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Developmental and hormonal regulation of protein H1° in rodents

(chromatin/development/regeneration)

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ABSTRACT **The tissue and cellular distribution and regulation of the chromatin protein H1° has been examined in developing and adult mouse and in rat. The protein appears in specific cell types of solid tissues only when the cells have terminated their maturation. This was found for brain, retina, striated and cardiac muscle, and liver. In tissues that depend on hormones for their function and maintenance, the expression of H1° is dependent on the continued presence of the specific maintenance hormone. In regenerating rat liver the amount of H1° decreases to one-third after the onset of DNA synthesis. The possible role of H1° is discussed in light of these results.**

Proteins with properties similar or identical to those of the very lysine rich chromosomal protein H1° have been described in several mammalian tissues and in tissue culture cells (1–16). The protein isolated from ox or mouse liver has been shown to consist of two subfractions that have identical molecular weights (25,000) but differ slightly in charge (2, 3). The two subfractions appear to have similar or identical amino acid compositions and peptide patterns. Recently it has been shown that H1° has extensive sequence homology with histone H5 from goose erythrocytes (4).

H1° is of interest for several reasons. Its level in different tissues varies widely and it is predominantly found in tissues exhibiting little cellular proliferation (1, 6, 7, 9, 15, 17). In earlier work, using indirect immunofluorescence with monospecific antibodies, we showed that H1° was present in many but not all fully mature postmitotic cells, and therefore that its distribution in different cells of the adult mouse is heterogeneous (15). These data suggest that H1° may play an important role in cell regulation.

The concept that H1° is involved in developmental regulation is strengthened by the observations that in rat pancreas, liver, and spleen the protein appears after birth once cell proliferation in the organs has ceased (7, 8, 17). It has also been shown to decrease in regenerating rat pancreas and liver (5, 18).

We report here studies on the regulation of H1° in developing mouse and in adult mouse and rat. The presence of H1° was shown to be correlated with cell maturation during development and liver regeneration. It was also found to be hormonally regulated in several glands in the adult. The results suggest that H1° may play a role in the maintenance of the mature differentiated state of some cells.

MATERIALS AND METHODS

Animals. Strain 129 male mice were used unless otherwise indicated. Fisher or Wistar male rats (both normal and hypophysectomized) were purchased from Iffa Credo (Paris, France). Hormones were injected either intraperitoneally or subcutaneously as described in the text or figure legends.

Biochemical Methods. Lysine-rich histones were extracted from whole tissues with 5% perchloric acid as described (3). Proteins were analyzed by electrophoresis in polyacrylamide gels as described (10). After staining with Coomassie brilliant blue the gels were scanned at 590 nm and the intensity of band staining was estimated by integration of the peaks.

Tissue Sections, Fixation, and Staining. Animals were killed by cervical dislocation, and tissues were immediately removed and frozen on microtome tissue holders in Tissue-Tek freezing medium. Frozen sections (5–10 μm) were cut with a Slee cryostat. The sections were placed on microscope slides, rapidly air dried, and fixed as described (15). Antisera were prepared and indirect immunofluorescent staining was performed as described (15). Sera were diluted 1:200 for staining. Nuclei were rendered fluorescent by incubation in 1 μM 4',6-diamidino-2-phenylindol (DAPI) for 5 min. DAPI fluorescence does not interfere with the rhodamine used for the immunofluorescence. Fluorescence was viewed with a Leitz Orthoplan microscope and photos were taken manually (60 sec), using Ilford HP5 film.

Partial Hepatectomy. Male Wistar rats weighing 150–200 g were partially hepatectomized as described by Higgins and Anderson (19). One hour before sacrifice rats were injected with [*methyl*-^{3}H]thymidine in 1 ml of 0.15 M NaCl (80 μCi/100 g of body weight, specific activity 50 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels). Livers were excised and put in cold 0.15 M NaCl and nuclei were immediately prepared (20). For measurement of incorporated ^{3}H, 5×10^5 nuclei in a volume of 0.05 ml were mixed with 10 vol of tissue solubilizer. The mixture was concentrated by evaporation before being mixed with 10 ml of scintillation fluid.

