What are the properties of Genetic RNA?

by

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A note for the RNA Tie Club.

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Is he in heaven?
Is he in hell?
Dear damned elusive Pimpernel..

May 1960

What are the properties of genetic RNA?

By genetic RNA we mean the hypothetical RNA which is presumed to be made under the direction of the gene, and which itself determines (or at least in part) the amino acid sequence of proteins synthesized in the ribosomes of the cytoplasm.

~~Recent experiment in bacteria~~

We have been shown by Pardee, Jacob and Monod that when the z gene, which controls the enzyme β-galactosidase, is introduced from an Hfr to an F^- cell the synthesis of the protein starts within one minute at a high uniform rate. ~~This result~~ ~~must be explained~~ Three possible explanations suggest themselves:

① that the RNA of the z gene (or a single molecule of RNA carried with it) is the template for protein synthesis.

② that the z gene produces rapidly many copies of genetic RNA, which are incorporated into ribosomes, and that after a minute or so a control mechanism operates to prevent the production of further genetic RNA.

(3) that the ~~DNA of the~~ 2 gene continually produces RNA templates but that these are unstable, with an average life time of only a minute or so.

On various theoretical grounds the first two explanations seem unlikely. ~~In addition~~ Moreover recent work (~~Hall and~~ Spiegelman, ~~1960~~) have shown that β-galactosidase appears to be ~~synthesized~~ synthesized in ribosomes (making explanation (1) unlikely) and also ( ) that the continued presence of the gene seems necessary for the synthesis of the enzyme. It thus seems likely that the third explanation is the correct ~~one~~ one. We understand (personal communication) that this is the ~~one~~ explanation favoured by the authors.

~~Much experimental work has~~

~~It seems likely on many grounds~~

~~Without reviewing the~~

It seems to us unlikely ~~that it~~, for many reasons, that all the RNA of the microsomes is unstable. It thus seems ~~more~~ more reasonable to suppose that the genetic RNA is only a small part of the ribosomal RNA.

It has been known for some time from the work of Volkin and his other co-workers (

that immediately after ~~phage~~ the infection of E. Coli by a T even phage there is a rapid turnover of ~~RNA~~ part of the RNA, although there is very little net synthesis. This RNA appears to have the same base composition (expecting uracil to thymine) as the DNA of the infecting phage, and to be quite different from the ~~RNA~~ base ratios of Coli DNA & or RNA. The Recently of this RNA has been found in the ribosomes of the infected cell (, Hall and Spiegelman, 1960).

On the basis of these two sets of experiments we propose the following hypothesis:

① the genetic RNA makes up only a small part (say 10 to 20%) of the RNA of the ribosomes

② it has approximately the same base ratios as the DNA which controls it

③ it is ~~under or least~~ is very unstable, at least under some circumstances.

Such a hypothesis would explain a number of facts, such as the weak correlation between the base composition of ~~DNA~~ RNA and DNA observed in bacteria ( ); the ~~[illegible]~~ difference between the base-ratios of "nuclear ~~[illegible]~~ RNA" (which would be largely though not entirely Genetic RNA) and ~~[illegible]~~ ribosomal RNA in mammals; the lack of any unusual base-ratios of ribosomes in the silk-worm glands of insects ( ) and very recent results of the labelling of the RNA of yeast (Tian +) ~~and a number of others.~~ ~~It will not so [illegible] readily~~ in which they find the the base-ratios of the newly synthesized material appears to resemble those of yeast DNA.

~~explain~~ While there are a number of facts which are not so readily explained (protein synthesis in amoeba and acetabularia, reviewed by Brachet ; protein synthesis in bacteria without RNA turnover, - - - - - - - - - -) we feel nevertheless that the hypothesis is sufficiently attractive to merit serious study. It ~~[illegible]~~ should not be too difficult to either prove it or disprove it in the form stated.

