Regulation of Tyrosine Hydroxylase mRNA Stability by Protein-binding, Pyrimidine-rich Sequence in the 3′-Untranslated Region*

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The stability of mRNA for tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine synthesis, is regulated by oxygen tension in the pheochromocytoma-derived PC12 cell line. We previously identified a pyrimidine-rich 27-base-long protein-binding sequence in the 3′-untranslated region of TH mRNA that is associated with hypoxia-inducible formation of a ribonucleoprotein complex (hypoxia-inducible protein-binding site [HIPBS]). In this study, we show that HIPBS is an mRNA stabilizing element necessary for both constitutive and hypoxia-regulated stability of TH mRNA. The mutations within this sequence that abolish protein binding markedly decrease constitutive TH mRNA stability and ablate its hypoxic regulation. A short fragment of TH mRNA that contains the wild-type HIPBS confers the increased mRNA stability to the reporter chloramphenicol acetyltransferase mRNA. However, it is not sufficient to confer hypoxic response. The HIPBS element binds two isoforms of a 40-kDa poly(C)-binding protein (PCBP). Hypoxia induces expression of the isoform 1, PCBP1, but not the isoform 2, PCBP2, in PC12 cells.

Tyrosine hydroxylase (TH),¹ the rate-limiting enzyme in the biosynthesis of catecholamines, is expressed in specific populations of neurons in the central and peripheral nervous systems, in the neuroendocrine cells of the adrenal medulla and carotid body, and in cultured cell lines such as the pheochromocytoma-derived PC12 cell line. Regulation of TH gene expression at the level of gene transcription is well documented. Recently, there has been growing evidence that TH mRNA is also regulated at the level of mRNA turnover. We demonstrate that expression of the PCBP1 isoform is induced by hypoxia in PC12 cells.

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§ The abbreviations used are: TH, tyrosine hydroxylase; UTR, untranslated region; HIPBS, hypoxia-inducible protein-binding site; PCBP, poly(C)-binding protein; RPA, RNase protection assay; CAT, chloramphenicol acetyltransferase; PIPES, 1,4-piperazinediethanesulfonic acid; t½, half-life; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride; PAGE, polyacrylamide gel electrophoresis.

PC12 cells (1–3). It is enhanced during differentiation of neuroblastoma cells (1) and during stimulation of the protein kinase C pathway in PC12 cells (2). In contrast, the stability of TH mRNA does not change in PC12 cells during stimulation of TH mRNA expression by dexamethasone or forskolin (3). A recent study demonstrated substantial differences in basal TH mRNA turnover rates between different neuronal populations from as short a time as 6–7 h, in the dopaminergic neurons of the arcuate nucleus, to as long as 11–23 h, in the dopaminergic midhypothalamic neurons (4). In addition, TH mRNA is destabilized in the dopaminergic cells of the arcuate nucleus in a manner that corresponds to the rhythmic output displayed by these neurons (4).

Our laboratory demonstrated that hypoxia augments the stability of TH mRNA in PC12 cells (5). We identified a 27-base-long pyrimidine-rich sequence within the TH mRNA 3′-untranslated region (UTR) (1552–1578 bases of TH mRNA) that binds protein factors in a hypoxia-inducible manner (hypoxia-inducible protein-binding sequence [HIPBS]) in PC12 cells (6, 7), catecholaminergic cells of the superior cervical ganglia, and the dopaminergic cells of the carotid body (8). Mutational analysis revealed that the optimal protein-binding site is represented by the motif (UC/C/U/C/C) within the pyrimidine-rich sequence, where the underlined cytidines are the core binding site (7). This motif is conserved in TH mRNAs from different species. These results imply that formation of the ribonucleoprotein complex associated with HIPBS may be involved in physiological regulation of TH mRNA stability in catecholaminergic cells. Thus, identification of the role of this element in the regulation of TH mRNA stability is critically important to further investigations in this area.

In the present study, we investigated the role of HIPBS in regulating the stability of TH mRNA during normoxia and hypoxia. We show that HIPBS is a stabilizing element necessary for maintaining constitutive and hypoxia-regulated TH mRNA half-life. We also show that the HIPBS-binding protein is represented by two isoforms of a 40-kDa poly(C)-binding protein (PCBP, also known as αCP or hnRNPE (9–12)), and we demonstrate that expression of the PCBP1 isoform is induced by hypoxia in PC12 cells.

