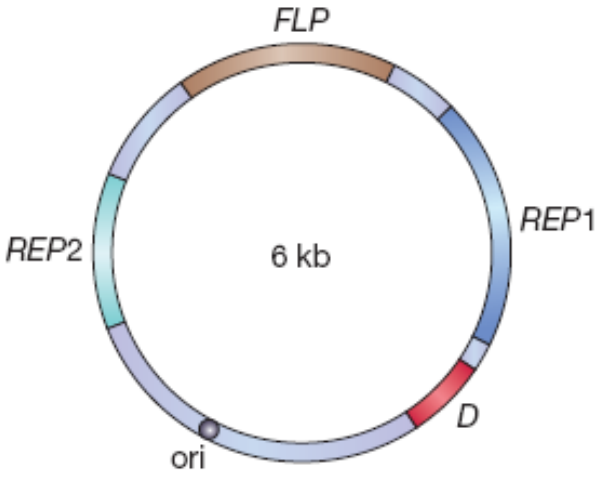
Yeast cloning and expression vectors

yeast 2 µm plasmid



The yeast 2 µm plasmid. *REP1 and REP2 are involved in replication of the* plasmid, and *FLP codes for a protein that can convert the A form of the* plasmid (shown here) to the B form, in which the gene order has been rearranged by intramolecular recombination. The function of *D is not* exactly known.

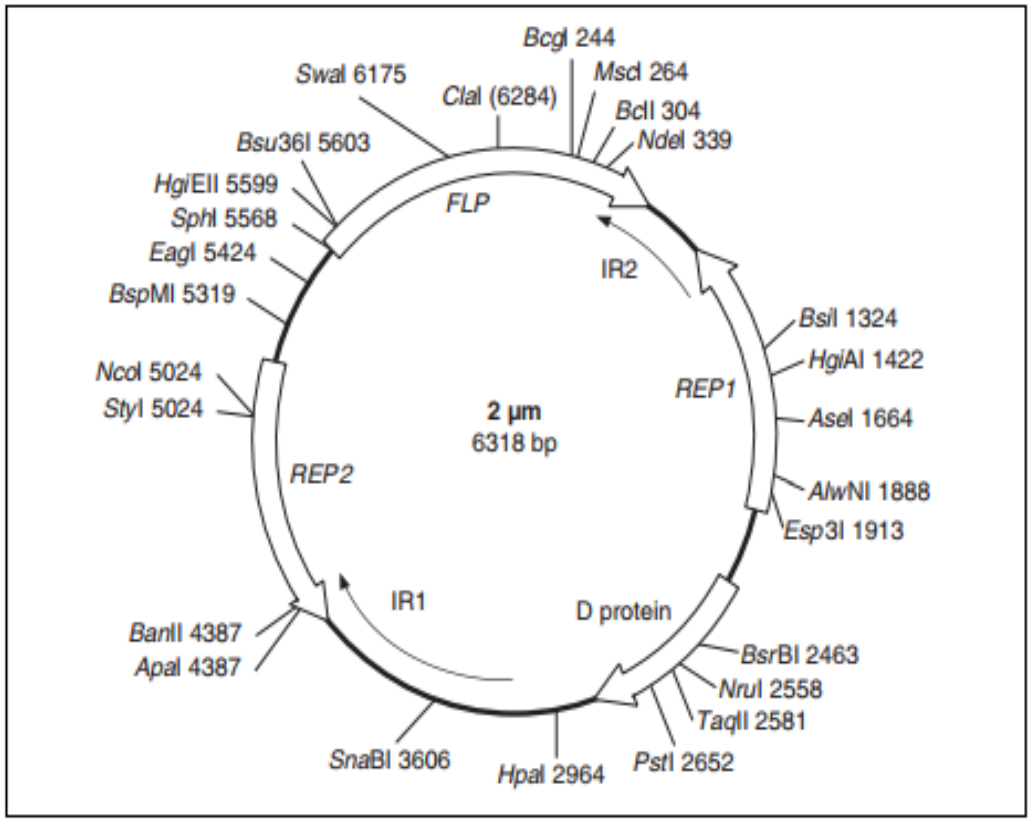


Figure 13.4.4 2µm plasmid. The 2µm circle is a naturally occurring DNA plasmid found in almost all strains of *S. cerevisiae*, with a copy number of ~20 to 80. The plasmid exists in two different forms, A and B (the former is shown above), due to intra-molecular recombination between two perfect 599-bp inverted repeats. Strains that carry this plasmid are called cir^+; strains missing the plasmid cir^0 have been identified or isolated (*see UNIT 13.9*). It is extremely stable mitotically, with a spontaneous loss rate in haploid cells of 10^{-4} per generation; during meiosis the plasmid is transmitted to all four spore products. The plasmid has been completely sequenced (Hartley and Donelson, 1980) and the sequence is available from GenBank (Plant: yscplasm).

