65. *Suppression of RNA Synthesis following Transferrin Removal in Chick Myotubes*

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Addition of chick transferrin (Tf) to basal culture medium (BCM) composed of 85% Eagle's minimum essential medium (MEM) and 15% horse serum is required for growth and differentiation of chick myogenic cells (Ozawa and Kohama, 1973, 1978; Kimura *et al.*, 1982). In the presence of Tf, myotubes synthesized and accumulated various kinds of proteins (Hagiwara *et al.*, 1981; Gerstenfeld *et al.*, 1984; Ozawa *et al.*, 1985), and accumulation of proteins resulted in myotube growth. Following Tf removal from the medium, large myotubes degenerated, becoming lean and flat, and their content of creatine kinase activity decreased. They were finally destroyed and detached from the culture dishes (Ozawa, 1977; Ozawa and Hagiwara, 1982). These morphological and biochemical analyses imply that there is disturbance in protein turnover in myotubes cultured under Tf-deficient conditions. In order to clarify the mechanism, we examined the metabolism of RNA and protein and found that their synthesis was greatly reduced following Tf removal.

Materials and methods. Procedures for preparation of chick myoblasts and purification of chick serum Tf were described elsewhere (Kimura *et al.*, 1982). Myogenic cells (initial cell density: 2×10^6 cells/dish) were cultured in BCM containing 10 μ g/ml chick Tf in 100 mm plastic culture dishes until the culture became almost confluent (9th day of incubation). On that day, experiments were started by changing the medium to the experimental and control media described below (day 0). DNA was measured by the method of Hinegardner (1971). Cytoplasmic RNA was isolated using guanidine hydrochloride as described by Chirgwin *et al.* (1979) and RNA content was determined by the orcinol method described by Brown (1946). RNA synthesis was determined assaying the amount of [5,6- 3 H]uridine (Amersham, 40–60 Ci/mmol) taken into the TCA insoluble fraction as described by Yu and Frigelson (1971). Cells at various stages were labeled for 60 min with 2 μ Ci/ml uridine. Total cytoplasmic RNA was size-fractionated with 1% agarose gel electrophoresis, according to the procedure of Macmaster and Carmichael (1977) and stained with ethidium bromide.

Results and discussion. Myotubes of 9 day culture were allowed to culture with control and experimental media for 4 more days. The control and experimental media were composed of BCM containing 10 μ g/ml Tf and BCM alone, respectively. Myotubes cultured with and without Tf are referred to as Tf(+) and Tf(–) myotubes, respectively.

By day 4 after the medium switch, cells became flat in the Tf(–) myotubes. However, there was no obvious difference between DNA content on day 4 and the content on day 0 in the two myotubes. In contrast to DNA, total content of their cytoplasmic RNA, most of which was probably rRNA, decreased in both myotubes. In Tf(+) myotubes, the content decreased by about half of the initial

Fig. 1. Time course of RNA content in cultured myotubes. RNA content in Tf(+) myotubes (●) and in Tf(-) myotubes (○). Each point denotes the average of at least three dishes.

content by day 4 (Fig. 1). Decrease in the content in a confluent myogenic cell culture may be a natural course. In Tf(-) myotubes, however, the decrease rate after day 2 was faster and the content on day 4 was less than one-tenth of the initial content.

Synthetic rate of the total RNA in Tf(+) myotubes decreased a little with the lapse of time (Fig. 2). In Tf(-) myotubes, the rate began to decrease by 18 h after Tf removal, reached less than half of the initial rate on day 1, and decreased gradually thereafter. Degradation rates of the RNA did not greatly differ, although that in Tf(-) myotubes was a little faster (data not shown).

Fig. 2. Uptake of ^3H -uridine into TCA insoluble fractions in Tf(+) myotubes (●) and Tf(-) myotubes (○). Each sample was spotted on a filter paper, washed with cold TCA four times and counted in a toluene scintillator. No radioactivity remained on the filter when it was washed with hot TCA.

We have observed that synthesis of myosin heavy chain and light chain 1 mRNA was also reduced following Tf removal. RNA synthesis, especially total RNA synthesis, increased again after Tf readdition (data not shown). The results, however, differed between experiments. It is assumed that the reversibility depends on the grade of progress of degenerative process at the time of Tf readdition. It is clear that Tf influences RNA synthesis either directly or indirectly, and reduction in RNA synthesis is the main cause of reduced RNA content in Tf(−)myotubes.

The ratio of 28S RNA to 18S RNA was less in Tf(−)myotubes than in Tf(+)myotubes on day 2 (Fig. 3), suggesting that Tf removal did not affect all kinds of RNAs equally.

Fig. 3. Denaturing agarose gel electrophoretic patterns of RNAs. RNAs from (A) Tf(−)- and (B) Tf(+) myotubes on day 2. The same amount of RNA sample was applied to each lane. (C) RNAs from *E. coli* as an internal marker.

In addition to the disturbance in RNA metabolism, uridine uptake into cells was reduced following Tf removal (data not shown). However, it was clear from the time course study that reduction in the size of nucleotide pools due to the decreased uptake was not the sole cause of the decreased RNA synthesis. Under these conditions, protein synthesis was also affected in Tf(−)myotubes; for example, the rate was about two-thirds of that in Tf(+)myotubes on day 2 (data not shown).

We conclude that Tf removal from culture medium gives rise to a decrease

in RNA synthesis of myotubes, which, in turn, results in a decrease in protein synthesis. This is responsible, at least partly, for degeneration of the myotubes.

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