Liver-specific deletion of IGF2 mRNA binding protein-2/IMP2 reduces hepatic fatty acid oxidation and increases hepatic triglyceride accumulation

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Insulin-like growth factor 2 mRNA-binding proteins 1–3 (IGF2BP1–3, also known as IMP1–3) contribute to the regulation of RNAs in a transcriptome-specific context. Global deletion of the mRNA-binding protein insulin-like growth factor 2 mRNA-binding protein 2 mRNA-binding protein 2 (IGF2BP2 or IMP2) in mice causes resistance to obesity and fatty liver induced by a high-fat diet (HFD), whereas liver-specific IMP2 overexpression results in steatosis. To better understand the role of IMP2 in hepatic triglyceride metabolism, here we crossed mice expressing albumin-Cre with mice bearing a floxed Imp2 gene to generate hepatocyte-specific IMP2 knockout (LIMP2 KO) mice. Unexpectedly, the livers of LIMP2 KO mice fed an HFD accumulated more triglyceride. Although hepatocyte-specific IMP2 deletion did not alter lipogenic gene expression, it substantially decreased the levels of the IMP2 client mRNAs encoding carnitine palmitoyltransferase 1A (CPT1A) and peroxisome proliferator–activated receptor α (PPARα). This decrease was associated with their more rapid turnover and accompanied by significantly diminished rates of palmitate oxidation by isolated hepatocyes and liver mitochondria. HFD-fed control and LIMP2 KO mice maintained a similar glucose tolerance and insulin sensitivity up to 6 months; however, by 6 months, blood glucose and serum triglycerides in LIMP2 KO mice were modestly elevated but without evidence of liver damage. In conclusion, hepatocyte-specific IMP2 deficiency promotes modest diet-induced fatty liver by impairing fatty acid oxidation through increased degradation of the IMP2 client mRNAs PPARα and CPT1A. This finding indicates that the previously observed marked protection against fatty liver conferred by global IMP2 deficiency in mice is entirely due to their reduced adiposity.

IGF2 mRNA binding proteins 1–3 (IGF2BP1–3, also called IMP1–3) participate in regulation of the RNA life cycle in a transcriptome-specific manner (1, 2). Each IGF2 mRNA binding protein (IMP)4 is able to bind diverse RNA species, including long noncoding RNAs and mRNAs, to control their splicing (especially IMP1), transport, translation, and stability. All three IMPs are expressed coordinately in the mouse embryo starting at ~E10.5 and peaking at ~E12.5. The expression of Imp1 and Imp3 is largely extinguished after birth, whereas Imp2 is widely expressed postnatally (3, 4).

Genome-wide association studies of many populations have identified SNPs in the second intron of the human IMP2 gene that occur in excess in individuals with type 2 diabetes (5–7). Because little was known about the biological functions of IMP2 in vivo, we characterized mice with global IMP2 deficiency (8). In contrast to Imp1-null mice, which are born small and show ~50% mortality at postnatal day 3 (9), Imp2-null mice appear normal at birth, and their body weight is similar to WT littermates until weaning; thereafter, however, Imp2-null mice gain less weight because of slower accumulation of both lean and fat mass. The reduced skeletal muscle mass seen with global IMP2 deficiency is due to a myocyte-intrinsic diminution in protein synthesis, attributable in part to diminished autocrine IGF2 production (10). The lesser fat mass of Imp2-null mice is especially marked on a high-fat diet and is accompanied by reduced circulating lipids, markedly less liver triglyceride accumulation, and much better glucose tolerance and insulin sensitivity. Regarding the mechanism(s) underlying resistance of mice with global IMP2 deficiency to fatty liver, the relative contribution of reduced FFA delivery versus altered hepatocyte triglyceride metabolism is not known. Notably, transgenic overexpression of IMP2 in mouse liver results in increased triglyceride deposition (11), perhaps in part through up-regulation of hepatocyte IGF2 expression (12, 13).

In addition to their altered metabolism, Imp2-null mice are long-lived and exhibit fewer malignancies at an advanced age (8). Considerable evidence implicates human IMP2 as a tumor promoter that is overexpressed in many common human cancers and associated with an adverse outcome (14–16). For

4 The abbreviations used are: IMP, IGF2 mRNA binding protein; E10.5, embryonic day 10.5; HFD, high-fat diet; AS1M, acid-soluble metabolite(s); IP, immunoprecipitate; QPCR, quantitative PCR.
example, the 62-kDa variant of the IMP2 polypeptide has been identified as an autoantigen in human hepatocellular carcinoma (HCC); its expression is low in normal adult liver but high in hepatocellular carcinoma nodules and fetal liver (17). In mice with liver-specific IMP2 overexpression, treatment with diethylnitrosamine is accompanied by an earlier onset and accelerated progression of hepatic tumorigenesis than in diethylnitrosamine-treated controls (18). It is unclear whether the steatosis accompanying hepatic IMP2 overexpression contributes to the pro-tumorigenic phenotype.

