

have now studied the products of various partial ribonuclease digestions. This has involved the development of a number of new techniques which will be described in detail later. From the large number of partial digestion products obtained, we deduced the unique sequence shown in Figs. 1 and 2.

Fig. 1 is drawn to show the residues which are believed to be involved in base-pairing. These base-paired regions were identified as four sequences which were particularly resistant to digestion by ribonucleases. The longest double-stranded region is believed to be formed by base-pairing between the two ends of the molecule, and there are also two smaller "loops". Base-pairing between the two ends of the molecule is also found in transfer RNA, but otherwise there is less base-pairing in 5S RNA than in transfer RNA.

It will be noted that two residues are shown as occupying position 13. It appears that there are two 5S RNAs, presumably controlled by separate genes, one having a G in position 13 and the other a U. This was found in one strain of *E. coli* (MRE600, obtained from Dr H. E. Wade of the MRE Experimental Station, Porton, Wiltshire), while in another strain (CA265, obtained from Dr S. Brenner of this laboratory) a difference has been found in another position. It is probable that there are also other minor heterogeneities and therefore Fig. 1 illustrates the structure only of the two principal components of 5S RNA in *E. coli*, MRE600.

There are two sequences of ten and eight residues, respectively, that are repeated twice in the molecule. In Fig. 2 the structure is written so that the common sequences are aligned. There is considerable homology between the two parts of the chain, indicated by the boxed regions. This observation suggests that the 5S RNA may have evolved from a smaller RNA by a duplication of a part of the DNA sequence within the gene. There also appears to be some homology between the two ends of the molecule as shown by the underlining in Fig. 2. This could be explained by a separate duplication.

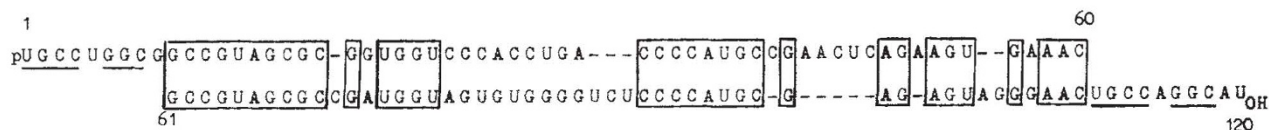


Fig. 2. Homologies between the two halves of the sequence of 5S RNA. The residues are numbered as in Fig. 1. Homologies are shown by the boxed areas. Dashes are where gaps have to be left in the sequence in order to maximize these homologies. The underlining shows similarities between the two ends of the molecule.

Previous work on RNA sequences has been confined to transfer RNAs and a number of complete sequences have been reported⁴⁻⁷. The 5S RNA is 120 residues long compared with 75-85 residues in the transfer RNAs, and the absence of "minor" bases makes interpretation somewhat more difficult. This work shows, however, that it is possible, using the small-scale techniques which we have developed, to determine the nucleotide sequence of an RNA labelled with phosphorus-32.

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¹ Rosset, R., Monier, R., and Julien, J., *Bull. Soc. Chim. Biol.*, **46**, 87 (1964).

² Sanger, F., Brownlee, G. G., and Barrell, B. G., *J. Mol. Biol.*, **13**, 373 (1965).

³ Brownlee, G. G., and Sanger, F., *J. Mol. Biol.*, **23**, 337 (1967).

⁴ Holley, R. W., Apgar, J., Everett, G. A., Madison, J. T., Marquisee, M., Merrill, S. H., Penswick, J. R., and Zamir, A., *Science*, **147**, 1462 (1965).

⁵ Zachau, H. G., Dutting, D., and Feldmann, H., *Hoppe Seyler's Z. Physiol. Chem.*, **347**, 212 (1966).

⁶ Madison, J. T., Everett, G. A., and Kung, H., *Science*, **153**, 531 (1966).

⁷ RajBhandary, U. L., Chang, S. H., Stuart, A., Faulkner, R. D., Hoskinson, R. M., and Khorana, H. G., *Proc. US Nat. Acad. Sci., Wash.*, **57**, 751 (1967).

BIOPHYSICS

Electron Spin Resonance in Biological Tissues

SEVERAL points arise from the recent communication by Dettmer, Driscoll, Wallace and Neaves¹.

First, the simple factors affecting electron spin resonance signals in tissues, which they set out to describe, seem to be only the rapid decay of free radical signals, as measured extensively by Commoner and Ternberg², Kerkut *et al.*³ and mentioned briefly as a source of error by Mallard and Kent⁴. Far from not having appeared in print, as stated in their first paragraph, it is seen that many workers have noted this effect.

Second, the techniques of lyophilization used by Dettmer *et al.* seem to us to be unnecessary and, in certain respects, dangerous. It was shown by Truby *et al.*⁵ that lyophilization can create unwanted free radicals in organic materials, and Varian Associates, Inc., even show an example of this in their literature. We think that it is unnecessary to use this technique because adequate techniques now exist for the study of whole tissues at both room temperature and low temperatures²⁻⁴.

That lyophilization has some effect can be shown from the spectra of lyophilized samples which Dettmer *et al.* show in their communication. These spectra, when observed in the light of the methods of analysis of Searle *et al.*⁶ and Lebedev⁷, are seen to represent asymmetric lines, possibly arising from *g*-value anisotropy. The lines have a Lorentzian shape and the anisotropic splitting in terms of individual line width is about 3.0.

On the other hand, the lines observed by other workers, although still asymmetric, can be shown to have a value of anisotropic splitting approximately equal to 2.0, which applies both at room and low temperatures down to 77° K. In addition, the spectra under these conditions result from individual lines of Gaussian shape. Because these Gaussian spectra are in the presence of water (and therefore an environment containing many protons) the Gaussian shape may result from the broadening effect of

this. The difference in the apparent anisotropic splitting between the Lorentzian and the Gaussian spectra may arise from a difference in individual line width between the two cases. Because no details of line widths are published by Dettmer *et al.*, however, it is not possible to follow this further.

Alternatively, it is possible that the differences described here arise merely because these authors are observing electron spin resonance signals generated by the lyophilization process, and this in itself represents a serious pitfall.

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¹ Dettmer, C. M., Driscoll, D. H., Wallace, J. D., and Neaves, A., *Nature*, **214**, 492 (1967).

² Commoner, B., and Ternberg, J. L., *Proc. US Nat. Acad. Sci.*, **47**, 1374 (1961).

³ Kerkut, G. A., Edwards, M. L., Leech, K., and Munday, K. A., *Experientia*, **17**, 497 (1961).

⁴ Mallard, J. R., and Kent, M., *Nature*, **210**, 588 (1966).

⁵ Truby, K. F., and Goldzieher, W. J., *Nature*, **182**, 1371 (1958).

⁶ Searle, J. W., Smith, R. C., and Wyard, S. J., *Proc. Phys. Soc.*, **78**, 1174 (1961).

⁷ Lebedev, Ya. S., *Zh. Strukt. Khimii*, **4**, 22 (1963).