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mRNA Silencing in Human Erythroid Cell Maturation

HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN K CONTROLS THE EXPRESSION OF ITS REGULATOR c-Src[*][S]

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Erythroid precursor cells undergo nuclear extrusion and degradation of mitochondria when they mature to erythrocytes. It has been suggested before that the reticulocyte 15-lipoxygenase (r15-LOX) plays an important role in initiating the breakdown of mitochondria in rabbit reticulocytes. The expression of rabbit r15-LOX is regulated by the heterogeneous nuclear ribonucleoproteins (hnRNP) K and E1 at the translational level. However, this mechanism has never been confirmed in human erythropoiesis. Based on K562 cells we have set up an inducible human erythroid cell system. We show that, during induction, K562 cells exhibit changes in morphology and protein expression that are characteristic for terminal erythroid maturation: nuclear exclusion, expression of endogenous human r15-LOX regulated by hnRNP K and hnRNP E1, and loss of mitochondria. Importantly, induction of terminal erythroid maturation in primary human CD34$^+$ cells recapitulated the results obtained in K562 cells. Employing the physiologically relevant K562 cell system we uncovered a new mechanism of interdependent post-transcriptional regulation of gene expression. The timely expression of the tyrosine kinase c-Src, which phosphorylates hnRNP K in later stages, is controlled by hnRNP K in early stages of erythroid maturation. hnRNP K binds to the 3′-untranslated region of the c-Src mRNA and inhibits its translation by blocking 80 S ribosome formation. In premature erythroid cells, small interfering RNA-mediated knockdown of hnRNP K, but not of hnRNP E1, leads to the de-repression of c-Src synthesis.

Post-transcriptional regulation of gene expression plays a key role in cellular differentiation and development (1–4). A striking example for such processes is the control of mRNA translation in erythroid precursor cells, which undergo nuclear extrusion when they mature to erythrocytes. In the bone marrow, committed erythroid progenitor cells mature to proerythroblasts in which hemoglobin synthesis can first be detected. Subsequently, cell division ceases, and the nucleus is extruded at the maturation level of the polychromatic/orthochromatic normoblast (5–9). Whereas all mRNAs have to be synthesized in the bone marrow erythroblast before this stage, those that are not translated until later in erythroid maturation are stored in translationally silent messenger ribonucleoprotein particles (10). Enucleated reticulocytes are released into the blood stream and mature until degradation of mitochondria is initiated and reticulocytes become functional erythrocytes. The breakdown of mitochondria at this final step is initiated by the enzyme r15-LOX,[3] which catalyzes the dioxygenation of phospholipids in mitochondrial membranes (11–13). To prevent synthesis of r15-LOX that would cause mitochondria degradation in premature cells, the timing of r15-LOX mRNA translation activation is controlled tightly (14, 15).

We have previously investigated the translation of rabbit r15-LOX in cell-free systems and in transfected HeLa cells. Initiation of rabbit r15-LOX mRNA translation is inhibited by hnRNP K and hnRNP E1, which bind, singly or together, to the differentiation control element (DICE) in the 3′-UTR of the r15-LOX mRNA (16, 17). How is r15-LOX mRNA translation activated? In transfected HeLa cells, hnRNP K, but not hnRNP E1, specifically activates the tyrosine kinase c-Src and is a substrate of this kinase (18). c-Src-dependent phosphorylation of tyrosine 458 in the hnRNP K homology (KH) domain 3 of hnRNP K leads to the loss of its DICE-binding activity and consequently its role as an inhibitor of rabbit r15-LOX mRNA translation *in vitro* (19). The asymmetric dimethylation of hnRNP K by protein arginine methyltransferase 1 (PRMT1) inhibits the activation of c-Src by hnRNP K in transfected HeLa cells (20, 21).

The model of r15-LOX mRNA translation regulation is built on experimental data that were obtained in cell-free systems

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Table S1.

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[3] The abbreviations used are: r15-LOX, reticulocyte-15-lipoxygenase; DICE, differentiation control element; ES cells, embryonic stem cells; 15-HETE, 15-hydroxyeicosatetraenoic acid; hnRNP, heterogeneous nuclear ribonucleoprotein; nt, nucleotide(s); PRMT1, protein arginine methyltransferase 1; RNAi, RNA interference; siRNA, small interfering RNA; TRITC, tetramethylrhodamine isothiocyanate; UTR, untranslated region; ORF, open reading frame; sORF, short open reading frame; CAT, chloramphenicol acetyltransferase; RT, reverse transcription; HPLC, high-performance liquid chromatography; DAPI, 4′,6-diamidino-2-phenylindole; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Erk, extracellular signal-regulated kinase.

and in transfected HeLa cells but has not been investigated in an erythroid cell system so far. To analyze the biochemical pathways that control r15-LOX expression, the development of an inducible erythroid cell system was required. For this purpose we made use of human K562 cells, which represent the proerythroblast stage (22).

Here we show that premature human K562 cells undergo terminal erythroid maturation when stimulated with butyrate and exhibit nuclear exclusion, expression of endogenous human r15-LOX regulated by hnRNP K and E1, and loss of mitochondria. Terminal erythroid maturation of primary $CD34^+$ cells recapitulated the results obtained in K562 cells. Further investigation of the factors involved in this regulatory pathway revealed an interesting feedback mechanism. Employing immunoprecipitation experiments, *in vitro* mRNA binding assays and *in vitro* translation studies as well as RNA interference (RNAi), we found that hnRNP K, but not hnRNP E1, regulates the timely expression of c-Src during erythroid maturation. To our knowledge, these data indicate for the first time that the expression of a tyrosine kinase that is important for erythropoiesis is regulated at the level of translation.

EXPERIMENTAL PROCEDURES

Plasmids—pET16b-hnRNP K, pET16b-hnRNP K 5RG, and pGEM-CAT were described previously (16, 17, 20). The 679-nt 3′-UTR of human r15-LOX mRNA was amplified by PCR from pBSII KS hr15-LOX (23) and inserted between XhoI and NotI of pGEM-CAT to generate pGEM-CAT hDICE. pBS II SK-DICE has been described previously (24). The c-Src mRNA 3′-UTR (2038 nt) was subdivided in three fragments: Src1 (nt 1–600), Src2 (nt 601–1200), and Src3 (nt 1201–2038), which were amplified by PCR from K562 cell total RNA and inserted between XbaI and EcoRI of pBS II KS (Stratagene) to generate pBS II KS Src1, Src2, and Src3, respectively. To generate pGEM-CAT Src3, Src3 was cleaved by XhoI and NotI from pBS II KS Src3 and inserted between XhoI and NotI of pGEM-CAT. pGEM sCAT was generated by removing amino acids 303–731 from the CAT-ORF. Synthetic oligonucleotides, which code for the peptide MDYKDDDDKPAVAAALELVDPGPEEGPAMAGPEEGP, were cloned into the BamHI site of pBS II KS (Stratagene) to create a short open reading frame (sORF). The sORF-hDICE cDNA contained, in addition to sORF, the 3′-UTR of the human r15-LOX mRNA (23), which was amplified by PCR from pBSII KS hr15-LOX (23) and cloned into the EcoRI site. The construct sORF-Src3 contained, in addition to sORF, the fragment Src3 of the c-Src mRNA 3′-UTR, which was cleaved from pBSII KS Src3 by XbaI and EcoRV, treated with Klenow enzyme, and cloned into EcoRV.

Cell Culture and Cytoplasmic Extract Preparation—K562 cells were maintained in RPMI 1640 (10% fetal bovine serum). Erythroid maturation was induced by the addition of 1.5 mM sodium butyrate. Cytoplasmic K562 cell extract was generated after treatment with 200 mM KCl prior to lysis (25). Human $CD34^+$ cells derived from growth factor mobilized peripheral blood were purchased from CellSystems. 2×10^5 $CD34^+$ cells were cultured in StemPro-34 SFM (Invitrogen) supplemented as described in a previous study (26). Terminal erythroid maturation was induced as reported before (26), but without addition of the dexamethasone antagonist ZK112993. Approximately 5×10^3 cells were used for immunostaining at days 0, 3, and 6 of induction (Fig. 4, *C–G*). RNA was isolated from 3×10^4 $CD34^+$ cells at days 0 and 6 of induction, respectively (Fig. 4*B*). Murine embryonic stem cells (ES) $PRMT1^{+/+}$ or $PRMT1^{-/-}$ were cultivated, and extracts were prepared as in a previous study (20).

Determination of Cell Viability and Hemoglobin Content—Cell viability and cell number were determined by trypan blue exclusion (27). The number of hemoglobin-positive cells was determined by benzidine staining (28).

In Vitro Transcription—For UV-cross-linking assays ^{32}P-labeled or competitor RNA was generated as described in a previous study (24). Capped mRNAs transcripts were generated as described before (17). For translation initiation assays capped ^{32}P-labeled mRNAs were synthesized and purified as described before (17).

UV Cross-linking—UV cross-linking assays were performed using the indicated amounts of His-hnRNP K (16, 24).

Expression of Recombinant hnRNP K—His-tagged proteins were expressed and purified as described previously (16, 19).

In Vitro Methylation Assay—*In vitro* methylation was performed using 44 μg of cytoplasmic K562 cell extract (20).

