The Appearance and Disappearance of the Post-Transcriptional Repressor of Tyrosine Aminotransferase Synthesis during the HTC Cell Cycle

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Abstract. The synthesis of the enzyme tyrosine aminotransferase in HTC cells (an established line of rat hepatoma cells) is inducible by glucocorticoid hormones only during the latter part of G1 phase and throughout S phase in the cell generation cycle. We have earlier shown that during the first few hours of G1 phase when the enzyme cannot be induced, its synthesis is constitutive, presumably using as template, preexisting messenger RNA. Our model for tyrosine aminotransferase gene regulation in eukaryotic cells entails a specific post-transcriptional repressor which is formed only during the periods in the cell cycle when tyrosine aminotransferase is inducible. This model predicts that during the noninducible period, G2, the tyrosine aminotransferase repressor would not be present and thus tyrosine aminotransferase synthesis would be constitutive. Data are presented which confirm this prediction in further support of the model.

Introduction. We have been studying the control of gene expression in mammalian cells using as a model system an established line of rat hepatoma cells (HTC) in culture in which adrenal steroid hormones induce the synthesis of the intracellular enzyme, tyrosine aminotransferase (TAT).

On the basis of information about both random and synchronized populations of HTC cells, we have proposed a model which describes the regulation of TAT biosynthesis throughout the cell generation cycle (Fig. 1). This model involves two genes: an "S" gene, which determines the structure of the induced enzyme, and a regulatory "R" gene, which codes for a labile post-transcriptional repressor of TAT biosynthesis. The behavior of other eukaryotic systems (for examples see ref. 6) suggests that a similar post-transcriptional mechanism of gene regulation might operate quite generally, at least in higher organisms.

The regulation of TAT synthesis and its relationship to the HTC cell generation are depicted in Figure 1. To account for the induction of TAT we propose that during the inducible period of the cycle, i.e., between the third hour of G1 (G1[3]) and the beginning of G2, both the structural and the regulatory genes for TAT are continuously transcribed. However, despite its constant production, the mRNA for TAT cannot, in the absence of inducer, accumulate to induced levels. Its accumulation is prevented by the post-transcriptional
repressor which both inhibits the translation of the TAT messenger and promotes its degradation. When inducing steroids are present, the repressor is antagonized, thereby activating and stabilizing the mRNA, and allowing its accumulation. The increased mRNA concentration is presumed to cause the observed increased rate of TAT synthesis.3, 6, 7

We have previously demonstrated4 that TAT is not inducible during G2. As shown in the model, we attribute this noninducibility, like that in early G1, to inhibition of the transcription of both the S and R genes. Therefore, somewhere near the junction between S and G2, repressor synthesis should stop. If this supposition is correct, there should be no repressor remaining during G2, since it turns over rapidly. If cells in G2 are actually free of repressor, TAT synthesis should be constitutive, and removing the inducer from fully-induced cells during the G2 period should not decrease their synthesis of TAT. The experiments described below show that TAT synthesis is, in fact, constitutive during G2, in accordance with the model.

Materials and Methods. Populations of HTC cells were grown and synchronized as described previously.1, 4 TAT was induced with Dexamethasone (Sigma), and its enzymic specific activity and isotope content were determined as described in earlier work.3, 6, 7 The incorporation of labeled thymidine into DNA was quantitated by measuring, in a liquid scintillation counter, the radioactivity precipitated from cell extracts by 10% trichloroacetic acid.4 Tritiated amino acid mixture and 3H-thymidine were purchased from New England Nuclear Corp.