Aliquots of nuclei from each regeneration time were suspended in digestion buffer [0.34 M sucrose in buffer A of Hewish and Burgoyne (20), pH 7.4, containing 1 mM $CaCl_2$ and 0.5 mM phenylmethylsulfonyl fluoride]. They were then briefly digested by micrococcal nuclease (20 units of nuclease for 10^7 nuclei for 1 min) to obtain a homogeneous suspension upon dissociation of nuclei by 2% $NaDodSO_4$ in 62.5 mM Tris·HCl, pH 6.8/5% (vol/vol) 2-mercaptoethanol. Proteins were denatured by immersing the samples for 2 min in boiling water.

RESULTS

Developmental Regulation of H1°. We have previously reported that H1° is present in some but not all fully differentiated

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Abbreviation: DAPI, 4',6-diamidino-2-phenylindol.

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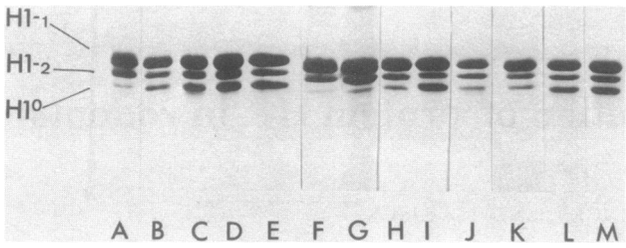

FIG. 1. $NaDodSO_4$ gel of 5% perchloric acid-extracted proteins from mouse tissues at various times after birth, stained with Coomassie brilliant blue. Lanes A–E, liver at 0, 3, 5, 6, and 16 weeks; lanes F–I, brain at 0, 3, 6, and 16 weeks; lanes J–M, kidney at 0, 3, 6, and 16 weeks.

cell types of solid tissues in mouse (15). It has also been demonstrated that H1° appears in some mammalian organs after birth (7, 8, 17). These findings suggest that the accumulation of H1° is correlated either with the termination of cell proliferation or with the maturation of postmitotic cells. In order to investigate these possibilities we examined mouse tissues at various stages of development either by extraction of the lysine rich proteins followed by electrophoresis in polyacrylamide gels or by indirect immunofluorescence with monospecific antisera to H1°. All tissues were also stained with antiserum to mouse histone H1 in order to ensure that cells were not negative because of failure of antibodies to penetrate the tissue sections. Preimmune sera were used for negative controls. Except where stated, immunofluorescent staining with either anti-H1° or anti-H1 serum was always localized in nuclei. The monospecificities of the sera were determined by immunoprecipitation of metabolically labeled total nuclear proteins of tissue culture cells and by radioimmunoassay using purified proteins from mouse liver, brain, and kidney (data not shown).

Fig. 1 shows the electrophoretic analysis of the 5% perchloric acid-extractible proteins from mouse liver, brain, and kidney at various stages of development. It may be seen that the appearance of H1° relative to histone H1 is tissue dependent. Whereas the three adult tissues (16 weeks) are all rich in H1°, organs from newborn animals contain very little of the protein. H1° accumulates rapidly in kidney and liver after birth, reaching adult levels within 3–6 weeks. In brain, however, H1° does not begin to accumulate until the 3rd week, and then it appears slowly until the 15th week. The levels of histone H1 did not vary significantly at the different developmental stages. Similar results have been reported for rat pancreas, in which H1° accumulation did not begin until cell proliferation had terminated (7).

Table 1 shows the percentage of H1°-positive cells in various mouse organs examined by indirect immunofluorescence at different stages of development. The only recognizable cell types containing detectable quantities of H1° in embryos are nucleated erythrocytes and the fiber cells of the lens, both of which are positive at the earliest developmental stage at which they could be detected. In the other tissues examined H1° became detectable only after birth, with the cellular accumulation varying from tissue to tissue. This can be seen with respect to both the percentage of positive cells and the intensity with which they stain.

The appearance of H1°-positive cells in the brain is particularly interesting. Although it is thought that the neuronal cells of the cortex are postmitotic at birth, their morphogenesis and biochemical maturation take place over a 3- to 4-week period after birth (22). However H1° begins to appear in these neurons only after 3 weeks, when most of the maturation is terminated. A more detailed analysis of H1° development in the brain will be presented elsewhere.

