Collagen-prolyl 4-hydroxylase (C-P4H) α-subunit is of regulatory importance in the assembling of C-P4H tetramers, which are necessary for the hydroxylation of procollagen chains. Change in collagen expression by hypoxia or iron diminishment is a significant issue in extracellular matrix remodeling. It was proposed that C-P4H-α (I) is regulated at the posttranscriptional level under these conditions. Here we report that the induction of C-P4H-α (I) in human fibrosarcoma cells HT1080 by the iron chelator 2,2-dipyridyl is predominantly caused by an enhancement of mRNA stability. This effect is mediated by an increased synthesis and binding of heterogeneous nuclear ribonucleoprotein (hnRNP)-A2/B1, which interacts with a (U)_n element located in the 3′-untranslated region of C-P4H-α (I) mRNA. Luciferase reporter gene assays demonstrating that C-P4H-α (I) 3′-untranslated region and co-transfection with hnRNP-A2/B1 provide evidence that the (U)_n element is necessary and sufficient for posttranscriptional control of C-P4H-α (I) synthesis under the analyzed conditions. Further indication for the significance of hnRNP-A2/B1 in C-P4H-α (I) induction was obtained by microarray experiments. In a data set representing 686 independent physiological conditions, we found a significant positive correlation between hnRNP-A2/B1 and C-P4H-α (I) mRNAs.

EXPERIMENTAL PROCEDURES

Cell Culture and RNA/Protein Isolation—Human fibrosarcoma line HT1080 (ATCC, passages 16–21) cells were maintained in Dulbecco’s modified Eagle’s medium (high glucose; PAA Laboratories GmbH) supplemented with 10% heat-inactivated fetal calf serum, 50 units/ml penicillin, 50 μg/ml streptomycin, 15 mM Hepes, and 2 mM/liter glutamine at 37 °C, 5% CO₂. Before use in experiments, cells were maintained in a medium containing 0.4% fetal calf serum for at least 24 h. Measurements started with the application of fresh medium containing 0.4% fetal calf serum. For 2,2-dipyridyl (2,2-DP, Sigma) treatment, the iron chelator was dissolved in ethanol and supplemented to a final concentration of 100 μM 2,2-DP and 0.1% ethanol. Control cells were also supplemented with ethanol (0.1% final concentration). For RNA and protein isolation, cells were washed with ice-cold phosphate-buffered saline. RNA was prepared using RNA-Beer (Biozol Diagnostica Vertrieb GmbH) according to the manufacturer’s protocol. Protein extracts (10,000 × g supernatants, S10) were isolated using lysis buffer (10 mM Tris, pH 7.5, 140 mM NaCl, 1 mM EDTA, 25% glycerol, 0.1% SDS, 0.5% proteinase K).
Nonidet P40, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1× Complete protease inhibitor mix (Roche Diagnostics).

RT-PCR—Primers were designed to bridge at least one intron. PCR conditions were used as follows: 3 min 95 °C, cycles: 30 s 95 °C, 30 s annealing, 30 s 72 °C, final elongation for 2 min at 72 °C; 2.5 mM MgCl₂. The primers were as follows: C-P4H-α (I), forward, 5′-CCACAGCAGAGGAATTTACAG, reverse, 5′-ACACTAGTCCAACTTTCAGG; actin, forward, 5′-TGAAGTGTAGTCTGACATC, reverse, 5′-GTGATGCTGCTGAGAG; glyceraldehyde-3-phosphate dehydrogenase, forward, 5′-CACCATCTTCCAGGACCG; reverse, 5′-GCCAGGCATTGCTGTG; hnRNP-A2/B1: Acris Antibodies GmbH, AF0210; anti-C-P4H-α: Acris Antibodies GmbH, BM4520; anti-β-actin: Acris Antibodies GmbH, BM4520) for 1.5 h. The membranes were washed three times with Tris-buffered saline with Tween and incubated with an anti-mouse secondary-antibody (Promega) for 1 h. Bands were detected using the ChemiGlow™ West detection kit (Alpha Innotech Corp.). Membranes were stripped for 5 min with distilled water, 5–15 min with 0.2M NaOH, and 5 min with distilled water and reprobed with anti-β-actin antibody (Chemicon) to detect relative β-actin levels as loading control.

