news and views

'Enigma Variations' of mammalian messenger RNA

from Beverly Griffin

THE significance of the sequences of the 3′-end of messenger RNAs in mammalian cells has for the past few years been a problem for cell biologists. Most messenger RNAs isolated from the cytoplasm of eukaryotic cells have been found to contain polyadenylic acid sequences of varying lengths at their 3′-ends. Attempted explanations for the function of the 3′-terminal poly (A) residues have had to take into account the fact that although most eukaryotic messenger RNAs contain them, not all do (*Nature*, **252**, 268; 1974). Now, before the significance of the unusual nature of the 3′-end of many messenger RNAs has been clarified, attention has been re-directed to the 5′-ends of these molecules by the discovery that they also have unusual nucleotide sequences.

Rottman, Shatkin and Perry (*Cell*, **3**, 197; 1974) described a nucleotide sequence at the 5′-terminus of a wide variety of messenger RNAs, which consisted of 7-methylguanosine linked through its 5′-hydroxyl group *via* a tri- (or pyro)-phosphate group to the 5′-hydroxyl of an O-methylated nucleoside. Although these authors gave a chemically incorrect structure to 7-methylguanosine, it seems nonetheless that their general ideas about the structure of the 5′-ends of eukaryotic messenger RNAs may be correct. A similar kind of structure at the 5′-ends of several low molecular weight nuclear RNAs of Novikoff hepatoma ascites cells had also been reported in an abstract by Ro-Choi *et al.* (*Fedn Proc.*, **33**, 1548; 1974). Between them, these two reports have stimulated a great deal of research.

Three papers in this issue of *Nature* by Muthukrishnan, Both, Furuichi and Shakin (**255**, 33; 1975), by Abraham, Rhodes and Banerjee (**255**, 37; 1975) and by Adams and Cory (**255**, 28; 1975) discuss the blocked (or capped) 5′-ends of messenger RNA from two viruses (reovirus and vesicular stomatitis virus (VS)) and from rabbit reticulocyte and mouse myeloma cells. In all three papers, 7-methylguanosine has been shown to be the nucleoside used by the cell as a block for the 5′-tri-(or pyro)-phosphate end of the messenger RNA. This is linked to an O-methyl nucleoside which may apparently be one of any of the four bases. It is postulated here (as in the transfer RNAs) that O-methylation may serve to protect the phosphodiester link of messenger RNA from the nuclease activities in the cell, with the protected nucleoside possibly playing an important rôle during the processing of the messenger. Similar results are now appearing, or will shortly appear, in the literature about the 5′-ends of other messenger RNAs, not only from viruses whose genomic and messenger information reside in the same molecules (reovirus, Furuichi *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 362, 742; 1975, and silkworm cytoplasmic polyhedrosis virus, Furuichi and Miura, *Nature*, **253**, 374; 1975), but also in viruses containing DNA as their genomes (vaccinia virus, Wei and Moss, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 318; 1975 and Urushibara *et al.*, *FEBS Lett.*, **49**, 385; 1975, Simian virus 40, Lavi and Shatkin, *Fedn Proc.*, **34**, 526; 1975, and adenovirus, see Muthu krishnan *et al.*), and in uninfected cells (including mouse L cells, monkey BSC-1 cells and HeLa cells, see Muthukrishnan *et al.*) The weight of the evidence suggests in fact that methylated blocked 5′-termini may prove to be a general feature of eukaryotic messenger RNAs.

Thus, before a biological explanation has been found for the nature of the 3′-end of mammalian messenger RNAs, a biological explanation must also be sought for the nature of the 5′-end. Some progress has already been made on the rôle of the structure at the 5′-end, notably by the group at the Roche Institute of Molecular Biology (Muthukrishnan *et al.*, page 33, and Both, Banerjee and Shatkin, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1189; 1975). They show first that methylated reovirus and VSV messenger RNAs stimulate protein synthesis (in wheat germ or mouse L cell protein synthesising systems) to a greater extent than unmethylated messenger RNAs. They also show that messengers synthesised *in vitro* in the presence of S-adenosylmethionine by virion-associated RNA polymerases contain the 5′-terminal structures found *in vivo*, and moreover that the *in vitro* synthesised messengers are translated with fidelity in the protein synthesising system. Their conclusions from these, and studies carried out with aurintricarboxylic acid (which inhibits polypeptide chain initiation) and sparsomysin (which inhibits chain elongation), are that methylation (at least with reovirus and VSV) is required for translation and probably occurs at the initiation step of protein synthesis. They do not exclude the possibility that 5′-terminal methylation is also important in the processing of eukaryotic messenger RNA.

None of the studies to date offers any explanation for the choice of 7-methylguanosine as the blocking group for the 5′-end of mammalian messenger RNA. The guanosine may be added to the 5′-end of the RNA by guanosine triphosphate, and this may in part explain the need for GTP in protein synthesis. Guanosine may then be methylated at N–7 by the machinery of the cell. (It is noteworthy that all the chemical studies on methylation of nucleosides show the high susceptibility of the 7-position of guanosine to methylation.) 7-methylguanosine, moreover, is a highly polar molecule at every *p*H at which it can be studied, and has never been isolated except in a hydrated state. Thus, this modified base on a messenger RNA would be expected not only to increase the hydrophilic nature of the macromolecule, but also to help it to form stable salt bridges in, for example, a protein-nucleic acid interaction complex.

Finally, it is conceivable that the blue fluorescence associated with 7-methylguanosine at *p*H 7 (Hendler *et al.*, *Biochemistry*, **9**, 4141; 1970) could be used as an assay for locating and purifying messenger RNAs. This marker might prove of value particularly in messengers lacking the 3′-poly (A) end (where the usual affinity chromatography procedures cannot be used for separating messenger from the bulk of other large cellular RNA molecules).

There seems little doubt that the finding of unusual sequences at the 5′-ends of eukaryotic messenger RNAs will prove a considerable stimulus to research, and may prove to be more illuminating than complicating.