The Translocation Step in Peptide-Bond Formation

By M. CANNON. [Department of Biochemistry, King's College (University of London), London W.C.2]

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.

Haenni, A. L. & Lucas-Lenard, J. (1968). *Proc. nat. Acad. Sci., Wash.*, **61**, 1363.

Hershey, J. W. B. & Monro, R. E. (1966). *J. molec. Biol.* **18**, 68.

Maden, B. E. H., Traut, R. R. & Monro, R. E. (1968). *J. molec. Biol.* **35**, 333.

Monro, R. E., Maden, B. E. H. & Traut, R. R. (1966). *Symp. Fed. Europ. biochem. Soc.* p. 179. Ed. by Shugar, D. London: Academic Press.

Traut, R. R. & Monro, R. E. (1964). *J. molec. Biol.* **10**, 63.

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

By P. J. HEALD and J. E. O'GRADY. (Department of Biochemistry, University of Strathclyde, Glasgow)

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.

until day 8, when significant increases were found in the implantation sites. RNA concentrations were constant until day 7, when concentrations increased in the implantation sites. The RNA/DNA ratio did not change until day 6, when it increased significantly in the implantation sites. The uptake of [^3H]uridine into RNA significantly increased on day 3 and again on days 6 and 7 in the implantation sites, but decreased on days 8 and 9 to that of the non-implanted areas. In contrast, there was no increased uptake into DNA on day 3 but a significant increase in the implantation areas on days 6–9. In view of the short duration of exposure to [^3H]uridine, the changes observed probably represent changes in nuclear metabolism (Hamilton, Widnell & Tata, 1968). The results are interpreted as indicating that at the commencement of implantation there is a rapid synthesis of RNA, which is essential for the further development of the implantation site.

The increase in RNA synthesis on day 3 is not understood at present.

This work was generously supported by a grant from the Medical Research Council.

Hamilton, T. H., Widnell, C. C. & Tata, J. R. (1968). *J. biol. Chem.* **243**, 408.

Heald, P. J., O'Grady, J. E. & Howie, A. (1969). *J. Reprod. Fertil.* (in the Press).

Psychoyos, A. (1960). *C.R. Acad. Sci., Paris*, **251**, 3037.

An Early Effect of Oestradiol-17 β on the Permeability of Rat Uterus to Ribonucleic Acid Precursors

By R. J. BILLING, B. BARBIROLI and R. M. S. SMELLIE. (*Department of Biochemistry, University of Glasgow*)

It has been shown that within 2 hr. of administration of oestradiol-17 β there is a fivefold stimulation in the incorporation of radioactive precursors into rat uterine RNA (Gorski & Nicolette, 1963; Means & Hamilton, 1966; Hamilton, Widnell & Tata, 1968). This is usually taken to indicate an increase in the rate of RNA synthesis, but it could be accounted for by other factors such as changes in the sizes or specific radioactivities of the pools of endogenous precursors.

Experiments have been performed to investigate the size of the pools of ribonucleoside and ribonucleotide precursors and changes in the radioactivity of these pools and of the RNA in rat uterus at various times after the administration of oestradiol.

Groups of immature female albino rats were injected with 1 μg . of oestradiol-17 β or 0.9% NaCl. Then 30 min. before death animals from each group

received a mixture containing 12.5 μC of each of [^3H]adenosine, [^3H]guanosine, [^3H]cytidine and [^3H]uridine intravenously. The uteri from each group were pooled and fractionated into acid-soluble and acid-insoluble material. The E_{260} of the acid-soluble material was determined and it was then fractionated by ion-exchange chromatography into ribonucleosides and ribonucleotides. The radioactivities of each fraction were determined.

The E_{260} of the acid-soluble fraction showed no significant increase until about 7 hr. after hormone administration, but the total radioactivity in the acid-soluble fraction and the specific radioactivity of the ribonucleotide pool increased sharply to maxima after 2–3 hr. Very similar changes occurred in the radioactivity of the acid-insoluble fraction.

From these observations it is concluded that most of the change in radioactivity of uterine RNA occurring in the first 5 hr. after oestradiol administration can be accounted for in terms of increases in the specific radioactivities of the pools of ribonucleotide precursors.

Gorski, J. & Nicolette, J. (1963). *Arch. Biochem. Biophys.* **103**, 418.

Hamilton, T. H., Widnell, C. C. & Tata, J. R. (1968). *J. biol. Chem.* **243**, 408.

Means, A. R. & Hamilton, T. H. (1966). *Proc. nat. Acad. Sci., Wash.*, **56**, 1594.

The Effect of Oestradiol-17 β on the Rate of Synthesis of Ribonucleic Acid in Rat Uterus

By R. J. BILLING, B. BARBIROLI and R. M. S. SMELLIE. (*Department of Biochemistry, University of Glasgow*)

It has been observed that when [^3H]adenosine is administered to rats the radioactivity of the pool of adenine nucleotides in the uterus is nearly constant over the period 3–11 hr. after administration of the precursor, and that the total radioactivity of the tissue also remains constant over this period. Advantage has been taken of this observation to investigate changes in the incorporation of adenine nucleotides into RNA from the acid-soluble pool in response to treatment with oestradiol-17 β . The hormone was administered to groups of animals 3 hr. after [^3H]adenosine, and the radioactivities of the acid-soluble and acid-insoluble fractions were determined at various times up to 8 hr. after hormone treatment. The radioactivity in the acid-soluble fraction of treated animals remained constant for the first 5 hr. after treatment and then declined between 5 and 8 hr. In the acid-insoluble fraction the radioactivity was only very slightly higher than that in the controls during the first 4 hr. after hormone treatment, but thereafter it increased