H1° accumulation in striated muscle also appears to be correlated with the termination of maturation. Although a few positive myotube nuclei are present in the first week after birth, the accumulation of H1° is not complete until 2–3 weeks. Although given adult myotubes contain mainly H1°-positive nuclei, a few negative nuclei are seen.

The relationship between the appearance of H1° and tissue development was further examined in mouse retina. Retinal development continues after birth, with the ganglion, bipolar, and sensory layers being formed by day 7, when the ganglion and bipolar cells are fully postmitotic. These two cell types ap-

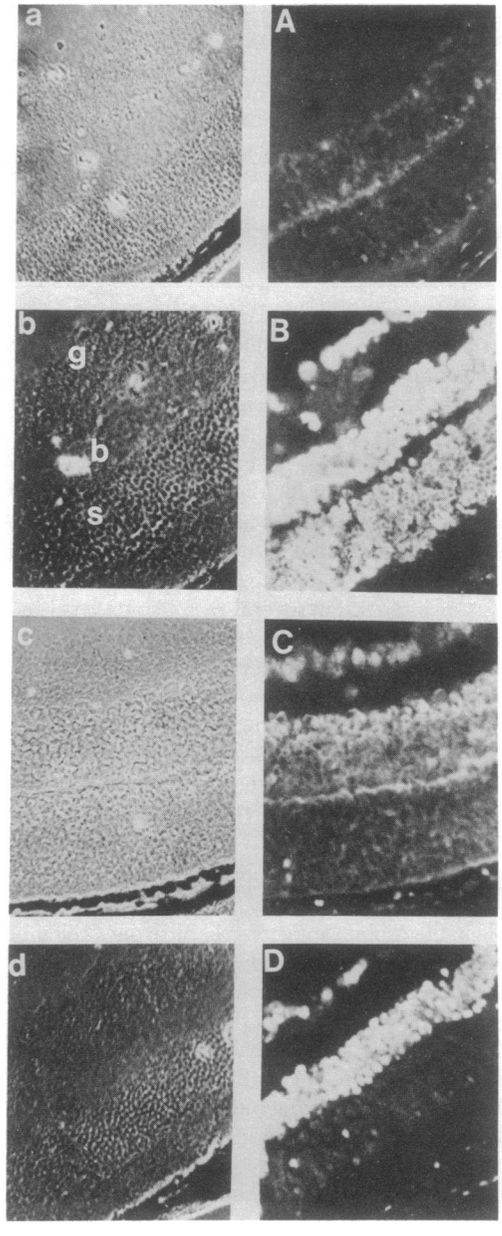

FIG. 2. Immunofluorescence microscopy of developing mouse retina. (*a–d*) Phase-contrast photos; (*A–D*) fluorescence photos. (*a*, *A*) Retina from 10-day-old mouse, stained with anti-H1° serum; (*b*, *B*) same retina stained with anti-H1 serum; (*c*, *C*) retina from mouse 24 hr after eye opening, stained with anti-H1° serum; (*d*, *D*) retina from mouse on day 5 after eye opening, stained with anti-H1° serum. Sensory, bipolar, and ganglia cell nuclei are indicated by s, b, and g, respectively.

Table 1. H1°-positive cells in mouse tissues

		H1°-positive cells, %				
		Embryo				
Tissue	Cell type	9-day	15-day	Birth	3 weeks	Adult
Brain	Neuronal			<5	5–10	90–100
(cortex)	Glial			<5	<5	<5
Retina	Ganglion		<1	<2	90–100	100
	Bipolar		<1	<2	90–100	90–100
	Sensory			<2	<2	<2
Lens	Epithelial		<5	5–10	70–85	80–90
	Fiber		80–100	80–100	100	100
Liver	Hepatocytes			10–15	50–65	90–100
Muscle						
Striated	Myotube			<10	50–60	80–95
Cardiac				5–10	60–75	80–90
Bone marrow	Erythroblast*					<2
Yolk sac	Erythrocyte	100				

* Identified as spectrin positive (21). Tissue sections were stained both by indirect immunofluorescence using rabbit anti-mouse H1° followed by rhodamine-labeled sheep anti-mouse IgG and by DAPI (1 μM). H1°-positive nuclei are given as the percentage of DAPI-labeled nuclei.

pear to be fully differentiated before the 10th postnatal day, when the eyes begin to open (23). To follow the appearance of H1° in retinal cells, mice were examined at 15 days before birth, at birth, and at various times thereafter (three mice per point). The results can be seen in Table 1 and Fig. 2. H1° does not appear in the ganglion and bipolar cell nuclei until after the animals have opened their eyes, and it could not be detected at any stage in the sensory cells. Preliminary results suggest that exposure to light is the signal for the appearance of H1° in the ganglion and bipolar cells.

