Modulation of Human Triosephosphate Isomerase Gene Transcription by Serum*

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We have monitored the level of mRNA encoding the glycolytic and gluconeogenic enzyme triosephosphate isomerase (TPI) during the growth arrest of cells by serum deprivation and the subsequent growth activation of cells by serum addition. This analysis has demonstrated that the steady state level of TPI mRNA changes 3-20-fold, depending upon the cell type, during the traversal of cells from a proliferative to a nonproliferative state and vice versa. These changes are largely attributable to changes in the rate of TPI gene transcription rather than to alterations in post-transcriptional processes as determined by nuclear run-on measurements. Following serum stimulation, the increase in TPI gene expression is maximal at or around the onset of DNA synthesis. We have also quantitated TPI mRNA throughout the cell cycle following cell synchronization with aphidicolin. Our results indicate that the steady state level of TPI mRNA is relatively constant throughout the division cycle of proliferating cells. Thus, while TPI gene expression is modulated during the traversal of cells to and from a nonproliferative state, it is not significantly modulated during the cycle of events that is characteristic of continuously proliferating cells.

The stimulation of cellular proliferation by serum has been widely used to identify and study genes that are growth regulated. Cells prepare for S phase during the G1 phase of the cell cycle (reviewed in Pardee (1989)). Since serum and rapid protein synthesis are not required during the final 2-h transit to S phase or during the rest of the cell cycle, those growth-regulated genes that are activated prior to these times during the serum-induced transition from a quiescent state to a proliferative state may function in transmitting the mitogenic stimulus to the DNA replication machinery (reviewed in Cross and Dexter (1991)). Examples of genes in this class include those for thymidine kinase (Pardee, 1989; Heikkila et al., 1987), thymidylate synthetase (Jehn et al., 1985), and hsp70 (Wu and Morimoto, 1985). Each of these genes is activated in response to serum stimulation at or around the start of S phase, and the first two encode a protein that functions to meet the demands for TTP in DNA replication. Each of these genes is also activated in continuously proliferating cells upon entry into the S phase of the cell cycle (Johnson, 1984; Kim et al., 1988; Knight et al., 1987; Milarski and Morimoto, 1986; Stewart et al., 1987). Other genes in this class are those that encode the cytoplasmic actins (Elder et al., 1984; Greenberg and Ziff, 1984), which are structural components of the cell. These genes are activated in response to serum stimulation well before the onset of S phase but after the activation of the c-fos and c-myc genes. They are not, however, regulated as a function of the division cycle in proliferating cells.

We have been studying the regulation of the gene encoding human triosephosphate isomerase (TPI), a glycolytic and gluconeogenic enzyme that catalyzes the interconversion of dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. Because glycolysis is required for cell growth and maintenance, this gene is functionally housekeeping. In the present report, we have undertaken an analysis of TPI gene expression in cultured cells that have been first rendered quiescent (in G0) by serum deprivation and subsequently stimulated to reenter the cell cycle (in G1) by the addition of serum. By RNA blotting, we have demonstrated that the level of TPI mRNA fluctuates 5-20-fold, depending upon the cell type, with the availability of serum. We have used nuclear run-on analysis to demonstrate that this fluctuation is attributed largely to changes in the rate of TPI gene transcription. Following serum stimulation, the increase in TPI gene expression is maximal at or around the onset of DNA synthesis. In an effort to distinguish regulatory events that operate during the stimulation of quiescent cells to reenter the cell cycle from processes of the cell cycle, we have monitored the steady state level of TPI mRNA after release from an aphidicolin-induced block at the G1/S boundary. These experiments have revealed that the level of TPI mRNA remains relatively constant throughout the cell cycle. Taken together, these results demonstrate that TPI gene expression is modulated by the regulatory events that govern the transition from G0 to G1.

