Thrombopoietin in Thrombocytopenic Mice: Evidence Against Regulation at the mRNA Level and for a Direct Regulatory Role of Platelets

By Ruedi Stoffel, Adrian Wiestner, and Radek C. Skoda

Thrombopoietin (TPO), originally described as an activity in the serum of thrombocytopenic animals that leads to increased production of platelets, has recently been isolated and cloned. Its closest relative in the cytokine superfamily, erythropoietin (EPO), is transcriptionally regulated during anemia, and it was expected that TPO would similarly be regulated during thrombocytopenia. We induced thrombocytopenia in mice and confirmed that TPO activity was upregulated, as determined by a bioassay. Liver and kidney were found to be the major sources of TPO mRNA. Surprisingly, TPO mRNA in these tissues was not upregulated in thrombocytopenic mice. Using a sensitive RNAse protection assay that can distinguish between TPO isoforms, we found no change in the profile of mRNA for these isoforms. A semi-quantitative reverse transcription-polymerase chain reaction assay also did not demonstrate upregulation of TPO mRNA in the spleen. Thus, the increase of TPO activity during thrombocytopenia is not caused by regulation at the level of TPO mRNA. Furthermore, isolated mouse platelets absorbed high amounts of bioactive TPO out of TPO-conditioned medium in a dose-dependent fashion. Our results are consistent with TPO protein being regulated at a posttranscriptional level and/or directly through absorption and metabolism by platelets.

© 1996 by The American Society of Hematology.

EARLY EXPERIMENTS have determined that the physiologic changes occurring in response to acute thrombocytopenia are mediated by a humoral factor called thrombopoietin (TPO). Such changes include increases in megakaryocyte number, size, and ploidy and will result in increased production of platelets. These experiments have determined that TPO activity is inversely related to platelet mass and have suggested that a feedback mechanism exists that can sense a decrease in platelet mass and cause a reciprocal increase in circulating TPO activity. Recently, the orphan cytokine receptor c-mpl has been used as a reagent to isolate and clone a ligand that had biologic activities resembling TPO. Several lines of evidence indicate that this ligand is, in fact, TPO: the purified mpl-ligand is a potent stimulator of thrombopoiesis in vivo, and soluble c-mpl receptor can abrogate TPO activity. Using bioassays for TPO, two other groups independently purified and partially sequenced the TPO protein and found it to be identical to the sequence of the mpl-ligand. TPO activity produced by human embryonic kidney (HEK) cells is also identical to mpl-ligand.

In analogy to the transcriptional activation of erythropoietin (EPO) mRNA in response to anemia, the increased levels of circulating TPO protein during thrombocytopenia may be due to upregulation of TPO mRNA. Alternatively, a model has been proposed in which TPO production is constant, and TPO activity is regulated by the binding and metabolism of TPO by platelets, which express c-mpl, the TPO receptor. Here we describe experiments that support the latter model.

MATERIALS AND METHODS

Induction of thrombocytopenia in mice. C57BL/6J mice were purchased from BRL, Fullinsdorf, Switzerland. Twelve mice were injected intraperitoneal with 0.1 mL of antiplatelet serum generated in rabbits (gift from Dr Jack Levin, University of California, San Francisco, CA). Groups of three mice were killed after 6 days and 9 days. The experiments have been approved by the local animal welfare committee.

Blood and tissue analysis. Blood was obtained by cardiac puncture without anticoagulants. Approximately 300 μL of blood was immediately mixed with EDTA, and blood counts were performed with an automated blood counter (model Tecnicon H-3; Miles Inc., Territown, NY). The remaining blood was allowed to coagulate, and serum was collected for the TPO proliferation assay. Bone marrow cells were prepared by flushing two femurs from each mouse with phosphate-buffered saline (PBS). Viable, trypan blue-excluding marrow cells were counted. For morphologic examination, bone marrow cells were concentrated on microscopic slides using a Shandon Cytopsin 3 centrifuge (Life Science International, Ostrnmore, UK) and stained with Wright stain. For histology, freshly dissected tissues were fixed in Optimal Fix (American Histology Reagent Co, Stockton, CA). Fixed specimens were embedded in paraffin, sectioned, and stained by the Transgenic Pathology Laboratory at the University of California at Davis, CA.

