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DIFFERENTIAL BEHAVIOR OF TWO DEXAMETHASONE INDUCED mRNA ACTIVITIES IN HTC CELLS IN RESPONSE TO CORDYCEPIN AND TO WITHDRAWAL OF HORMONE

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SUMMARY. - In HTC cells dexamethasone induces the accumulation of tyrosine aminotransferase mRNA activity in addition to a mRNA activity coding for a protein of a yet unknown function (DIP). The kinetics of decay of these two mRNA activities were followed in induced cells under the influence of cordycepin and in cells withdrawn from the hormone. For tyrosine aminotransferase mRNA activity a relative half life of approximately 1 hour was estimated in both cases. However, DIP mRNA activity decayed to 50 % of the fully induced level within 3 to 4 hours following withdrawal of the hormone, whereas after application of cordycepin to induced cells only a small and delayed decay could be observed. Application of cordycepin two hours after withdrawal of hormone caused a stabilization of DIP mRNA activity.

Administration of corticosteroids to rats stimulates specific enzyme synthesis in the liver (1-3) as a result of the accumulation of the respective mRNA activities (4-6). Experiments (7-9) support the assumption that the increased mRNA activity observed is at least partly due to an increase in the amount of the respective mRNA, preceded by increased transcription rates for these mRNA sequences.

Gene expression stimulated by corticosteroids, however, seems to be subject to regulation on many levels and little

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is known about the contribution of possible posttranscriptional control mechanisms in the modulation of enzyme activity. On the protein level, it has been demonstrated that cortisol blocks degradation of tyrosine aminotransferase (10) and that the deinduction of this enzyme is susceptible to inhibitors of RNA and protein synthesis (1,11,12). Enucleation experiments indicated the importance of newly synthesized RNA for the degradation of the enzyme protein (13). On the mRNA level we have recently shown, that inhibition of protein synthesis in cortisol induced rats by cycloheximide results in an accumulation of tyrosine aminotransferase mRNA activity over that in the hormone treated animals (12). In other systems, factors controlling the half life of the induced mRNA (14,15), seem to contribute considerably to the accumulation of induced proteins and also accelerate the deinduction process following withdrawal of the hormone.

This has tempted us to perform an analysis of half lifes of dexamethasone induced mRNA activities using in vitro translation of poly(A)-containing RNA in a wheat germ system. We examined the decay of TAT[3] mRNA activity and of a mRNA activity coding for a yet unidentified, dexamethasone inducible protein (DIP[4]), following removal of the steroid and after application of cordycepin. As well known, cordycepin (3'-deoxyadenosine) prevents the polyadenylation at the 3' end of pre-mRNA in the nucleus as well as the appearance of mature mRNA in the cytoplasm (16,17).

MATERIALS AND METHODS:

L-(^{35}S)Methionine (580 Ci/mmol) was purchased from

[3] TAT: tyrosine aminotransferase or L-tyrosine:2-oxoglutarate aminotransferase (EC 2.6.1.5.);

[4] DIP: dexamethasone inducible protein

Amersham Buchler, Braunschweig, dexamethasone from Sigma Munich, and oligo(dT)-cellulose (grade T2, chain length of 11 nucleotides) from Collaborative Research, Waltham, Massachusetts. All other chemicals were of analytical grade, obtained from Merck, Darmstadt and Serva, Heidelberg. Fresh wheat germ was a kind gift of GEG, Mannheim.

Tissue culture cells. HTC cells were kindly supplied by Dr. J.P. Beck, Straßbourg. Cells were grown in suspension culture in a modified Swim's 77 medium supplemented with 10 % newborn calf serum (18). For all experiments cells were centrifuged from growth media while in late log phase (usually at a cell density of 6×10^5 cells per ml) and suspended at the same density in "induction medium". This medium was identical to growth medium except that the serum was treated with charcoal to eliminate traces of hormones.

Preparation of cytoplasmic poly(A)-containing RNA. Dexamethasone was supplied to the cell suspension at the early stationary phase (about 10^6 cells/ml) at a final concentration of 10^{-7}M. A stock solution of 10^{-2}M dexamethasone in dimethylformamide was used.

After 20 hours the fully induced cells were treated with cordycepin (5 µg/ml) and/or were withdrawn from the hormone by changing the culture medium. Unstimulated as well as fully induced cells served as controls.

