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Shapiro *et al.* (*1*), after an extensive review of our experimental data, suggest changes of two nucleotides in the nucleotide sequence of the alanine RNA (*2*). One change involves deleting a nucleotide from the octanucleotide C-U-C-C-C-U-U-I- to give a heptanucleotide, and the second change involves adding a nucleotide to the octanucleotide G-G-G-A-G-A-G-U*- (the asterisk indicates that the position is occupied by a mixture of uridine and dihydrouridine) to give a nonanucleotide. The basis for the suggestion is that, even though the observed nucleotide composition analyses (*3*) are within experimental error of calculated values for octanucleotides, the agreement would be improved if the fragment were a heptanucleotide and a nonanucleotide.

Until we determined the sequences of the two octanucleotides it was our assumption also that the fragments were a heptanucleotide and a nonanucleotide, respectively. However, during determination of the sequences, it was found that both fragments were octanucleotides. Thus, Shapiro *et al.* have retraced paths that we took ourselves. In our judgment, they have not considered adequately the additional experimental data that exclude their suggestions.

It may be worthwhile to summarize the evidence that was considered conclusive, particularly since it serves to emphasize the power of the method (*4*) that was used for sequence determination of oligonucleotides composed of five to eight nucleotide residues. In the sequence determinations, an oligonucleotide was first treated with alkaline phosphatase to remove the terminal 3′-phosphate. Snake-venom phosphodiesterase was then used to obtain a mixture of stepwise degradation products, formed by the successive removal of nucleotide residues from the 3′-end. Chromatography of the mixture, under conditions that separate the products primarily on the basis of charge, gave a series of peaks. The size of the oligonucleotide was obtained by counting the number of peaks. The nucleotide sequence was obtained from analyses of the successive peaks for their terminal nucleosides. With each of the above octanucleotides, the method gave a set of evenly spaced peaks, and the number of peaks indicated that the starting material was an octanucleotide.

The suggestion that the octanucleotide C-U-C-C-C-U-U-I- is perhaps a heptanucleotide requires that one ignore the fact that the chromatographic pattern shows that there is one more stepwise degradation product than would be obtained from a heptanucleotide.

The suggestion that the octanucleotide G-G-G-A-G-A-G-U*- is a nonanucleotide requires that the chromatogram contain one more peak than was observed, or that a peak corresponding to a stepwise degradation product be missing. In the chromatogram (*4*), the observed peaks are approximately evenly spaced, with no indication of a gap corresponding to a missing peak. The spacings between peaks are 7, 8.5, 7, 7.5, and 8.5 tubes, respectively, starting at the trinucleoside diphosphate and proceeding to the octanucleoside heptaphosphate. The last peak in the series is known to be the octanucleoside heptaphosphate because it contains the terminal pyrimidine. Actually this peak contains two components, because of the mixture of terminal uridine and dihydrouridine. As an added control in the chromatogram, the presence of a terminal 3′-phosphate is shown to displace the chromatographic peak 11.5 tubes to the right (comparison of the position of the octanucleoside heptaphosphate with that of the octanucleotide), which is exactly what would be predicted for the addition of two negative charges without the addition of any nucleoside. The removal of two nucleotide residues without the presence of a detectable intermediate would result in a gap in the pattern with a width of more than 11.5 tubes. The chromatogram shows no such feature. In conclusion, the chromatographic pattern is exactly what would be expected from an octanucleotide and is clearly inconsistent with a nonanucleotide pattern.

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Adenovirus "Helpers"

In their report [*Science* **149**, 754 (1965)] on adenovirus-associated defective virus particles (AAV), Atchison, Casto, and Hammon establish three important facts: (i) AAV multiplies only in the presence of mulitplying simian adenovirus type 15 (SV 15). (ii) Only SV 15 and human adenovirus type 2, of several viruses tested, can serve as "helper" for AAV. (iii) AAV and SV 15 are serologically unrelated. The authors then draw an analogy between AAV and Rous sarcoma virus, another defective virus. The Rous sarcoma defect involves the inability of the virus to manufacture its own coat protein; in the presence of helper, Rous sarcoma is produced by utilizing coat protein provided by the helper. Consequently Rous sarcoma and its helper virus are serologically identical. A better analogy can be drawn between AAV and a defective plant virus, the so-called satellite of tobacco necrosis virus (TNV). The satellite cannot multiply alone, is helped only by strains of TNV, and is serologically unrelated to TNV. Concerning both AAV and the TNV satellite, the major question that remains unanswered is: what restricts the choice of helper virus?

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