Construction of plasmid vectors. TPO cDNA was generated by reverse transcription-polymerase chain reaction (RT-PCR) using first-strand cDNA synthesized from mouse liver RNA using the sense primer 5'-TCAAGAGTGGCCGAGATGGAGCTGACTG-3' and the antisense primer 5'-ATAAAGATCTGCTATGTTTC-3' and the antisense primer 5'-ATAAAGATCTGCTATGTTTC-3'. The fragments were subcloned into pBluescript KS (Stratagene, La Jolla, CA) and completely sequenced. For expression in COS cells, the full-length TPO cDNA was cut with HindIII and Bgl II and subcloned into the pcDNA1 vector (In-vitrogen, San Diego, CA). For stable transfections, mouse TPO and c-mpl cDNAs were subcloned into the pGEM expression vector as an XhoI/NotI or BclI fragment, respectively.

RNA isolation, ribonuclease protection assay, and RT-PCR analysis. Tissues were homogenized in 4 mol/L guanidium isothiocyanate with a Polytron homogenizer, and RNA samples were prepared by the acid phenol method. For ribonuclease (RNAse) protection assays, we used a 24-hr labeling of full-length TPO cDNA with [32P]CTP (ICN, Costa Mesa, CA). The sense primer 5'-ATAAAGATCTGCTATGTTTC-3' and the antisense primer 5'-ATAAAGATCTGCTATGTTTC-3' were used for an XhoI/NotI or BclI fragment, respectively. For ribonuclease (RNAse) protection assays, we used a 24-hr labeling of full-length TPO cDNA with [32P]CTP (ICN, Costa Mesa, CA). The sense primer 5'-ATAAAGATCTGCTATGTTTC-3' and the antisense primer 5'-ATAAAGATCTGCTATGTTTC-3' were used for an XhoI/NotI or BclI fragment, respectively.

RNA isolation, ribonuclease protection assay, and RT-PCR analysis. Tissues were homogenized in 4 mol/L guanidium isothiocyanate with a Polytron homogenizer, and RNA samples were prepared by the acid phenol method. For ribonuclease (RNAse) protection assays, we used a 24-hr labeling of full-length TPO cDNA with [32P]CTP (ICN, Costa Mesa, CA). The sense primer 5'-ATAAAGATCTGCTATGTTTC-3' and the antisense primer 5'-ATAAAGATCTGCTATGTTTC-3' were used for an XhoI/NotI or BclI fragment, respectively.

From the Department of Pharmacology, Biozentrum of the University of Basel, Basel; and the Division of Hematology, Department of Research, University Hospital, Basel, Switzerland.

Submitted April 26, 1995; accepted August 30, 1995.

Supported by Grants No. 31-37760.93 and 32-35503.92 (to R.C.S.) and 3135-040025.94 (to A.W.) from the Swiss National Science Foundation.

Address reprint requests to Radek C. Skoda, MD, Department of Pharmacology, Biozentrum, University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1996 by The American Society of Hematology.

0006-4971/96/8702-0$3.00/0
analysis,\textsuperscript{22} we constructed a riboprobe for the detection of all of the known TPO mRNA isoforms by subcloning a 398-bp Sal I-Sca I fragment of mouse TPO cDNA into pBluescript. The resulting vector was digested with Xhol, transfected with T7 RNA polymerase, and hybridized to 30 pg of total RNA at 50°C as described.\textsuperscript{23} This riboprobe protects a 398-nucleotide (nt) fragment for TPO-1 mRNA. In addition, a 310-nt fragment can be detected for TPO-3, a 240-nt fragment for both TPO-2 and TPO-4 isoforms, and a 147-nt fragment for TPO-2. If genomic DNA was present in the RNAs, fragments of 168, 160, and 72 nt would be generated, because two introns are present in the genomic region spanned by this riboprobe. RNA loading was normalized with a riboprobe for mouse hypoxanthine-guanine phosphoribosyl transferase (HPRT), a housekeeping gene. A Sca I-HindIII fragment representing nucleotides 679 to 840 of mouse HPRT cDNA\textsuperscript{24} was subcloned into pBluescript. This riboprobe protects a 161-nt fragment. HPRT riboprobe was mixed with the TPO probe and added as an internal standard to each sample. Protected fragments were separated on 6% polyacrylamide/8 M urea sequencing gels. Dried gels were exposed on film or on phosphorimager screens, and quantitations of radioactive bands were performed on a PhosphorImager 425 using the ImageQuant software (Molecular Dynamics Inc, Sunnyvale, CA).