In Vitro Translation—10-μl reactions contained 500 fmol of capped CAT mRNA and 4 μl of cytoplasmic K562 extract and were adjusted to a final concentration of 120 mM potassium acetate, 0.7 mM magnesium acetate, 0.1 mM amino acids without methionine, 4 units of RNasin (Promega, Madison, WI), 50 μg/ml creatine kinase, 44 μM hemin, 30 mM creatine phosphate, 800 μM ATP, 100 μM GTP, and 5 μCi of [^{35}S]methionine (10 μCi/μl). Translation reactions were incubated at 30 °C for 1 h.

Sucrose Gradient Analysis of Translation Initiation Complexes—Cytoplasmic K562 extract was incubated with 500 fmol of ^{32}P-labeled mRNA in the presence of 1 mM cycloheximide for 15 min. Translation initiation complexes were resolved on linear 5–25% sucrose gradients (17).

Western Blot Assays—Western blot assays were performed as described previously (18).

RNA Isolation and RT-PCR—Total RNA was isolated using TRIzol (Invitrogen). For reverse transcription 2 μg of RNA (Figs. 1*A*, 2*A*, and 3*D*) or equal volumes of RNA (Figs. 4*B* and 5*A*), random primers, and 150 units of M-MLV-RT (Promega) were used. Aliquots of the reverse transcription were used for PCR with GoTaq-Flexi DNA polymerase (Promega) and the respective primer pairs (supplemental Table S1).

R15-LOX Activity Assay—For measurements of the enzymatic activity of human r15-LOX, 0.1 ml of cytoplasmic K562 extract (20 μg/μl) was diluted with 0.2 ml of phosphate-buffered saline and incubated with 100 μM arachidonic acid for 20 min at room temperature. The hydroperoxy fatty acids formed were reduced with sodium borohydride to stabilize 15-hydro(pero)xyeicosatetraenoic acid, the primary reaction product of r15-LOX, as 15-hydroxyeicosatetraenoic acid (15-HETE). The reaction mixture was acidified, and proteins were precipitated by addition of 0.7 ml of methanol. The pellet was spun down, and aliquots of the clear supernatant were subjected to reversed-phase HPLC, which was performed on a Shimatzu

HPLC system connected to a Hewlett Packard diode array detector 1040. Analytes were separated on a Nucleosil C-18 column (Macherey-Nagel, KS-system, 250 × 4 mm, 5-μm particle size) coupled to a guard column (30 × 4 mm, 5-μm particle size). The absorbance was recorded at 235 nm in a methanol/water/acetic acid (80:20:0.1, v/v) solvent system. Fractions co-eluting with 15-HETE were collected, the solvent was evaporated, and the residue was reconstituted in hexane. The enantiomer composition of 15-HETE was analyzed by chiral phase HPLC on a Chiracel OD column using the solvent system hexane/2-propanol/acetic acid (100:5:0.1, v/v). Again the absorbance was recorded at 235 nm.

Immunoprecipitation—20 μl of protein A-Sepharose coupled with the hnRNP K or Fyn antibody was incubated with 100 μg of cytoplasmic K562 cell extract for 1.5 h at 4 °C in IPP buffer (20 mM HEPES, pH 7.4, 100 mM KCl, 5 mM magnesium acetate, 1 mM dithiothreitol, 0.025% Triton X-100, and protease inhibitors). Beads were washed twice in IPP buffer and boiled in SDS sample buffer, and the supernatant was analyzed in Western blot assays. For RT-PCR co-immunoprecipitated RNA was isolated from the pellet with TRIzol (Invitrogen).

Immunofluorescence Microscopy—K562 cells or $CD34^+$ cells were spun on poly-L-lysine-coated coverslips. Staining was performed as described previously (20). Where indicated, Phalloidin-TRITC (Invitrogen) was used to stain the actin cytoskeleton. Cells were mounted with ProLong Gold Antifade reagent with DAPI (Invitrogen). Mitochondria were stained with MitoTracker orange (Invitrogen). For microscopy an E600 (Nikon) or Axiovert 200M (Zeiss) microscope equipped with cameras (Hamamatsu, Zeiss) was used. Images were acquired by Lucia (Nikon) or AxioVision (Zeiss) software and compiled with Corel Technical Suite 12. 700 K562 cells were counted from three independent experiments to determine the number of cells lacking the nucleus and mitochondria.

Antibodies—Antibodies were purchased from Santa Cruz Biotechnology (hnRNP K, Lyn, Fyn, p-Tyr, CD34, glycophorin A), Oncogene (v-Src), Abcam (GAPDH and PRMT1) and Sigma (α-tubulin) and used according to the manufacturer's protocol. The r15-LOX antibody was raised in guinea pig (29). For hnRNP E1 detection in Western blots an antibody provided by B.-J. Thiele (dilution 1:5000) was used. For immunofluorescence microscopy of K562 cells an antibody against the hnRNP E1 peptide ^{231}SPLDLAKLNQVAR^{243} (Eurogentec), which discriminates hnRNP E1 from hnRNP E2 (30) (dilution 1:200) was used. For immunofluorescence microscopy of $CD34^+$ cells an hnRNP E1 antibody (Santa Cruz Biotechnology) was used that allowed co-staining of CD34.

The monoclonal antibody against non-methylated hnRNP K (non-R^{met} K) was generated against the internal sequence of human hnRNP K, bearing non-methylated arginine residues (^{255}MRGRGGFDRMPPGRGGRP^{272}). The peptide was synthesized and coupled to keyhole limpet hemocyanin or ovalbumin (PSL, Heidelberg). Lou/c rats were immunized with 50 μg of peptide-keyhole limpet hemocyanin using CPG 2006 and incomplete Freunds adjuvant as adjuvant. Supernatants were tested in a differential enzyme-linked immunosorbent assay. Peptide-specific antibodies were analyzed by Western blotting and immunoprecipitation through the use of extracts from ES $PRMT1^{+/+}$ and $PRMT1^{-/-}$ cells and recombinant hnRNP K (20). The monoclonal antibody MRGR 3D1 (rat IgG2a, dilution 1:10) recognized specifically the non-methylated hnRNP K (see Fig. 3, *A* and *B*).

RNAi—K562 cells (1×10^6 cells in RPMI without fetal bovine serum and antibiotics) were transfected by electroporation at 0.36 kV, 100 microfarads (GenePulser II, Bio-Rad) with 500 pmol of a mixture of siRNAs against hnRNP K: 5′-AGACUACAAUGCCAGUGUUTT-3′, 5′-CUGUGGAAUGCUUAAAUUATT-3′, 5′-GGAACAAGCAUUUAAAAGTT-3′, 500 pmol of a mixture of siRNAs against hnRNP E1: 5′-CUACUCGAUUCAAGGACAATT-3′, 5′-UGAACCAGGUGGCAAGACATT-3′, and 5′-UCACCAUUCCAAAUAACUUTT-3′ or 500 pmol of a nonspecific control siRNA: 5′-AGGUAGUGUAAUCGCCUUGTT-3′ (all MWG-Biotech). Cells were harvested 48 h post-transfection for immunofluorescence microscopy, Western blot assays, and RT-PCR.

RESULTS

Human K562 Cells Induced for Erythroid Maturation Show Enucleation and Loss of Mitochondria—Erythroid maturation of K562 cells can be induced with sodium butyrate (31, 32). The synthesis of fetal ζ-globin, adult α-globin, and hemoglobin, detected by benzidine staining, served as a suitable marker for initiation of erythroid-specific gene expression (33). Morphological changes associated with the maturation of erythroid cells have not been studied in cell culture. Enucleation and loss of mitochondria have so far only been demonstrated in isolated reticulocytes (34, 35).

When K562 cells were induced with sodium butyrate, the α-, β-, γ-, ε-, and ζ-globin genes were up-regulated (Fig. 1*A*, *lanes 1–5*) and globin synthesis increased from day 2 of erythroid maturation (Fig. 1*B*, *lanes 2–5*). The number of hemoglobin-positive cells increased from 1.4% at day 0 to 85% at day 8 of the induction period (Fig. 1*C*). Trypan blue exclusion indicated that 98% of the cells were viable at day 8 (data not shown), consistent with the finding that maturing erythroid cells are resistant to apoptosis (9, 36).

To investigate the morphological changes that characterize terminal erythroid maturation, we examined the cells from day 0 to 8 of induction. Importantly, enucleated cells, in which nuclear DAPI staining was not detectable, while cytoplasmic α-tubulin was visible, appeared at day 4 (Fig. 1*D*, *arrows*). Because the nucleus is surrounded only by a narrow layer of cytoplasm, the loss of the nucleus causes a significant reduction of the cell volume. At day 8 the nucleus was extruded from 29% of the cells, and in an additional 6% the nucleus was fragmented. Declining of mitochondria staining started at day 6 of the maturation period and at day 8 22% of the enucleated cells lacked mitochondria, as indicated by the staining with MitoTracker (Fig. 1*D*, *arrowheads*). Nuclear extrusion and the loss of mitochondria strongly indicated terminal erythroid maturation.