At the appropriate time periods 0.5 g aliquots of cells were harvested by centrifugation and washed once in cold PBS (137 mM NaCl, 3 mM KCl, 10 mM Na_2HPO_4/KH_2PO_4, pH 7.4). Cells were lysed in polysome buffer (6) containing 1 % (w/v) of both deoxycholate and Triton X-100 in a Dounce homogenizer with a s-fitting pestle. Immediately after the preparation of the 30.000 g supernatant fraction 2 mg/ml of heparin were added. Cytoplasmic ribonucleoproteins were precipitated from the 30.000 g supernatant fractions by $MgCl_2$ as described in Ref. 6.

Poly(A)-containing RNA was isolated from the pellet by phenol-chloroform-extraction, followed by affinity chromatography on oligo(dT)-cellulose (6).

In vitro translation and identification of the translational products. We have used the wheat germ system as described (6). (^{35}S)Methionine was used as labelled amino acid. In all cases 6 µg of RNA were translated per 50 µl assay.

The levels of tyrosine aminotransferase were determined by immunoprecipitation of the in vitro formed subunits by the respective antibodies (19), subsequent electrophoretic separation of the precipitate in polyacrylamide slab gels and fluorography (6). The levels of DIP were examined directly in the protein patterns obtained from electrophoretic separations of the total proteins synthesized in vitro.

The relative amounts of products were determined by scanning the fluorograms with a Vernon photometer equipped with an automatic integrator.

Actin was labelled in vitro with (^{14}C)-formaldehyde as described by Rice and Means (20).

RESULTS AND DISCUSSION

We initially intended to restrict our experiments to the study of the decay of TAT mRNA. In the course of our experiments we detected in the protein patterns of the in vitro synthesized products a protein (DIP) of approximately 40.000 daltons, similarly induced by dexamethasone. We therefore considered in the present study both TAT and DIP mRNA activities.

HTC cells were fully induced for 20 hours by dexamethasone and the fully induced culture was either a) treated with 5 µg/ml cordycepin or b) the hormone was withdrawn by changing the medium or c) the hormone was withdrawn and 2 hours thereafter cordycepin was added. At appropriate time intervals aliquots of cells were harvested, cytoplasmic poly(A)-containing RNA was prepared and relative mRNA activities were determined as described in Methods. DIP mRNA activity was measured directly by analysis of the electrophoretic profile of the total products of translation. We estimate that DIP constitutes approximately 1 % of total proteins synthesized. In order to get directly comparable values, care was taken that all mRNAs analyzed had similar biological activities in the wheat germ assay with maximum deviations of 10 %.

Within 1 to 1.5 hours after removal of the hormone from the culture medium TAT mRNA activity had decayed to 50 % of the maximum induced values. If cordycepin is added 1 hour after removal of the hormone or is given in the presence of hormone the same decay kinetics of TAT mRNA activity were observed (Fig. 1 and 2).

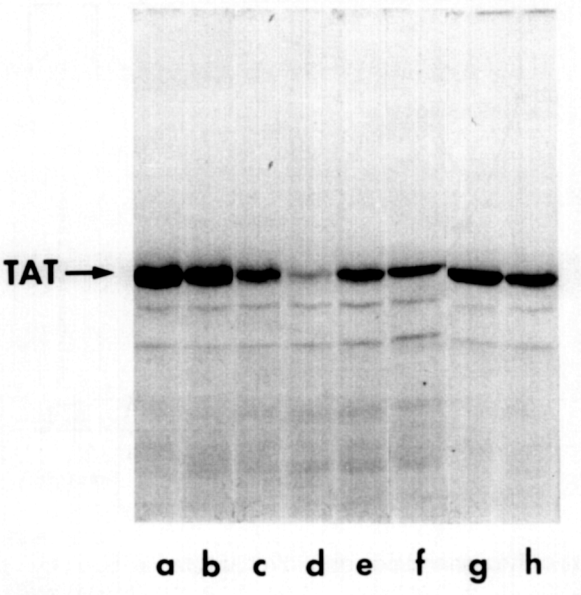

Figure 1

Decay of dexamethasone induced TAT mRNA activity in HTC cells following withdrawal of the hormone from the culture medium and cordycepin application.