For RT-PCR analysis, oligo(dT)-primed first strand cDNA was synthesized from 2 ng total RNA using RNaseH- MuLV reverse transcriptase (Stratagene), in a reaction volume of 20 pl under conditions recommended by the manufacturer. The reaction mixture was diluted to 50 pl with H2O, heated to 95°C for 5 minutes to inactivate reverse transcriptase, and then quickly chilled on ice for 10 minutes. PCR was performed with 2 pl of the first strand cDNA as a template in a final reaction volume of 20 pl. The reaction was performed in 50-mmol/L KCl, 1.5 mmol/L MgCl2, 20 mmol/L Tris pH 8.3, 0.25 mmol/L deoxynucleotide triphosphates (dNTPs), 0.75 \mu mol/L of each primer, and 0.05 UKpl Taq polymerase (Life Technologies, Gaithersburg, MD). The primer pair for mouse TPO was 5'-GCTGGTGAAAAGGACCTCT-3' (forward) and 5'-CAACAATCCAGAAGTCCT-3' (reverse), amplifying a 608-bp product, and for HPRT, 5'-GCCTGTGAAAGGACACTCT-3' (forward) and 5'-CACAGGACTAGAACACCTGC-3' (reverse), amplifying a 259-bp product.\textsuperscript{27} We performed 30 cycles for TPO and 28 cycles for HPRT, each consisting of 60 seconds at 94°C, 60 seconds at 55°C, and 60 seconds at 72°C using a DNA thermal cycler (Perkin Elmer, Norwalk, CT). The PCR products were electrophoresed on 1.5% agarose gels and then transferred to Hybond N+ membrane (Amer sham, Buckinghamshire, UK). Blots were probed with internal 32P-labeled oligonucleotides for TPO 5'-AGGACCTCTGGAAGAAGG-3' or HPRT 5'-GATATGCCCTCTGACTATAA-3'. The hybridizations were performed overnight at 55°C in 7% dodecyl sulphate (SDS), 1 mmol/L EDTA, 0.5 mol/L sodium phosphate buffer pH 7.2, and 1% bovine serum albumin (BSA), and the blots were washed three times for 15 minutes with 6X saline sodium citrate (SSC), 0.1% SDS at 55°C.

Cell transfections and proliferation assay. Transient transfections of COS cells with the pcDNA1-TPO expression vector were performed by the diethyl aminoethyl (DEAE)-Dextran method.\textsuperscript{28} To generate a cell line responsive to TPO, BaF3 cells were electroporated with 40 \mu g of pGD-mlp DNA at 250 V/960 \mu F in PBS and plated in serial dilutions. Clones were selected in 0.6 mg/mL G418 beginning at 24 hours, and G418-resistant clones were assayed for expression of mpl protein by Western blot using polyclonal rabbit anti-mlp antibodies.\textsuperscript{29} BaF3/mlp clone TM17 expressed the highest level of mlp protein and grew well in conditioned medium from transiently transfected COS cells secreting mouse TPO. This clone was chosen for the TPO proliferation assays. To assess the levels of TPO in serum from thrombocytopenic mice, TM17 cells were washed out of interleukin (IL)-3-containing medium, incubated for 16 hours in medium without IL-3, and plated in 96-well plates at 10^4 cells per well in 100 \mu L of medium containing dilutions of mouse serum. After 22 hours, 1 \mu Ci of 3H-thymidine was added to each well, and incorporation of 3H-thymidine was measured after 6 hours in a \beta-counter. Alternatively, XTT, a colorimetric tetrazolium dye,\textsuperscript{29} was used to determine TPO activity in conditioned media. Cells (5 \times 10^4 per well) were seeded, and after 3 days of stimulation, 50 \mu L of a 1 mg/mL stock solution of XTT with 5 mmol/L phenazine methosulfate (PMS), an electron coupling agent, was added to each well. The product of XTT reduction by viable cells, reflecting the number of cells per well, was measured at 4 hours at 450 nm. A stable cell line producing mouse TPO was generated by electroporating NIH/3T3 cells with a pGD-TPO expression construct followed by selection in 0.5 mg/mL G418 as above. TPO-secreting clones were identified using the TM17 proliferation assay. Adsorption of TPO by platelets. Mouse platelets were isolated from five mice as described.\textsuperscript{30} The platelets were washed with PBS and counted in a Neubauer chamber. The automated platelet count of the purified platelet preparation was 166 \times 10^9/\mu L, with no detectable white blood cells (WBCs) and no red blood cells (RBCs). In the stained cytopsin of this purified platelet preparation with approximately 500,000 platelets, we counted a total of 108 RBCs and no WBCs. Specified numbers of platelets were then pelleted for 5 minutes at 1,300g and incubated while rotating for 1 hour at 37°C with 50 \mu L of conditioned medium containing TPO or IL-3. Platelets were removed by 5 minutes of centrifugation at 1,300g, and TPO activity of the supernatants was assessed by the BaF3/mlp cell proliferation assay.