Vectors based on the 2 um plasmid—

- *Yeast episomal plasmids*
- *Yeast integrative plasmids*
- *Yeast replicative plasmids*

Yeast episomal plasmids

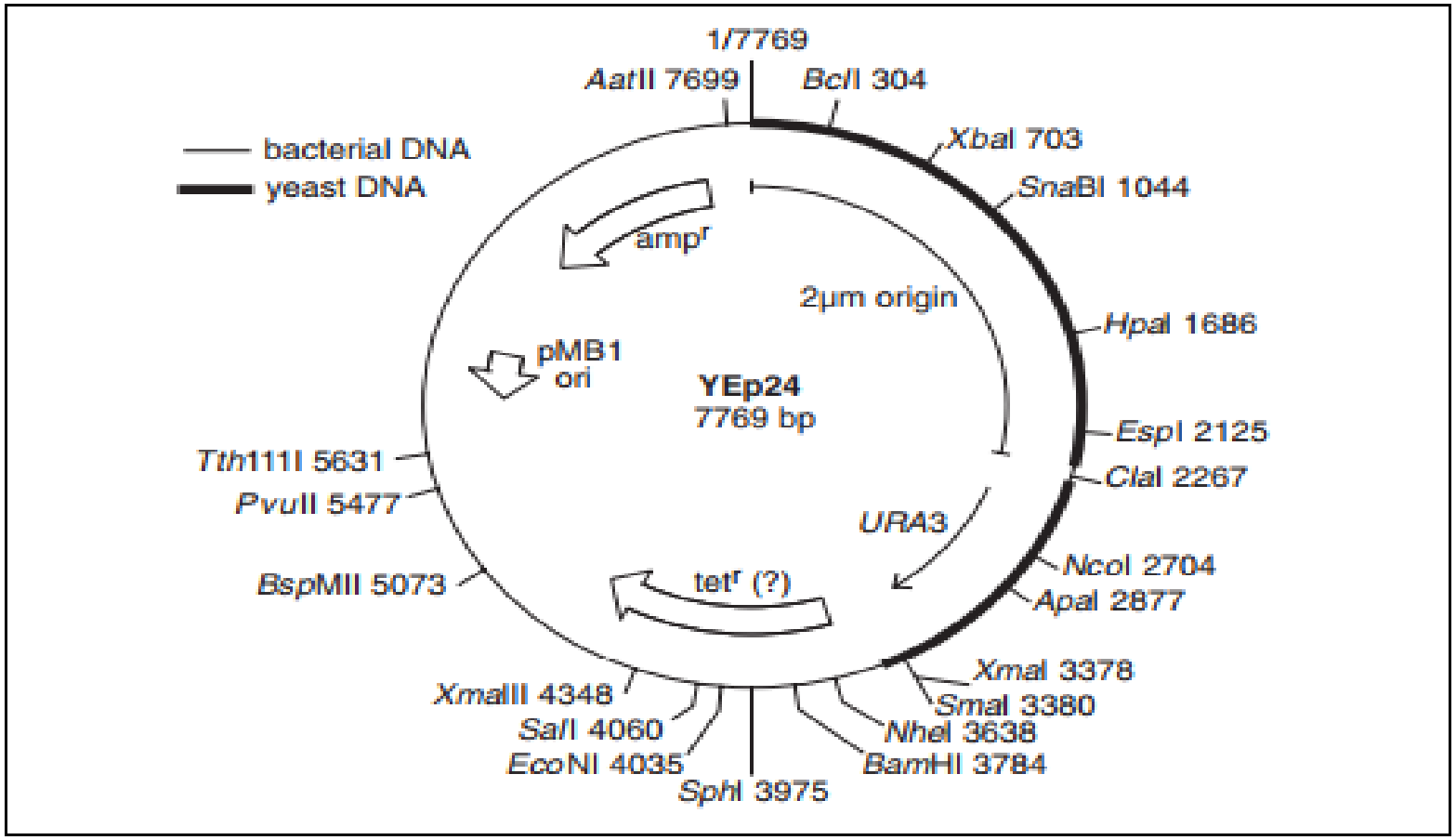


Figure 13.4.3 **YEp24.** YEp24 has the 2.2-kb *Eco*RI fragment of the B form of the 2 µm plasmid and the 1.1-kb *Hin*dIII *URA3* gene inserted into the *Eco*RI and *Hin*dIII sites, respectively, of pBR322 (Botstein et al., 1979). The expression of the tet^r gene is variable among different isolates of this plasmid. YEp24 is mitotically stable in cir$^+$ strains at a copy number of about 20 but is unstable in cir^o strains. The complete sequence of YEp24 is available from the Vecbase database (file name: Vecbase.Yep24) and a detailed restriction map can be found in the New England Biolabs catalog.