Erythroid Maturation of K562 Cells Displays Control of Human r15-LOX mRNA Expression—Because mitochondria degradation could be detected from day 6 on (Fig. 1*D*) and r15-LOX has been implicated in the maturational breakdown of mitochondria in rabbit reticulocytes (12), we profiled the

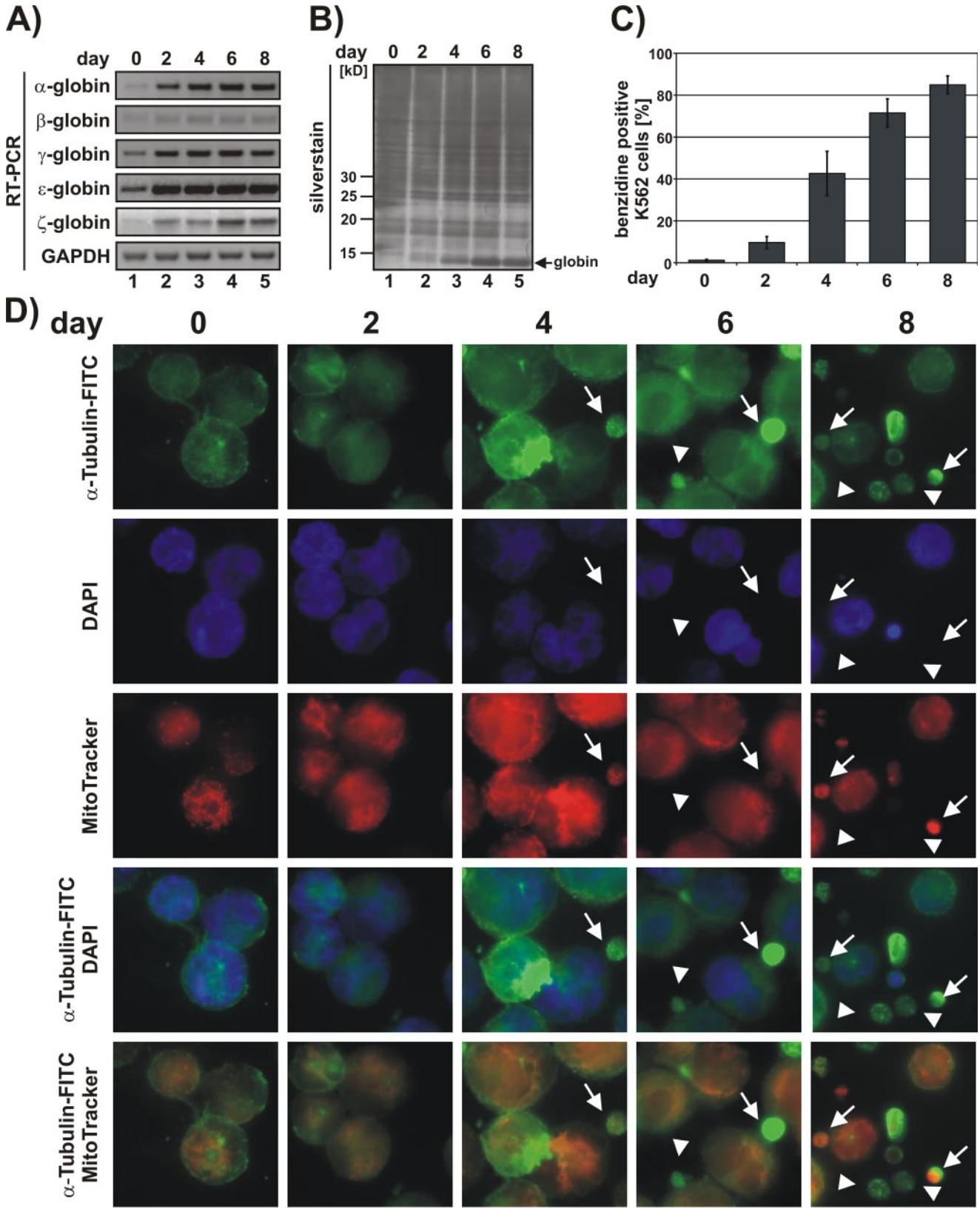

FIGURE 1. **Globin expression, hemoglobin formation, enucleation, and loss of mitochondria during induced erythroid maturation of K562 cells.** *A*, the level of globin mRNAs and GAPDH mRNA was analyzed by RT-PCR. *B*, cytoplasmic K562 cell extract was resolved by SDS-PAGE and analyzed by silver staining. Globin as a marker for erythroid-specific protein expression is indicated. *C*, benzidine staining of hemoglobin during erythroid maturation. *D*, immunofluorescence microscopy of K562 cells during erythroid maturation. An α-tubulin antibody was used to localize the cytoplasm, nuclei were detected by DAPI staining and mitochondria were visualized by MitoTracker orange that stains functional mitochondria. Cells lacking the nucleus are indicated by an *arrow*, and the loss of mitochondria staining is indicated by an *arrowhead*, respectively. Cellular integrity was checked by phase contrast microscopy (data not shown).

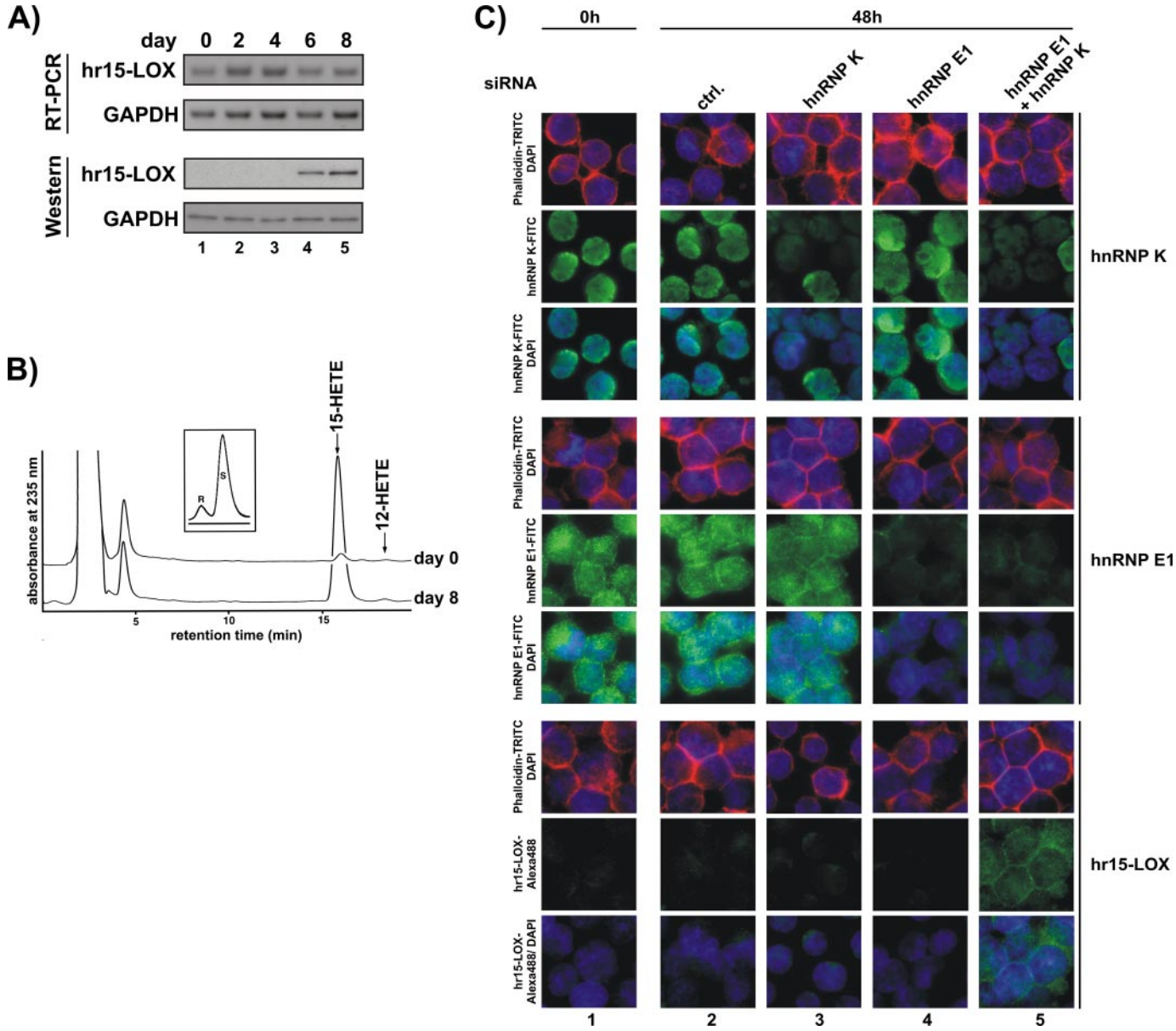

FIGURE 2. **Expression and enzymatic activity of human r15-LOX (hr15-LOX) during induced erythroid maturation of K562 cells.** *A*, detection of endogenous hr15-LOX or GAPDH mRNA and protein in cytoplasmic extracts of K562 cells by RT-PCR and Western blot assays. *B*, hr15-LOX activity assays and HPLC analysis of the specific reaction product 15-HETE at days 0 and 8. *Inset*: the enantiomer composition of 15-HETE was analyzed by chiral phase HPLC. The profile indicates the preferential formation of the specific r15-LOX product 15(*S*)-HETE. *C*, non-induced K562 cells were transfected with control siRNAs (*ctrl.*) or siRNAs against hnRNP K and E1, as indicated. Cytoplasm was visualized by phalloidin-TRITC, nuclei by DAPI staining. hnRNP K, hnRNP E1, and hr15-LOX were detected by specific antibodies. The knockdown of hnRNP K and E1 was verified by a Western blot assay shown in Fig. 6*A*.

expression of human r15-LOX mRNA and protein in our human erythropoiesis model. Human r15-LOX mRNA was present during the entire maturation period (Fig. 2*A*, *lanes 1–5*), whereas human r15-LOX protein could only be detected at days 6 and 8 (Fig. 2*A*, *lanes 4* and *5*). In contrast, GAPDH mRNA and protein were both detected at day 0 (Fig. 2*A*, *lane 1*). These data indicate repression of human r15-LOX mRNA translation at early maturation stages. To explore whether the induced human r15-LOX was enzymatically active we incubated cytoplasmic extracts from cells at days 0 and 8 in the presence of arachidonic acid and analyzed the reaction products (Fig. 2*B*). At day 8, human r15-LOX converted arachidonic acid to 15-HETE. In contrast, when extracts of non-induced cells (day 0) were used, only small amounts of 15-HETE were detected, which also appeared in the absence of cell extract (data not shown). Because at day 8 maturing erythroid cells contain large amounts of hemoglobin, we confirmed that the 15-HETE did not originate from heme catalysis (37). 15(*S*)-HETE strongly dominated the enantiomer mixture as shown by chiral phase HPLC (Fig. 2*B*, *inset*), and thus, heme catalysis could be excluded (38). This proves that human r15-LOX expressed at day 8 is enzymatically active.