RESULTS

To examine the effects of thrombocytopenia on the levels of TPO mRNA, we injected mice intraperitoneally with 0.1 mL of rabbit anti-mouse platelet serum (RAMPS). Groups of three mice were killed at various times and analyzed (Fig 1A). After 4 hours, the platelet counts decreased more than 40-fold to a mean of 22 \times 10^5/\mu L and remained low at 24 and 48 hours. Two of the three mice killed after 24 hours had normal platelet numbers (not shown). These two nonresponders were not further examined. To assure that the animals were killed at the relevant time, we also determined TPO activity in serum. We used a proliferation assay with BaF3/mlp cells that express the mouse c-mpl and proliferate in response to TPO. Only the transfected BaF3/mlp cell line responded to the sera from thrombocytopenic mice, but not the parental untransfected BaF3 cells. Proliferation of BaF3/mlp cells was measured by incorporation of 3H-thymidine (Fig 1A). The values for the untreated controls were indistinguishable from background incorporation into BaF3/mlp cells in TPO-free medium. Thus, serum TPO concentrations in normal mice are below the limit of detection of this assay. TPO increased to measurable levels after 4 hours and was elevated at 24 and 48 hours. RAMPS does not lead to destruction of megakaryocytes.\textsuperscript{31} This was confirmed by cyto- spin analysis of bone marrow cells and histopathology of the spleens from these mice (not shown). The average numbers of megakaryocytes per spleen section increased from 30 in the controls, and 33 at 4 hours, to 52 at 24 hours, and to 109 at 48 hours (not shown).

To examine if megakaryocyte mass is important for the regulation of TPO mRNA, we also analyzed mice pancytopenic after TBI with 8 Gy. Platelet levels in mice treated with
TBI decreased slowly and reached a mean of $43 \times 10^3/\mu L$ at day 9 (Fig 1B). Conversely, TPO levels were measurable at day 6 and increased further at day 9. No megakaryocytes were found on histopathologic examination of spleens at days 6 and 9 (not shown).

We devised a sensitive RNase protection assay that can distinguish between four TPO isoforms (Fig 2). The TPO riboprobe spans the region between nucleotides 330 and 728 of the mouse TPO cDNA sequence.\(^1\) RT-PCR from mouse liver RNA yielded the full-length TPO cDNA (TPO-1) and two shorter isoforms, TPO-2 and TPO-3, that have been described previously.\(^2,3\) In addition, we found a fourth isoform, TPO-4, which has a deletion of 197 bp from position 569 to 766 of the mouse TPO cDNA.\(^4\) We analyzed expression of the TPO isoforms and found the highest levels in liver and kidney, which express the highest levels of TPO (Fig 2), and spleen, small amounts of TPO produced in these organs may regulate megakaryopoiesis in a paracrine fashion. By RNase protection assay, TPO was not detectable in 30 $\mu g$ total RNA from spleen or bone marrow (Fig 2A) or in 8 $\mu g$ of polyA+ RNA from spleen (Fig 2B). Therefore, we analyzed expression of TPO by RT-PCR. TPO mRNA was detectable in bone marrow. However, the transcript seems to be present at very low abundance, because amplification was not reliable despite consistent amplification of the HPRT transcript in the same samples (not shown). In contrast, TPO was amplified consistently from spleens from thrombocytopenic mice (Fig 4). We used 30 cycles of PCR with the TPO primers and normalized the results with the PCR products obtained with HPRT primers after 28 cycles. We could not detect any significant increase in TPO mRNA in spleens during thrombocytopenia induced with either RAMPs or TBI.