EXPERIMENTAL PROCEDURES

Materials—All chemicals were purchased from either Fisher or Sigma and enzymes from Promega or Life Technologies, Inc.

Cell Culture—PC12 cells were grown in Dulbecco’s modified Eagle’s medium/F-12 supplemented with 15 mM HEPES buffer, 10% fetal bovine serum, 100 units/ml penicillin, and 100 mg/ml streptomycin, as described previously (5, 6).

Plasmid Constructs—The 5′-marked TH cDNA constructs were obtained by inserting full-length TH cDNA (see Fig. 1A), which had either a wild-type or mutated protein-binding site, into the BamHI site of pC3-M1 expression vector (Invitrogen). Expression of TH mRNA from these vectors results in exogenous TH transcripts containing 25 bases of
the TH 5′-UTR region and an additional 26-base-long sequence (BamHI-HindIII) upstream from the plasmid DNA (see Fig. 1A).

The chimeric CAT-TH constructs were generated by inserting the KpnI-ApaI fragment of TH 3′-UTR that contains either a wild-type or mutated protein-binding site into the respective restriction sites behind the chloramphenical acetyltransferase (CAT) gene within the pcDNA3-CAT vector (see Fig. 3A). The derived chimeric mRNAs are referred to, respectively, as CAT-wTH (wild-type) or CAT-mTH (mutant).

Identification of TH mRNA Stability Element

The TH mRNA expression measured in the same experiment. Expression of the endogenous TH and CAT mRNAs was directly normalized to the radioactivity measured in the 18 S ribosomal band. The measurements of mRNA half-life (t1/2) were performed using actinomycin D (5 μg/ml) as described previously (5).

RNA-Protein-binding Reactions (6, 7)—Forty micrograms of the S-100 fraction of cytosolic proteins and the [γ-32P]ATP-labeled RNA transcripts (50,000 cpm) or [γ-32P]ATP-labeled HIPBS oligoribonucleotide (20,000 cpm) were incubated on ice for 20 min in 10 mM HEPES, pH 7.9, 3 mM MgCl2, 50 mM KCl, 200 mM/μl Echerichia coli TRNA, 10% glycerol, 1 mM DTT, 0.5 mM PMSF and then treated with RNase T1 and heparin as described (6–8). For the competition experiments, the competitor RNA was added at the same time. RNA-protein complexes were resolved on 7% native acrylamide gel. UV light cross-linking was performed by irradiating the binding reactions with 1 × 10^6 J/cm2 UV light (Fisher). Samples were boiled in SDS-sample buffer with 100 mM DTT and electrophoresed on 8% SDS-polyacrylamide gels as described previously (6).

Poly(C)-Agarose Affinity Purification—This procedure was performed essentially as described previously (9). The S-100 protein fraction (15–20 mg of protein extract) from PC12 cells was treated with micrococcal nuclease (500 units; Worthington) in the presence of 1 mM CaCl2 for 1 h at room temperature. The reaction was stopped by adding 5 mM EGTA, and extracts were incubated for 1 h at 4 °C with E. coli tRNA (40 μg/ml), heparin (5 mg/ml), DTT (100 mM), and poly(U) RNA (5 μg/ml) with constant rocking. Extracts were centrifuged at 10,000 × g for 30 min. The extracts were treated with RNasin (40 units; Promega) and incubated four times for 1 h each with 100 μl of poly(C)-agarose (Sigma). The poly(C)-agarose with the bound proteins was washed five times with a 10× volume of wash buffer (100 mM KCl, 20 mM HEPES pH 7.9, 1 mM DTT, 0.5 mM PMSF, 1 mM heparin) and eluted with salt gradient (0.5, 1, 1.5, and 2 mM KCl, 20 mM HEPES, pH 7.9, 1 mM DTT, and 0.5 mM PMSF) for 15 min each at room temperature. The eluted fractions were concentrated, washed twice with the wash buffer (Microcon-10 microconcentrators; Amicon), and resuspended in the storage buffer (50 mM KCl, 20 mM HEPES, pH 7.9, 10% glycerol, 1 mM DTT, 0.5 mM PMSF).