~~Insert A here~~ page 5 ~~This idea [illegible] in discussions with Dr. Francois Jacob and Dr. [illegible] Maaloe, to whom we should express [illegible] really [illegible] and our hypothesis is [illegible] elaboration of ideas already held [illegible] by them. We should like to thank Dr. [illegible] for allowing us to see their paper before publication.~~

If correct it has one interesting consequence. If it turns out that most of the genetic RNA is unstable, then a particular ribosome ~~making~~ which is ~~making~~ synthesizing a particular protein at one moment is likely to be making quite a different protein a few minutes later. On this picture the ribosome constitute the main part of the machinery for protein synthesis ~~than~~ into which genetic instruction can flow. These have obvious implications for control mechanisms, but these are outside the scope of this note.

We cannot claim any particular novelty for ~~this~~ these idea, but as we found them exciting we taught them worth a limited circulation. ~~Our~~ Our hypothesis arose in discussion with Dr. François Jacob and Dr. O. Maaloe, and is really an elaboration of ideas already held by them (and their colleagues). We should like to Drs Yeas and , ~~whose ...~~ for allowing us to see their paper before publication.

Introduction

It seems virtually certain that very detailed genetic information is passed from the nucleus to the cytoplasm. More precisely, ~~from a the gene in the nucleus~~ we believe that a major part of this information determines (or at least in part) the amino acid sequence of proteins, and that the information passes from the gene ~~to~~ in the nucleus (or "nuclear area") to the ribosomes in the cytoplasm. It is unnecessary here to review the whole body of information which drives us to this conclusion. In brief there is now good evidence for both mammalian cells and for bacteria that a substantial part of the protein synthesis in a cell is associated with the ribosomes. ~~Moreover we have two cases~~ There is the large body of evidence associating genes with proteins, and in particular in two cases (mammalian haemoglobin and bacterial β-galactosidase) there is evidence that while the ~~protein is coded~~ structure of the protein

is controlled by a gene (or genes) it is actually synthesised in the cytoplasm.

So far, so good. On the other hand the problem of <u>how</u> this information is passed is ~~however~~ in a very unsatisfactory state. It is natural to ~~see~~ suppose that it is carried by RNA, or by an RNA-protein complex. Moreover ~~this~~ there is ~~good~~ suggestive evidence from a number of sources (for amoeba, for plant cells, for neurospora, etc) that RNA passes ~~for~~ from the nucleus to the cytoplasm. ~~more~~ ~~precisely that in a labelling experiment the label~~ However when ~~the~~ more detailed questions are asked the picture becomes obscure. For example the base-ratios of the RNA of the nucleus, which is labelled quickly, differs from that of the cytoplasm. Detailed studies of the ~~pa~~ kinetics of labelling are not easily compatible with the idea

that the nuclear RNA is the precursor of the ribosomal RNA.

When we widen our questions we find rather too many ~~things~~ properties of ribosomes are unexpected from our naive point of view. If the RNA of ribosomes were the template for protein synthesis one would reasonably expect the amount of RNA ~~to vary~~ per particle to vary considerably, since proteins appear to come in all sizes, ~~whereas the amount per part~~ whereas in fact ~~a~~ ~~is rather~~ the amount ~~constant~~ appears to be tolerably constant. Again in ~~cells~~ ~~such as~~ ~~the~~ the silk gland of insects, which produce one protein with a very peculiar amino acid composition (largely glycine and alanine) one might expect the base-ratios of the ribosomal RNA to be different from that of other organs of the same animal; but this is not the case. ~~Finally~~ We have the peculiar fact that in bacteria the base-ratios of the DNA ~~is~~ are ~~very vari~~ very different in different

species, while the RNA ratios are largely the same. Finally when we ask the question " is the RNA template stable, or is it constantly supplied by the gene ? " we find a very confusing position. In amoeba and acetabularia, considerable protein synthesis appears goes on after the removal of the nucleus. In bacteria, protein synthesis (under special conditions) appears to continue without any turn-over of RNA (see a recent paper by). Yet there is evidence, which we shall discuss more fully in a moment, that the the continued presence of a gene is necessary for the synthesis of a protein.

A reasonable unprejudiced person could reasonably conclude that RNA had nothing to do with protein synthesis. Fortunately our prejudices are still in a healthy state, and we are prepared to resist this conclusion.