We examined if platelets can remove TPO activity when preincubated with TPO using the BaF3/mpl cell line (Fig 5). To control for any inhibitory activity released during the incubation with isolated platelets, we used IL-3-containing WEHI-3 conditioned media that were treated the same way. We observed a dose-dependent decrease in TPO activity in supernatants incubated with mouse platelets. IL-3 activity was unaffected under the same conditions, and no inhibition was observed. Untransfected parental BaF3 cells did not respond to the TPO-conditioned media (not shown). To exclude the possibility that platelets incubated in medium containing calcium and other platelet-activating agents may release proteases to which TPO might be more sensitive than to IL-3, we performed the following control experiment. Purified platelets were incubated with medium for 1 hour. This medium was then separated from platelets by centrifugation, mixed 1:1 with TPO or IL-3-containing conditioned media to give a final concentration of 2.5%, and assayed for activity. No decrease in activity of either TPO or IL-3 was observed, indicating that the decrease in TPO activity after incubation with platelets is not due to release of proteases or other agents that interfere with the TPO assay (not shown). Therefore, platelets bind TPO, presumably through mpl, and may be involved in regulating the free circulating TPO concentration.

**DISCUSSION**

We have analyzed TPO mRNA levels during thrombocytopenia in mice and have found no upregulation despite a measurable increase of TPO activity in serum (Fig 1). EPO, the closest homologue of TPO, can be upregulated more than 100-fold at the mRNA level,\(^18,20\) and it was suspected that a similar mechanism would exist for TPO. Liver and kidney, which express the highest levels of TPO (Fig 2), were examined by an RNase protection assay (Fig 3). With the same RNase protection assay, we tested the possibility that TPO isoforms, which are believed to be generated by alternative or aberrant splicing, might play a role in the...
regulation of TPO activity (Fig 3A and B). Two TPO isoforms have been described. TPO-2 has a deletion of the four amino acids LPPQ at position 112 to 115, and TPO-3 is produced by an internal splice in the last exon. The proteins for TPO-2 and TPO-3 were expressed but not secreted by transfected cells lines, suggesting that these proteins are retained in the secretory pathway. As these isoforms are conserved between humans, pig, and mouse, it was suspected that they might play a regulatory role. Interestingly, no splice variants have been described for EPO, which shares a highly homologous gene structure with TPO. However, we found no changes in mRNA levels for TPO-2 or TPO-3 during thrombocytopenia (Fig 3A and B). Therefore, regulation of TPO does not occur at the mRNA level in liver and kidney and does not involve changes in the ratios of TPO isoform mRNAs.

Because we found no expression of TPO in spleen and bone marrow by RNase protection (Fig 2) and these organs are the sites of megakaryopoiesis in mice, upregulation of TPO produced in situ in a paracrine fashion could have a major effect on megakaryopoiesis. By RT-PCR, we were able to detect TPO mRNA in spleen (Fig 4) and, less reliably, also in bone marrow (not shown). However, there was no significant increase of TPO mRNA detectable by RT-PCR (Fig 4). Although this assay is not as quantitative as RNase protection, we should have been able to detect a 5- to 10-fold mRNA increase. Plasma from thrombocytopenic animals was sufficient to induce accelerated platelet production, and purified thrombopoietin caused an up to fivefold increase in platelet count when injected into normal recipients. This demonstrates that TPO is a potent humoral stimulator. As the levels of expression were very low and we could not detect upregulation, the physiologic importance of locally produced TPO in the spleen remains unclear.

It has been proposed that platelets might be directly involved in the regulation of circulating TPO activity. Platelets express mpl protein. Purified fractions of megapoietin, which was shown to be identical with TPO, lost activity when first incubated with sheep platelets and tested after removal of platelets by centrifugation. Megapoietin activity was assessed measuring increase in megakaryocyte ploidy of isolated rat megakaryocytes. We used the proliferation assay with BaF3/mpl cells to more directly measure TPO exposed to various concentrations of isolated mouse platelets (Fig 5). When TPO-conditioned media were exposed to 10^6 platelets per microliter, the optical density 450-nm reading, reflecting the number of proliferating BaF3/mpl cells, decreased by 40% (Fig 5). As in this range the dose response curve of the assay is linear with the logarithm of the TPO.