mRNA Stability—To test mRNA stability, cells were incubated for 18 h with or without the iron chelator 2,2-dipyridyl (100 μM). Native cytosolic extracts were isolated using a lysis buffer (20 mM Tris, pH 7.4, 150 mM KCl, 30 mM MgCl₂, 0.25% Nonidet P40, 1 mM dithiothreitol, 1× Complete protease inhibitor mix) at 0–4 °C. Following the isolation step, the native extracts were incubated at room temperature up to 4 h. RNA was prepared using RNA-Bee (Biozol Diagnostica Vertrieb GmbH) according to the manufacturer’s protocol and reverse-transcribed using random hexamer primers. The estimation of mRNA concentration was performed by RT-PCR. To compare the RNA decay under control conditions and in the lack of iron ions, mRNA levels were adjusted to equal amounts at the starting point (0 h).

C-P4H-α (I) UTR-dependent Reporter Gene Constructs—For reporter gene assays, the luciferase vector pGL3-promoter (Promega, constitutive SV40 promoter) was used. The vector-specific 5′- and 3′-UTRs of luciferase mRNA were replaced by the human C-P4H-α (I) UTRs (GenBankTM gi:63252885). The 5′-UTR of C-P4H-α (I) mRNA (133 nt) was cloned using the vector-specific HindIII (5′-end) and NcoI (3′-end) restriction sites and the 3′-UTR (999 nt, including the poly-A signal) using the vector-specific XbaI (5′-end) and BamHI (3′-end) restriction sites. These restriction sites were added to the UTR sequences by primer extension. 3′-UTR variants correspond to: 3′-A, first 500 nt of 3′-UTR; 3′-B, terminal 522 nt of 3′-UTR. Deletion of the U-rich element was performed by PCR technique. Modified vectors were confirmed by sequencing. The resulting vector constructs represent constitutively transcribed luciferase transcripts with or without the specific C-P4H-α (I) UTRs/UTR variants.

C-P4H-α (I) UTR-dependent Reporter Gene Assays—HT1080 cells were cultured in 96-well plates (μClear Platte 96k, Greiner Bio-One GmbH) and were co-transfected with the firefly luciferase pGL3-promoter vector (Promega), as well as its transformed variants, and the Renilla-luciferase pRL-TK vector using the FuGENE 6 Transfection Reagent (Roche Diagnostics) according to the manufacturer’s protocol. After 6 h, the transfection medium was removed, and measurements started after the addition of fresh medium. Co-transfection with expression vectors encoding RNA-binding proteins (hnRNP-A2/B1, pReceiver-M02 vector, GeneCopeia™, Inc., Ex F0171-M02; hnRNP-E1, pG55 vector, gift from A. Ostareck-Lederer, Martin Luther University, Department of Biochemistry and Biotechnology, Halle, Germany)
was used in a ratio of 1:3 (firefly luciferase vector:hnRNP expression vector). Luciferase activity was detected using the Dual-Glo™ luciferase assay system (Promega) and a luminometer (Labsystems Luminoscan RS) programmed with individual software (Luminoscan RII, Ralf Mrowka). The co-transfection with the Renilla-luciferase expression vector served as a control.

RNA-Protein Interaction Studies—In vitro transcripts representing the 5′- or 3′-UTR of C-P4H-α (I) mRNA were radioactively labeled using [α-32P]uridine-5′-triphosphate and [α-32P]adenosine-5′-triphosphate (800 Ci/mmol, MP Biomedicals Germany GmbH).

UV Cross-linking Experiments—1–2 ng of [α-32P]U/C/A- or [α-32P]cTP-labeled in vitro transcripts representing 100,000 cpm were incubated with 35 μg of cytosolic protein extract for 30 min at room temperature in 10 mM Hepes pH 7.2, 3 mM MgCl2, 5% glycerol, 1 mM dithiothreitol, 150 mM KCl, and 2 units/μl RNaseOUT (Invitrogen) in the presence of rabbit rRNA (0.5 μg/μl). For competition assays, a 50-fold excess of unlabeled in vitro transcripts was added. Then the samples were exposed to UV light (255 nm, 1.6 J, UV-Stratalinker) on ice, treated with RNase A (30 μg/ml final concentration) and RNase T1 (750 units/ml final concentration) for 15 min at 37 °C, and subjected to 12% SDS-PAGE and autoradiography.