Previously we have shown that hnRNP K and E1 singly or together repress rabbit r15-LOX mRNA translation in a cell-free system (16). To investigate their role in the control of endogenous human r15-LOX expression in the inducible cell system we performed RNAi against the endogenous hnRNP K and hnRNP E1 singly or together. Interestingly, only the simul-

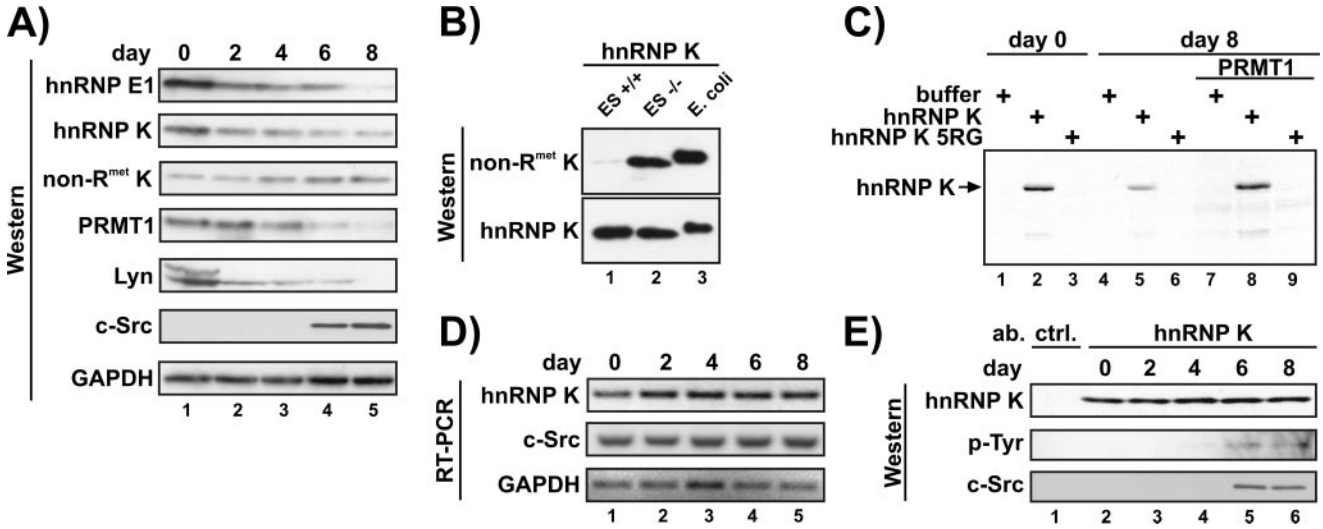

FIGURE 3. **Expression of hr15-LOX mRNA translation regulators during induced erythroid maturation of K562 cells.** *A*, cytoplasmic extracts of K562 cells from days 0 to 8 were resolved by SDS-PAGE. The expression of hnRNP E1, total hnRNP K, non-methylated hnRNP K, PRMT1, Lyn, c-Src, and GAPDH as a loading control was determined by specific antibodies in Western blot assays. *B*, the antibody non-R^{met}K recognizes specifically non-methylated hnRNP K in extracts from ES cells that do not express PRMT1 (*ES*$^{-/-}$) (*lane 2*) and recombinant hnRNP K expressed in *E. coli* (*lane 3*), but not asymmetrically dimethylated hnRNP K in (*ES*$^{+/+}$) cells (*lane 1*). A commercial monoclonal antibody against hnRNP K detects both, the methylated and non-methylated hnRNP K (*lanes 1-3*). *C*, 9 pmol of recombinant hnRNP K (*lanes 2*, *5*, and *8*) or the arginine substitution variant hnRNP K 5RG as a specificity control (*lanes 3*, *6*, and *9*) were incubated with [^{14}C]*S*-adenosylmethionine and cytoplasmic K562 cell extract at day 0 (*lanes 1–3*) or day 8 (*lanes 4–9*) of induction in the absence (*lanes 1–6*) or presence (*lanes 7–9*) of 2.3 pmol of recombinant PRMT1, followed by SDS-PAGE and autoradiography. *D*, the level of mRNAs coding for hnRNP K, c-Src, and GAPDH was analyzed by RT-PCR. *E*, immunoprecipitation of total hnRNP K with the commercial monoclonal antibody from cytoplasmic extracts of K562 cells followed by SDS-PAGE and Western blot assays. The level of immunoprecipitated protein was detected with the hnRNP K antibody (*hnRNP K*), and the phosphorylated protein was detected with an anti-phosphotyrosine antibody (*p-Tyr*) and co-immunoprecipitated c-Src with the c-Src antibody (*c-Src*).

taneous knockdown of both proteins by siRNAs resulted in a de-repression of human r15-LOX synthesis in non-induced cells (Fig. 2*C*, *panel 5*), indicating that either protein inhibits the translation of human r15-LOX mRNA. Taken together, the data indicate that sodium butyrate-stimulated K562 cells constitute a suitable human erythropoiesis model system that adequately mirrors essential key features of *in vivo* erythropoiesis, such as nuclear extrusion, mitochondria degradation, and translational control of human r15-LOX mRNA regulated by hnRNP K and E1.

Post-translational Modifications of hnRNP K Are Altered during Erythroid Maturation—Because it was not known how the factors involved in r15-LOX mRNA translational control are regulated during erythroid maturation, we employed the inducible K562 cell system to analyze their expression pattern and changes in functional post-translational modifications. We first analyzed the expression of the endogenous proteins hnRNP E1 and K (Fig. 3*A*). The level of hnRNP E1 and K decreased continuously starting at day 2 of induction, whereas GAPDH did not change (Fig. 3*A*, *lanes 1–5*), indicating that both proteins are degraded during erythroid maturation.

The regulatory activity of hnRNP K not only depends on its intracellular concentration, but also on its methylation state. hnRNP K is quantitatively asymmetrically dimethylated on five arginine residues (Arg^{256}, Arg^{258}, Arg^{268}, Arg^{296}, and Arg^{299}) by PRMT1 (20). The non-methylated form of hnRNP K interacts with c-Src and activates the kinase more efficiently than the methylated protein (20, 21). To discriminate between methylated and non-methylated hnRNP K, we generated a monoclonal antibody that specifically recognizes non-methylated hnRNP K. The antibody reacts with hnRNP K synthesized in murine embryonic stem cells, which do not express PRMT1 (ES−/−), or in *Escherichia coli*, but not with asymmetrically dimethylated hnRNP K expressed in wild-type ES cells (Fig. 3*B*, *lanes 1–3*). During erythroid maturation the amount of non-methylated hnRNP K increased, although the level of total hnRNP K decreased. This was paralleled by a decrease of PRMT1 (Fig. 3*A*, *lanes 1–5*). Consequently, the methylation activity of the extract was significantly reduced to 35% at day 8 compared with day 0, when recombinant hnRNP K was used as a substrate and [^{14}C]*S*-adenosylmethionine as a methyl donor (Fig. 3*C*, *lanes 2* and *5*). The methylation activity of the extract from day 8 could be restored by addition of recombinant PRMT1 (Fig. 3*C*, compare *lanes 2*, *5*, and *8*). In contrast, the arginine substitution variant hnRNP K 5RG (20) was not methylated (Fig. 3*C*, *lanes 3*, *6*, and *9*). hnRNP K mRNA was present at a constant level during induction (Fig. 3*D*, *lanes 1–5*) and could presumably be translated, but due to the lack of PRMT1, the only methyltransferase that acts on hnRNP K (20) newly synthesized hnRNP K was not methylated (Fig. 3*A*, *lanes 1–5*).

Next we analyzed the expression of the Src tyrosine kinase family members Lyn and c-Src (Fig. 3*A*). Consistent with the finding that Lyn is present in erythroblasts and is degraded during erythroid maturation (39), the expression level of Lyn decreased from day 2 of induction (Fig. 3*A*, *lanes 2–5*). In contrast, c-Src was detectable only at day 6 and thereafter (Fig. 3*A*, *lanes 4* and *5*).