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1 The abbreviations used are: TPI, triosephosphate isomerase; MEM-α, modified Eagle’s medium-α; FBS, fetal bovine serum; bp, base pair(s).
Cell Culture, Cell Synchronization, RNA and DNA Purification, and Measurements of DNA Synthesis—Synchronous cell populations were obtained by either of the two forms of growth blocks. Cell synchronization at the G1/S boundary was achieved by serum deprivation. Human 143 cells were seeded at approximately 1.5 \times 10^6 cells/150-mm plate in modified Eagle's medium-α (MEM-α, Gibco) plus 15% fetal bovine serum (FBS). After 24 h, cells were washed three times with serum-free MEM-α and then incubated for 48 h in MEM-α plus 0.5% FBS. Cells were released from this growth block by the addition of fresh medium and the 15% medium of MEM-α plus 0.5% FBS. At specified times throughout the serum deprivation (MEM-α plus 0.5% FBS) and serum stimulation (MEM-α plus 15% FBS) phases of the experiment, cells were harvested; for each point, three-quarters of the harvested cells were used in the preparation of nuclei for nuclear run-on analysis, and the remaining one-quarter of the harvested cells was used in the preparation of total cellular RNA for blot analysis. Briefly, cells were lysed in guanidinium isothiocyanate, and RNA was purified from DNA by centrifuging the lysate through cesium chloride (Chirgwin et al., 1979).

In experiments using human L153 cells to correlate DNA synthesis and the level of TPI mRNA with the addition of serum, [3H]thymidine (113 Ci/mmol) was added to 2 μCi/ml, and DNA and RNA were purified from the entire culture (Chirgwin et al., 1979). The DNA was dialyzed and quantitated spectrophotometrically at A260 nm, and isotopic incorporation was determined by liquid scintillation. The RNA was analyzed by blotting.

Cell synchronization at the G1/S boundary of the cell cycle was achieved with an aphidicolin block. HeLa cells were seeded at approximately 2.5 \times 10^5 cells/150-mm plate in MEM-α plus 15% FBS. After 24 h, the medium was removed and replaced with fresh medium containing aphidicolin (Natural Products Branch, National Cancer Institute) at a final concentration of 5 μg/ml. After a period of 20 h, the aphidicolin block was removed by three washes in aphidicolin-free medium and the subsequent addition of fresh medium (MEM-α plus 15% FBS). Cells were harvested at specified times prior to the aphidicolin block (exponentially growing cells), prior to release from the aphidicolin block (0 h), and at 2-h intervals subsequent to release from the aphidicolin block. One hour before harvesting, [3H]thymidine (115 Ci/mmol) was added to 2 μCi/ml. DNA and RNA were purified and analyzed as described above.

DNA Probes—TPI mRNA was detected using a 720-bp PstI-EcoRI fragment (codons 1–239) from the human TPI cDNA of pHTPI5A (Maquat et al., 1985). Histone H4 mRNA was detected using a 595-bp HindII-EcoRI fragment from the human histone H4 gene of pUC50, a pUC19 derivative that harbors a 1.74-kilobase pair HindII-EcoRI fragment including the histone H4 gene and flanking sequences (Sierra et al., 1983). For RNA blot analysis, each fragment was 32P-labeled by random priming (Feinberg and Vogelstein, 1983).

For nuclear run-on analysis, the TPI fragment was subcloned into the PstI and EcoRI sites of pUC13, and the histone fragment was subcloned into the HindII and EcoRI sites of pUC13. The resulting plasmids, pUC-TPI and pUC-H4, were first linearized and subsequently slot-blotted onto nitrocellulose for measurements of transcription in isolated nuclei.

RNA Blot Analysis—RNA (20 μg per time point) was denatured with glyoxal, electrophoresed through a 1.5% agarose gel (McMaster and Carmichael, 1977), and transferred to a nylon membrane (Zeta-bond). The transferred RNA was first visualized by short wave ultraviolet irradiation to assess the integrity of 28 S and 18 S rRNAs and subsequently hybridized (Church and Gilbert, 1984) simultaneously to both TPI and histone H4 DNA probes that had been 32P-labeled by random priming (Feinberg and Vogelstein, 1983). The membranes were exposed to x-ray film, and the resulting autoradiographs were quantitated by densitometry.

Nuclear Run-on Analysis—Nuclei were prepared from 143 cell monolayers (Almendral et al., 1988). The entire nuclear preparation was used for the run-on reaction according to the protocol of Lamers et al. (1981). Equivalent counts of 32P-labeled RNA (3 × 10^6 cpm) were hybridized in a total volume of 3 ml for 3 days to nitrocellulose strips on which were immobilized slot-blotted plasmid DNAs (2 μg of each) carrying TPI or histone H4 sequences. The strips were exposed to x-ray film, and the autoradiographs were quantitated by densitometry.