Results and Discussion. Populations of random HTC cells grown in eight glass prescription bottles in growth medium1 were induced by 5 × 10⁻⁷ M Dexamethasone10 for 20 hours, after which a mixture of 3H-amino acids (12.5 µCi/ml medium) was added. The cells were labeled for four hours under growth conditions, and then mitotic cells were harvested and pooled from all eight bottles (zero time sample). The interphase cells from one bottle, detached by stirring, served as the "zero time" sample of the interphase population. The
remaining nonmitotic cells (in the seven bottles) were "deinduced" by adding back 50 ml of steroid-free medium (lacking serum) containing $2 \times 10^{-7} M$ Colcemid, 50 $\mu g/ml$ Neomycin, and 0.02 per cent CaCl$_2$, but no labeled amino acids. At two-hour intervals thereafter, the mitotic cells which had accumulated were harvested, and at the same time the interphase cells from one bottle were also collected. The mitotic indices of the selectively harvested synchronized populations were all greater than 85 per cent. The cells were rapidly chilled, washed with chilled phosphate buffered saline, and quickly frozen. The TAT specific enzymic activities and the radioactivity remaining in the specifically immunoprecipitated TAT of the extracts are plotted in Figure 2 as a function of the time after removing the inducer. This time represents the position in the cell cycle (in hours preceding metaphase) at which the harvested cells were located when the inducer was removed. Thus, metaphase cells detached four hours after removing inducer have presumably transversed almost the entire G2 phase in the absence of steroid. From the upper portion of the figure it is apparent that, as usual, the TAT enzymic activity in the interphase cells immediately fell upon removing inducer. However, the enzymic activity in the cells located in G2 at the time of inducer removal, i.e., those cells four to five hours prior to metaphase, did not decline significantly.

The lower portion of Figure 2 clearly shows that the rates of degradation of TAT, measured radioimmunologically, in both G2 and random interphase cells were identical. Thus, the failure of TAT activity to decline during G2 must be due to its continued synthesis at the induced rate, even in the absence of the steroid. Therefore, TAT synthesis is constitutive in cells traversing G2 phase.
We have earlier shown that Colcemid does not affect the viability of HTC cells or the constitutive synthesis of TATs during mitosis and G1.

As seen previously,\(^6,\)\(^10\) the decline of TAT enzymic activity in the "interphase" populations in the present experiments directly paralleled the degradation of TAT measured radioimmunologically, and thus TAT synthesis in these populations was promptly repressed upon removing inducer.

To be sure that the constitutive period actually coincides with G2, we ascertained, for the cells being harvested at metaphase, their exact position in the cell cycle with respect to DNA synthesis when the steroid was removed. For this purpose, in the experiment shown in Figure 3, random populations of cells were induced on glass bottles and the inducer removed 20 hours later. As in the experiment depicted in Figure 2, Colcemid and induction medium were returned to the bottles at zero time, but in addition, \(^3\)H-thymidine was present for the first two hours. Mitotic and interphase cells were harvested separately two, four, and six hours after deinduction. The TAT specific enzymic activities and the incorporation of \(^3\)H into the DNA of the cell extracts were determined.

The data in Figure 3 demonstrate that those mitotic cells detached at two and four hours after removing inducer (deinduction) contained negligible \(^3\)H in their DNA and were thus in G2 at the time of deinduction. As expected, their TAT specific activities remained elevated (not shown). The cells harvested at metaphase six hours after deinduction contained large amounts of \(^3\)H in their DNA and thus were in the DNA synthetic (S) phase at the time of \(^3\)H-thymidine exposure (0 to 2 hr after deinduction). The TAT of the interphase cells from the same vessels showed the expected rapid fall of enzyme activity (as in Fig. 2) and, as is depicted in Figure 3, they incorporated high levels of \(^3\)H into their DNA.

These results permit us to conclude that cells in the G2 phase do not require steroid hormone to maintain a previously induced rate of TAT synthesis. Therefore, TAT synthesis during G2, as in early G1, is constitutive, consistent with the predicted absence of the post-transcriptional repressor. Furthermore, the apparent absence of repressor in G2, demonstrated here, coincides with the absence of inducibility during G2, shown earlier.\(^4\) This is consistent with the
suggestion that the post-transcriptional repressor (Fig. 1) is the (direct or indirecct) site of inducer action.

Abbreviations: HTC, rat hepatoma cells; TAT, tyrosine aminotransferase.

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