STUDIES ON THE BREAKDOWN OF MESSENGER RNA

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One of the characteristics of bacterial messenger RNA originally postulated (Jacob,and Monod, 1961) was its rapid turnover. Experiments readily verified the prediction that a particular RNA fraction which incorporated labelled precursors at a rapid rate did indeed exist with most of the expected properties of an information carrier (Brenner et al, 1961 ; Gros et al, 1961). It remained however to be shown that this RNA fraction also undergoes breakdown in vivo, and to establish what sort of correlation exists, if any, between this breakdown and protein synthesis. Using actinomycin D to prevent completely RNA synthesis, (Levinthal et al, 1962) have shown that the rapidly labeled RNA fraction of B. subtilis is decomposed into acid soluble fragments and that its functioning during protein synthesis is catalytic. E. coli is not sensitive to actinomycin D but we shall report on two other substances, dinitrophenol and proflavine, which behave like specific inhibitors of DNA transcription in this organism.

Experiments

Addition of DNP to an exponentially growing culture at a final concentration of 5×10^{-3} M leads to the rapid cessation of incorporation of a uracil label into cold 5 % TCA insoluble material. Assimilation from a radioactive internal pool is also subject to this inhibition as shown by resuspending the cells in a medium containing C^{12} uracil prior to the addition of DNP. Figure 1 illustrates the time dependent disappearance of

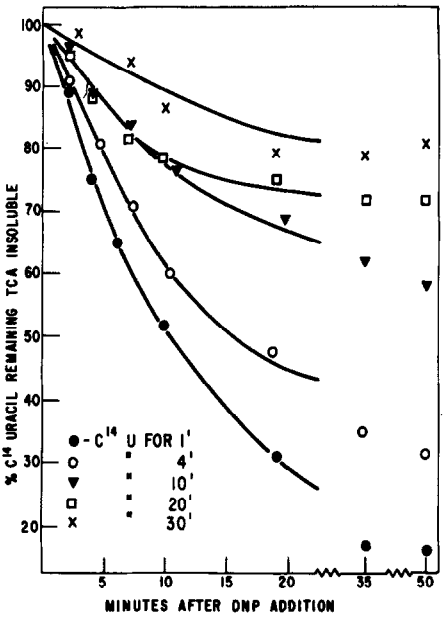

Figure 1

labelled TCA insoluble material after DNP terminated C^{14} uracil pulses of various durations. It can be summarized as follows :

1 - The decay is approximately first order and a half life period of about 8 minutes can be derived from the data for the one minute pulse.

2 - There is a residual stable fraction of 20 % which is probably a minimal value since it can also be observed after pulses as short as 10 seconds.

3 - The percentage of stable material increases with increasing pulse length reaching 85 % for a pulse of one generation time (60 minutes).

Characterization of the labile RNA fraction.

The following observations suggest that the RNA disappearing after treatment with DNP includes what is generally recognized as messenger RNA.

1 - The 14 S peak which can be demonstrated in a sucrose gradient after a short exposure to a labeled RNA precursor (Gros et al, 1961) is markedly reduced if the cells are incubated with DNP prior to extraction. Furthermore there is no corresponding net increase of radioactivity in the remainder of the profile.

2 - The ability of extracted RNA to stimulate in vitro amino acid incorporation diminishes dramatically after exposure of the cells to dinitrophenol as shown in Table 1.

TABLE I

Time after DNP addition	0'	15'	20'	30'	60'
% remaining TCA insoluble C^{14}	100	35	26	17	11
% remaining incorporating activity	100	12	16	9	not detectable

3 - Extracts from cells incubated in the presence of DNP have a very low endogenous capacity to incorporate aminoacids in vitro but are stimulated by Poly U or by RNA (Nirenberg and Matthaei, 1961) obtained from non-DNP treated cells. In addition if dialysed, such extracts support T_2 DNA primed amino acid incorporation (Wood and Berg, 1962).

In vivo protein synthesis in the presence of DNP.

Figure 2 illustrates the fact that the rate of incorporation of C^{14} valine (10^{-2} M) into hot TCA insoluble material diminishes as a function of the time of exposure of the culture to DNP. Essentially the same data are obtained using the formation of β-galactosidase as an index of specific protein synthesis. If DNP and inducer are added simultaneously there is no detectable increase in enzyme. However, if DNP is added three minutes after inducer, enzyme synthesis can be demonstrated for at least 10 minutes although the rate of this synthesis is initially lower than the control and decreases with time.