mRNA Level Correlation Study—Normalized human mRNA expression levels for hnRNP-A2/B1 mRNA/C-P4H-α (I) mRNA were obtained from the Stanford Microarray data base as used in Stuart et al. (17), which contains a collection of different independent investigations. The difference of the sum of all microarrays in the data base and the number of data points used for the correlation analysis in this study results from the fact that some entries for hnRNP-A2/B1 and C-P4H-α (I) were missing in the microarray data sets. Regression and correlation analysis was performed using the math module of the open source/GPL program xmgrace, and the null hypothesis was rejected at the 0.05 level.

Statistical Analysis—Autoradiographic signals were scanned and quantified using the Scion Image software (Scion Corp.). Results appear as means, and in Figs. 1–3, 6, and 7, error bars represent the standard deviation (S.D.). Data were analyzed using the Student’s t test, and the null hypothesis was rejected at the 0.05 level.

RESULTS

Human HT1080 fibroblasts were treated with or without 100 μM 2,2-DP, and synthesis of C-P4H-α (I) was analyzed at the mRNA and protein level. As demonstrated in Fig. 1, the lack of Fe2+ ions resulted in an ~4-fold increase at the mRNA level and an ~6.5-fold increase at the protein level after 18 h. The analysis of time-dependent C-P4H-α (I) mRNA decay confirmed that the elevated mRNA concentration was not only attributable to an increase at the transcriptional level but also to an enhanced mRNA stability (Fig. 2). The mRNA half-life time under control conditions is 1.9 h (±0.3 h, S.D.) but was elevated after 18 h of Fe2+ diminishment to 4.2 h (±0.8 h, S.D.). The data indicated that in response to the lack of Fe2+ ions, the C-P4H-α (I) mRNA stability increased about 2-fold. Interestingly, we did not observe an alteration in C-P4H-α (I) mRNA half-life time after 7 h of 2,2-DP treatment, indicating the requirements of trans-acting factors that have to be induced. However, the mRNA turnover of glyceraldehyde-3-phosphate dehydrogenase (Fig. 2B), C-P4H-β, or C-P4H-α (II) (data not shown) was not altered under the same conditions. To confirm the posttranscriptional regulation of C-P4H-α (I) expression, we performed UTR-dependent reporter gene assays (pGL3-promoter vector) in which the 5′- and/or 3′-UTR of luciferase mRNA were replaced by specific 5′- and 3′-UTRs of C-P4H-α (I) mRNA or by artificial 3′-UTR variants (for a schematic illustration, see Fig. 3A). The transcription rate is controlled by the constitutive SV40 promoter. Therefore, differences in luciferase activity depend only on posttranscriptional control, mediated by the UTRs, and involve mainly mRNA stability and translational efficiency.

2,2-DP treatment neither changed luciferase activity resulting from the original pGL3-promoter vector (control) nor changed luciferase activity in the case when luciferase mRNA contains the C-P4H-α (I) 5′-UTR. By contrast, the C-P4H-α (I) 3′-UTR significantly enhanced luciferase activity (Fig. 3B), indicating a 3′-UTR-mediated posttranscriptional control. Interestingly, the combination of 5′- and 3′-UTRs showed a significantly stronger increase in luciferase activity. This observation correlated with a higher increase at C-P4H-α (I) protein level when compared with the mRNA level after 18 h of 2,2-DP treat-

![Figure 2. Influence of 2,2-dipyridyl on C-P4H-α (I) mRNA stability.](image-url)
hnRNP-A2/B1 Modulate C-P4H-α (I) mRNA Stability

