Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 365–368, January 1982
Biochemistry

Induction of tyrosine aminotransferase mRNA by glucocorticoids and cAMP in fetal rat liver

(development/gene regulation/hormones)

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Communicated by Joseph G. Gall, October 5, 1981

ABSTRACT **Tyrosine aminotransferase (L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) enzyme and mRNA activity were not detectable in day 20 fetal rat liver. Precocious induction of catalytic activity by *in utero* injection of dibutyryl cAMP was a direct consequence of the *de novo* appearance of translatable tyrosine aminotransferase mRNA. In contrast, *in utero* injection of hydrocortisone acetate failed to elicit fetal liver enzyme activity. This failure was due to the inability of the steroid hormone to induce the appearance of tyrosine aminotransferase mRNA activity. In fetal rat liver explants, either compound was capable of stimulating the synthesis of adult levels of enzyme and mRNA. However, catalytic and mRNA activity comparable with that seen *in vivo* 24 hr after birth required the concerted action of both inducers.**

One of the central problems in the study of development is the determination of the factors responsible for differential gene expression and the appearance of tissue-specific proteins. The enzymatic differentiation of developing rat liver has proved to be a useful model for these studies. During development, most rat liver enzymes accumulate rapidly and in discrete groups or clusters: the late fetal cluster (16–22 days of gestation), the neonatal cluster, and the late suckling cluster (1).

Tyrosine aminotransferase (L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) is one of the enzymes appearing in the neonatal cluster (1). Enzyme activity, virtually absent in fetal rat liver, starts to increase 2 hr after birth, reaching a maximum of at least twice the adult level by 12 hr. The catalytic activity then decreases to adult level by 2 days after birth (2, 3). Premature appearance of tyrosine aminotransferase can be elicited in fetal rats by *in utero* injection of glucagon (4, 5) or dibutyryl cAMP (6); glucocorticoid injection does not precociously induce catalytic activity (4, 6). However, all three compounds induce enzyme activity *in vitro* (7–9).

As part of our studies on the regulation of tyrosine aminotransferase gene expression, we are investigating the process responsible for the appearance of tyrosine aminotransferase in newborn rats. We present evidence that the appearance of enzyme activity after precocious induction *in utero* is a direct consequence of the *de novo* accumulation of mRNA coding for tyrosine aminotransferase. In addition, we have used fetal rat liver explants to correlate tyrosine aminotransferase mRNA and enzyme activity during induction by dibutyryl cAMP and hydrocortisone acetate.

MATERIALS AND METHODS

Chemicals. Cortef acetate (hydrocortisone acetate, 50 mg/ml) was provided by Upjohn. BGJb medium, Fitton–Jackson modification, and penicillin/streptomycin (penicillin, 10,000 units/ml; streptomycin, 10,000 μg/ml) were from GIBCO. Translation grade [^{35}S]methionine (specific activity, 1350–1450 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) was from Amersham. Cordycepin, protein A-Sepharose, and other biochemicals were from Sigma.

Animals. Sprague–Dawley (CD) rats from Charles River Breeding Laboratories were mated overnight. The morning that sperm were found in a vaginal smear was designated day 1 of pregnancy or gestation.

***In Vivo* Procedures.** Timed pregnant females in day 20 of gestation were anesthetized with ether and the uteri were exposed. Each fetus was injected intraperitoneally through the uterine wall (6) with 0.05 ml of 20 mM dibutyryl cAMP, 7.9 mM hydrocortisone acetate, or 0.9% NaCl. The dams were sacrificed by cervical dislocation 6 hr later. The fetuses were removed from the uterus, weighed, and decapitated. Fetal livers were removed, rinsed in cold water, blotted dry, and frozen in liquid nitrogen. Samples were stored at −70°C.

Organ Culture. The organ explant system used was that described by Simkins *et al.* (10) with modifications. After excision, fetal livers were rinsed in cold culture medium and manually cut into 1.5-mm cubes. Each cube was cultured on a 7-mm-diameter Millipore filter (0.45 μm) in a 16-mm well (Costar, Cambridge, MA) containing 0.2 ml of medium. The culture medium was BGJb medium, Fitton–Jackson modification/20 mM Hepes, pH 7.6, supplemented with penicillin at 100 units/ml and streptomycin at 100 μg/ml. Before use, the medium was equilibrated at 37°C with water-saturated 95% air/5% CO_2. Fresh medium was provided every 24 hr.