Non-methylated hnRNP K, which interacts with c-Src and activates the kinase (20) and c-Src that, in turn, phosphorylates hnRNP K (18, 19) were detected in late stages of erythroid maturation (Fig. 3*A*, *lanes 4* and *5*) when human r15-LOX mRNA became translated (Fig. 2*A*, *lanes 4* and *5*). Therefore we asked the question: Is hnRNP K tyrosine phosphorylated by c-Src? To

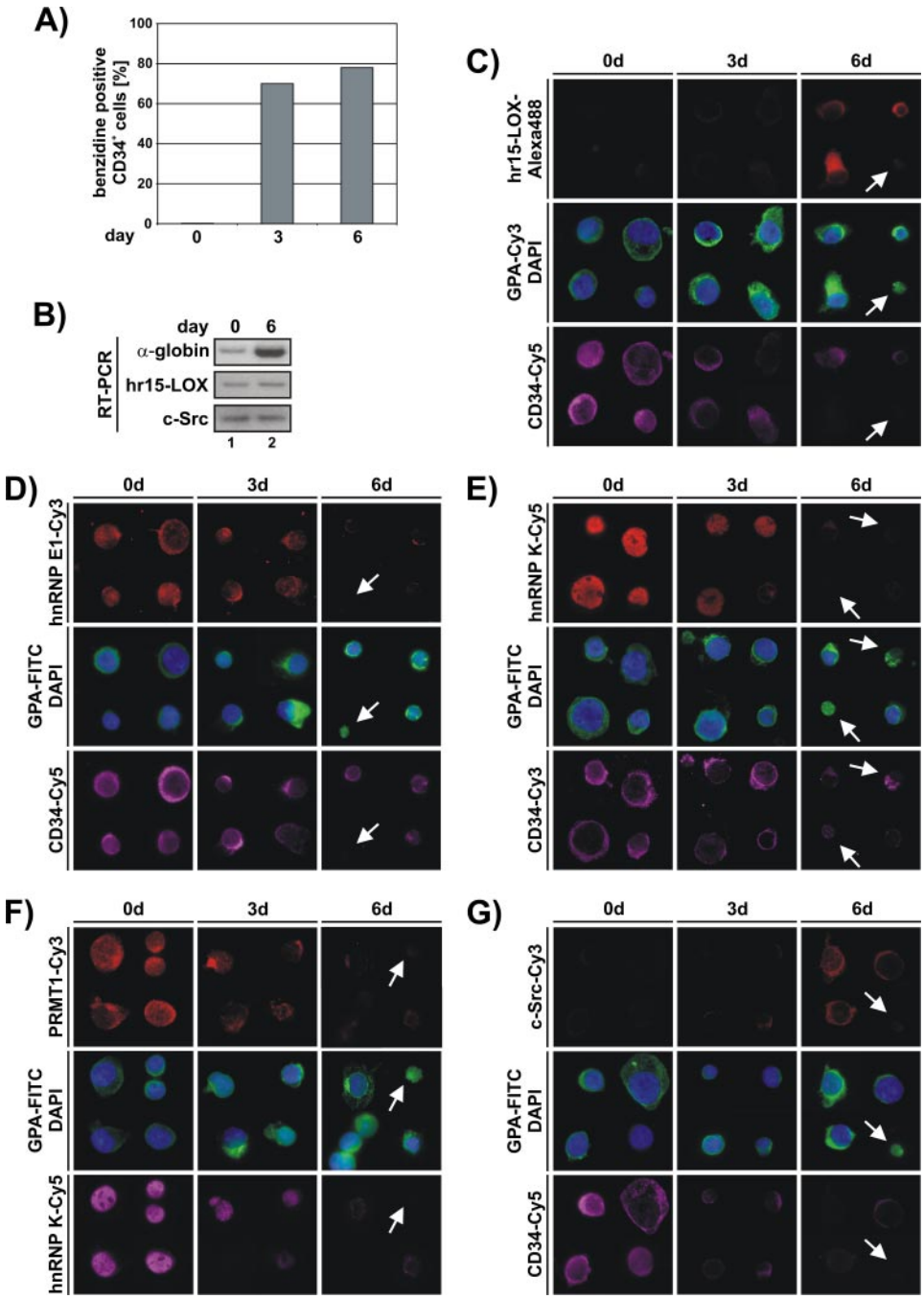

FIGURE 4. **Expression of hr15-LOX and its translational regulators in human CD34$^+$ cells induced for terminal erythroid maturation.** *A*, benzidine staining of hemoglobin during erythroid maturation. *B*, the level of endogenous α-globin, hr15-LOX, and c-Src mRNAs in non-induced CD34$^+$ cells and at day 6 of the induction period was analyzed by RT-PCR. *C–G*, immunostaining of CD34$^+$ cells during induction. Nuclei were stained with DAPI. Erythroid maturation was monitored by staining of glycophorin A (*GPA*) and CD34 (except *F*; both the CD34 and the PRMT1 antibody were raised in rabbit). Four representative cells are shown in every panel. Enucleated cells are marked by an *arrow*. *C*, immunostaining of hr15-LOX. *D*, detection of hnRNP E1 with a specific antibody. *E*, a specific antibody was used to detect hnRNP K. *F*, immunostaining of PRMT1 and hnRNP K (instead of CD34). *G*, detection of c-Src by immunostaining.

answer this, hnRNP K was immunoprecipitated from cytoplasmic extracts (Fig. 3*E*, *lanes 2–6*), and the precipitated proteins were analyzed with a phosphotyrosine-specific antibody and an antibody against c-Src. Importantly, phosphorylation of hnRNP K was observed only at days 6 and 8 of maturation (Fig. 3*E*, *lanes 5* and *6*) when c-Src co-immunoprecipitated with hnRNP K (Fig. 3*E*, *lanes 5* and *6*). Taken together, these data explain how the post-translational modifications of hnRNP K, which have an impact on its potency to regulate r15-LOX mRNA translation, are altered during induced erythroid maturation.

Interestingly, c-Src mRNA could be detected from day 0 to 8 (Fig. 3*D*, *lanes 1–5*), whereas c-Src protein was present only at days 6 and 8 of induction, (Fig. 3*A*, *lanes 4* and *5*). This strongly indicates that c-Src expression is also regulated at the translational level.

Post-transcriptional Regulation of hr15-LOX and c-Src Synthesis during Erythroid Maturation Can Be Recapitulated in CD34$^+$ Cells—To verify that human K562 cells provide a physiologically relevant system to study the translational regulation of hr15-LOX and c-Src synthesis, we analyzed their expression in primary human CD34$^+$ cells, which can be induced for terminal erythroid maturation in the presence of erythropoietin (26). CD34$^+$ cells continued to divide on differentiation initiation only for one to two divisions, thus only a small number of terminally maturing cells could be obtained. Therefore we analyzed mRNA levels by RT-PCR at days 0 and 6 of induction and detected the proteins by immunofluorescence microscopy at days 0, 3, and 6. Only 2% of the cells were hemoglobin-positive before induction, indicating that CD34$^+$ cells were not differentiated. Hemoglobin accumulation strongly increased until day 6 of induction (Fig. 4*A*), consistent with the formation of hemoglobin in K562 cells (Fig. 1*C*). Whereas the amount of α-globin mRNA increased during induction, hr15-LOX mRNA and c-Src mRNA levels remained unchanged as in K562 cells (compare Figs. 4*B*, 1*A*, 2*A*, and 3*D*). Importantly, although hr15-LOX mRNA was already present in non-induced CD34$^+$ cells (Fig. 4*B*), the protein could only be detected at day 6 of induction (Fig. 4*C*). As in K562 cells, the expression of hnRNP E1 and K that regulates hr15-LOX mRNA translation strongly decreased until day 6 (compare Fig. 4*D* and 4*E* with Fig. 3*A*). In addition the level of PRMT1 decreased (Fig. 4*F*). As in K562 cells, c-Src mRNA was already present in non-induced