FIGURE 3. Influence of C-P4H-α (I) mRNA UTRs on luciferase (Luc) expression. HT1080 cells were transfected using the pGL3-promoter vector (SV40 promoter) and transgenic variants in which vector-specific 5′- and 3′-UTRs of luciferase mRNA were replaced by C-P4H-α (I) UTRs. The influences of 2,2-DP (100 μM, 18 h) on luciferase activity of original vector (pGL3p) and vector constructs with C-P4H-α (I) 5′- and/or 3′-UTR sequences are shown as black bars. Values are related to control levels (gray bars). A, schematic illustration of reporter gene constructs. The dotted lines represent the original UTRs of luciferase-mRNA. Boxes labeled 5′UTR or 3′UTR represent the specific C-P4H-α (I) UTRs. The 3′-UTR was experimentally divided into two parts, termed 3′-A and 3′-B, to test individual influences. Boxes labeled Luciferase represent the coding sequence of luciferase transcript. & statistical analyses of results. n = 12. **, p < 0.01.

ment. These data indicated the involvement of translational control in the adaptation of C-P4H-α (I) expression in response to Fe²⁺ diminishment. Polysomal gradient analyses, however, indicated that translational control is of minor importance (data not shown). Thus, the 3′-UTR-mediated posttranscriptional control in the lack of Fe²⁺ ions correlated mainly with an increased mRNA stability and is attributed to the 3′-terminal ~500 nt of the 3′-UTR (called 3′-B, Fig. 3B). We concluded that the enhanced mRNA stability is attributed to a cis-element within the terminal half of the 3′-UTR.

Further experiments concentrated on the investigation of C-P4H-α (I) 3′-UTR-protein interaction, with the aim to detect possible mRNA stabilization factors. As demonstrated in Fig. 4A, UV cross-linking assays revealed that two 3′-UTR-binding proteins with a molecular mass of ~36 and ~38 kDa showed an increased binding behavior in response to 18 h of Fe²⁺ diminishment. These trans-acting factors were identified by affinity chromatography and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analyses as RNA-binding proteins hnRNP-A2 and hnRNP-B1. Both proteins represent splicing variants resulting from one gene. Western blotting analyses showed that the concentrations of both proteins were elevated under Fe²⁺ deficiency after 18 h (Fig. 5B), which correlated well with the increased binding seen in cross-linking experiments (Fig. 4A). The enhanced protein concentrations of the RNA-binding proteins hnRNP-A2 and -B1 can be attributed to an elevated mRNA concentration (Fig. 5A). We did not observe a significant increase in hnRNP-A2/B1 mRNA stability (data not shown), indicating that the induction of hnRNP-A2/B1 expression in response to Fe²⁺ diminishment is controlled mainly at the transcriptional level.

Trans-factors such as hnRNP-A2/B1 interact with discrete cis-mRNA elements or specific three-dimensional mRNA structures. UV cross-linking signals depend on the direct interaction of trans-acting factors with cis-acting elements. The observed signal intensity depends on the quality and quantity of nucleotides involved in the RNA-protein interaction. The separate labeling of C-P4H-α (I) 3′-UTR transcripts with the four possible nucleotides [³²P]U/C/A or [³²P]GTP is an approach to get detailed information concerning which nucleotides are preferentially represented in the binding site. Such labeling experiments revealed that both hnRNP-A2 as well as hnRNP-B1 only interacted with uracil in the recognition element (Fig. 4B). There was no label transfer from C-P4H-α (I) 3′-UTR to hnRNP-A2/B1 proteins when the other possible nucleotides were used for the labeling reaction. From the UTR-dependent reporter gene assays, however, it was evident that only the 3′-terminal half of the 3′-UTR is responsible for mRNA stabilization. UV cross-linking competition assays in which non-labeled U(3–7) elements (see Fig. 7A, sequence) or terminal 522 nt (3′-B) were added in an excess when compared with the radioactive labeled 3′-UTR showed that hnRNP-A2/B1 significantly interacted only with the 3′-terminal part of the 3′-UTR (Fig. 4C). Although both 3′-UTR parts contain several U(3–7) elements (see Fig. 7A, sequence), only the 3′-B part contains a U-rich element, which consists of a continuous stretch of 16 uridines (U₁₆ element). As shown by UTR-dependent reporter gene assays, the deletion of the 3′-UTR-located U₁₆...
element significantly suppressed the posttranscriptional influence in response to the diminishment of Fe^{2+} ions by 2,2-dipyridyl (Fig. 6). These data confirm that the increased binding amount of hnRNP-A2/B1 to the 3'-UTR-located U(16) element is of crucial importance in the adaptation of C-P4H-α (I) gene expression under hypometabolic conditions, caused by the lack of Fe^{2+} ions.