Assays. Tyrosine aminotransferase activity was assayed by a procedure adapted from Granner and Tomkins (11). One unit of enzyme catalyzes the formation of 1 nmol of *p*-hydroxyphenylpyruvate per min at 37°C. Protein content was determined by the Lowry method. Actual tyrosine aminotransferase activity was distinguished from tyrosine deamination by aspartate aminotransferase (EC 2.6.1.1) (12) by incubating part of the liver homogenate before assay with goat anti-rat tyrosine aminotransferase IgG to inhibit tyrosine aminotransferase catalytic activity. The difference in activity between the antibody-treated and the untreated homogenates is a measure of true tyrosine aminotransferase activity.

RNA for translation was isolated by a modification of the guanidine hydrochloride extraction procedures of Chirgwin *et al.* (13) and Ullrich *et al.* (14). Two grams of liver from injected animals or 22 explants was homogenized in extraction buffer (6 M guanidine hydrochloride/25 mM Tris·HCl/5 mM EDTA, pH 7.5). The homogenate was adjusted to 33.3% ethanol, and the nucleic acids were precipitated overnight at −20°C. The sample was centrifuged at 20,000 × *g* for 15 min and the pellet was suspended in fresh extraction buffer. This was layered over a CsCl cushion (5.7 M CsCl/0.1 M EDTA, pH 7.5) and cen-

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trifuged at 125,000 × g for 16 hr. The RNA pellet was suspended in sterile water and precipitated and washed in ethanol. Poly(A)$^+$RNA was isolated by passing the guanidine hydrochloride extracts through an oligo(dT)-cellulose column (15). The RNA was reprecipitated, washed, dried under nitrogen, resuspended in sterile water (minimum volume), and stored at −70°C.

Tyrosine aminotransferase mRNA activity was quantitated by translation in the rabbit reticulocyte lysate system (16) followed by immunoprecipitation of *in vitro*-synthesized radioactive tyrosine aminotransferase. Immunoprecipitation was by the two-step indirect method described by Ivarie and Jones (17) except that protein A-Sepharose was used in place of *Staphylococcus aureus* cells. Immunoprecipitates were analyzed by electrophoresis in 10% NaDodSO$_4$/polyacrylamide gels. Frozen gels were sliced and radioactivity was extracted and assayed in 3% Protosol in Econofluor (New England Nuclear). The amount of [^{35}S]methionine incorporated into tyrosine aminotransferase was quantitated by summing the radioactivity of the gel slices containing the peak of the *in vitro*-synthesized enzyme subunit and subtracting a background estimated from the radioactivity in the slices on either side of the peak. RNA concentrations were chosen so that the cpm incorporated into the enzyme subunit was proportional to the amount of tyrosine aminotransferase mRNA added to the translation system. The use of this assay to quantitate basal and hormone-induced levels of tyrosine aminotransferase mRNA in adult rat liver has been well established (18).

RESULTS

Two inducers of adult liver tyrosine aminotransferase were tested for their ability to precociously induce enzyme activity in fetal rat liver. After correcting for the presence of aspartate aminotransferase (12), virtually no tyrosine aminotransferase activity was detected in the livers of day 20 fetal rats (Table 1). As previously reported (6), *in utero* injection of dibutyryl cAMP resulted in the appearance of catalytic activity within 6 hr. Hydrocortisone acetate injection failed to elicit the same response 6 hr after treatment (4, 6).