RESULTS

To monitor the accumulation of TPI mRNA during exit from and entry into the cell cycle, 143 cells that were growing exponentially in medium supplemented with 15% FBS were first shifted to medium supplemented with 0.5% FBS for a period of 48 h (serum-deprived) and subsequently shifted back to medium supplemented with 15% FBS (serum-stimulated). Cells were harvested at various times throughout the course of this experiment, and total cellular RNA was isolated for analysis by RNA blot hybridization to TPI cDNA. To assess the movement of cells to and from a nonproliferative state, RNA samples were simultaneously analyzed for the presence of histone H4 mRNA, the accumulation of which is coupled to DNA synthesis during the S phase of the cell cycle (Borum et al., 1975; Capasso et al., 1981; Dietze et al., 1979; Heintz et al., 1983; Lührer et al., 1985; Marzullo and Pandey, 1988; Sittmann et al., 1983).

As expected, the steady state level of histone H4 mRNA declined during the period of serum deprivation, consistent with the movement of cells from a proliferative to a nonproliferative state. After 48 h of deprivation, the level of histone H4 mRNA was reduced approximately 10-fold relative to the level observed in exponentially growing cells (Fig. 1A). Upon stimulation of these cells by the addition of serum, the level of histone H4 mRNA increased, reaching a steady state level that was similar to that observed in exponentially growing cells (Fig. 1A). This finding indicates that the accumulation of histone H4 mRNA is a reflection of the rate of DNA synthesis, which is coupled to the S phase of the cell cycle.

![Fig. 1. Kinetic analysis of the steady state TPI mRNA level (A) and the TPI gene transcription rate (B) during serum deprivation and subsequent serum stimulation of human 143 cells. A, RNA blot. Human 143 cells, exponentially growing in culture medium supplemented with 15% FBS, were shifted to culture medium supplemented with 0.5% FBS for a period of 48 h and then shifted back to culture medium supplemented with 15% FBS. Total cellular RNA (20 μg), extracted at the indicated times, was denatured, electrophoresed, transferred to a nylon membrane, and hybridized to a 720-bp PstI-EcoRI fragment (codons 1–239) from the human TPI cDNA of pHTPI5A (Maquat et al., 1985) and a 595-bp HindII-EcoRI fragment from the human histone H4 gene of pUC50 (Sierra et al., 1983). B, nuclear run-on analysis. For each time point in the experiment represented in panel A, three-quarters of the cells were used for the isolation of nuclei. RNA was extracted following transcription in vivo in isolated nuclei (Lamers et al., 1982), and equivalent counts of 32P-labeled RNA (3 × 10^6 cpm) were hybridized to a nitrocellulose strip on which were immobilized slot-blotted plasmid DNAs carrying TPI or histone H4 sequences. EXP, cells exponentially growing.

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**MATERIALS AND METHODS**

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of histone H4 mRNA increased, reaching a maximum at 12 h that represented an 8-fold increase over the level of histone H4 mRNA observed at 48 h of serum deprivation. Between 12 and 48 h after serum addition, the steady state level of histone H4 mRNA exhibited a cyclical fluctuation that weakened in intensity with time, consistent with the cells traversing from one S phase to the next while approaching confluency and, therefore, growth arrest (data not shown). The relatively long time lapse (12 h) between the addition of serum and an increase in histone H4 mRNA is characteristic of cells that have been arrested in a quiescent G0 state during serum deprivation.

Similarly, the steady state level of TPI mRNA declined by 48 h of serum deprivation to approximately 10-fold the level in exponentially growing cells (Fig. 1A). Following the stimulation of cells by serum addition, the level of TPI mRNA rose, reaching a maximum at 12 h that represented a 5-fold increase over the level observed at 48 h of serum deprivation (Fig. 1A and data not shown). Between 12 and 48 h after serum addition, the steady state level of TPI mRNA gradually declined as the cells approached confluency. Notably, unlike the decline for histone H4 mRNA, this decline was not characterized by a cyclical fluctuation, suggesting that TPI mRNA is not regulated by the phase of the cell cycle in proliferating cells (see below). These results demonstrate that the steady state level of TPI mRNA in 143 cells fluctuates in response to the availability of serum. This fluctuation was also observed in L153 cells, a nontransformed human diploid fibroblast cell line (Fig. 2). However, while the kinetics of TPI mRNA accumulation in response to serum availability was similar in both cell lines, the magnitude of this response was 5-fold larger in L153 cells; the serum-induced peak in the steady state level of L153 TPI mRNA occurred 12 h following serum stimulation of quiescent cells and represented a 20-fold increase over the steady state level in cells that had been deprived of serum for 48 h (Fig. 2). An analysis of the incorporation of [3H]thymidine into DNA during the course of this experiment revealed that this peak coincided with or slightly preceded the onset of DNA synthesis (Fig. 2) and, therefore, was not a consequence of DNA synthesis. The difference between the magnitude of the serum-induced peak of the two cell lines could derive from the fact that 143 cells, because of their transformed phenotype, continue to grow for a longer period under serum-deprived conditions than L153 cells. Consistent with this possibility, the persistence of a significant level of histone H4 mRNA in 143 cells after 48 h of serum deprivation (Fig. 1A) suggested that a significant fraction of these serum-deprived cells was, in fact, still cycling. Therefore, the accumulation of TPI mRNA following serum stimulation of 143 cells may be partially masked by incomplete withdrawal from the cell cycle following serum deprivation. Nevertheless, these experiments demonstrate that the steady state level of TPI mRNA fluctuates approximately 5–20-fold with the availability of serum and, presumably, as a function of the proliferative state of the cell.