Some Experiments

In such a situation the only reasonable way to proceed is to select one of two pieces of evidence, ignoring the rest, and see where this leads one.

Consider, then Let us then restrict ourselves in the first place to bacteria, and let us choose an experiment which bears directly on the transfer of information. We shall consider the protein β-galactosidase as in E. Coli K12. At background we have the following evidence

(1) the amino acid sequence of the protein is controlled (at least in part) by the Z gene. Direct evidence for amino acid changes in a mutant protein is lacking, but the indirect evidence — that several mutations at the Z locus produce an altered protein — is good.

(6) the enzyme appears to be synthesized at ribosomes. When fully induced the amount of β-galactosidase in the cell may amount to as much as 5% of all the protein of the cell.

Now the crucial experiment is that of Pardee, Jacob and Monod.

They have shown that when the Z gene is introduced into the

F⁻ by the Hfr the synthesis of β-galactosidase takes place at

~~Synthesised~~ at a high constant rate within about 1 minute of the

introduction of the gene. This is not what one would have expected before

the experiment was done. One might have expected that the gene would

produce RNA templates at a roughly constant rate, and that as

the number of these templates increased the rate of

synthesis of the protein would steadily increase.

Three explanations ~~of this~~ suggest themselves.

(1) the DNA of the gene is the template (or, alternatively, a

single molecule of RNA carried in by the gene); that is, there is

only one template. This seems highly unlikely. Such a template

would have to act extremely fast. ~~For because~~ Moreover it neglects

the existence of microsomes and the suggestive ~~evid~~ evidence that these are the sites of β-galactosidase synthesis.

(2) The ~~gene~~ gene makes RNA templates ~~very rapidly (~~ very rapidly and these are ~~the~~ then incorporated into ribosomes. ~~& very rapidly~~ After a minute or so a control mechanism shuts off the making of templates.

This does not seem very plausible. ~~A~~ ~~I~~ A theoretical investigation of a simple mechanism along these lines suggests that ~~a~~ the amount of protein produced would <u>not</u> be proportional to the number of ~~genes~~ copies of a gene in the cell.

(3) The genes make RNA templates which pass into microsomes, but these templates are unstable and are destroyed in a time not longer than a minute or so.

This is the explanation we shall favour, and we understand ~~this is th~~ that it is the one now preferred by the authors of the experiment.

We now ask: is there some other example in bacteria of an unstable RNA ~~the~~? One immediately recalls the work of Volkin and coworkers on the ~~RNA~~ turn-over of RNA after phage infection. The background facts are as follows. After ~~the~~ the infection of E. Coli with a T even bacteriophage, the total amount of protein synthesis proceeds at approximately the normal rate. There is no further net synthesis of RNA. At the beginning of phage infection protein synthesis is necessary (probably to produce a set of special enzymes); later on a large part of the protein being synthesized by the cell is the protein of the bodies of the phage.

Now what Volkin found by using isotopic labelling is that there is a rapid RNA turnover (without appreciable net synthesis) after phage infection, and that the new RNA made appeared to have ~~the~~ a base composition & similar to that of phage DNA, and quite different from that of Coli DNA or RNA. More recent experiments make it

not very likely then that RNA is a direct precursor of phage DNA.

~~Using the~~ We can now ~~give~~ give

The Hypothesis

We now boldly combine the conclusions of the two experiments and generalize them to produce the following hypothesis.

(1) Genetic RNA has the same over-all base-ratios as genetic DNA.

(2) it passes into the ribosomes, but it is only a minor component (10-20% ?).

The major part of ribosomal RNA is not genetic RNA

(3) Genetic RNA is / or can in some circumstances / unstable". That is, it may have only a limited life.

Brenner very recently it has been shown that, provided they are isolated in a medium with a high enough concentration of Mg^{++}, the ribosomes of the intact cell contain this special RNA

We thus arrive at a picture of the action of ribosomes which differs in important respects from the one we used to hold. On the old picture a ribosome made a single type of protein and continued to do so while it remained intact, (though possibly at varying rates). On the new picture a ribosome may be making one protein at one moment, and a quite different protein a few minutes later. Its "structural" protein and most of the RNA remains the same, but the "genetic RNA" from the nucleus may change. Then the population of microsomes as may be looked upon as the machinery for protein synthesis through which flow the instructions from the genes as to which proteins are to be made. This obviously allows the control mechanism to be operate at the gene level, but we shall not pursue this aspect of the problem here.