Knowing the specific activity of valine, the percentage of bulk protein which it constitutes, the amount of RNA broken down, and assuming that 3 % of the total RNA is messenger (Gros et al, 1961 b), it can be calculated that a triplet of bases must be used about six times in protein synthesis. This indicates that there is probably no obligatory destruction

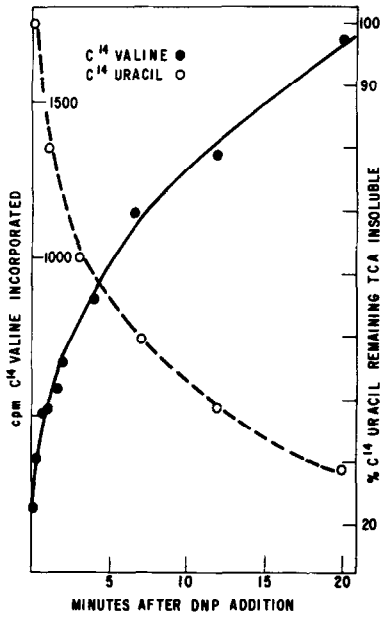

Figure 2

of a molecule of messenger RNA after it specifies the synthesis of a molecule of protein. That there is, however, a relationship between the rate of breakdown of the label RNA and the synthesis of protein, is implied by the fact that chloramphenicol reduces by a factor of three the rate of decay of RNA after a DNP terminated uracil pulse.

In vivo effect of proflavine.

Figure 3 shows the effect of adding proflavine sulphate at a final concentration of 70 μg/ml to an exponentially growing culture 45 seconds after C^{14} uracil. Disappearance of cold acid insoluble material is logarithmic and the half-life is 5.4 minutes. There is a stable fraction of 22 % which can be systematically subtracted from the data to yield an exponential decay with a half-life thus corrected, of 3.5 minutes.

Mechanism of action of dinitrophenol and proflavine.

Hurwitz et al. (1962) had already shown that proflavine at high concentrations inhibits the activity of the DNA primed RNA synthesis in vitro. Table 2 provides additional evidence that proflavine acts at the level of

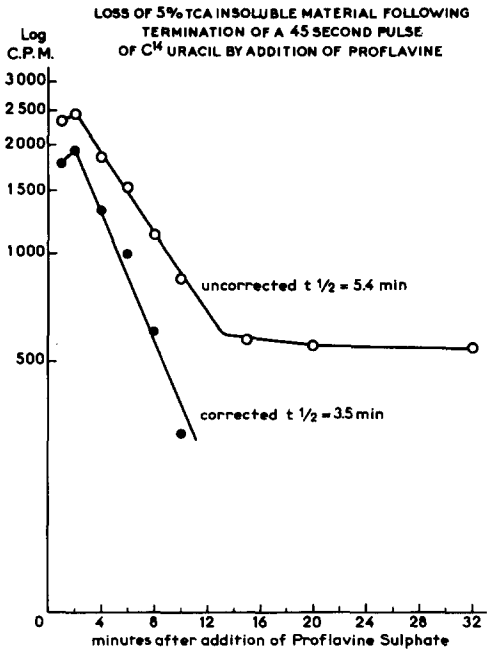

Figure 3

the transcription of information from DNA to RNA and that this is also the case for dinitrophenol. Thus each agent can be seen to inhibit DNA directed amino acid incorporation in a cell free system at concentrations, which if anything are stimulatory for the poly U primed synthesis of polyphenylalanine by the same extract. These data suggest that the observed effects on RNA and protein synthesis in vivo, are a consequence of the cessation of DNA primed RNA synthesis.

TABLE II

Effect of DNP and Proflavine on DNA and Poly U Dependent Amino Acid Incorporation

	T_2 DNA directed Threonine incorporation	Poly U directed Phenylalanine incorporation
Complete system	6578	1088
Minus T_2 DNA	166	-
Minus Poly U	-	160
Plus DNP-Final Concentration 4×10^{-3} M	3231	1480
Plus DNP " " 1.6×10^{-3} M	4320	1513
Plus DNP " " 0.8×10^{-3} M	6515	1186
Plus Proflavine sulphate final concentration 40 µg/ml	108	1651

The extract was an extensively dialysed alumina preparation from a DNP treated culture of ML 308.

The complete reaction mixture for DNA directed threonine incorporation contained the following in μm in a total volume of 0.5 ml : Tris buffer pH 7.8, 21 ; $MgSO_4$, 6 ; $MnSO_4$, 0.5 ; KCl, 18 ; UTP, 0.2 ; CTP, 0.2 ; GTP, 0.35 ; ATP, 1.5 ; PEP, 5 ; 19 unlabelled l-amino acids, 0.05 each ; β-mercaptoethanol, 1.5 ; l-threonine, 0.025 (specific activity 10 μc/μm) ; pyruvate kinase, 15 μg ; extract 200 μl (ca 4.6 mg protein) ; T_2 DNA, 43.5 μg.

The complete mixture for phenylalanine incorporation did not include $MnSO_4$, UTP, CTP, or T_2 DNA. It contained in μm : Tris pH 7.8, 6 ; $MgSO_4$, 21 ; GTP, 0.1 ; β-mercaptoethanol, 1 ; l-phenylalanine, 0.023 (specific activity 10.7 μc/μm ; poly U, 20 μg, but was otherwise identical to that described for threonine incorporation. Incubation : 45 minutes at 37°.

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