The Translocation Step in Peptide-Bond Formation

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Puromycin reacts with ribosomal-bound peptidyl-t-RNA* to form peptidyl-puromycin. Nascent peptides are thus cleaved from the ribosomes and growth ends prematurely. Puromycin reacts with nascent chains on washed ribosomes in the absence of added supernatant factors, the reaction being catalysed by an enzyme, peptidyltransferase, that is an integral part of the 50s ribosomal subunit. The reaction is stimulated by GTP and supernatant factors and this suggests that the mechanism of protein synthesis could involve two ribosomal binding sites. Peptidyl-t-RNA bound to the donor site reacts with puromycin and requires only peptidyltransferase (Monro, Maden & Traut, 1966). Peptidyl-t-RNA bound to the acceptor site must first be translocated to the donor site in a process requiring GTP and supernatant factor before puromycin can act (Traut & Monro, 1964).

More recent work (Haenni & Lucas-Lenard, 1968) has shown that if *N*-acetylphenylalanyl-t-RNA is bound to ribosomes such ribosomes will bind phenylalanyl-t-RNA in the presence of polyuridylic acid, and on addition of GTP and supernatant factors a peptide bond is formed. If, however, 5'-guanylmethylenediphosphonate, a specific inhibitor of the GTP reaction (Hershey & Monro, 1966), replaces GTP no peptide bond is formed although phenylalanyl-t-RNA becomes bound to the ribosome. This raises the possibility that GTP may be necessary for functional binding of phenylalanyl-t-RNA and has interesting implications for the puromycin reaction. The stimulation of this reaction by GTP may not be explainable on the two-site model, since GTP could aid functional binding of puromycin, thus increasing its reactivity towards peptidyl-t-RNA.

Experiments have been carried out to examine the above possibilities. If the two-site model is correct, ribosomes carrying nascent protein and then incubated with GTP and supernatant factors should undergo translocation. If such ribosomes are then salt-washed extensively to remove bound supernatant factors (Maden, Traut & Monro, 1968), incubation with puromycin should remove more nascent protein than from control ribosomes not previously treated with GTP and supernatant factors. If the two-site model is incorrect and GTP is involved in functional binding of puromycin we would expect both sets of ribosomes above to react

similarly with puromycin. Experiments have also been carried out with 50s ribosomal subunits.

The results will be presented and discussed in relation to GTP function and the two-site model for peptide-bond formation.

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The Uptake of [³H]Uridine into the Nucleic Acids of Rat Uterus During Early Pregnancy

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After a fertile mating, the fertilized rat ovum develops in the oviduct until 3.5 days later it is shed into the uterus. This blastocyst implants in the uterine wall at some period between midnight on the fifth day and the morning of the sixth day after mating. The site of implantation is clearly distinguishable from the rest of the uterus.

There have been few attempts to examine quantitatively the biochemical events occurring during these processes. In the present work, the uptake of [³H]uridine into RNA and DNA of the uterus and specifically of the implantation sites has been examined *in vivo* during the first 9 days of pregnancy. Female rats were mated and the morning on which sperm was found in the vaginal smears was designated day 1. [³H]Uridine (100 μ C; 30 C/m-mole) was injected via the femoral vein under ether anaesthesia, followed by 1.0 ml. of a suspension of ⁵¹Cr-labelled rat erythrocytes in 0.9% (w/v) NaCl. Then 50 min. later 2 ml. of a solution of Niagara Blue 2B [2% (w/v) in 0.9% (w/v) NaCl] was injected via a tail vein to render the implantation sites visible (Psychoyos, 1960). The animals were killed 10 min. later, and samples of uterus were taken for the determination of RNA and DNA (Heald, O'Grady & Howie, 1969). All values for radioactivity were adjusted on the basis of the counts determined in the acid-soluble fractions.

From day 1 to day 5 there was no change in the dry matter or the acid-soluble radioactivity, but from day 6 to day 9 there was a highly significant increase of 30–40% in the water content and the acid-soluble radioactivity in the implantation sites. At all times the contamination of acid-soluble radioactivity by radioactivity from blood was negligible. DNA concentrations did not change

* Abbreviation: t-RNA, transfer RNA.