Fluorograms of the immunoprecipitated material synthesized in a wheat germ system under the direction of cytoplasmic poly(A)-containing RNA obtained after various incubations of aliquots from the cell culture:

a) fully induced: 20 hours after application of 1 x 10^{-7}M dexamethasone

b), c), d) 60, 120, 180 min after removal of the hormone by changing the culture medium

e) like c) second independent estimation

f) 120 min after removal of the hormone and 60 min after addition of cordycepin (5 µg/ml)

g), h) 30, 90 min after application of cordycepin (5 µg/ml) to the culture medium in the presence of hormone

(TAT →) position of marker tyrosine aminotransferase

Since cordycepin inhibits the appearance of mature mRNA in the cytoplasm (16, 17), we should measure the half life of TAT mRNA activity prevailing under induced conditions, when cordycepin is given in the presence of the hormone. As in this case the kinetics of decay are very similar to the kinetics after withdrawal of the hormone, we conclude that deinduction of TAT mRNA activity is not accompanied with significant changes in the half life of this mRNA.

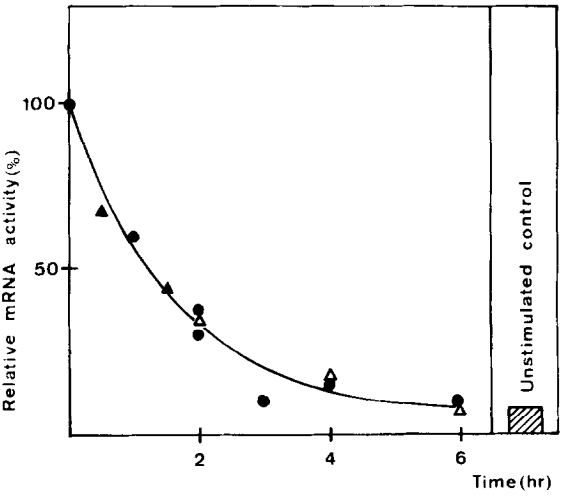

Figure 2
Decay of dexamethasone induced TAT mRNA activity in HTC cells after withdrawal of the hormone from the culture medium or after cordycepin application.
Photographic evaluation of two fluorograms of which an example is depicted in Fig. 1.
(●) fully induced cells withdrawn from the hormone by changing the medium
(Δ) withdrawn from the hormone; cordycepin (5 µg/ml) added 1 or 2 hours later, respectively
(▲) cordycepin (5 µg/ml) added in the presence of hormone.

These data are in accordance with earlier reported experiments of Stiles et al. (21) and Steinberg et al. (11), who reached the same conclusions by more indirect estimations, measuring the rates of translation in vivo.

As is suggested by the relative large DIP mRNA activity observed after dexamethasone stimulation, this mRNA activity decays much slower than TAT mRNA activity. 50 % of the fully induced levels are reached only 3 to 4 hours after withdrawal of the hormone (Fig. 3 and 4). When cordycepin is given to fully induced cultures in the presence of hormone, only a very small and delayed decay can be observed within 4 hours. Even when cordycepin is given 2 hours after withdrawal of the hormone a stabilization of DIP mRNA activity is observed.