Liver Regeneration. Because H1° is found predominantly in chromatin of mature functional cells in stable tissues of mouse and rat, it might be expected that the protein would be lost during tissue regeneration. Indeed, a decrease in H1° had been shown to accompany the regeneration of rat pancreas after treatment of animals with ethionine (5). During rat liver regeneration the cells leave their G_0 state and divide (24), after which they appear to dedifferentiate with the appearance of fetal-specific proteins, such as type III pyruvate kinase and α-fetoprotein (25, 26). Both proteins are maximally produced 48–72 hr after partial hepatectomy, and their levels return to control values after 5–6 days. It was therefore of interest to determine whether the H1° content changed during liver regeneration and if so whether the changes correlated with the early events (onset of

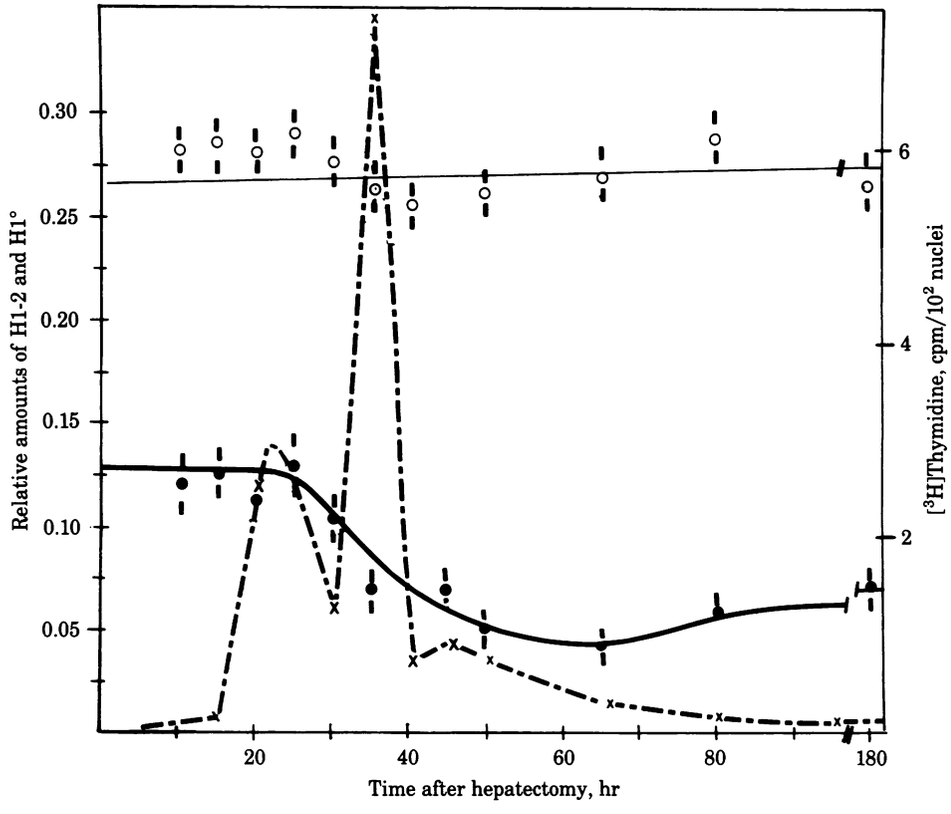

FIG. 3. DNA synthesis and amount of H1° (●) and H1-2 (○) in regenerating liver. The amounts of H1-2 and H1° were estimated relative to H4. ×, Incorporation of [^{3}H]thymidine.

DNA synthesis) or occurred after DNA replication.