Electroelution of Proteins from Ribonucleoprotein Complexes—The binding reactions were performed using 15 μg of unlabeled HIPBS oligoribonucleotide and 1.5–2 mg of protein extract from PC12 cells in a 300-μl reaction volume. The control reactions were identical except for the RNA. Each 300-μl reaction was loaded into a 35-mm-wide well of a preparative nondenaturing gel. One well contained the binding reaction with the labeled RNA for further identification of the RNA-protein complex. The gel was exposed to x-ray film, and the fragments of gels containing unlabeled complexes co-migrating at the same level as the labeled complexes were excised, macerated, and loaded into the elution

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Fig. 1. Steady-state levels of wild-

type (wt) or mutated (mut) exogenous (ex) TH mRNAs in PC12 cells. A, re-

stricted map of rat TH cDNA (open bar) in the pcDNA3 expression vector. Lo-

cations of the HIPBS element (hatched region) with wt and mut sequences and of the marker sequence used to generate the riboprobe (bracket) are shown. CMV, cytomegalovirus promoter; BGH, bovine growth-hormone polyadenylation signal. B, RPA analysis of expression of wt (lanes 4 and 5) or mut (lanes 6 and 7) exogenous TH mRNAs (exTH mRNAs) as compared with the endogenous TH mRNA (endTH mRNA). M (lane 1), RNA markers; FP (lane 2), free probes for TH and 18 S (ar-

rows); V (lane 3), only endogenous TH mRNA is detected in PC12 cells transfection

with an empty pcDNA3 vector. The bottom panel showing lighter, exposed 18 S bands indicates equal loading of total mRNA. C, quantification (mean ± S.E.) of the constitutive expression of wt (black) or mut (gray) exogenous TH mRNA analyzed by RPA represented as percent expression of the endogenous TH mRNA.
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**RESULTS**

Stability of TH mRNA Is Decreased by Mutation within the Pyrimidine-rich Site—To determine the role of HIPBS in the regulation of TH mRNA stability, we stably expressed, in PC12 cells, the full-length exogenous TH mRNA with either wild-type or mutated HIPBS. The exogenous mRNAs were marked by a short sequence located in the 5′-end of the message (HindIII-BamHI, Fig. 1A) to differentiate exogenous from endogenous TH mRNAs and to measure both in the same sample using RPA (Fig. 1B).

First we analyzed the constitutive expression of exogenous...

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**Fig. 1.**...
as compared with endogenous TH mRNA. The steady-state level of exogenous wild-type TH mRNA was at 64.8 ± 6.9% (n = 5) of the endogenous TH mRNA (Fig. 1, B, lanes 4 and 5, and C). In contrast, the steady-state level of the mutated TH mRNA was significantly decreased and was only 37.3 ± 1% (n = 4, p < 0.01) of the endogenous TH mRNA (Fig. 1, B, lanes 6 and 7, and C).

Next we analyzed degradation rates of the wild-type and mutated exogenous TH mRNAs after inhibition of transcription with actinomycin D (Fig. 2). During normoxia the \( t_{1/2} \) of the wild-type TH mRNA was 9.2 ± 0.4 h (n = 3; Fig. 2A, solid squares), and it increased almost 2-fold to 17.2 ± 0.5 h (n = 2, p < 0.01; Fig. 2A, open squares) during hypoxia. This is similar to the half-life measured for endogenous TH mRNA (7.5 ± 0.4 h (n = 5) in normoxia and 15 ± 0.7 h (n = 5) in hypoxia; p < 0.01). The half-life of the mutated TH mRNA was significantly lower at 4 ± 0.8 h (n = 3, p < 0.01; Fig. 2B, solid squares), and it not only failed to increase during hypoxia but showed a small decrease to 2.9 ± 0.3 h (n = 5, p = 0.035, Fig. 2B). These data indicate that HIPBS is an mRNA stabilizing element.

**HIPBS Is a mRNA Stabilizing Element in the Context of a Heterologous mRNA**—To further characterize the role of HIPBS in regulation of mRNA stability, we stably expressed in PC12 cells chimeric mRNA that have fragment of TH 3′-UTR cloned downstream from the CAT reporter gene (Fig. 3A). The chimeric mRNA had either a wild-type (CAT-wtTH mRNA) or mutated, inactive (CAT-mutTH mRNA) HIPBS site.

