New observations on the mode of action of ribonuclease on living root-tips

We have previously shown[1-3] that ribonuclease penetrates into living onion root-tip cells, where the enzyme produces an almost complete inhibition of protein synthesis and of incorporation of amino acids into proteins; this inhibition can be overcome by the addition of an excess of RNA[2]. The recovery of amino acid incorporation is complete in the ribonuclease-treated onion roots provided that the action of ribonuclease is not too long and that yeast RNA is allowed to act for a sufficiently long period: if the inhibition of protein synthesis by ribonuclease is 60–70 %, a 24-h treatment with yeast RNA often leads to complete recovery of the incorporation of amino acids into proteins[4]. RNA extracted from the onion roots themselves is about 4 times as active as commercial yeast RNA, while the nucleotides present in a ribonuclease digest of yeast RNA have no appreciable effect[4]. Since protein synthesis in the ribonuclease-treated onion roots is already strongly inhibited 30 min after the addition of the enzyme, and since the total RNA content does not decrease appreciably during this lapse of time, it was concluded that the inhibition is presumably due to the formation in the living roots of a complex between ribonuclease and intracellular RNA[3].

More recent work by HOAGLAND *et al.*[5] has shown the importance of one special fraction of RNA in protein synthesis: it is the *soluble* RNA, to which the activated amino acids are transferred. In the experiments of HOAGLAND *et al.*[5], *in vitro* this early step of protein synthesis is particularly sensitive to ribonuclease.

These results of HOAGLAND *et al.*[5] have led us to a re-examination of the mode of action of ribonuclease in living onion root-tips. We have tested the possibility that, in this case, the inhibition of protein synthesis might be due to a breakdown of soluble RNA, without marked effect on the RNA which is bound to cell particles.

Onion root-tips were treated with ribonuclease (Armour; 1 mg/ml) for periods of time varying from 30 min to 2 h. The treated and the control root-tips were then homogenized in sucrose and submitted to differential centrifugation. The RNA content of the various fractions was then estimated by the OGUR AND ROSEN[6] method, while their protein content was measured with the technique of LOWRY *et al.*[7].

No significant difference between normal and ribonuclease-treated roots could be found for any of the fractions which could be spun down by the fractional centrifugation (cell membranes, nuclei, mitochondria and microsomes). On the other hand, a considerable decrease in the ratio between RNA and protein was found in the soluble fraction, as in indicated in Table I.

The decrease of the RNA/protein ratio is essentially due to a decrease in soluble RNA content, the protein content of the soluble fraction remaining constant within the experimental errors of measurement. It is worth noting that, although the RNA/protein ratio varies considerably from one experiment to the other (in correlation with physiological changes in the onions during the year), the general trend remains the same: the ribonuclease treatment reduces the content in soluble RNA by about 45 %. In Expts. 2, 3 and 4, soluble RNA and proteins were estimated at time intervals of 30 min, 1 h and 2 h: the decrease in the soluble RNA content was 39 %

Abbreviation: RNA, ribonucleic acid.

TABLE I

RNA/PROTEIN RATIO IN THE SOLUBLE FRACTION

T_0, R_0: values obtained at zero time. T_1, R_1: values after 1 h treatment with distilled water or ribonuclease (1 mg/ml) respectively.

Expt.	T_0	T_1	R_0	T_1	*Decrease in soluble RNA* %
1	1.43	1.77	1.54	0.87	44
2	0.67	0.63	0.63	0.33	48
3	0.20	0.22	0.24	0.12	56
4	0.26	0.23	0.24	0.14	42

after 30 min, 47 % after 1 h and 49 % after 2 h. In other words, a ribonuclease treatment of 30 min is sufficient to destroy about 40 % of the soluble RNA, without exerting any significant effect on the RNA of the other cell fractions. Since growth and protein synthesis in onion roots are almost completely inhibited by ribonuclease treatment for 30 min and since further action of the enzyme has little additional effect[2], it can be concluded that the inhibition of protein synthesis in living onion roots treated with ribonuclease is apparently due to partial breakdown of soluble RNA by the enzyme.

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