Furthermore, the influence of the trans-factors hnRNP-A2/B1 by interaction with the C-P4H-α (I) 3'-UTR cis-element on gene expression was demonstrated by UTR-dependent reporter gene assays. As shown in Fig. 7B, overexpression of hnRNP-A2/B1 lead to a significant increase in C-P4H-α (I) 3'-UTR-dependent luciferase activity. This influence was abolished by the deletion of the U(16) element. In contrast, another known RNA-binding protein, hnRNP-E1, did not alter C-P4H-α (I) 3'-UTR-mediated reporter gene activity, neither if the U(16) element was present nor if it was deleted.

To test the hypothesis whether hnRNP-A2/B1 is also important as a regulatory factor under other physiological or pathophysiological conditions, we performed an hnRNP-A2/B1 mRNA/C-P4H-α (I) mRNA correlation study based on microarray data, which depend on 686 different experimental conditions (17). The elevated hnRNP-A2/B1 expression level was associated with an increased mRNA concentration (Fig. 5A) and has a positive influence on C-P4H-α (I) mRNA stability. Thus, an elevated hnRNP-A2/B1 mRNA level should cause on average an elevated C-P4H-α (I) mRNA level. Therefore, we would expect a positive correlation between both mRNA signals. Indeed, statistical analysis revealed a significant positive correlation between hnRNP-A2/B1 and C-P4H-α (I) mRNA levels (Fig. 8). This supports the view that hnRNP-A2/B1 is not only a factor of C-P4H-α (I) mRNA stabilization under conditions of Fe^{2+} deficiency but is also relevant for a large variety of other changes in cell metabolism.

**DISCUSSION**

Posttranscriptional control affecting mRNA properties such as mRNA localization, translational efficiency, or mRNA stability modulates the expression rate, probably of each gene. All eukaryotic mRNAs contain 5'- and 3'-UTRs of different length. The human average of the
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FIGURE 6. Influence of 3′-UTR localized U_{16} element on luciferase (Luc) expression in response to the lack of Fe^{2+} ions. HT1080 cells were transfected with original pGL3-promoter (pGL3p) or transgenic variants, in which the original luciferase UTRs were replaced by C-P4H-α (I) 3′-UTRs, and incubated under control conditions or 2,2-DP treatment (18 h). The deletion of the U_{16} element (3′-UTR) del localized within the 3′-UTR, significantly suppresses the mRNA stabilization-related enhancement of luciferase activity. n = 6, **, p < 0.01.

5′-UTR is 300 nt (median: 240 nt), and that of the 3′-UTR is ~770 nt (median: 400 nt) (18). mRNA UTRs constitute a “hotspot” of regulatory elements (cis-elements), which interact with RNA-binding factors (trans-factors) to form specific ribonucleoprotein (RNP) complexes (19–22). mRNP complexes are dynamic, and their influence on C-P4H-α (I) 3′-UTR-dependent luciferase activity, independently of whether the U_{16} element is present or not. n = 8, **, p < 0.01.

The lack of Fe^{2+} ions mimics hypoxia/anoxia by inhibiting the respiratory chain, which subsequently leads to hypometabolism. Consequently, iron chelators such as desferrioxamine or 2,2-dipyridyl are used when studying hypoxia response pathways (29–31). However, the adaptation of C-P4H-α (I) gene expression in the lack of Fe^{2+} ions is different when compared with hypoxic conditions (1% oxygen) and seems to be mediated by different mechanisms. Hypoxia induces C-P4H-α synthesis mainly by activation of transcription and increases the efficiency of translation (32, 33). As we show here, Fe^{2+} diminishment induces C-P4H-α (I) synthesis by a different molecular effect, namely by increasing mRNA stability via RNA-binding proteins hnRNP-A2/B1, which interact with a U_{16} motif in the 3′-UTR.