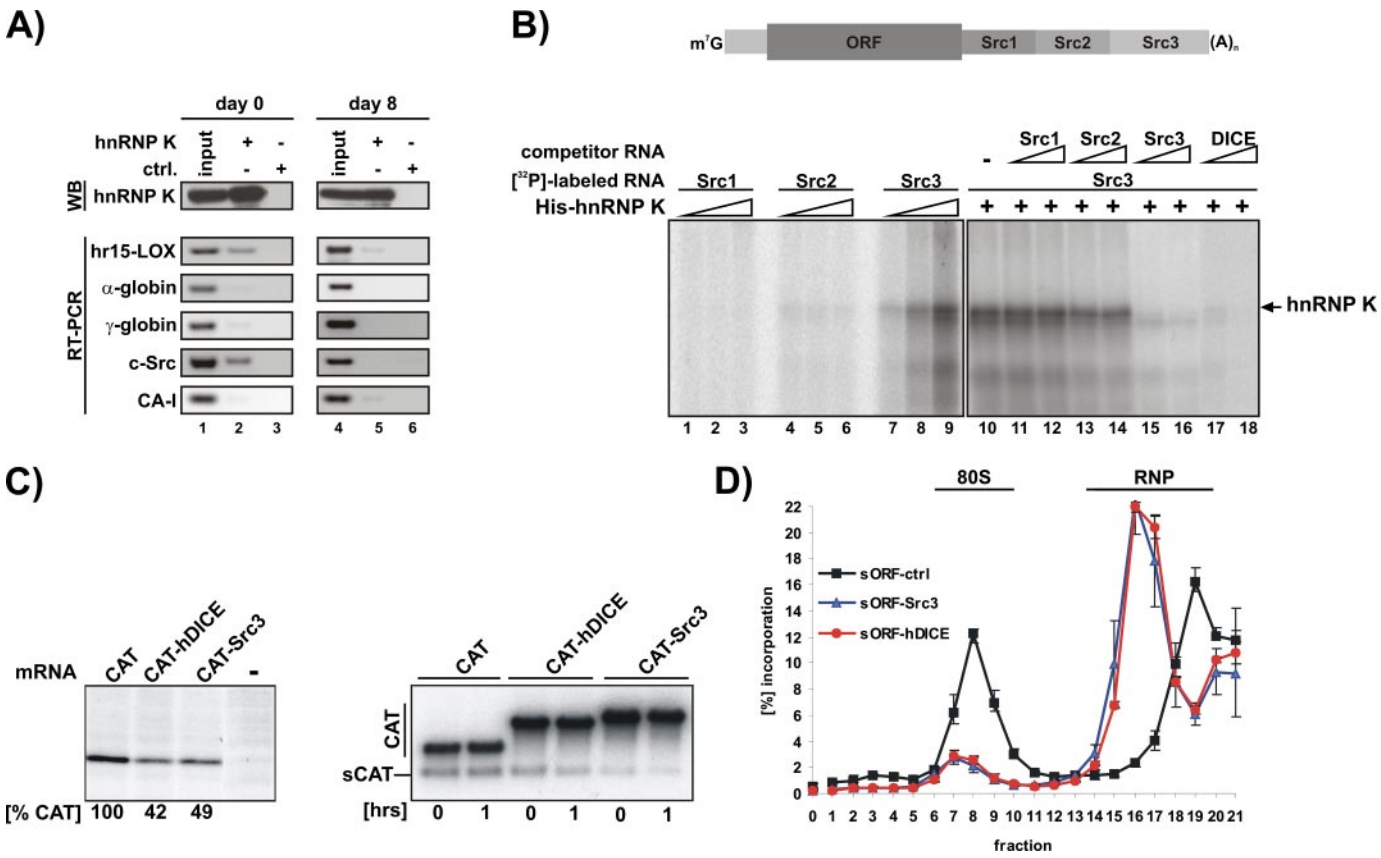

FIGURE 5. **hnRNP K directly binds to the c-Src mRNA 3′-UTR, which mediates translational inhibition by blocking 80 S complex formation.** *A*, immunoprecipitated hnRNP K from cytoplasmic extract from non-induced cells (*lane 2*) and from cells at day 8 of induction (*lane 5*) was detected by Western blotting. For the control immunoprecipitation (*ctrl.*) an unrelated monoclonal antibody was used (*lanes 3* and *6*). Co-immunoprecipitated mRNAs were detected by RT-PCR as indicated. *B*, *upper panel*: schematic representation of the c-Src mRNA. *Lower panel*: ^{32}P-labeled fragments of the c-Src mRNA 3′-UTR, Src1 (nt 1–600, *lanes 1–3*), Src2 (nt 601–1200, *lanes 4–6*), or Src3 (nt 1201–2038, *lanes 7–9*) were incubated with 1.8, 3.6, and 7.2 pmol of recombinant hnRNP K. The ^{32}P-labeled Src3 was incubated with 7.2 pmol of hnRNP K (*lane 10*) in the presence of 10- or 100-fold molar excess of unlabeled competitor RNAs Src1 (*lanes 11* and *12*), Src2 (*lanes 13* and *14*), Src3 (*lanes 15* and *16*), or DICE (*lanes 17* and *18*). *C*, cytoplasmic extract of non-induced K562 cells was used in cell-free translation reactions programmed with ^{32}P-trace-labeled reporter mRNAs CAT, CAT-hDICE, CAT-Src3, or no RNA. Translation reactions were carried out in the presence of [^{35}S]methionine. Translation products were resolved by SDS-PAGE and analyzed by autoradiography. The percentage of translated CAT protein is indicated below. ^{32}P-trace-labeled mRNAs were extracted from the translation reactions at time point 0 and after 1 h, as indicated. The ^{32}P-trace-labeled short CAT (*sCAT*) mRNA was used as an extraction control. The extracted mRNAs were separated on an agarose gel and analyzed by autoradiography. *D*, fractionation of translation initiation reactions containing cytoplasmic extract of non-induced K562 cells and ^{32}P-labeled reporter mRNAs sORF-ctrl, sORF-Src3, or sORF-hDICE in the presence of cycloheximide on 5–25% sucrose gradients and analysis by scintillation counting. Positions of 80 S and RNP-containing fractions are indicated.

CD34$^+$ cells, but c-Src protein could only be detected at day 6 of the induction period (compare Figs. 4*B* and 4*G* with Figs. 3*A* and 3*D*). This indicates that expression of c-Src is regulated at the translational level in induced CD34$^+$ cells as well.

During erythroid cell maturation the level of CD34 decreases (40), whereas glycophorin A is augmented (40, 41). From days 0 to 6 of erythroid maturation we observed impairment in CD34 staining (Fig. 4, *C*, *D*, *E*, and *G*), whereas the glycophorin A signal increased (Fig. 4, *C–G*). Furthermore, enucleated cells appeared at day 6 (indicated by *arrows*, Fig. 4, *C–G*). Mitochondria staining could not be reproducibly analyzed, because the MitoTracker reagent produced a high background signal in the small volume of CD34$^+$ cells, which did not allow clear distinction between stained (mitochondria containing) and unstained cells (no mitochondria). Taken together, the induction of terminal erythroid maturation of primary CD34$^+$ cells confirms the results obtained in induced K562 cells.

hnRNP K Binds Directly to the c-Src mRNA 3′-UTR and Inhibits c-Src Expression in Non-induced K562 Cells—Having shown that c-Src expression is regulated post-transcriptionally in induced K562 and CD34$^+$ cells, we asked the question: Is hnRNP K involved in this process? To address this, hnRNP K was immunoprecipitated from cytoplasmic K562 extracts at days 0 and 8 of induction (Fig. 5*A*, *lanes 2* and *5*) and interestingly the c-Src mRNA co-precipitated specifically with hnRNP K at day 0, but not at day 8. Human r15-LOX mRNA also co-precipitated specifically at day 0. In contrast, other erythroid-specific mRNAs coding for α-globin, γ-globin, and carboanhydrase I did not co-precipitate (Fig. 5*A*, *lanes 2* and *5*). This result suggests that hnRNP K may act as a translational regulator of the c-Src mRNA as well.

The 5′-UTR and 3′-UTR of the c-Src mRNA were screened for the presence of potential hnRNP K binding elements. The 3′-UTR of the c-Src mRNA contains UCCC motifs, which were identified previously as the sequence motif in the DICE that interacts with KH domain 3 of hnRNP K (19, 42). To analyze whether hnRNP K directly interacts with the c-Src mRNA 3′-UTR we performed UV-cross-linking experiments with the ^{32}P-labeled c-Src mRNA 3′-UTR subdivided in three fragments (Src1, -2, and -3) (Fig. 5*B*, *lanes 1–9*). Recombinant hnRNP K

was bound directly to Src3 (Fig. 5*B*, *lanes 7–9*). Only Src3 and the r15-LOX DICE did compete for the hnRNP K-Src3 interaction (Fig. 5*B*, *lanes 15–18*), indicating that hnRNP K binds to the same sequence motif in the 3′-UTRs of c-Src and r15-LOX mRNA.

To investigate whether the fragment Src3 of c-Src mRNA 3′-UTR mediates translation inhibition, we performed *in vitro* translation assays. For this purpose translation-competent extracts were generated from non-induced K562 cells. The DICE of the human r15-LOX mRNA served as positive control. The ^{32}P-trace-labeled CAT reporter mRNAs bearing either the human DICE (CAT-hDICE) or Src3 (CAT-Src3) were translated in these extracts in the presence of [^{35}S]methionine (Fig. 5*C*). Translation of CAT-hDICE and CAT-Src3 mRNA was reduced to 42% or 49%, respectively, compared with CAT control mRNA (Fig. 5*C*, *left panel*). A complete translational silencing cannot be expected, because the exogenously added mRNAs compete with the endogenous human r15-LOX mRNA and c-Src mRNA for hnRNP K. The reduced CAT expression from mRNAs bearing either the DICE or Src3 was not due to mRNA degradation as demonstrated by mRNA isolation at time point 0 and after 1 h of translation (Fig. 5*C*, *right panel*). This result indicates that the Src3 fragment mediates translation inhibition *in vitro*.

To delineate the mechanism of translational control, we performed translation initiation reactions with ^{32}P-labeled mRNAs (sORF-ctrl or sORF-Src3). As a positive control a human DICE-bearing mRNA (sORF-hDICE) was employed. The mRNAs were incubated in translation extracts in the presence of cycloheximide that inhibits the peptidyltransferase reaction (43) and stalls 80 S ribosomes at the AUG. Translation initiation complexes were resolved on linear 5–25% sucrose gradients, in which 80 S ribosomes can be separated from RNPs (Fig. 5*D*). The incorporation of radiolabeled sORF-Src3 mRNA and sORF-hDICE mRNA in the 80 S ribosome-containing fractions was significantly reduced compared with that of the control mRNA (sORF-ctrl) (Fig. 5*D*), indicating that the Src3 fragment and the human r15-LOX mRNA DICE mediate translation inhibition at the level of initiation by preventing 80 S ribosome formation. The latter confirms the experiments previously shown for the rabbit r15-LOX mRNA DICE in a cell-free system with added recombinant hnRNP K and E1 (16).

hnRNP K Silences Expression of c-Src in Erythroid Precursor Cells—The mRNA binding studies with recombinant hnRNP K and the translation experiments in extracts generated from non-induced K562 cells suggest that hnRNP K inhibits c-Src mRNA translation in erythroid precursor cells. To test whether hnRNP K acts as a cellular inhibitor of c-Src mRNA translation as well, we performed RNAi with siRNAs against hnRNP K and E1, as a control, or a non-related siRNA (Fig. 6, *A* and *B*). Transfection of non-induced K562 cells with specific siRNAs strongly suppressed the expression of hnRNP K and E1, respectively (Fig. 6, *A* and *B*). However, only the siRNA-mediated knockdown of hnRNP K, but not hnRNP E1, resulted in a de-repression of c-Src synthesis in non-induced K562 cells (Fig. 6, *A* and *B*).