In the same experiment, the level of functional tyrosine aminotransferase mRNA was assayed to determine its relationship to catalytic activity. Poly(A)$^+$RNA was prepared from fetal and adult liver samples and translated in the rabbit reticulocyte lysate translation system. Newly synthesized tyrosine aminotransferase was immunoprecipitated and analyzed by NaDodSO$_4$/polyacrylamide gel electrophroesis (Fig. 1). Translatable tyrosine aminotransferase mRNA was not detectable in saline- or hydrocortisone-treated fetal rat livers (Fig. 1*B*). However, mRNA activity was present in the livers of animals treated with dibutyryl cAMP, as shown by the presence of radioactive tyrosine aminotransferase (Fig. 1*A*). Table 1 summarizes the tyrosine aminotransferase catalytic and mRNA activities found for the various liver samples. For comparison, basal adult values are included. The sensitivity of the translational assay is such that it is impossible to state unequivocally that tyrosine aminotransferase mRNA activity is completely absent in fetal rat liver. However, it is clear that dibutyryl cAMP markedly increased the level of translatable mRNA coding for tyrosine aminotransferase. The failure of hydrocortisone acetate to precociously induce fetal liver enzyme activity *in utero* can be attributed to its inability to elicit the appearance of translatable mRNA coding for tyrosine aminotransferase. Neither dibutyryl cAMP nor hydrocortisone acetate significantly altered the total mRNA template activity in fetal liver.

Fetal liver explants were used to examine in more detail the hormonal factors regulating the development of tyrosine ami-

Table 1. Effect of dibutyryl cAMP and hydrocortisone acetate on levels of tyrosine aminotransferase catalytic and mRNA activity in fetal rat liver

Treatment	*n*	Catalytic activity, units/mg of protein	mRNA activity: Total (×10^{-6})*	mRNA activity: Tyrosine aminotransferase†
Saline	24	0.07 ± 0.03	6.9	ND
cAMP	27	3.19 ± 0.56	8.9	803
Saline	24	0.06 ± 0.6	6.4	ND
Hydrocortisone	39	0.12 ± 0.04	7.8	ND
Basal adult		15.8	6.7	2546

Results are based on data from Fig. 1. Catalytic activity is expressed as mean ± SEM of the specific activity. ND, not detectable.

* cpm of [^{35}S]methionine incorporated into total protein per 7 μg of poly(A)$^+$RNA.

† cpm of [^{35}S]methionine incorporated into tyrosine aminotransferase per 7 μg of poly(A)$^+$RNA.

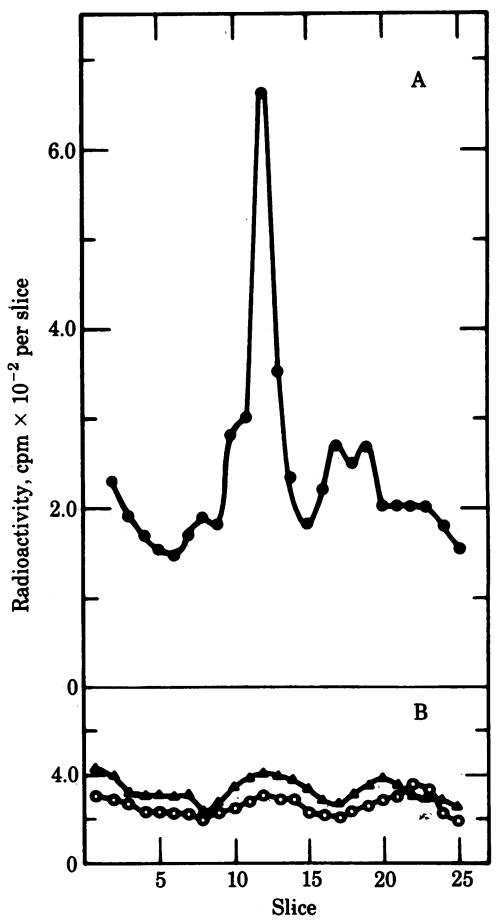

FIG. 1. NaDodSO$_4$/polyacrylamide gel electrophoretic profile of tyrosine aminotransferase synthesized *in vitro*. Poly(A)$^+$RNA was prepared from the livers of day 20 fetal rats treated *in utero* with dibutyryl cAMP (1.0 μmol per fetus for 6 hr) or hydrocortisone acetate (0.4 μmol per fetus for 6 hr). mRNA preparations were translated in the rabbit reticulocyte lysate system; newly synthesized radioactive tyrosine aminotransferase was immunoprecipitated, and the immunoprecipitates were washed, solubilized, and analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis. Radioactivity was measured in the immunoprecipitates after translation of 7 μg of poly(A)$^+$RNA from fetal rats treated with dibutyryl cAMP (*A*) or fetal rats treated with saline (○) or hydrocortisone acetate (▲) (*B*).