The artificial division of the 3′-UTR as described in the legend for Fig. 3 is marked as follows. The dotted line indicates a sequence that is present in both 3′-A and 3′-B, and the underlined part represents the 3′-B part. 8, HT1080 cells were transfected with the original pGL3-promoter (pGL3p) vector or transgenic variants, in which the original luciferase 3′-UTR was replaced by the specific C-P4H-α (I) 3′-UTR, with or without the U_{16} element. Furthermore, cells were co-transfected with expression vectors encoding the hnRNP-A2/B1 or hnRNP-E1 proteins. Controls were co-transfected with empty expression vectors. The influence of hnRNP-A2/B1 or hnRNP-E1 overexpression on luciferase activity, depending on C-P4H-α (I) 3′-UTR (Luc-3′-UTR) or 3′-UTR without the U_{16} element (Luc-3′-U(16) del), was measured. Relative values were normalized to the influence of hnRNPs on the original pGL3-promoter vector. hnRNP-A2/B1 co-transfection leads to a significant increase in C-P4H-α (I) 3′-UTR-dependent luciferase activity (p < 0.01), and the influence is abolished by deletion of the U_{16} element. hnRNP-E1 shows no significant influence on C-P4H-α (I) 3′-UTR-dependent luciferase activity, independently of whether the U_{16} element is present or not. n = 8, **, p < 0.01.

FIGURE 7. Influence of 3′-UTR localized U_{16} element on luciferase (Luc) expression in response to overexpression of hnRNP-A2/B1 or hnRNP-E1. A, cDNA sequence of C-P4H-α (I) 3′-UTR. The U_{16} element is shown in bold print. The artificial division of the 3′-UTR as described in the legend for Fig. 3 is marked as follows. The dotted line indicates a sequence that is present in both 3′-A and 3′-B, and the underlined part represents the 3′-B part. 8, HT1080 cells were transfected with the original pGL3-promoter (pGL3p) vector or transgenic variants, in which the original luciferase 3′-UTR was replaced by the specific C-P4H-α (I) 3′-UTR, with or without the U_{16} element. Furthermore, cells were co-transfected with expression vectors encoding the hnRNP-A2/B1 or hnRNP-E1 proteins. Controls were co-transfected with empty expression vectors. The influence of hnRNP-A2/B1 or hnRNP-E1 overexpression on luciferase activity, depending on C-P4H-α (I) 3′-UTR (Luc-3′-UTR) or 3′-UTR without the U_{16} element (Luc-3′-U(16) del), was measured. Relative values were normalized to the influence of hnRNPs on the original pGL3-promoter vector. hnRNP-A2/B1 co-transfection leads to a significant increase in C-P4H-α (I) 3′-UTR-dependent luciferase activity (p < 0.01), and the influence is abolished by deletion of the U_{16} element. hnRNP-E1 shows no significant influence on C-P4H-α (I) 3′-UTR-dependent luciferase activity, independently of whether the U_{16} element is present or not. n = 8, **, p < 0.01.
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of an hnRNP-A2/B1-binding sequence. A quite different type of hnRNP-A2/B1-binding motif of 11 bases, which is not AU-rich, was described in myelin basic protein mRNA. There it plays a role in mRNA trafficking (39). The up-regulation of hnRNP-A2/B1 by Fe²⁺ deficiency has an interesting feature. Both proteins are overexpressed at an early stage in a variety of tumors and have been proposed as early markers for cancer (44).

Our observation that the elevated hnRNP-A2/B1 expression starts with an enhanced mRNA concentration enables us to test the hypothesis whether hnRNP-A2/B1 modulate the C-P4H-α (I) mRNA stability not only after Fe²⁺ diminishment. We have identified a positive correlation between hnRNP-A2/B1 and C-P4H-α (I) gene expression in a large data set of 686 independent microarray experiments. The data include a broad variety of physiological and pathophysiological conditions such as the human cell cycle, response to serum, response to infectious challenges, and profiles of multiple cancers. This supports independently the idea of hnRNP-A2/B1 as a positive effector of C-P4H-α (I) gene expression.

Taken together, we have shown that hnRNP-A2/B1 regulate C-P4H-α (I) mRNA stability. The RNA-protein interaction is mediated by a U-rich element located within the 3′-UTR of the mRNA. Thus, hnRNP-A2/B1 are demonstrated as factors participating in collagen synthesis and extracellular matrix remodeling.