The finding that IMP2 hepatic overexpression results in steatosis whereas global IMP2 deficiency is accompanied by a marked reduction in hepatic triglyceride suggested that IMP2 may favor liver triglyceride deposition. To examine the role of endogenous IMP2 in hepatocyte triglyceride balance, Imp2ff mice were crossed with mice expressing cre recombinase driven by the albumin promoter. Liver-specific Imp2ff knockout (LIMP2 KO) mice exhibit a marked loss of IMP2 expression in postnatal liver. When consuming a high-fat diet, LIMP2 KO mice unexpectedly exhibited increased hepatic triglyceride accumulation and, ultimately, elevated blood triglyceride levels. We demonstrate that this results from a reduction in hepatic fatty acid oxidation caused by more rapid turnover and decreased abundance of the IMP2 client mRNAs encoding the CPT1A and PPARα polypeptides.

Results

Generation of hepatocyte-specific IMP2 knockout mice

During mouse development, expression of IMP2 mRNA in the liver peaks around E12.5 and diminish sharply after birth (Fig. 1A); however, at E12.5, the liver is primarily a hematopoietic organ, and hepatoblasts are relatively few (22). Liver IMP2 polypeptide expression in WT and LIMP2 KO mice was examined by immunoblot at 2 months age; liver IMP2 protein was greatly reduced in homozygous LIMP2 KO mice (Fig. 1B). Additional immunoblot studies of LIMP2 KO with tissues from fat, muscle, and brain showed no significant
difference in IMP2 protein level compared with control animals, confirming the tissue specificity of Cre expression (data not shown).

Deletion of Hepatocyte IMP2 promotes triglyceride deposition

Mice were placed on a high-fat diet (HFD) from weaning. No significant differences in body weight or body composition of WT and LIMP2 KO mice were observed, either at weaning or after 30 weeks on normal chow or on the high-fat diet (Fig. S1). Although hepatic triglyceride content in WT and LIMP2 KO male mice at 10 weeks age is similar (Fig. S2A), by 30 weeks of age, the livers of LIMP2 KO male mice on the HFD contained 60% more triglyceride than control livers (Fig. 1C). In female mice, although a tendency toward higher triglyceride levels was observed in LIMP2 KO livers of mice on both normal chow (Fig. S2B) and the HFD (Fig. S2C), liver triglycerides were highly variable and not statistically significant. Thus, hepatocyte expression of IMP2 is protective against development of fatty liver in male mice, whereas the effect in females remains to be determined. Subsequent studies focused on male mice.

Down-regulation of lipid oxidation genes in the livers of LIMP2 KO mice

To explore the mechanisms underlying increased lipid accumulation in the livers of male LIMP2 KO mice, we performed real-time PCR for mice fed a HFD at 30 weeks. The analysis revealed that albumin-Cre–mediated deletion of the Imp2 gene in the liver had no effect on the relative abundance of a cohort of mRNAs encoding elements important for lipogenesis (ACC1, ACC2, FAS, ACLY, SREBP1c, SREBP2, and ChREBP) and triglyceride synthesis (CD36, SCD1, GPAT, AGPAT, Lipin, DGAT, ApoB, and MTP), apart from a 62% reduction in PPARα mRNA (Fig. 1D). By contrast, IMP2 deletion resulted in significant down-regulation of several mRNAs encoding elements critical for fatty acid oxidation: a 60% decrease in CPT1A mRNA, a 64% decrease in PPARα mRNA, a 50% decrease in FGF21 mRNA, and a nonsignificant 24% decrease in CPT2 mRNA (Fig. 1E). Western blots of the PPARα, PPARγ, and Cpt1A polypeptides confirmed the reduced abundance of these polypeptides, whereas CPT2 was unaltered (Fig. 1F). Altogether, these data suggest that IMP2 promotes lipid oxidation without affecting neutral lipid synthesis.

Liver IMP2 deficiency results in diminished oxidation of palmitate

The ability of primary mouse hepatocytes (Fig. 2A) and liver mitochondria (Fig. 2B), prepared from the livers of 6-week-old male LIMP2 KO and WT mice, to carry out oxidation of [1-14C]palmitate was determined. In hepatocytes, generation of 14CO2 and 14C-labeled acid-soluble metabolites (ASM) from [1-14C]palmitate by LIMP2 KO cells was 33% and 36% less than in WT hepatocytes, whereas essentially identical levels of 14CO2 and 14C ASM were generated from LIMP2 KO and WT hepatocytes when they were pretreated briefly with the CPT-1
inhibitor etomoxir (Fig. 2A). Thus, hepatocyte IMP2 deficiency reduces mitochondrial fatty acid oxidation by intact cells. This was confirmed by an assay of $[1^{-14}C]$palmitate oxidation by KO mitochondria was reduced by 15% ($p < 0.05$; **, $p < 0.01$) compared with isolates from WT mice and LIMP2 KO mice were similar (Table 1). In addition, glucagon, leptin, cholesterol, FFA, and triglycerides of control and LIMP2 KO mice did not differ at 12 and 30 weeks and 14 and 32 weeks of age, respectively (Fig. 4, A–E). However, at 30–32 weeks of age, LIMP2 KO mice developed hypertriglyceridemia and slight hyperglycemia by 30 weeks of age.