It is regrettable that one cannot formulate a more explicit hypothesis ~~for the~~ about the instability of the postulated genetic RNA.

Three possibilities suggest themselves.

(1) Genetic RNA is broken down each time a protein is made i.e. it is only used once. This seems to us unlikely.

(2) genetic RNA is broken down after, say, 10 copies of the protein ~~are~~ have been made. (One can imagine a ribosome being filled up with ~~p~~ new protein molecules until it disaggregates, ~~reals~~ thus releasing the new protein and ~~destroying~~ allowing its genetic RNA to be destroyed.

(3) genetic RNA may be inherently stable but there is a special mechanism which breaks it down in certain circumstances; e.g., either at special moments in the ~~a~~ life of the cell. ~~or for the special only~~ ~~certain gene~~ ~~Alter~~ Alternatively some genetic RNA's may be stable ~~a~~ or others unstable. Or they might be a special ~~breakdown~~ breakdown or inactivation mechanism for each particular ~~the~~ type of genetic RNA.

It is clear that although these ideas are attractive they run into formidable difficulties. Learning as one rides the higher organisms such as amoeba and acerabularia it is surprising that if genetic RNA exists and is so often turns over that it has not been picked up before. We suspect that some of these difficulties may have arisen because it is unstable, and others because it is perhaps apt to leak off one of ribosomes unless special precautions are taken. However we feel that if it indeed does exist it should soon show itself in properly designed experiments.

We do not feel confident that our hypothesis is correct, but if it turns out to be right we shall not be surprised.

Acknowledgements

Although the combination of ideas presented here is new to us we cannot fairly call the ideas themselves new. There are precedents for each one of them. Moreover it is clear that a number of the authors of the work described must be thinking along very similar lines.

The ideas arose during discussions at Cambridge with Dr. François Jacob, following the 1960 meeting of the Society of General Microbiology, and also with Dr. O Maaloe; they were strongly influenced by the work and opinions of the Pasteur Groups. It is because it has so radically altered our own thinking that we have thought it worth while to put this particular formulation onto paper.

~~Redundant~~

X . . . -
Y . . -
A — T
↓ X' . . - ↑
Y' - - -
T — A

assume equal abundance

$\frac{2}{3}$

comp

A	$\frac{1}{6}$	$+\frac{1}{6}$	2
T	$\frac{1}{6}$	$+\frac{1}{6}$	2
G		$+\frac{1}{6}$	1
C		$+\frac{1}{6}$	1

$\cancel{\frac{1}{6}\cdot\frac{1}{6}} = 1$

$\cancel{\frac{1}{6}\cdot\frac{1}{6}} = R$

$\frac{1}{2}\cdot\frac{1}{4} = \frac{1}{8}$

$\frac{1}{4}\cdot\frac{1}{4} = \frac{1}{16}$

$\frac{1}{2}\cdot\frac{1}{2} = \frac{1}{8}$

$\frac{5}{16}$

	T	A	C	G
A	$\frac{5}{48}$	$\frac{5}{48}$	$\frac{1}{16}$	$\frac{1}{16}$
T	$\frac{5}{48}$	$\frac{5}{48}$	$\frac{1}{16}$	$\frac{1}{16}$
G	$\frac{1}{16}$	$\frac{1}{16}$	$\frac{1}{48}$	$\frac{1}{48}$
C	$\frac{1}{16}$	$\frac{1}{16}$	$\frac{1}{48}$	$\frac{1}{48}$
	$\frac{16}{48}$	$\frac{16}{48}$	$\frac{8}{48}$	$\frac{8}{48}$

✓

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G T $\frac{1}{16}$

G T $\frac{1}{8}$

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5	5	3	3
5	5	3	3
3	3	1	1
3	3	1	1