Steady-state levels of the CAT-wtTH mRNA were substantially increased compared with CAT mRNA lacking the TH insert (Fig. 3B, lanes 4–6 compared with lane 3). In contrast, steady-state levels of the CAT-mutTH mRNA (lane 7) were significantly lower than either CAT-wtTH mRNA (lanes 4–6) or CAT mRNA alone (lane 3). Importantly, the change in the constitutive levels of chimeric mRNAs was also reflected in the comparable differences in the levels of the derived protein measured using CAT assays (not shown).

Measurements of the degradation rates of CAT, CAT-wtTH, and CAT-mutTH mRNAs after transcriptional inhibition with actinomycin D (Fig. 4) show that insertion of the \( \omega \)TH sequence into the 3′-UTR of the CAT gene increased the half-life of the chimeric mRNA from 5 ± 0.2 (n = 3) to 11.6 ± 1 h (n = 5, p < 0.01). On the other hand, insertion of the same sequence with a mutation that abolished protein binding (7) resulted in a substantial destabilization of the chimeric mRNA and a decrease in the mRNA \( t_{1/2} \) to 2.3 ± 0.05 h (n = 2). This is significantly lower (p < 0.01) than the half-life of the CAT-wtTH mRNA.

To determine whether expression of chimeric CAT-wtTH mRNA is regulated during hypoxia, the mRNA levels were measured during normoxia and hypoxia in the absence or presence of actinomycin D. Unexpectedly, hypoxia failed to regulate expression of the CAT-wtTH mRNA in PC12 cells (data not shown). Further, even insertion of the full-length 3′- UTR and 5′-UTR of TH mRNA downstream and upstream, respectively, from the CAT gene did not confer hypoxic regulation to this chimeric mRNA (data not shown). Thus, the HIPBS is an mRNA stabilizing element that is necessary but not sufficient for the O2-dependent regulation of TH mRNA turnover. Moreover, additional regulatory elements involved in the hypoxic regulation are most likely located within the coding region of TH mRNA.

**Characterization of the RNA-Protein Complex**—The TH mRNA-protein complexes were analyzed by UV light cross-linking of TH mRNA-protein-binding reactions and separation by SDS-PAGE under reducing conditions (100 mM DTT). Two
large complexes were identified at approximately 80 and 50 kDa (Fig. 5A, lane 1). Addition of poly(U) competitor blocked, to a large extent, the 80-kDa complex, revealing a major complex at 50–55 kDa (Fig. 5A, lane 2), but it did not affect formation of the complex identified in the RNA gel shift assays as shown previously (6). Formation of the 50–55-kDa complex in the presence of poly(U) was abolished by addition of the unlabeled 162-base-long TH transcript (TH tr, lane 3), HIPBS oligoribonucleotide (lane 4), or poly(C) RNA (lane 5). Only the 50–55-kDa complex was formed when HIPBS oligoribonucleotide was used as a probe (lane 6). The 50–55-kDa complex (arrow) is formed by wild-type (WT) TH transcript (lane 1) but not by TH mRNA mutated in the protein-binding region (MUT, lane 2). C, the formation of the 50–55-kDa complex is induced in cells exposed to 5% O₂ (hypoxia, lane 2) as compared with cells exposed to 21% O₂ (normoxia, lane 1).

Identification of the Protein Factor in the Complex with the Pyrimidine-rich Sequence in TH mRNA—Because poly(C) RNA is such an effective competitor of the complex formation (6, 7), poly(C)-aggressive affinity was used to purify the binding factor from the S-100 protein extracts from PC12 cells (Fig. 6A). Proteins bound to poly(C)-agarose were fractionated by elution with increasing concentrations of KCl (0.5–2 M). The presence of the TH mRNA-binding activity was determined by Northern and Western analysis of protein extracts before incubation with poly(C)-agarose (lane 3, poly(C)-depleted protein extracts after incubation with poly(C)-agarose; lane 4, poly(C) eluate-pooled protein fractions eluted from the poly(C)-agarose. Arrow indicates the 50–55-kDa complex. Bracket indicates mobility of the free probe.

Protein fractions eluted with the salt gradient were pooled and analyzed for their ability to form complexes with TH mRNA. The combined eluted fractions formed a 50–55-kDa complex with TH transcript that migrated with the same mobility (Fig. 6B, lane 4) as the complex formed by the total S-100 fraction (Fig. 6B, lane 2). The 30-kDa HIPBS-binding protein was identified in the eluates (Fig. 6A, lanes 2–5). This protein was further identified as the 40-kDa PCBP (also known as αCP or hnrNPE (10–12)) with a specific polyclonal antibody (Fig. 6A, lanes 7–10).