To determine the relative contribution of transcriptional versus post-transcriptional processes to this fluctuation, the rate of TPI gene transcription was measured by run-on analysis in nuclei isolated from an aliquot of the same 143 cells that had been harvested for RNA blot analysis as described above. For comparative purposes, the rate of histone H4 gene transcription in these nuclei was determined similarly by run-on analysis. Measurements indicated that the rate of histone H4 gene transcription in exponentially growing cells decreased by no more than 2-fold after 48 h of serum deprivation and increased by 2.5-fold 12 h following the stimulation of these cells by serum addition (Fig. 1B). The observation that changes in the rate of histone H4 gene transcription are insufficient to account for the changes in the steady state level of histone H4 mRNA in response to serum availability is consistent with the contribution of post-transcriptional processes in determining the abundance of this mRNA as a function of serum concentration (Heintz et al., 1983).

By comparison with histone H4 gene transcription, the rate of TPI gene transcription was subject to greater fluctuation as a function of serum. By 48 h of serum deprivation, the rate of TPI gene transcription decreased approximately 4-fold relative to the rate observed in exponentially growing cells. Upon stimulation of cells by serum addition, the rate of TPI gene transcription subsequently increased; by 12 h of serum stimulation, the rate of transcription rose to approximately 4-fold above that at 48 h of serum deprivation (Fig. 1B). These results demonstrate that the observed fluctuation in the steady state level of TPI mRNA in response to the availability of serum can be attributed almost entirely to changes in the rate of TPI gene transcription. Additionally, the half-life of TPI mRNA can be estimated to be 20 h.

In an effort to distinguish processes that affect the abundance of TPI mRNA during cell traversal to and from the cell cycle from processes within the cell cycle, we monitored the steady state level of TPI mRNA as a function of the cycle. For this purpose, cells were synchronized by treatment with aphidicolin, a heterocyclic diterpenoid that permits cells to traverse the G2, M, and G1 phases of the cycle but prevents replicative DNA synthesis through the selective inhibition of DNA polymerase α (Huberman, 1981). The use of this agent permits the isolation of a cell population that is reversibly...
arrested at the G1/S border (Huberman, 1981).

In this experiment, RNA blot hybridization was performed using total cellular RNA prepared from HeLa cells prior to aphidicolin treatment, prior to release from a 20-h aphidicolin block, and at 2-h intervals throughout a 24-h period post-release. The phase of the cell cycle throughout the course of this experiment was assessed by following the incorporation of \([^{3}H]tHymidine\) into newly synthesized DNA and by analyzing the steady state level of TPI mRNA. After release from the aphidicolin block, the cells rapidly underwent two successive rounds of replication as indicated by increases in the incorporation of DNA precursors (Fig. 3). The first round was initiated immediately after release from the aphidicolin block and continued for 6–8 h post-release, while the second round was initiated at approximately 18 h post-release and continued until 22–24 h post-release. Consistent with the pattern of \([^{3}H]tHymidine\) incorporation, S phase-regulated histone H4 mRNA exhibited two periods of transient increase, one 2–6 h and the other 18–22 h post-aphidicolin release. Taken together, the kinetics of \([^{3}H]tHymidine\) incorporation and histone H4 mRNA accumulation suggest that, upon release from the aphidicolin block, the cells progressed largely in unison through one complete cell cycle.

