Giant Messengers Repeated

from our Molecular Genetics Correspondent

ALTHOUGH it is now more than a decade since messenger RNA was identified in bacteria, and even though a few specific messengers have recently been isolated in eukaryotic cells, the production of eukaryotic mRNA remains obscure in all but outline. Very large molecules of RNA are found in the eukaryotic nucleus; much of their content seems to be unstable and is degraded rapidly so that only a small amount matures—sometimes by a process involving the addition of a sequence of polyadenylic acid—and is transported to the cytoplasm to become the messenger RNA of polysomes. But the maturation of heterogeneous nuclear RNA to mRNA has usually been followed only *en masse*; and problems such as the size of the unit of transcription and the relationship of the non-coding to coding sequences of the precursor RNA can probably be solved only by following the maturation of individual messengers.

Two options have been explored recently in attempts to isolate specific messengers and their precursors. One is to use cells which predominantly synthesize one protein product, so that most of their informational RNA falls into only one class. An ingenious alternative has been reported by Daneholt (*Nature New Biology*, **240**, 229; 1972), who has made use of the salivary gland cells of *Chironomus tentans*. In common with other Diptera, these glands contain giant polytene chromosomes which consist of a many-fold replication of the genetic material and in which active sites can be identified by the "puffs" which extrude from the chromosomes where RNA is synthesized. One of the largest of these puffs, BR2 (Balbiani Ring 2), can be isolated from fixed cells by micromanipulation so that its RNA alone can be obtained and characterized. This offers the unusual advantage that an RNA coded by a known genetic locus can be studied; because the BR2 region is active only in salivary glands and not in any other tissue, its RNA presumably codes for a specific salivary polypeptide, and if this can be identified it may become possible to follow every stage of gene action in this one cell system.

By incubating salivary glands *in vitro* with ^{3}H-uridine or cytidine, Daneholt obtained BR2 ^{3}H-labelled RNA from the isolated puff region. By using very mild conditions of extraction the RNA could be characterized on gels; it has a single predominant peak which appears to correspond to a molecular weight of about 35×10^6. The RNA sediments at about 75S on gradients, which corresponds to a molecular weight of about 15×10^6. Salivary gland chromosomes are organized into a series of bands, each of which seems to function as a unit of transcription, because a puff always extrudes from a single band. The average band in *C. tentans* contains DNA with a molecular weight of about 60×10^6; the RNA transcript of BR2 (which is of about average size in band length) may therefore correspond to the entire length of DNA in the chromosome band. If this conclusion proves to be general, it will solve the old problem of finding a function for the large amount of DNA in a band—a reasonable estimate for the order of the size of a gene is 10^6 daltons and because this is much smaller than the content of a band it has been difficult to decide whether (as appearances suggest) each band contains only one genetic function, consisting of either many genes making up the entire band length or one gene corresponding to only part of it; or whether the genetic organization of the band bears no relation to its apparent function.

But the idea that the band is the unit of transcription raises a similar question. Does all the RNA code for protein? If so, does the large 75S RNA consist of a repetition of many identical genes or of an operon-like structure containing many different, but related, genes? If only some of the RNA codes for protein, what are the structure and function of the rest? These problems can be answered only by following the fate of the RNA after transcription. The size of the BR2 transcript is of the same order as the heterogeneous nuclear RNA which is rapidly labelled in the cytoplasm of a variety of eukaryotic cells. Does it share in common with these molecules the degradation of much of its sequence?

Because the BR2 transcript originates from a specific genetic locus, it can be assayed by cytological hybridization *in situ* with salivary glands. Lambert (*Nature*, **242**, 51; 1973) has used this criterion to follow the progress of the transcript from the puff to the cytoplasm. After labelling with tritiated precursors, he isolated Balbiani rings themselves, nuclear sap and cytoplasm. The labelled RNA was extracted from each fraction and annealed with salivary gland chromosomes. Both BR2 RNA and nuclear sap RNA hybridized strongly with the BR2 chromosome puff; cytoplasmic RNA also bound to the BR2 region, but less actively. These ingenious experiments therefore show that the BR2 RNA, or at least a substantial part of it, is indeed transported from nucleus to cytoplasm.

That the BR2 RNA hybridizes with its corresponding genetic locus indicates that it must, in part at least, contain repetitious sequences of nucleotides, because a unique sequence for the BR2 region could anneal only much more slowly than is allowed in conditions of cytological hybridization. One possible interpretation is that some of the BR2 RNA has a highly repeated sequence and does not code for protein, while the rest of the molecule is unique. An alternative is that the whole RNA codes for protein and consists of a repetition of many copies of one gene; of course, if this gene codes for a repeating sequence of amino-acids, there may also be internal repetitions.

That the complete sequence of the RNA is transported to the cytoplasm (and therefore presumably codes for protein) is suggested by the recent results of Daneholt and Hosick (*Proc. US Nat. Acad. Sci.*, **70**, 442; 1973). The RNAs synthesized by the four chromosomes of *Chironomus* range in size from 15–100S. Nuclear sap, however, contains a predominant peak at 75S which may be the transcript of BR2. By following the progress of this peak into the cytoplasm, Daneholt and Hosick have found that the kinetics of movement of 75S RNA are consistent with the idea that it is releasd into the nuclear sap on transcription and then passes intact, after only a short delay, into the cytoplasm. This may indeed be a general phenomenon because the full range of nuclear RNA sizes is seen in the cytoplasm.

Quantitative experiments suggest that 75S RNA makes up about 1.5 per cent of the total RNA and is comparatively stable; it must therefore code for some protein predominant in the salivary gland cell. The presence of 75S RNA in the cytoplasm suggests that most if not all of its sequence codes for protein and that the degradative maturation processes of other eukaryotes do not apply in the salivary glands. The repetitious structure of the 75S BR2 transcript promises that its complete characterization may throw an important light on the vexed question of the organization of eukaryotic DNA, in particular on the function of repeated sequences and the numbers of individual genes in the genome.

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