To confirm that the purified poly(C)-binding protein is present in the HIPBS-associated protein complex, proteins were electroeluted from the unlabeled complex identified by the gel shift assay, separated on SDS-PAGE, and screened by Western analysis with a specific antibody against the two isoforms of PCBP (Fig. 7A). As controls, the proteins were eluted from corresponding gel slices in which the binding reaction did not include RNA. HIPBS bound to a protein factor electroeluted from the complexes formed in the presence of RNA (Fig. 7A, B).
Identification of TH mRNA Stability Element

The major finding from this study is that HIPBS is an mRNA stabilizing element required for constitutive and hypoxia-regulated control of TH mRNA. A four-point mutation that abolished the protein-binding site within the full-length TH mRNA resulted in a 2-fold destabilization of the mutated mRNA and a corresponding 2-fold decrease in mRNA steady-state levels. In addition, mutation of HIPBS abolished the O$_2$-dependent regulation of TH mRNA stability. We further demonstrated that a short fragment of the TH 3'-UTR containing the HIPBS was sufficient to confer augmented mRNA stability on the heterologous mRNA, which in turn resulted in augmented steady-state levels of the chimeric mRNA and derived protein. Importantly, the mutated HIPBS conferred destabilization to chimeric CAT-TH mRNA. The decrease in the half-life because of the mutation in the protein-binding site was very similar for both the chimeric CAT-TH mRNA or full-length exogenous TH mRNA.

Interestingly, whereas HIPBS is a necessary element for the O$_2$-dependent stabilization of TH mRNA in the context of the full-length TH mRNA and whereas it binds a protein factor in a hypoxia-inducible manner, HIPBS alone is not sufficient to confer hypoxic regulation to the CAT mRNA. Neither are the full-length 3'- and 5'-UTRs of TH mRNA. This result is similar to previously published data regarding hypoxic regulation of VEGF mRNA stability (13). In that study, the full-length 3'-UTR of VEGF mRNA did not confer hypoxic regulation on the heterologous mRNA (13). Thus, it is possible that additional necessary regulatory elements are located within the coding region of hypoxia-regulated mRNAs. Work is in progress to identify these additional elements in TH mRNA.

Sequences similar to that in TH mRNA were reported to regulate the stability of other mRNAs. First, the stability of the erythropoietin-specific $\alpha$2-globin mRNA is regulated by three short cytidine-rich repeats similar to the TH protein-binding motif (14). Second, a pyrimidine/cytidine-rich sequence within the chromosome 9-UTR containing the HIPBS was involved in the regulation of the stability of this mRNA and forms a similar ribonucleoprotein complex with protein factors (15). Mutations in these elements destabilize globin and collagen $\alpha1(1)$ mRNAs. In our study, however, we show not only that the mutations destabilize TH mRNA but also that the short fragment of mRNA containing the protein-binding site is capable of conferring increased mRNA stability to a heterologous mRNA.

The increased expression of chimeric CAT-TH mRNA was accompanied by a corresponding increase in expression of functional protein. An increase in expression of functional protein (data not shown), an indication that translation of the chimeric mRNA was mainly not affected. This observation is important because pyrimidine-rich sequences binding similar protein factors were reported to regulate translation (12, 16, 17).

Analysis of the RNA-protein complexes after UV light cross-linking and SDS-PAGE analysis revealed the presence of one major broad 50–55-kDa complex. This finding extends and complements the results of our previous study (6), in which we detected two TH mRNA-protein complexes: the major 74- and a minor 53-kDa complex. We currently view the 50–55-kDa complex as the major one. The importance of this complex became clear only after competition with poly(U), which blocked protein binding at 80 kDa, and after analysis of complexes formed specifically with HIPBS oligoribonucleotide in comparison with the complexes formed with the longer TH transcript. Thus, the