DISCUSSION

In this study we demonstrate that butyrate-stimulated K562 cells constitute a useful human model for studying terminal erythroid maturation. This erythroid model system mirrors essential features of late erythroid development, such as nuclear exclusion and mitochondria degradation (Fig. 1). Furthermore it serves as a powerful system to analyze changes in protein expression controlled at the post-transcriptional level, to monitor post-translational modifications, and to identify translational regulated mRNAs. We found that hnRNP K not only functions as a translational regulator of human r15-LOX mRNA (Fig. 2) but also represses expression of c-Src (Figs. 5 and 6), an important regulator of cell cycle control, proliferation, and differentiation (44, 45).

Human CD34$^+$ cells isolated from peripheral blood recapitulated the results obtained with K562 cells following induction of terminal erythroid maturation (Fig. 4). The expression pattern of hr15-LOX and the regulators of hr15-LOX mRNA translation, hnRNP E1, hnRNP K as well as the post-transcriptional regulation of c-Src in induced CD34$^+$ cells and K562 cells prove that K562 cells provide a physiologically relevant system to study the mechanisms of human erythropoiesis. Our results discussed below are summarized in a model (Fig. 7) that describes how hnRNP K controls the timely expression and activity of its regulator c-Src and its impact on the expression of human r15-LOX in erythroid maturation.

The role of the r15-LOX for erythroid maturation has been studied in rabbits (12). In young rabbit reticulocytes the r15-LOX mRNA is present, but expression of the enzyme is silenced by binding of hnRNP K and E1 to the DICE in the r15-LOX mRNA 3′-UTR (16). However, there is little experimental evidence suggesting that the mechanism identified for rabbits may also be of physiological relevance in humans. When we compared the 3′-UTRs of the rabbit and human r15-LOX mRNA, we found that the rabbit r15-LOX mRNA 3′-UTR bears a DICE that consists of 10 tandem copies of a 19-nt CU-rich sequence motif (46), whereas the human DICE bears only 4 repeated elements, which are not consecutively arranged (23). Here we report for the first time that in a human model of erythroid maturation the expression of human r15-LOX follows a similar scenario as in isolated erythroid cells from rabbits. The expression and activity of human r15-LOX is restricted to late stages of maturation at days 6 and 8 (Fig. 2, *A* and *B*), when loss of mitochondria could be detected in enucleated cells (Fig. 1*D*). Importantly, only the simultaneous knockdown of hnRNP E1 and hnRNP K by RNAi led to an activation of human r15-LOX expression in non-induced K562 cells (Fig. 2*C*), indicating that either protein inhibits human r15-LOX mRNA translation. Although hnRNP E1 has been detected in the cytoplasm already in non-induced K562 cells, hnRNP K initially mainly nuclear, became localized to the cytoplasm from day 4 of induction (data not shown). It has been shown that Erk-dependent phosphorylation of serines 284 and 353 in hnRNP K mediates its cytoplasmic accumulation (47). Consistent with this finding, treatment of K562 cells with the Erk kinase inhibitor PD98059 caused nuclear retention of hnRNP K during induction (data not shown).

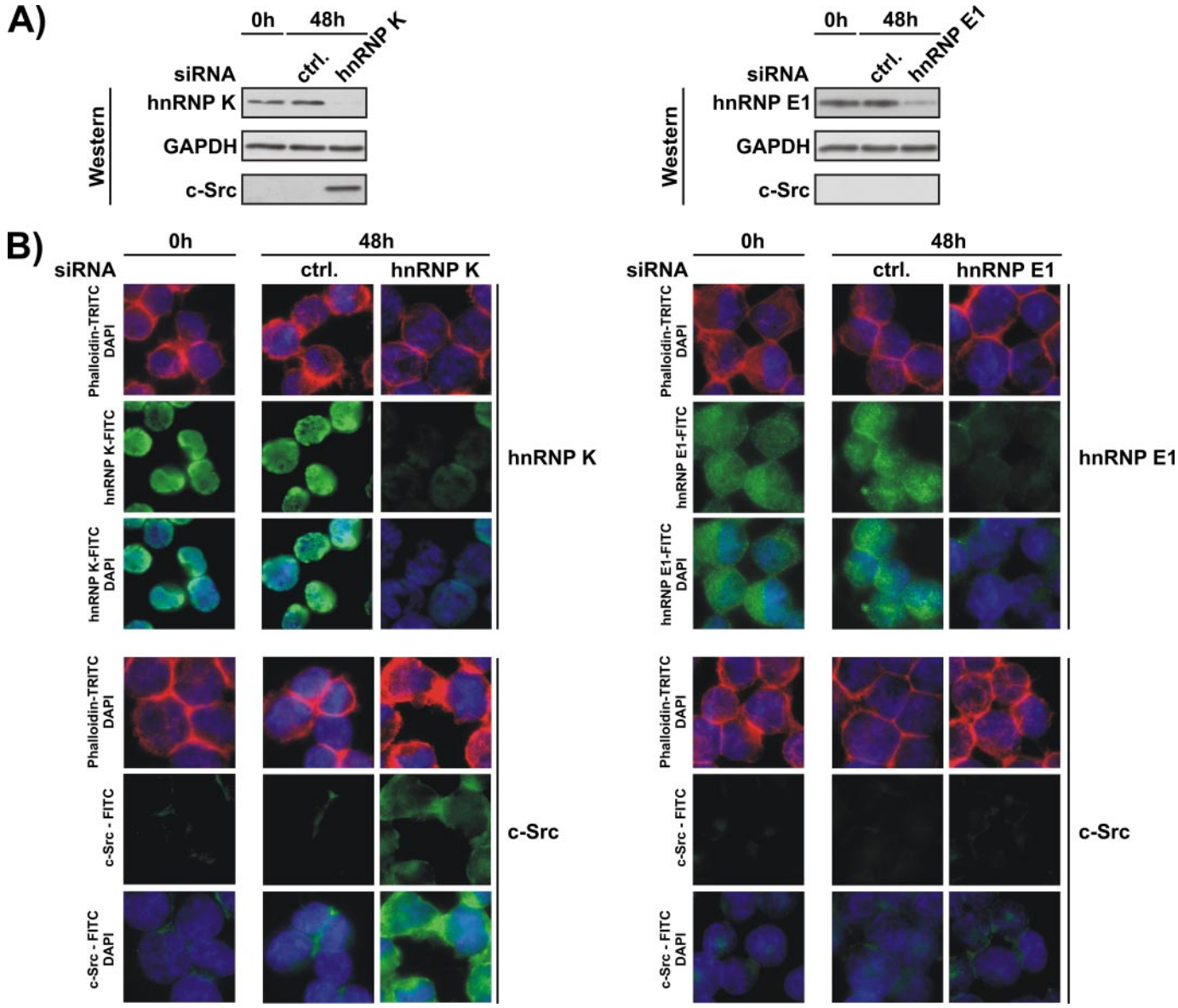

FIGURE 6. **SiRNA-mediated knockdown of hnRNP K, but not hnRNP E1, causes de-repression of c-Src synthesis.** *A* and *B*, non-induced K562 cells were transfected with control (*ctrl.*), hnRNP K, or hnRNP E1 siRNAs as indicated. *A*, detection of endogenous hnRNP K, hnRNP E1, GAPDH, or c-Src protein in cytoplasmic extracts of K562 cells at 0 and 48 h by Western blot assays. *B*, immunostaining of hnRNP K and E1 as in Fig. 2*C*. c-Src was detected with a specific antibody. Staining of the cytoplasm and nuclei are as in Fig. 2*C*.

Human r15-LOX mRNA co-precipitated specifically with hnRNP K from cytoplasmic extracts of non-induced K562 cells, whereas α-globin mRNA did not (Fig. 5*A*). It has been shown before that α-globin mRNA 3′-UTR directly interacts with hnRNP E1 and its homolog hnRNP E2 but not with hnRNP K (48, 49). In contrast to the human r15-LOX mRNA, the α-globin mRNA is continuously translated and stabilized by the α-complex formed at the CU-rich sequence in its 3′-UTR (50).

Surprisingly, in non-induced K562 cells c-Src mRNA is already present, whereas c-Src protein could only be detected at days 6 and 8 of induction (Fig. 3, *A* and *D*). Furthermore, c-Src mRNA co-precipitated with hnRNP K from cytoplasmic extract of non-induced cells, but not at day 8 of induction (Fig. 5*A*), and hnRNP K specifically interacted with the fragment Src3 of the c-Src mRNA 3′-UTR (Fig. 5*B*). Translation of a reporter mRNA bearing the fragment Src3 in its 3′-UTR was inhibited at the level of initiation in a cell-free system derived from non-induced K562 cells. Translation inhibition was not due to reduced mRNA stability (Fig. 5, *C* and *D*). In addition, siRNA-mediated knockdown of hnRNP K, but not of hnRNP E1, led to the de-repression of c-Src expression in non-induced K562 cells (Fig. 6, *A* and *B*). This indicates that only hnRNP K is involved in the regulation of the c-Src mRNA translation, in contrast to r15-LOX mRNA that is inhibited by hnRNP K and E1. Thus, we have identified the c-Src mRNA as a new target of hnRNP K-mediated translational control during erythroid cell maturation.