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REFERENCES

1. Myllyharju, J., and Kivirikko, K. I. (2004) Trends Genet. 20, 33–43
2. Kivirikko, K. I., Myllyla, R., and Pihlajaniemi, T. (1989) FASEB J. 3, 1609–1617
3. Kivirikko, K. I., Helaakoski, T., Tasanen, K., Vuori, K., Myllyla, R., Parkkonen, T., and Pihlajaniemi, T. (1990) Ann. N. Y. Acad. Sci. 580, 132–142
4. Myllyharju, J. (2003) Matrix Biol. 22, 15–24
5. Berg, R. A., Kao, W. W., and Kedesha, N. L. (1980) Biochem. J. 189, 491–499
6. Kukkola, L., Hieta, R., Kivirikko, K. I., and Myllyharju, J. (2003) J. Biol. Chem. 278, 47685–47693
7. Nissi, R., Autio-Harmainen, H., Marttila, P., Sormunen, R., and Kivirikko, K. I. (2001) J. Histochem. Cytochem. 49, 1143–1153
8. Wang, Y., Liu, C. L., Storey, J. D., Tibshirani, R. J., Herschlag, D., and Brown, P. O. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 5860–5865
9. Raghavan, A., Ogilvie, R. L., Reilly, C., Abelon, M. L., Raghavan, S., Vasudevan, J., K Rothwoll, M., and Bobijiana, P. R. (2002) Nucleic Acids Res. 30, 5529–5538
10. Bevilacqua, A., Ceriani, M. C., Capaccioli, S., and Nicolin, A. (2003) J. Cell. Physiol. 195, 356–372
11. Yang, E., van Nimwegen, E., Zavol, M., Rajevsky, N., Schroeder, M., Magnasco, M., and Darnell, J. E. Jr. (2003) Genome Res. 13, 1863–1872
12. Wilusz, C. J., and Wilusz, J. (2004) Trends Genet. 20, 491–497
13. Lagnado, C. A., Brown, C. Y., and Goodall, G. J. (1994) Mol. Cell. Biol. 14, 7984–7995
14. Zuhiga, A. M., Belasco, J. G., and Greenberg, M. E. (1995) Mol. Cell. Biol. 15, 2219–2230
15. Bakheet, T., Frevel, M., Williams, B. R., Greer, W., and Khabar, K. S. (2001) J. Histochem. Cytochem. 49, 211–220
16. Brooks, S. A., and Rigby, W. F. (2000) Nucleic Acids Res. 28, E49
17. Stuart, J. M., Segal, E., Koller, D., and Kim, S. K. (2003) Science 302, 249–255
18. International Human Genome Sequencing Consortium (2001) Nature 409, 860–921
19. Pesole, G., Liuni, S., Grillo, G., and Saccone, C. (1997) Gene (Amst.) 205, 95–102
20. Dreyfuss, G., Kim, V. N., and Kataoka, N. (2002) Nat. Rev. Mol. Cell. Biol. 3, 195–205
21. Lasko, P. (2003) Science’s STKE 2003, R6
22. Kuersten, S., and Goodwin, K. S. (2001) Nat. Rev. Genet. 4, 626 –637
23. Tourriere, H., Gallouzi, I. E., Chelbi, C., Capony, J. P., Mouaikel, J., van der G., and Tazi, J. (2001) Mol. Cell. Biol. 21, 7747–7760
24. Amara, F. M., Chen, F. Y., and Wright, J. A. (1993) Nucleic Acids Res. 21, 4803–4809
25. Sierra, J. M., and Zapata, J. M. (1994) Mol. Membr. Biol. 11, 211–220
26. Levy, A. P., Levy, N. S., and Goldberg, M. A. (1996) J. Biol. Chem. 271, 2746–2753
27. McCabe, E. C., Rondon, I. J., and Beckman, B. S. (1997) J. Biol. Chem. 272, 8628–8634
28. Spicher, A., Guichet, O. M., Duret, L., Aslanian, A., Sanjines, E. M., Denko, N. C., Giaccia, A. J., and Blau, H. M. (1998) Mol. Cell. Biol. 18, 7371–7382

8 M. Fähling, unpublished observations.