notransferase activity. Uniform cubes of day 20 fetal rat liver were cultured in a chemically defined medium in the absence of serum and hormones. The morphological and functional integrity of these explants has been established (10). The response of fetal liver explants to hydrocortisone and dibutyryl cAMP was tested several times during 60 hr of culture. The extent of tyrosine aminotransferase induction observed at these times is shown in Fig. 2. Addition of dibutyryl cAMP to fresh explants (0 hr in culture) produced small but reproducible increases in activity within 12 hr. Explants cultured for 24 or 48 hr prior to dibutyryl cAMP treatment were more responsive to the inducer. Tyrosine aminotransferase activity in these explants approached basal adult levels. Significant catalytic activity did not develop spontaneously in untreated control explants through 60 hr in culture. In contrast to its lack of effect *in utero*, hydrocortisone acetate was capable of inducing tyrosine aminotransferase activity in cultured liver explants. Measurable increases in enzyme activity were observed after hydrocortisone treatment of fresh explants. Again, explants cultured for 24 or 48 hr before treatment were more responsive.

The time course of induction of tyrosine aminotransferase activity in 24-hr explants is shown in Fig. 3. Enzyme activity was evident <2 hr after the addition of dibutyryl cAMP and continued to increase over the next 22 hr. After a 2-hr lag, a similar increase in enzyme activity occurred after hydrocortisone acetate treatment. The action of the steroid hormone and cAMP was prevented by inhibitors of RNA and protein synthesis, indicating that the increase in enzyme activity mediated by either inducer is the result of *de novo* synthesis of tyrosine aminotransferase and its mRNA (data not shown). Simultaneous addition of dibutyryl cAMP and hydrocortisone acetate resulted in a dramatic increase in the rate of appearance of enzyme activity. This led to induced levels at 12 or 24 hr after treatment that were several times greater than the sum of the individual increases mediated by either inducer alone. The synergism between steroid hormone and cAMP action has been observed in adult liver (19).

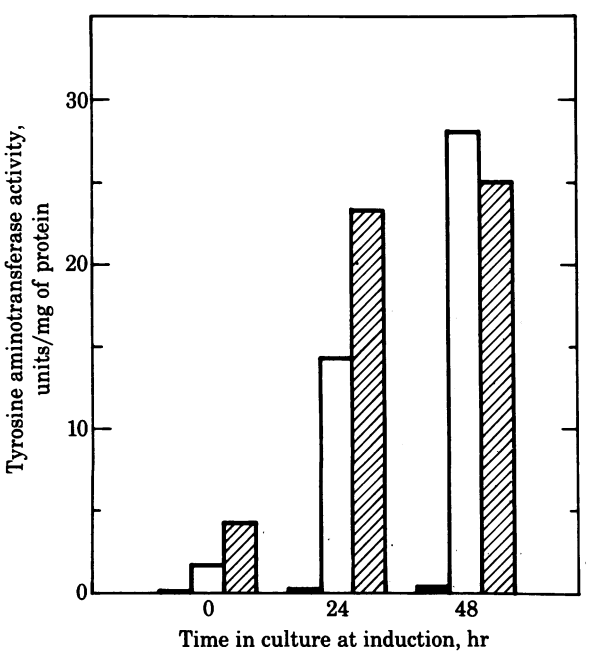

FIG. 2. Induction of tyrosine aminotransferase in fetal rat liver explants. Explants were incubated for 0, 24, or 48 hr before the addition of 0.5 mM dibutyryl cAMP (open bars) or 0.6 μM hydrocortisone acetate (striped bars). Control explants (solid bars) received no additions. Enzyme activity was determined 12 hr after treatment.