The catalytic activity of c-Src is down-regulated by a series of intramolecular interactions that impose conformational constrains on the catalytic domain, making it inaccessible for the substrate (44, 45). hnRNP K in its non-methylated form interacts with c-Src and functions as an activator of that kinase (20,

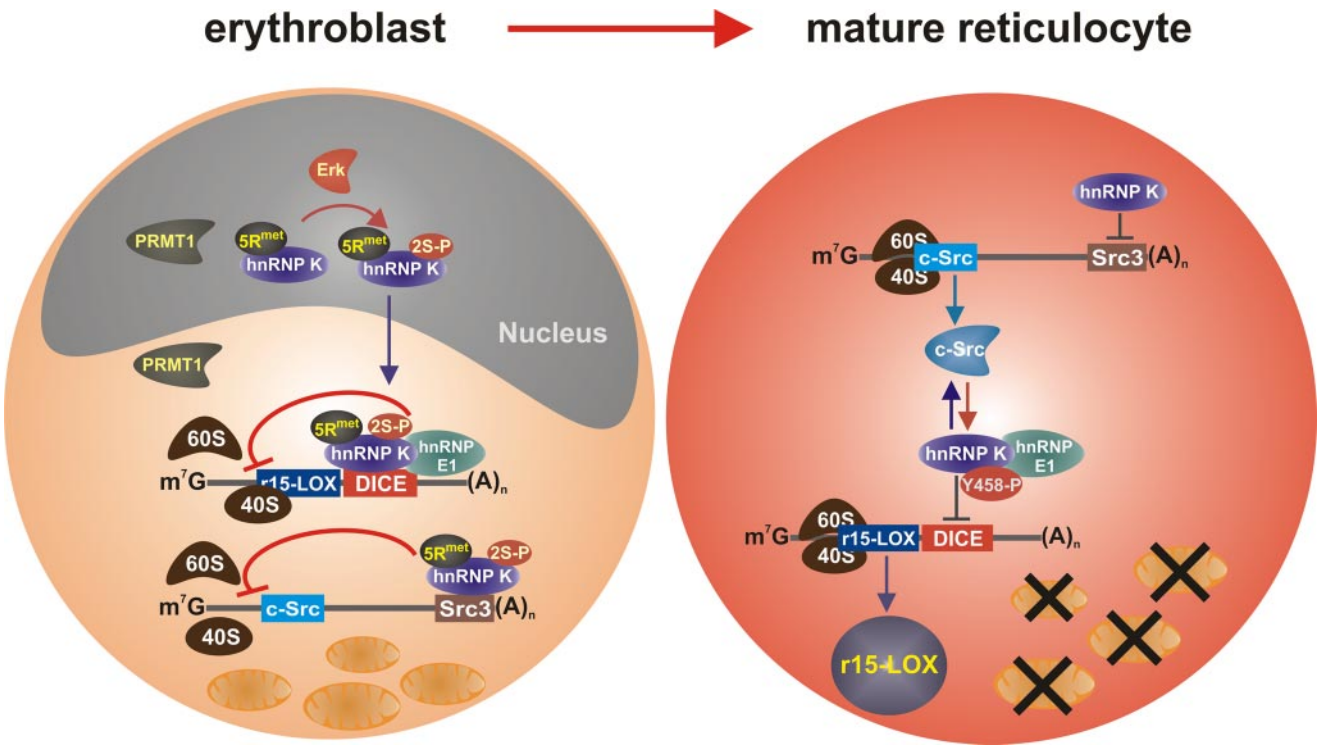

FIGURE 7. **Model of post-transcriptional regulation of gene expression in erythroid cell maturation.** r15-LOX is a key enzyme in erythroid cell maturation. The synthesis of r15-LOX is restricted to mature reticulocytes, where the enzyme initiates the degradation of mitochondria as a prerequisite for erythrocyte formation (12). *Left*: in erythroblasts the translation of r15-LOX mRNA is silenced, because the formation of translation competent 80 S ribosomes on r15-LOX mRNA is inhibited by hnRNP K and E1, which bind to the 3′-UTR DICE. This complex blocks the joining of the 60 S ribosomal subunit to the 40 S subunit at the AUG (16, 17). hnRNP K also binds to the c-Src mRNA 3′-UTR element Src3 and inhibits the c-Src mRNA translation by blocking 80 S ribosome formation (this study). hnRNP K is quantitatively asymmetrically dimethylated on five arginine residues by PRMT1 (20). Erk-dependent phosphorylation of hnRNP K on Ser^{284} and Ser^{353} leads to its cytoplasmic accumulation (47). *Right*: in mature reticulocytes hnRNP K is released from the element Src3 in the c-Src mRNA 3′-UTR, and the kinase is synthesized. Methylated hnRNP K is exchanged by the non-methylated form during erythroid maturation (this study) and non-methylated hnRNP K functions as a specific activator of c-Src (20, 21). The c-Src-dependent phosphorylation of Tyr^{458} in KH domain 3 of hnRNP K leads to the loss of DICE binding activity and consequently its role as an inhibitor of r15-LOX mRNA translation (19). r15-LOX mRNA translation is activated, and the newly synthesized enzyme catalyzes the dioxygenation of phospholipids in mitochondrial membranes (11–13).

21). hnRNP K activates only c-Src, but not the other members of the Src tyrosine kinase family Lyn, Fyn, or Lck (21). In non-induced K562 cells hnRNP K is barely detectable by an antibody that specifically recognizes non-methylated hnRNP K, but the amount of non-methylated hnRNP K increased until day 8 of induction (Fig. 3*A*). hnRNP K mRNA was present at a constant level and presumably translated (Fig. 3*D*), but PRMT1, the only enzyme that specifically catalyzes the asymmetric dimethylation of hnRNP K decreased until day 8 of induction (Fig. 3*A*). Therefore it is conceivable that the pool of methylated hnRNP K is exchanged by the non-methylated protein, as shown for histone H3.3 (51, 52). Consistent with this result, hnRNP K was phosphorylated at days 6 and 8 of erythroid maturation (Fig. 3*E*), when c-Src was expressed and the non-methylated hnRNP K interacted with c-Src (Fig. 3, *A* and *E*). The c-Src-dependent phosphorylation of Tyr^{458} in KH-domain 3 of hnRNP K leads to the loss of DICE-binding activity and abolishes its function as an inhibitor of r15-LOX mRNA translation (19).

Human r15-LOX expression and activity is restricted to late stages of erythroid maturation (Fig. 2, *A–C*), when the inhibition of c-Src mRNA translation by hnRNP K is released. Activation of c-Src mRNA translation is mediated by a so far unknown mechanism. It is possible that either the decreased level of hnRNP K is no longer sufficient to inhibit c-Src mRNA translation and/or non-methylated hnRNP K interacts less efficiently with c-Src mRNA and therefore the kinase is expressed.

Interestingly, in erythroid cells three mRNAs have been identified, r15-LOX, c-Src, and α-globin, that contain CU-rich elements in their 3′-UTRs. These mRNAs are regulated by interacting protein complexes at the post-transcriptional level during erythroid maturation. The mRNA 3′-UTR-protein complexes share two proteins, hnRNP K and/or hnRNP E1: r15-LOX mRNA interacts with hnRNP E1 and hnRNP K (16), c-Src mRNA is regulated by hnRNP K, but not hnRNP E1 (this study), and α-globin mRNA binds hnRNP E1, but not hnRNP K (48, 49). Additional components that differ between individual mRNA-protein complexes presumably specify their function in mRNA stabilization (α-globin mRNA) and translational regulation (r15-LOX mRNA and c-Src mRNA). Interesting insight in the molecular mechanism of mRNA-specific post-transcriptional control can be expected from the identification of additional components associated with hnRNP K in complex with the human r15-LOX mRNA and c-Src mRNA in this inducible erythroid cell system.

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Supplemental Data

Primers used for RT-PCR

mRNA	forward	reverse	(bp)
α-globin	GAGAGGATGTTCCTGTCCTT	CAGTGGCTTAGGAGCTTGAA	224
β-globin	GTGGATGAAGTTGGTGGTGA	TTGCCCAGGAGCCTGAAGTT	258
γ-globin	AAACCCTGGGAAGGCTCCT	TTCCCAGGAGCTTGAAGTTC	246
ε-globin	TGGGCAGACTCCTCGTTGTT	TACCCAGGAGCTTGAAGTTC	240
ζ-globin	TCTGGAGAGGCTCTTCCTCA	GGACAGGAGCTTGAAGTTGA	223
hnRNP K	TTAAGGCTCTCCGTACAGAC	TCCACAGCATCAGATTCGAG	199
hr15-LOX	TGAGCTGCAGTCTCATCTTC	CAGTGCTCATTATCTGGTCG	197
GAPDH	ACAGTCAGCCGCATCTTCTT	CTGGAAGATGGTGATGGGAT	290
c-Src	ACTATGAGTCTAGGACGGAG	CCTTTCGTGGTCTCACTTTC	274
CA-I	AACCGATCAGTGCTGAAAGG	CGTGAAGCTCGGCAGAATAT	229

mRNA Silencing in Human Erythroid Cell Maturation: HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN K CONTROLS THE EXPRESSION OF ITS REGULATOR c-Src

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