**Table 1. Relative Amounts of TPO Isoforms in Mouse Tissues**

<table>
<thead>
<tr>
<th>TPO Isoform</th>
<th>Liver</th>
<th>Kidney</th>
<th>Testis</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPO-1</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>TPO-2</td>
<td>44</td>
<td>52</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>TPO-3</td>
<td>7</td>
<td>3</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>TPO-4</td>
<td>9</td>
<td>7</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

Radioactive bands from Fig 2A and B were quantified with a phosphorimager. The relative abundance of TPO isoforms is expressed as percent of TPO-1 for each organ. The values were normalized with the number of uridines in the protected fragments. Values for TPO-4 were calculated by subtracting TPO-2 from TPO-2 + 4.
concentration, this represents removal of more than 40% of TPO activity. Using the more sensitive ³H-thymidine assay, we measured an incorporation in the range of 6,000 cpm with thrombocytopenic sera (Fig 1) and 60,000 cpm with saturating concentrations of 5% TPO-conditioned medium (not shown). Because the values measured with our thrombocytopenic sera are in the region where the curve becomes nonlinear, we cannot accurately assess the TPO concentration. However, we can make an approximate estimate and find that our TPO-conditioned medium at 2.5% contained at least a 100-fold excess of TPO activity compared with thrombocytopenic sera. These results demonstrate the ability of platelets to downregulate the free serum TPO concentration. The capacity of mouse platelets to adsorb TPO in our assay appears to be higher than the calculated binding capacity of sheep platelets. However, these results are not directly comparable, because the assays used to measure TPO were not the same. Isolated platelets may be activated and display a higher binding than platelets under physiologic conditions.

We show that the increase in TPO activity during thrombocytopenia is not mediated by changes in TPO mRNA abundance. Our data is consistent with TPO regulation at a translational or posttranslational level and/or with regulation

| FIG 3. Determination of TPO mRNA levels in liver and kidney from thrombocytopenic mice. TPO isoforms were detected by RNase protection analysis of total RNA. The values for TPO-1 in the controls were set to 100 for each organ. The mean values from three mice ± SEM are given except for RAMPS at 24 hours postinjection. Co, untreated controls. (A) Numbers above the lanes indicate time in hours after injection of RAMPS. (B) Mice treated with 8 Gy TBI: d6 and d9, days 6 and 9 after irradiation, respectively. |

| FIG 4. Determination of TPO mRNA levels in spleens from thrombocytopenic mice. TPO mRNA was detected by RT-PCR and compared with HPRT mRNA. Autoradiograms of Southern blots hybridized with ²⁵P-labeled TPO, and HPRT-specific internal oligonucleotides are shown. Co, untreated controls; numbers above the lanes indicate time in hours after injection of RAMPS; d6 and d9, days 6 and 9 after 8 Gy irradiation; —, no template DNA. The bands were quantified on a phosphorimager and are expressed in arbitrary units. The values for TPO-1 in the controls were set to 100. The mean values from three mice ± SEM are given, except for RAMPS at 24 hours postinjection. |
directly through metabolism by platelets. A number of other steps of TPO biosynthesis and metabolism may be important as well, as will be determined through detailed studies of the TPO protein in the future.

ACKNOWLEDGMENT

We thank Michael V. Wiles for help with the TBI and many helpful discussions, Robert D. Cardiff for reviewing the histopathology, Jack Levin for the antiplatelet antisemur, André Tichelli for the automated blood counts, and David C. Seldin for helpful comments on the manuscript.

REFERENCES


20. Goldberg MA, Gauth CC, Bunn HF: Erythropoietin mRNA levels are governed by both the rate of gene transcription and post-transcriptional events. Blood 77:271, 1991


tion by acid guanidium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156, 1987
Thrombopoietin in thrombocytopenic mice: evidence against regulation at the mRNA level and for a direct regulatory role of platelets

R Stoffel, A Wiestner and RC Skoda