An analysis of TPI mRNA throughout the course of this experiment revealed no more than a 2-fold fluctuation (Fig. 3). Thus, while the abundance of TPI mRNA is modulated during the traversal of a cell to and from a nonproliferative state, it is not significantly modulated during the cycle of events that is characteristic of continuously proliferating cells.

**DISCUSSION**

In this report, we have demonstrated that the abundance of TPI mRNA is modulated in response to the availability of serum. We have observed that following the serum stimulation of quiescent L153 cells, the steady state level of TPI mRNA is maximal at or around the onset of DNA synthesis. Since the cellular events that commit and prepare a cell for S phase take place in G1, and since serum and rapid protein synthesis are found to be required only prior to the final 2-h transit to S phase (reviewed in Pardee (1989)), the increase in the level of TPI mRNA at or around the time of cell doubling probably reflects the cellular housekeeping role that TPI occupies as an enzyme in the glycolytic pathway and, thus, probably also reflects a cellular adjustment from a quiescent to a growing state. While post-translational processes may also contribute an increase in TPI activity, the increase in TPI mRNA abundance is certainly an important growth response. Consistent with these ideas is the demonstration that the stimulation of rat fibroblasts by serum and, in particular, by epidermal growth factor increases the abundance of the mRNAs for four glycolytic enzymes, including TPI (Matrisian et al., 1985). It has been proposed that epidermal growth factor participates in the second step of the two-step process whereby quiescent cells are advanced to proliferate in response to serum (Stiles et al., 1979). The first step occurs in response to "competence" or "early response" factors such as platelet-derived growth factor, and the second step commits cells to DNA synthesis by the separate action of "progression" factors such as epidermal growth factor (reviewed in Cross and Dexter (1991)).

Like TPI and the cytoplasmic actins, most molecules in a proliferating cell are made continuously to fulfill housekeeping or structural functions (reviewed in Cross et al. (1989) and Pardee (1989)). All molecules in proliferating cells must double with each cell cycle in order to produce new cells. Our data suggest that the relatively large (5–20-fold) increase in the level of TPI mRNA is evident after cells are started from Go, because TPI mRNA is lower in abundance in quiescent cells than in cycling cells, possibly due to its degradation in Go.

An observation to emerge from this study is that the modulation of TPI mRNA abundance in response to serum is controlled largely at the level of transcription. Our finding that the rate of TPI gene transcription declines during the period of serum deprivation suggests that the factor(s) that mediates the increase in TPI gene transcription in response to serum is a factor that mediates constitutive transcription of the TPI gene in proliferating cells. Consistent with this idea, there is no apparent similarity to a known serum response element in the 595 bp that reside immediately upstream of the TPI gene transcription initiation site (designated +1) (GenBank and EMBL data bases; Benton (1990)).

We have previously demonstrated that accurate and efficient constitutive TPI gene transcription derives from the combinatorial association of multiple positive and negative regulatory elements that reside exclusively upstream of the +1 site (Boyer et al., 1989). In human cell lines, maximal TPI promoter activity is associated with sequences spanning positions −920 and −6. An inhibitory element(s) directly upstream of position −920 reduces by approximately 7-fold the level of transcription achieved with the maximally active promoter. More recently, we have described the sequence and factor requirements for minimal constitutive TPI gene transcription (Boyer and Maquat, 1990). The minimal transcription initiation unit spans promoter positions −65 and −6 and encompasses a single GC box (positions −53 to −48) that binds Sp1, a transcription factor IID-responsive TATATAA box (positions −27 to −21), and a novel cap proximal element (positions −18 to −6) that binds a 110-kDa nuclear factor that is present in HeLa cells.

Given the heterogeneous composition of factor binding sites throughout the TPI promoter, either of two alternative hypotheses may be proposed to account for the transcriptional
response to serum. First, such a response could be mediated through one (or more) of the factors that confer minimal constitutive TPI gene transcription. Modulation by serum of the level or activity of Spl, transcription factor IID, the cap proximal element-binding protein, or RNA polymerase II could alter the rate of TPI gene transcription, provided that the factor is functionally limiting for TPI gene transcription. Alternatively, the regulation of TPI gene transcription in response to serum could be achieved through modulation of the level or activity of a factor(s) that associates with the upstream of the minimal promoter. Mutational analysis of the TPI promoter should delineate the sequences and, by implication, the cellular activity that mediate the response of TPI gene transcription to serum. The identification of this activity should provide a significant contribution toward a more complete description of the molecular events that are a part of the process of cellular growth.

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REFERENCES