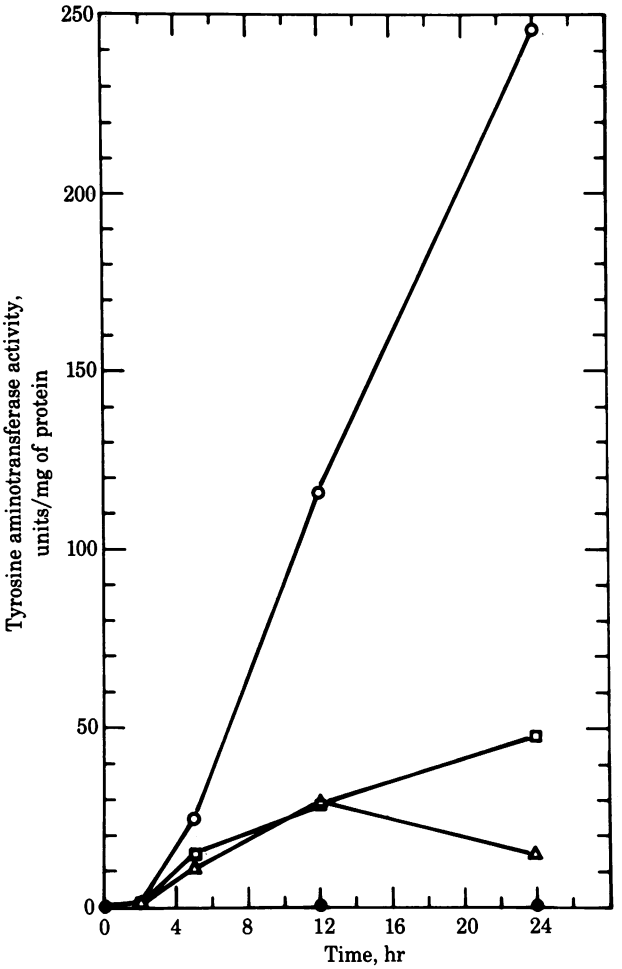

FIG. 3. Time course of tyrosine aminotransferase induction. Explants were incubated for 24 hr before the addition of 0.5 mM dibutyryl cAMP (△), 0.6 μM hydrocortisone acetate (□), or both inducers together (○). Control explants (●) received no additions.

The time course of induction of mRNA and enzyme activity in fetal liver explants is shown in Fig. 4. With either inducer, the appearance of tyrosine aminotransferase mRNA always preceded the induction of enzyme activity. In general, the changes in catalytic activity paralleled mRNA activity except for the biphasic appearance of mRNA induced by dibutyryl cAMP (Fig. 4A). The synergistic induction of enzyme activity resulting from the combined action of dibutyryl cAMP and hydrocortisone acetate was also observed at the level of tyrosine aminotransferase mRNA activity (Fig. 4). Thus, the mechanism of induction of tyrosine aminotransferase activity by glucocorticoids and cAMP in developing rat liver is identical to that in the adult (18).

DISCUSSION

Several important features of the control of tyrosine aminotransferase gene expression have emerged from our studies. No detectable pool of translatable mRNA in day 20 fetal rats was found that could account for the dramatic increase in enzyme activity seen in the newborn (Table 1). However, we cannot exclude the possibility that a rapid accumulation of tyrosine aminotransferase mRNA occurs during the remaining 2 days of gestation.

The precocious induction of enzyme activity achieved by *in utero* injection of dibutyryl cAMP was found to be a direct consequence of the *de novo* appearance of translatable mRNA coding for tyrosine aminotransferase (Table 1). Whether this mRNA