Rats were partially hepatectomized and at various times thereafter they were sacrificed and the remaining liver tissue was analyzed for DNA synthesis and chromatin protein composition as described in *Materials and Methods*. The results are shown in Fig. 3. Very little [^{3}H]thymidine was incorporated into DNA before 15 hr after hepatectomy. After this time two waves of incorporation with peaks at 25 and 36 hr were observed. By 80 hr the incorporation of [^{3}H]thymidine reached the 15-hr level. The H1° content of the regenerating tissue was determined relative to histone H4. This was also done for histone H1-2, a subclass of histone H1 that is present in all tissues and whose relative abundance in rat liver is about twice that of H1°. The relative H1-2 content remained constant throughout the regeneration period. However, the relative H1° content began to decrease at 30 hr and reached minimal levels 50–65 hr after surgery, at which time it was one-third of the control value. Thus the decrease in H1° content of the regenerating liver does not precede the onset of thymidine incorporation but rather appears to follow it, and the lowest levels of H1° are seen when DNA synthesis is nearly complete. This suggests that H1° is not actively removed from the chromatin prior to DNA replication but rather that its synthesis is decreased or arrested and that the protein is diluted among the daughter cells. It thus appears that the decrease in H1° during regeneration is correlated more with postreplicative events than with the onset of DNA replication.

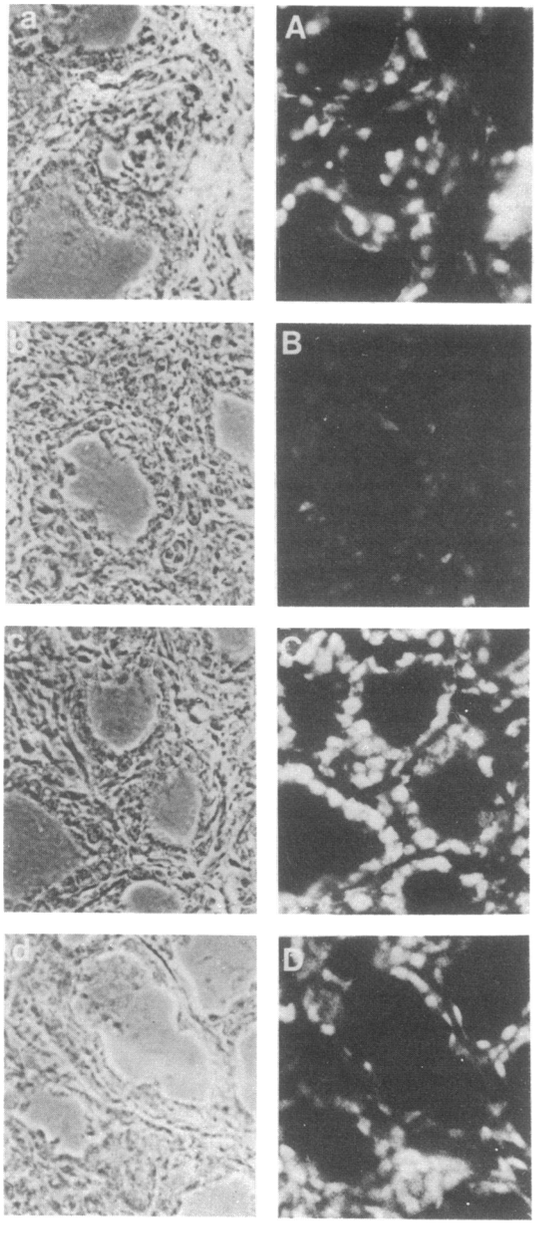

FIG. 4. Sections of rat thyroid. (*a–d*) Phase-contrast photos; (*A–D*) fluorescence photos. (*a*, *A*) Normal thyroid, stained with anti-H1° serum; (*b*, *B*) thyroid 4 days after hypophysectomy, stained with anti-H1° serum; (*c*, *C*) thyroid 4 days after hypophysectomy, stained with anti-H1 serum; (*d*, *D*) thyroid from hypophysectomized animals (4 days) subsequently injected with thyrotropin for 4 days, stained with anti-H1° serum.

