Effect of Tri-iodothyronine on the Transcription of Deoxyribonucleic Acids
of Different Degrees of Redundancy in Frog Larvae

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Two days after the injection of tri-iodothyronine into bullfrog tadpoles there is a quantitative stimulation of nuclear RNA synthesis in the liver, and this is followed by a transfer of RNA to the cytoplasm (Tata, 1967). Hybridization experiments revealed a slight increase in number of kinds of DNA-like RNA (D-RNA), but most of the newly synthesized RNA was ribosomal RNA (Wyatt & Tata, 1968). These results suggested that the effect of the hormone may be to stimulate the formation of ribosomes, which in turn could account for the transfer of more D-RNA from the nuclei to the cytoplasm. Church & McCarthy (1970) have shown that certain D-RNA molecules that are restricted to the nuclei in rabbit uterine cells are found in the cytoplasm after stimulation with oestradiol. Exposure of bullfrog larvae to tri-iodothyronine for 2 days causes the synthesis in the liver of ribosomal, transfer and DNA-like RNA, according to Nakagawa & Cohen (1967).

One purpose of the present investigation was to examine by DNA–RNA hybridization the populations of D-RNA in nuclei and intact cells of untreated larvae and those exposed to tri-iodothyronine to ascertain whether the hormone acted to translocate more kinds of nuclear D-RNA to the cytoplasm. The other aim was to determine the extent of transcription from DNA of different degrees of reiteration after treatment with tri-iodothyronine.

Several thousand frog larvae of *Rana pipiens* (stage 25; Shumway, 1940) were placed in boiled tap water for 6 days, and a comparable number were placed in boiled tap water containing tri-iodothyronine at a concentration of 0.001% for a similar time. The stock concentration of tri-iodothyronine (0.01%) was dissolved in 1% NaOH. One half of the control and one half of the hormone-treated larvae were washed, nuclei were isolated (Daniel & Flickinger, 1971) and RNA was extracted from the nuclei. The various RNA preparations were ³H-labelled *in vitro* by using [³H]dimethyl sulphate (Smith *et al.*, 1967; Greene & Flickinger, 1970).

Frog DNA was isolated from erythrocytes of adult frogs (Greene & Flickinger, 1970). A small amount of labelled frog DNA was obtained by cutting 100 tail-bud embryos into dorsal axial and belly regions (Flickinger *et al.*, 1967), incubating them for 24 h in

Niu & Twitty (1953) saline solution containing [¹⁴C]-thymidine (5 μ Ci/ml) and then isolating the DNA (Greene & Flickinger, 1970). Frog erythrocyte DNA was adjusted to a specific radioactivity of 80–130 d.p.m./ μ g with a small amount of the ¹⁴C-labelled DNA. The DNA was sheared at 275 MN/m² (40 000 lbf/in²) in a French pressure cell (Aminco) and then denatured at 98°C for 5 min, and the three repetition frequencies were separated by allowing renaturation to occur at various Cot [(mol of nucleotides/l) $\times$ time (s)] values and separating the renatured from denatured DNA with a hydroxyapatite column (Britten & Kohne, 1968). The sheared and denatured DNA that reassociated at Cot 0.2 was termed the highly reiterated DNA, the remainder that reassociated at Cot 300 was the moderately reiterated DNA, and the denatured DNA remaining after the Cot 300 incubation was the least reiterated DNA fraction. The three ¹⁴C-labelled DNA fractions were trapped on nitrocellulose filters in 1.2 M-NaCl–0.12 M-sodium citrate (2.5–3.0 μ g of DNA/filter), then hybridization was carried out in an incubation medium consisting of 1 M-NaClO₄ and 50% formamide in 10 mM-tris-HCl buffer, pH 7.2, containing 2.5 mM-sodium verenate at 37°C. The amounts of ³H-labelled RNA added varied from 600 μ g/ml to 23 mg/ml, and the incubation times ranged from 10 min for the highly reiterated DNA hybridizations to 5–16 h for the moderately reiterated DNA to 2–5 days for the least reiterated DNA hybridizations. The time and concentration were varied in order to attain Cot values of 10 for the hybridizations with highly reiterated DNA, 10 000 for the moderately reiterated DNA and 168 000 for the least reiterated DNA.

Much of the sheared DNA is lost from the filters (Flickinger *et al.*, 1972), but the amount of sheared DNA present at the end of the incubation can be determined by the specific radioactivity of the ¹⁴C-labelled DNA, and the amount of RNA hybridized is determined by the specific radioactivity of the ³H-labelled RNA. Hence the percentage hybridization can be calculated. The reliability and disadvantages of this method, as well as the operational procedures for the hybridizations, have been discussed (Flickinger *et al.*, 1972).

There is little change in the extent of hybridization

Table 1. *Effect of tri-iodothyronine on the transcription of DNA fractions of different degrees of redundancy in frog tadpoles*

Stage-25 larvae of *Rana pipiens* were kept in water (controls) or water containing tri-iodothyronine (0.001 %) for 6 days. RNA was prepared from isolated nuclei and whole larvae, and the RNA was labelled *in vitro* with [³H]dimethyl sulphate and hybridized to highly repetitious, moderately repetitious and least repetitious fractions of denatured frog DNA. Values are means $\pm$ s.d. of results obtained in four experiments.

	DNA hybridized (%)		
	Highly repetitious DNA	Moderately repetitious DNA	Least repetitious DNA
Nuclear RNA			
Control larvae	2.8 $\pm$ 0.30	20.3 $\pm$ 0.30	10.7 $\pm$ 0.38
Tri-iodothyronine-treated larvae	2.4 $\pm$ 0.45	19.8 $\pm$ 0.82	9.6 $\pm$ 0.64
Intact larval RNA			
Control larvae	3.0 $\pm$ 0.31	17.9 $\pm$ 0.26	7.5 $\pm$ 0.48
Tri-iodothyronine-treated larvae	3.5 $\pm$ 0.28	24.0 $\pm$ 2.2	9.8 $\pm$ 0.63

of nuclear RNA of untreated and tri-iodothyronine-treated larvae to the three reiteration classes of DNA (Table 1). The percentage hybridization to the DNA fractions is slightly greater for labelled RNA of hormone-treated intact larvae, compared with RNA of untreated larvae. The results suggest that tri-iodothyronine causes a slight increase in number of kinds of D-RNA accumulating in intact larvae and a slight decrease in the variety of D-RNA molecules in the nuclei. These results correlate with those of Tata (1967) and Church & McCarthy (1970), which show that hormone treatment can cause D-RNA previously restricted to nuclei to move to the cytoplasm. It is possible that an increase in ribosome formation plays a role in this translocation (Tata, 1967).

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