Yeast integrative plasmids

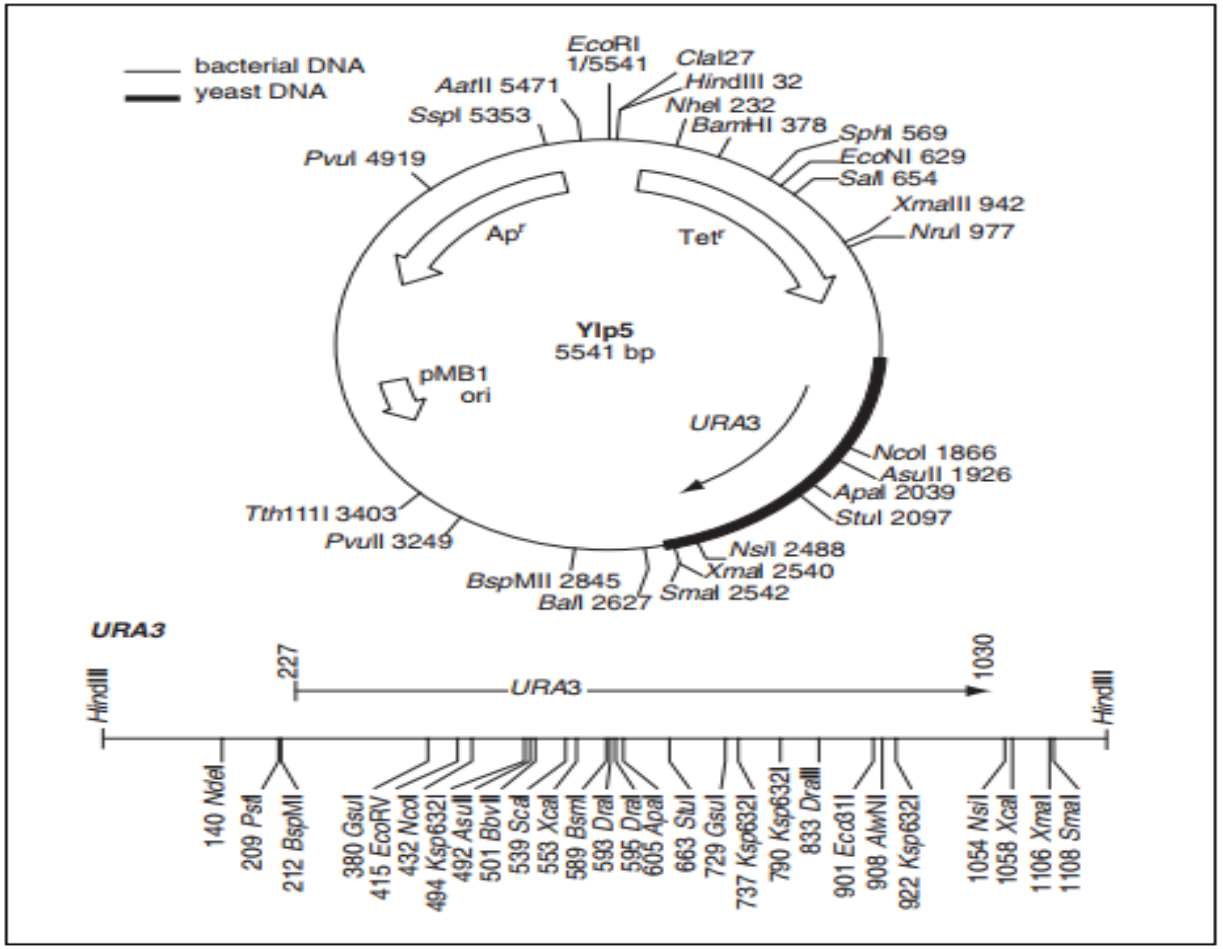
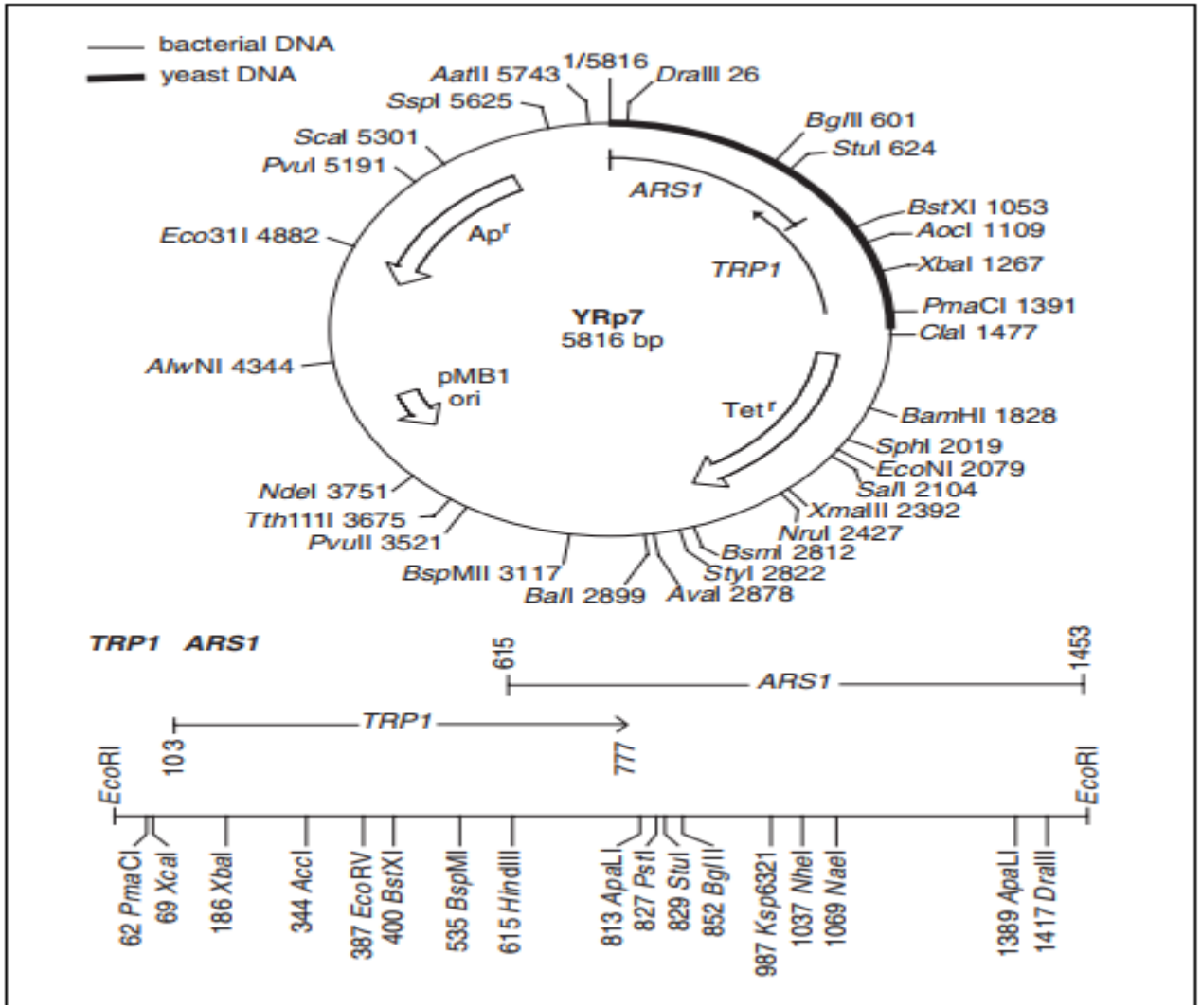


Figure 13.4.1 **Yip5**. Yip5 contains the 1.1-kb *Hin*dIII *URA3* gene cloned into the *Ava*I site of pBR322 via the addition of poly(dG-dC) tails (Struhl et al., 1979). Since this plasmid does not contain a yeast origin of replication, transformants occur by integration into the yeast genome at the *URA3* locus; the frequency of transformation can be increased by linearization of the plasmid within the *URA3* insert. The complete nucleotide sequence is available from the Vecbase database (file name: Vecbase.Yip5) and a detailed restriction map can be found in the New England Biolabs catalog.

