961 by three scientists: Mary Lyon, Liane Russell, and Ernest Beutler.
  - Interphase cells of XX (normal) females have a single, stainable nuclear body called a *Barr body*. (See Figure 14.18. The Instructor's Resource CD-ROM includes a micrograph of a cell with a visible Barr Body.)
    - The Barr body is the condensed, inactive X chromosome.
  - A female with the normal two chromosomes will have one Barr body.
  - An abnormal XXXX female will have three Barr bodies.

- An abnormal XXY male has one.
- Addition of a methyl group ($-CH_3$) to cytosine on DNA may be involved with the inactivation.
- The inactive X has one less-methylated gene, *XIST*, while the rest are heavily methylated, and, except for the *XIST* gene, are not transcribed.
- The other activated chromosome genes *are* transcribed and have the potential for expression.
- The RNA transcribed from *XIST* is not an mRNA and remains in the nucleus.
- It binds the X chromosome that transcribes it and triggers inactivation.

A DNA sequence can move to a new location to activate transcription

- Some gene expression is regulated by gene movement to another area of the chromosomes.
- *Saccharomyces cerevisiae*, a yeast species, has two mating types, *a* and α, which fuse to create a diploid zygote.
- Physically separate sites exist: the *a*, α, and MAT regions.
- The mating type of a given yeast cell depends on which copy, *a* or α, exists at the MAT site.
- Whichever allele is inserted at the MAT region is expressed.
- Usually the types are transcriptionally silent because of a repressor binding them.
- When transportation occurs of *a* or α to the MAT site, that choice of mating type switches on.
- DNA rearrangement is also important in producing the highly variable proteins that make up the human repertoire of antibodies.
- In some cancers, inactive genes move to be adjacent to active promoters.

Selective gene amplification results in more templates for transcription

- The process of increasing the number of copies of a gene in a cell is called *gene amplification*.
- One cell can make more gene product than another cell by this process.
- Mature frog and fish eggs have up to a trillion ribosomes, which are used for massive protein synthesis following fertilization.
- To make this number, ribosomal rRNA gene clusters are selectively amplified and copied until there are a million copies in just one cell.
- These DNA sequences go from being 0.2% to 68% of the total genomic DNA.
- Later, after cell division begins, the number of copies returns to normal.
- The mechanism for this overreplication of a single gene is not clearly understood.

Posttranscriptional Control

- Pre-mRNA can be processed in the nucleus by cutting and splicing.
- The longevity of mRNA in the cytoplasm can also be regulated.

Different mRNA's can be made from the same gene by alternate splicing

- Alternate splicing of a specific pre-mRNA can generate different proteins from a single gene.
- For example, cells in five different tissues slice the pre-mRNA for the structural protein tropomyosin into five different mRNA's.
- As a result, each of the five tissues has a different form of tropomyosin. (See Figure 14.20.)

The stability of mRNA can be regulated

- DNA must remain stable and has elaborate mechanisms for its repair.
- RNA has no repair system.

- After entering the cytoplasm, mRNA is subject to digestion by ribonucleases in both the cytoplasm and lysosomes.
- However, different mRNA's have different life spans.
- An interesting example of cellular modulation of mRNA life span is with tubulin.
  - When the tubulin concentration is high, some tubulin molecules bind to tubulin mRNA, accelerating the breakdown of tubulin mRNA.

Translational and Posttranslational Control

- Only a third of the genes coding for mRNA in yeast have a positive correlation between the amount of mRNA and the amount of protein synthesized.
- Two-thirds of the proteins coded showed no relationship.
- Proteins can affect translation by binding to mRNA in the cytoplasm.
- This is important to prevent the production of too many unnecessary proteins.
- Cyclin, which stimulates the cell cycle, must be shut off after it has done its job. If not, inappropriate cell division may lead to a tumor.

The translation of mRNA can be controlled

- The capping mechanism on mRNA may be a way to control translation.
- Before mRNA can be translated in eukaryotes, it must have a modified 5′ guanosine molecule to serve as a cap. (See Figure 14.11.)
- The oocytes of the tobacco hornworm moth offer an example of mRNA's that are stored by delaying modification of the G cap.
  - When the mRNA is needed, after fertilization, the cap gets modified and the mRNA's are translated.
- Ferritin, an iron storage protein in mammalian cells, increases with elevation in Fe^{2+}.
  - However, the amount of ferritin mRNA remains constant.
  - When iron is low, a translational repressor protein binds to ferritin mRNA and prevents translation.
  - When iron levels rise, excess iron binds to the repressor and alters its three-dimensional structure, causing it to detach from the mRNA. Translation is then able to proceed.
- Hemoglobin consists of four polypeptide chains and a nonprotein pigment, heme.
  - If heme synthesis does not equal globin synthesis, some polypeptide chains stay free in the cell.
  - Excess heme in the cell increases the rate of translation of globin mRNA by removing a block to initiation of translation at the ribosome.
  - This helps to maintain the balance among hemoglobin components.

The proteasome controls the longevity of proteins after translation

- Most gene products (proteins) are modified after translation.
- Regulating the lifetime of a protein is a way to control its actions.
- Proteins involved in cell division (cyclins) are hydrolyzed at the correct time to control cell cycle events.
- Proteins identified for breakdown are often linked to a 76-amino acid protein, ubiquitin.
- The protein–ubiquitin complex then binds a complex called a *proteasome*. (See Figure 14.21.)
- The protein is cleaved from the ubiquitin and then enters a hollow cylinder nicknamed the "molecular chamber of doom."
- Three different proteases digest it there into small peptides and amino acids.
- Overall, concentrations of proteins depend on rates of synthesis and rates of digestion.

The Instructor's Resource CD-ROM includes a micrograph of a cell with a visible Barr Body.