pathway is best defined in HeLa cells. By utilising an electrophoretic analysis of nucleolar RNAs, Weinberg and Penman (*J. molec. Biol.*, **47**, 169–178; 1970) found that the 45S primary transcript is cleaved to a 41S molecule, which in turn is split into a 32S and a 20S RNA; the 32S molecule then matures into 28S rRNA and the 20S molecule matures into 18S rRNA. By analysing the oligonucleotides into which these species are broken by ribonuclease, Maden, Salim and Summers (*Nature new Biol.*, **237**, 5–9; 1972) confirmed that the 41S precursor contains both 28S and 18S sequences and that the 32S and 20S precursors respectively contain sequences of only the 28S and 18S rRNA molecules. Because oligonucleotides were identified by their content of labelled methyl groups, these experiments showed also that all the methyl groups added to 45S RNA are located in regions of the molecule destined to become mature rRNAs.

The identification by Seeber and Busch (*J. biol. Chem.*, **246**, 7151–7158; 1971) of common 5′ terminal sequences in 45S, 32S and 28S RNAs of Novikoff hepatoma cells showed that the 28S rRNA sequences is located at this end of the precursors which contain it. The elegant analysis of RNA secondary structures reported by Wellauer and Dawid (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 2827–2831; 1973) achieved a more precise location of ribosomal RNA sequences in the precursors. The 45S precursor contains a 5′ terminal structure corresponding to 28S rRNA, with the 18S rRNA located within the remaining sequences; the 41S RNA structure is similar but lacks all the material on the 3′ side of the 18S sequence. The cleavage that generates 32S and 20S precursors must take place within the central non-rRNA region, since 32S RNA has 5′ terminal 28S sequence and 3′ terminal non-rRNA sequence; and 20S RNA has a 5′ non-rRNA sequence with 18S rRNA located 3′ terminally.

Each ribosome contains one 5S rRNA molecule in addition to the major RNAs and this seems to be coded by genes dispersed in the genome whose transcription is independent of that of the main precursor. The presence of another small RNA was first revealed by Pene, Knight and Darnell (*J. molec. Biol.*, **33**, 609–623; 1968), when they found that denaturing treatments release a small fragment from 28S rRNA. Originally characterised as 7S RNA, this molecule must be covalently linked to 28S rRNA, and more accurate characterisation has led to its recent description as 5.8S RNA. Synthesis of 5.8S RNA seems to be linked to that of the large rRNAs—kinetic experiments suggest its derivation by cleavage from the 32S precursor in HeLa cells. The sequence of the HeLa 5.8S RNA has now been examined by Maden and Robertson (*J. molec. Biol.*, **87**, 227–236; 1974), who have compared the oligonucleotides released by T1 ribonuclease with those cleaved from the rRNA precursors. When 28S rRNA is prepared by cold phenol extraction, it retains the 5.8S fragment; but heat treatment separates 28S rRNA from its small attached molecule. Correspondingly, upon T1 ribonuclease analysis, these two preparations of 28S RNA differ in the oligonucleotides characteristic of 5.8S RNA. One equivalent of the 5.8S RNA sequences is present for every equivalent of 28S RNA.

Fingerprints of the 32S RNA precursor show that it possesses the oligonucleotides constituting 5.8S RNA, again in molar yields which suggest the presence of one 5.8S RNA sequence in each precursor molecule. Some evidence for the presence of these fragments in 45S RNA is also presented. Although not yet precisely placed within the precursors, the 155 nucleotide long 5.8S RNA presumably lies between

Multifunctional gene in a eukaryote

On page 630 of this issue of *Nature* Bollon presents a genetical analysis of a "multifunctional" eukaryotic gene, threonine deaminase, concerned with both structure and regulation.

Working with the yeast, *Saccharomyces cerevisiae*, Bollon found that the *ilv1* gene not only encodes for a gene product that is catalytically active (that is, L-threonine deaminase; EC4.2.1.16) but also that the gene product is involved in multivalent repression of the other isoleucine–valine enzymes. It has thus been possible, by careful genetical analysis, to discriminate between the nucleotide sequences that encode for that structural (=catalytic) and regulatory (=multivalent repression) functions of the *ilv1* gene. The advantage of using the yeast system, for studying regulatory phenomena, is that yeasts lend themselves well for intragenic complementation. Using many different *ilv1 mutants*—impaired in threonine deaminase activity—intragenic complementation and fine structure analyses have been carried out.

Goldberger (*Science*, **183**, 810–816; 1974); Levinthal *et al.* (*Nature new Biol.*, **246**, 65–68; 1973) and Kasai (*Nature*, **249**, 523–527; 1974) have provided excellent background information about gene regulation. The first suggestion of an enzyme participating in its own regulation—by repression—was made by Vogel (in *The Chemical Basis of Heredity*, edit. by McElroy and Glass, Johns Hopkins Press, Baltimore 1957, page 276); it is also pertinent to note that the yeast system developed by Bollon and Magee (*Proc. natn. Acad. Sci. U.S.A.*, **68**, 2169–2173; 1971; *J. Bact.*, **113**, 1333–1344; 1973) offered the first evidence *in vivo* for the regulatory role of threonine deaminase, or for some form of the *ilv1* gene as Bollon shows in this issue. Of course, Umbarger and his many colleagues, as well as Hatfield's group, have provided a vivid picture of the role of *ilvA* (which specifies threonine deaminase) in multivalent repression in bacteria.

Although the *ilvl* gene product in yeast seems to be involved in multivalent repression, other elements are involved too. As already mentioned, Bollon and Magee noted that the involvement of leucine in multivalent repression may be unique (compare Levinthal *et al.*, 1973) and they suggest that leucine may function in repression by way of leucyl-tRNA; thus the *ilv1* gene product may be only one component of the multivalent machinery. One vital feature of the yeast work which may have been overlooked is that the genes specifying its various isoleucine–valine enzymes are found on different chromosomes (compare *ilvADE*, *ilvB* and *ilvC* in *Escherichia coli*).

The principal points of Bollon's work can be summarised as follows:

- A multifunctional gene (*ilv1*) has been analysed in detail in a eukaryotic organism for the first time.
- The *ilv1* product, threonine deaminase, catalyses the conversion of L-threonine→α-ketobutyrate in yeast.
- The regulatory role of the *ilv1* gene product is considered a positive effector for the derepression of the isoleucine-valine enzymes.
- Thus *ilv1* is truly 'multifunctional' with catalytic and regulatory parts being played by threonine deaminase.
- Intracistronic discrimination denoting the catalytic and regulatory functions of *ilv1* is presented.

Gerard A. O'Donovan