![Fig. 7. PCBP1 is induced by hypoxia. A, analysis of the protein factors electroeluted from an RNA-protein complex (lanes 2, 4, and 6) or a control gel slice that contains only proteins but no RNA (lanes 1, 3, and 5) by Northwestern (NW) assay using labeled HIPBS RNA (lanes 1 and 2) and by Western (W) using specific anti-PCBP1 (lanes 3 and 4) or anti-PCBP2 (lanes 5 and 6) antibodies, respectively. B, Western blot analysis of two independent sets of normoxic and hypoxic cytoplasmic extracts demonstrating the strongest (3-fold, lanes 1 and 2) and the weakest (1.8-fold, lanes 3 and 4) induction of the PCBP1 by hypoxia. Lane 5, assessment of the antibody specificity using antigenic peptide in the Western reaction. C, Western blot analysis of protein factors electroeluted from the HIPBS-associated ribonucleoprotein complex formed with proteins from hypoxic (5% O$_2$, lane 2) or hypoxic (21% O$_2$, lane 1) PC12 cells.

lane 2) but not to the proteins electroeluted from the gel slices in the absence of RNA (Fig. 7A, lane 1). Subsequent reprobing of the same blot with an antibody specific for the isoforms PCBP1 and PCBP2 revealed that both isoforms are actually present in the complex (Fig. 7A, lanes 4 and 6, respectively). PCBP2 migrates slightly higher than PCBP1 detected by Western blot, which may represent some post-translational modifications and a somewhat higher molecular weight of PCBP2 (a nine-amino acid difference).

To determine whether hypoxia induces expression of the PCBP isoforms in PC12 cells, cytoplasmic protein extracts were analyzed by Western blot using antibodies specific for each isoform. A 2.4 ± 0.3-fold induction in the expression of PCBP1 was measured in four independent sets of PC12 protein extracts (Fig. 7B). In contrast, no induction (or inhibition) of PCBP2 was measured (data not shown). To confirm the increased amount of PCBP1 protein in the complex, proteins were electroeluted from the HIPBS-associated complexes formed by normoxic or hypoxic extracts (Fig. 7C). Indeed, proteins elec-
80-kDa complex most likely represents nonspecific binding of protein factors to sequences other than HIPBS fragments within the 162-base-long TH transcript. The 50–55-kDa complex should correspond, after subtracting the molecular mass corresponding to the 28 bases of RNA (8 kDa), to the protein factor with an approximate molecular mass of 42–47 kDa.

In view of the strong affinity of the binding proteins for poly(C) RNA, poly(C) RNA-agarose was used to purify the protein. A similar procedure was used previously to purify the protein factor binding to the cytidine-rich elements in α2-globin mRNA (9, 10). As expected, we purified the 40-kDa poly(C)-binding protein that corresponds to PCBP (12), also known as αCP (9, 10) or hnRNPE (17, 18), as identified by the specific antibodies. The eluate from the poly(C)-agarose restored fully formation of the complex with HIPBS RNA or the TH transcript in the binding reactions. This finding differs from previously reported data showing that the poly(C)-eluted proteins did not restore formation of the complex associated with α2-globin stability element and was thus considered necessary but not sufficient for the complex formation (10). Further, formation of the globin-associated complex seems to require additional protein factors such as AUF1 (hnRNPD) protein (19). So far we did not find any evidence for the presence of AUF protein in the HIPBS-associated complex. The protein-binding motifs within the TH and α2-globin mRNAs show some potentially important differences. Whereas the essential PCBP-binding motifs are very similar in the two mRNAs (11), in the case of important differences. Whereas the essential PCBP-binding motifs are very similar in the two mRNAs (11), in the case of motifs are very similar in the two mRNAs (11), in the case of important differences. Whereas the essential PCBP-binding...2

The molecular mechanism by which binding of PCBP to the HIPBS element regulates mRNA stability, especially in the context of increased TH mRNA stability during hypoxia, is presently unknown. A possible mechanism would be protection of a nuclease cleavage site within the region associated with HIPBS in the TH mRNA. Protein binding to the wild-type sequence may protect this site from nuclease activity. When protein binding is prevented by mutation, the site becomes accessible to cleavage, resulting in a rapid degradation of mRNA. The fact that a fragment of TH mRNA containing mutated HIPBS confers decreased mRNA stability to the chimeric mRNA may favor such a possibility. On the other hand, formation of the complex associated with the globin mRNA stability element indicates its potential role in stabilization via the poly(A) tails (20, 21). To evaluate this possibility, a determination of the length of poly(A) tails of TH mRNA during normoxia and hypoxia is under way.

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