Hormonal Regulation. In order to determine whether the presence of H1° is correlated with the functional activity of specialized cells, we examined mouse and rat glands that depend on hormones for their activity and maintenance. The thyroid, adrenal cortex, and testes of rats were examined 4 days after hypophysectomy. In all cases examined, H1° was lost, although there was no appreciable loss of H1 or atrophy of the tissue. Similar results were obtained with mouse prostate and vas deferens after castration (data not shown). These findings indicate that the presence of H1° is correlated with the expression of differentiated functions by the cells. In order to confirm this, rats hypophysectomized 4 days previously were injected daily (for 4 days) with 2 units of thyrotropin (intraperitoneally). After sacrifice their thyroids were examined for H1° and H1. The results are shown in Fig. 4. It can be seen that injection of the hormone resulted in the reappearance of H1°. Identical results were found with mouse prostate and vas deferens after subcutaneous injection of testosterone into mice castrated 3 days previously. Hypophysectomy, castration, or reinjection of the maintenance hormones caused no detectable changes in the H1° content of kidney, retina, skeletal muscle, or nontarget glands. It is of interest that H1° appears to be regulated by both peptide and steroid hormones.

DISCUSSION

There is considerable variation in the amounts of H1° in adult tissues and cells. Its presence is dependent on the cell type, the developmental stage of the cell, and in some cases on hormonal stimulation. In early embryos the only identified cells that contain detectable quantities of the protein are the fiber cells of the lens and nucleated erythrocytes, both of which are terminally differentiated mature cells. The finding that nucleated fetal erythrocytes are H1° positive is of interest in light of the striking similarity demonstrated between this protein and H5, the erythrocyte-specific histone of birds, fish, reptiles, and amphibians (4). It is also interesting that mature nucleated erythroid precursors in adult mice do not contain detectable H1° (15).

During postnatal development the accumulation of H1° in brain, retina, muscle, liver, pancreas, and lens appears to be correlated with termination of cell maturation after proliferation has ceased.

H1° is also subject to regulation in adult animals. The most striking example of this is seen in glands that require specific hormones for their maintenance and activity. In all the cases that we have examined, H1° was rapidly lost during hormone deprivation and was shown to reappear after hormone injection. The loss of H1° in the hormone-deprived glands is specific, as judged by the relative stability of histone H1 in the glands. This hormonal regulation occurs in the absence of proliferation, because in all of the tissues examined cell division is hormone dependent.

The presence of H1° is also regulated during tissue regeneration. This has been shown both for pancreas (5) and liver (18).

In the regenerating rat liver the amount of H1° decreases to one-third, and this decrease follows rather than precedes the onset of proliferation, suggesting that the H1° regulation during regeneration is associated with the postreplication events rather than with the regulation of proliferation.

The loss of H1° in maintenance hormone-deprived glands and in regenerating liver may occur by different mechanisms. The kinetics shown in Fig. 3 indicate that the protein may be diluted into daughter cells by proliferation during liver regeneration. In the glands it is lost in the absence of cell division. If this is true there would have to be active mechanism for removing H1° in the hormone-deprived gland cells, or it may be continually turned over in these cells, with its synthesis being hormone dependent.

It has been suggested that H1° may play a role in the regulation of cell proliferation (1, 2, 4, 5, 10). Although our results do not rule out this possibility, they suggest that the accumulation of H1° is not the primary mechanism by which proliferation is blocked. In the developing tissues examined here and by others (7, 8, 17) H1° appears after proliferation has terminated. In some tissues, such as retina, cerebral cortex, and skeletal muscle, this occurs long after proliferation has ceased. Furthermore, we have reported that variant Friend erythroleukemia cells continue to divide even though they contain large amounts of the protein IP_{25}, which is identical to H1° (3, 12). This indicates that the presence *per se* of H1° is not sufficient to block cell proliferation.

Our results are consistent with the idea that H1° may play a role in the termination of the differentiation process or the maintenance of the differentiated state of some cell types. This is suggested by the correlation of H1° appearance with the termination of cell maturation during development and regeneration, and also by its presence only in hormonally stimulated gland cells. Also, the fact that H1° is present in some highly differentiated postmitotic cell types but absent in others indicates that it may play a specific role.

At present our ideas concerning the role of H1° are based on correlations between its presence in cells and their developmental and physiological states. Clearly more information concerning its interactions with the chromatin and its regulation is required in order to determine the molecular role of this fascinating protein.

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