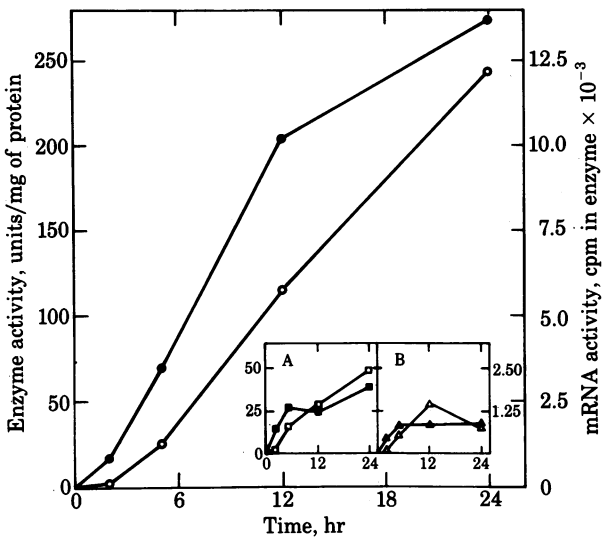

FIG. 4. Time course of induction of tyrosine aminotransferase mRNA in fetal liver explants. Explants were incubated for 24 hr before the addition of 0.5 mM dibutyryl cAMP and 0.6 μM hydrocortisone acetate. At the indicated times, tyrosine aminotransferase enzyme (○) and mRNA (●) activities were determined. mRNA activity is expressed as cpm of [^{35}S]methionine incorporated into tyrosine aminotransferase per 5 × 10^6 cpm in total protein. (*Inset*) Extent of induction by dibutyryl cAMP (*A*) or hydrocortisone acetate (*B*) alone (□ and △, enzyme activity; ■ and ▲, mRNA activity).

results from stimulation of gene transcription or from the maturation of precursor sequences to functional mRNA cannot be determined at this time. Likewise, *in utero* injection of hydrocortisone acetate did not induce enzyme activity because the treatment failed to elicit the appearance of functional tyrosine aminotransferase mRNA. This cannot be due to the inability to induce tyrosine aminotransferase mRNA *per se*; dibutyryl cAMP induces both enzyme and mRNA activity (Table 1). The defect seems to be in the glucocorticoid-mediated portion of the induction process. Yeoh *et al.* (20) recently reported that isolated hepatocytes prepared from fetal rats injected *in utero* with the synthetic glucocorticoid, dexamethasone, developed (in culture) tyrosine aminotransferase activity that was significantly higher than that of control cells. Enzyme activity appeared in the absence of added inducers and despite the presence of actinomycin D in the medium. They postulated that dexamethasone stimulated the synthesis of mRNA that could not be translated until a translational block was removed under the culture conditions. We do not observe the accumulation of translatable mRNA coding for tyrosine aminotransferase in fetal rat liver after glucocorticoid treatment. If a translational block exists *in utero*, it must be specific for glucocorticoid action because dibutyryl cAMP induces functional tyrosine aminotransferase mRNA activity.

Hydrocortisone acetate and dibutyryl cAMP elicit weak but reproducible induction of enzyme activity in fresh fetal liver explants, although the response becomes stronger with increased time in culture. It is unlikely that this represents ongoing differentiation of the fetal hepatocyte since it has been shown that developmental changes in serum protein synthesis in fetal mouse liver explants are arrested at the time of explantation (21). Rather, it seems that the explants are adapting to the *in vitro* culture conditions. The observation that hydrocortisone induces tyrosine aminotransferase catalytic and mRNA activity *in vitro* but not *in utero* suggests that culturing relieves the restrictions imposed by the uterine environment with respect to glucocorticoid action. Contrary to other reports, *in vitro* culture conditions alone were insufficient to stimulate enzyme activity in the absence of added inducers (22–24).

Either dibutyryl cAMP or hydrocortisone acetate was capable of inducing enzyme and mRNA activity in fetal liver explants to adult levels within 12 hr. However, activity comparable with that seen *in vivo* in the 24 hr after birth (2 or 3 times the adult level) required the concerted action of both inducers (Fig. 4). The presence of the steroid hormone and the cyclic nucleotide may be necessary for the dramatic increase in enzyme activity seen immediately after birth. Greengard (6) suggested that glucagon secreted in response to postnatal hypoglycemia initiates enzyme accumulation in neonatal liver. Experiments showing that premature delivery (5) or *in utero* injection of glucagon (4, 5) precociously induced tyrosine aminotransferase favor this hypothesis. In addition, glucose injection 2 hr after birth, when hypoglycemia is maximal, partially prevented the appearance of enzyme activity (5). In support of a role for glucocorticoids, Sereni *et al.* (2) found that amphenone B, an inhibitor of corticosteroid synthesis, delayed the appearance of tyrosine aminotransferase when injected immediately after birth. Neonatal adrenalectomy also prevented the induction of enzyme activity. This effect was reversed by injection of glucocorticoids. Perhaps it is the synergistic interaction between glucocorticoids and glucagon (or cAMP), clearly observed in fetal liver explants, that leads to the dramatic appearance of tyrosine aminotransferase in neonatal rat liver.

This work was supported by National Institutes of Health Grants AM 22030 and HD 07180.

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