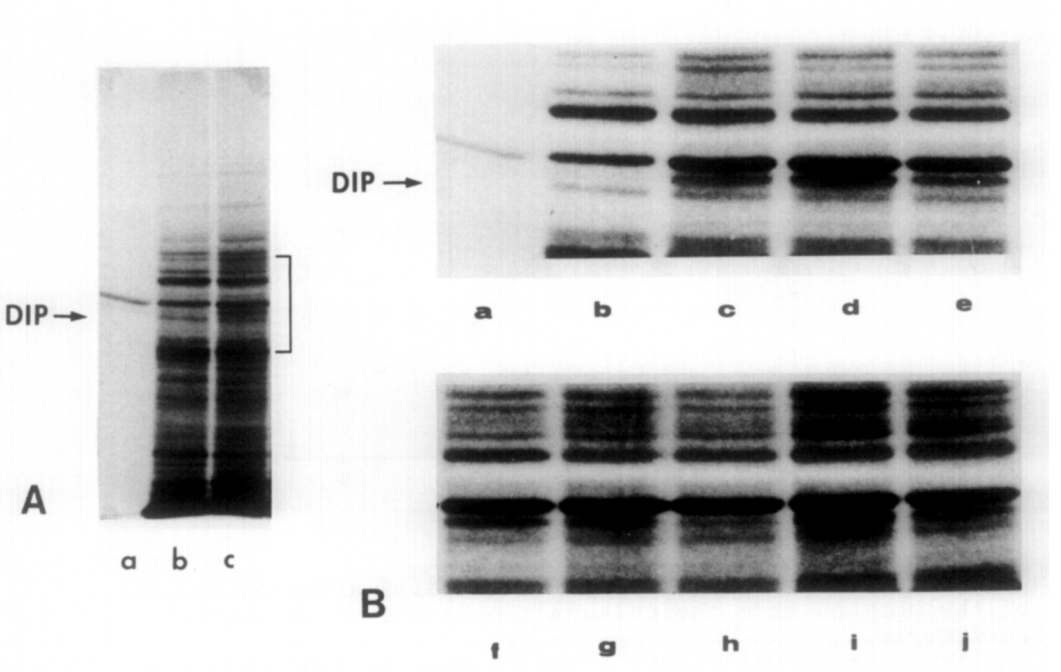

Figure 3

Decay of DIP mRNA activity in HTC cells after withdrawal of the hormone from the culture medium and application of cordycepin.

Fluorogram of the material synthesized in a wheat germ system under the direction of cytoplasmic mRNA obtained after various incubations of aliquots from the cell culture.

A) Total synthesized material separated on a SDS-polyacrylamid gel

B) Cut out from slots of total synthesized material (see bracket in A)
   a) actin marker
   b) unstimulated control
   c) fully stimulated
   d), e) 2, 4 hours after application of cordycepin (5 µg/ml) to the culture medium in the presence of the hormone
   f), g), h) 2, 4, 6 hours after withdrawal of the hormone by changing the culture medium
   i), j) 4, 6 hours after withdrawal of the hormone and 2 and 4 hours after cordycepin application, respectively

(DIP →) Position of the dexamethasone inducible protein.

It should be stressed that in our measurements not absolute but relative half lifes of mRNA activities were measured. TAT and DIP mRNA activities are expressed in percentage of the total mRNA activity. We do not know the average half life of the total mRNA activity, but

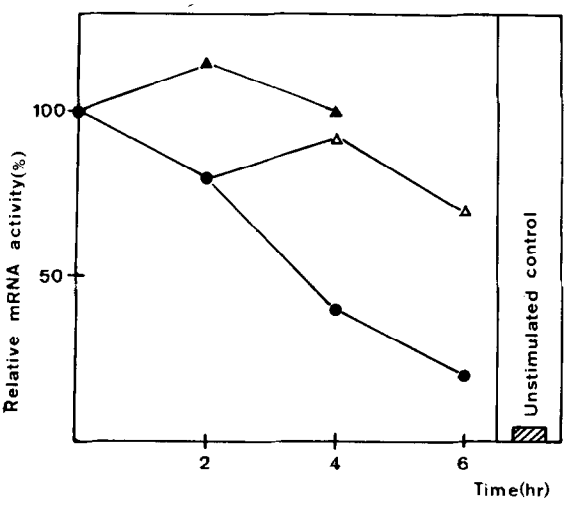

Figure 4
Decay of DIP mRNA activity in HTC cells after withdrawal of the hormone from the culture medium and application of cordycepin.
Photometric evaluation of the fluorogram depicted in Fig. 3.
(●) withdrawn from the hormone by changing the medium
(Δ) withdrawn from the hormone; cordycepin (5 µg/ml) was added 2 hours thereafter
(▲) cordycepin (5 µg/ml) was added in the presence of the hormone.

on the basis of the yield of poly(A)-containing RNA obtained from the same amount of cells, we conclude, that there is less than 20 % degradation of total mRNAs within 4 hours after addition of cordycepin. Therefore the observed effects are specific for DIP mRNA activity.

It is difficult to decide definitely whether the prolonged DIP mRNA activity observed after application of cordycepin reflects the normal half life during action of dexamethasone or is due to a secondary effect of the inhibitor. If the former is the case, it would suggest an additional mechanism initiated by withdrawal of the hormone and acting specifically on the degradation of DIP mRNA activity. This would implicate that such a degrading factor must have a rapid turnover,

since the same effect is seen when cordycepin is given 2 hours after withdrawal of the hormone. In addition it could not be a protein, because inhibition of protein synthesis by cycloheximide does not lead to a similar stabilization of DIP mRNA (unpublished results).

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