Yeast replicative plasmids



Choice of vectors

Three factors come into play when deciding which type of yeast vector is most suitable for a particular cloning experiment.

1.T**ransformation frequency,**

YEps> YRps>YIps

YEps have the highest transformation frequency, providing between 10,000 and 100,000 transformed cells per ug.

YRps are also quite productive, giving between 1000 and 10,000 transformants per ug,

YIp yields less than 1000 transformants per ug, and only 1–10 unless

special procedures are used.

The low transformation frequency of a YIp reflects the fact that the rather rare chromosomal integration event is necessary before the vector can be retained in a yeast cell.

Choice of vectors

2. Copy number.

YEps and YRps have the highest copy numbers: 20–50 and 5–100, respectively.

In contrast, a YIp is usually present at just one copy per cell. These figures are important if the objective is to obtain protein from the cloned gene, as the more copies there are of the gene the greater the expected yield of the protein product.

Choice of vectors

3. Stability

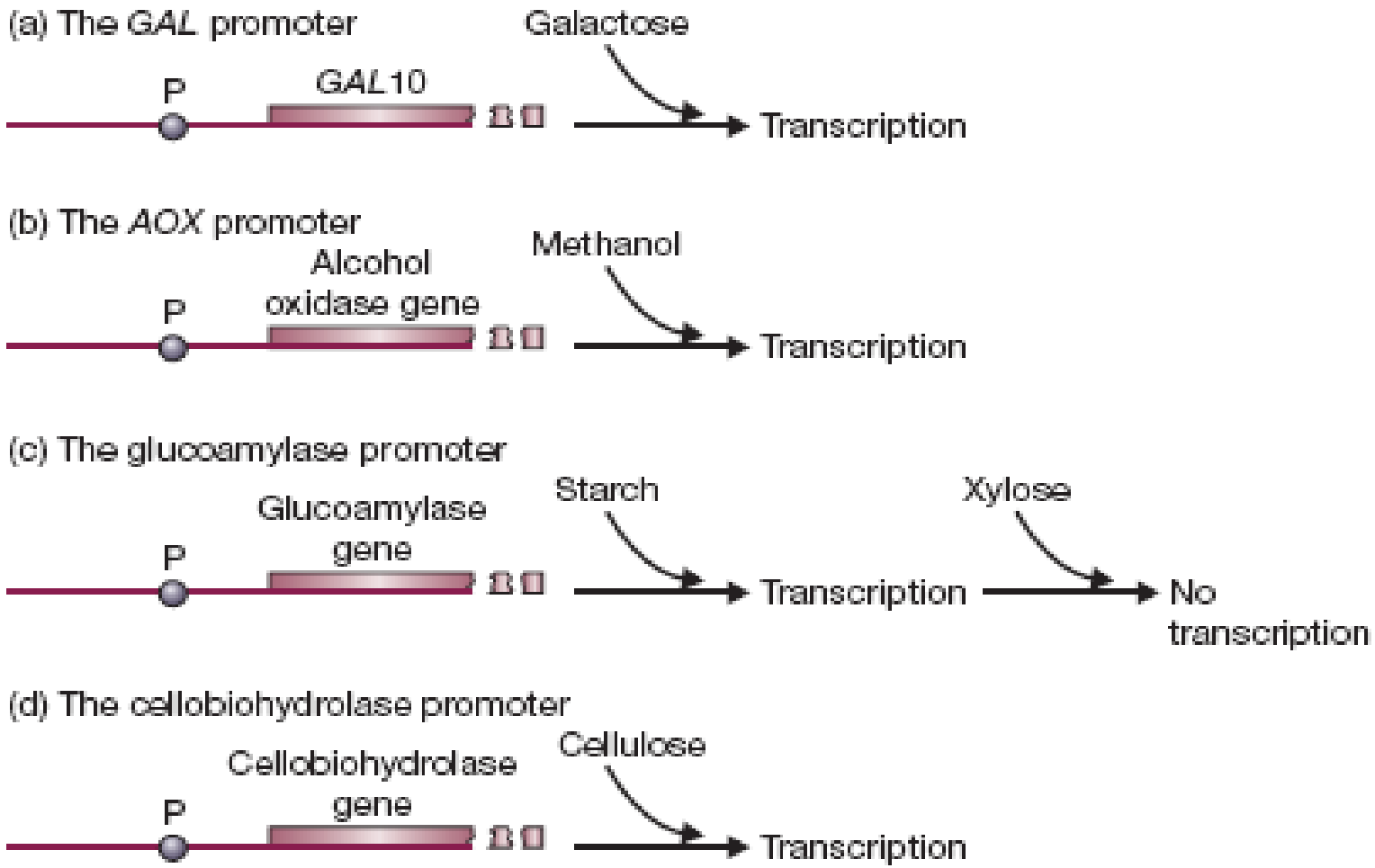
YIps produce very stable recombinants, as loss of a YIp that has become integrated into a chromosome occurs at only a very low frequency.

In contrast, YRp recombinants are extremely unstable, the plasmids tending to congregate in the mother cell when a daughter cell buds off, so the daughter cell is non-recombinant.

YEp recombinants suffer from similar problems,though an improved understanding of the biology of the 2 fm plasmid has enabled more stable YEps to be developed in recent years

Expression in Yeast

choice of promoters



Host Strains

- ***Saccharomyces cerevisiae-*** It is currently the most popular microbial eukaryote for recombinant protein production.
  - (Problems- unable to glycosylate animal proteins correctly, often adding too many sugar units ("hyperglycosylation"), it also lacks an efficient system for secreting proteins into the growth medium.

- ***Pichia Pastoris-*** *It* is able to synthesize large amounts of recombinant protein (up to 30% of the total cell protein) and its glycosylation abilities are very similar to those of animal cells.
  - The only significant problem with *P. pastoris is that it sometimes degrades recombinant proteins before they can be* purified, but this can be controlled by using special growth media.

- Other yeasts that have been used for recombinant protein synthesis include *Hansenula polymorpha, Yarrowia lipolytica, and Kluveromyces lactis. The last of these has the attraction that it* can be grown on waste products from the food industry.

Pichia pastoris- Glycosylation

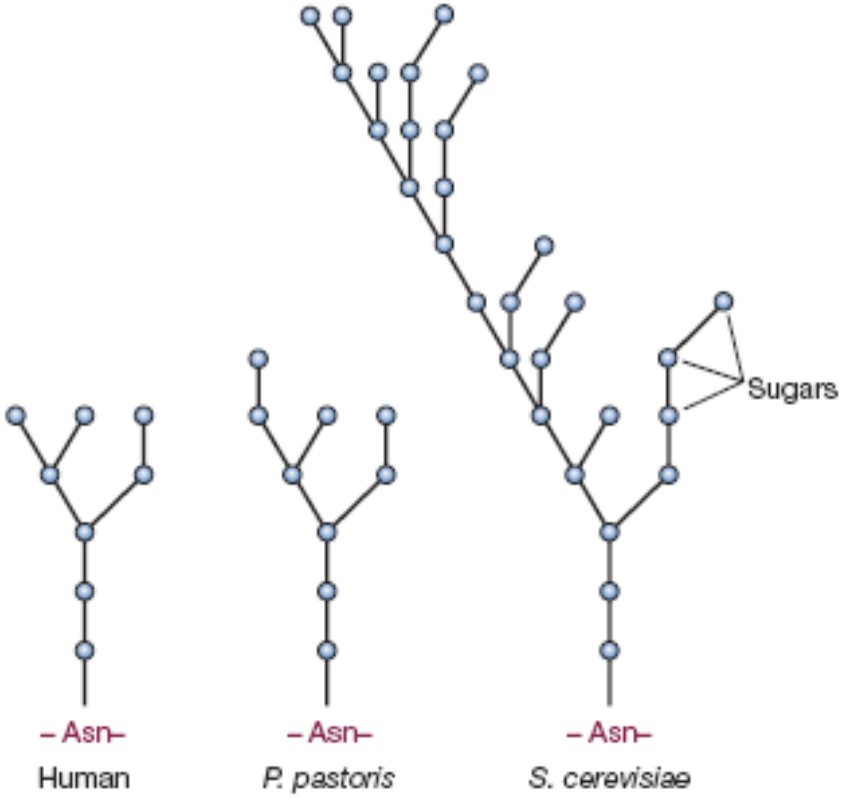


Figure 13.17

Comparison between a typical glycosylation structure found on an animal protein and the structures synthesized by *P. pastoris* and *S. cerevisiae*.