**IMP2 binds and stabilizes CPT-1A and PPAR$\alpha$ mRNAs and promotes CPT-1A mRNA translation**

IMP2 is a RNA-binding protein that can regulate the life cycle of client mRNAs through altered transport, stability, and translation. To identify IMP2 mRNA clients relevant to fat metabolism in the liver, immunoprecipitates (IPs) were prepared from extracts of hepatocytes isolated from 6-week-old male LIMP2 KO and WT mice. Generation of $^{14}CO_2$ by LIMP2 KO mitochondria was reduced by 15% ($p < 0.002$) and that of $[1^{-14}C]$ASM by 18.8% ($p = 0.05$) compared with isolates from WT livers (Fig. 2B).

**LIMP2 KO mice develop hypertriglyceridemia and slight hyperglycemia by 30 weeks of age**

At 10 weeks of age, the levels of blood glucose, insulin, glucagon, leptin, cholesterol, FFA, and triglycerides of control and LIMP2 KO mice were similar (Table 1). In addition, the glucose tolerance and insulin sensitivity of LIMP2 KO and WT mice did not differ at 12 and 30 weeks and 14 and 32 weeks of age, respectively (Fig. 4, A–E). However, at 30–32 weeks of age, LIMP2 KO mice displayed increased serum

**Hepatocyte IMP2 stabilizes CPT1A and PPAR$\alpha$ mRNAs**

Figure 3. The effect of Imp2 inactivation in AML12 hepatocytes on the overall abundance, half-life, and polysomal abundance of selected mRNAs. A, the effects of CRISPR-catalyzed Imp2 inactivation in AML12 hepatocytes on the abundance of selected mRNAs and polypeptides. RNA was extracted from AML12 hepatocytes transduced with CRISPR reagents directed at Imp2 or GFP, and the abundance of the indicated mRNAs was estimated by QPCR. The ratio of Imp2 CRISPR/GFP CRISPR is shown. Bottom panel, immunoblots of IMP2 and other polypeptides. B, mRNA half-life in IMP2 CRISPR and GFP CRISPR AML12 cells. Cells were treated with actinomycin ($1 \mu M$) for 12 h. RNA was extracted at $t = 0$ and every 2 h thereafter, and the abundance of the indicated mRNAs was determined by QPCR. The rate of decline was plotted by least squares using the first three time points shown in the bottom panel, and the time to 50% decrease of the initial value is shown in the top panel. C, the percentage of mRNA residing in polysomes in IMP2 CRISPR/GFP CRISPR AML12 cells. Total RNA and post-mitochondrial extracts were prepared from equal numbers of rapidly growing cells. The post-mitochondrial extracts were subjected to density gradient centrifugation. Total RNA and RNA from the pooled polysomal fraction of the gradients were quantified by QPCR, and the ratio of polysomal RNA/total RNA $\times 100$ is shown. *, $p < 0.05$; **, $p < 0.01$.
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Table 1
Some serum values in WT and LIMP2 KO mice.
Values in bold highlight statistically significant differences.

| Genotype | 8–10 weeks of age | 28–30 weeks of age |
|----------|-------------------|-------------------|
|          | WT | LIMP2 KO | p value | WT | LIMP2 KO | p value |
| Males, 6 pairs, HFD, 6-h fast |     |           |         |     |           |         |
| Blood glucose (mg/dl) | 127.6 | 128.3 | 0.48 | 218.4 | 236.7 | 0.02 |
| Serum insulin (ng/dl) | 0.43 | 0.41 | 0.52 | 5.1 | 4.9 | 0.56 |
| Serum glucagon (pg/ml) | 0.01 | 0.01 | 0.65 | 0.06 | 0.05 | 0.26 |
| Serum leptin (ng/ml) | 0.9 | 0.8 | 0.43 | 7.2 | 7.3 | 0.83 |
| Serum cholesterol (mg/dl) | 86.4 | 88.2 | 0.11 | 172.4 | 210.4 | 0.06 |
| Serum triglyceride (mg/dl) | 26.2 | 27.8 | 0.26 | 120.4 | 153.8 | 0.03 |
| Serum FFA (mmol/l) | 0.2 | 0.2 | 0.82 | 1.2 | 1.1 | 0.68 |

Males, 4 pairs, HFD, 6-h fast
Alanine aminotransferase (normal = 10–190 units/liter) | 69.3 | 53.8 | 0.28 |
Aspartate aminotransferase (normal = 10–380 units/liter) | 119.3 | 94.4 | 0.28 |

triglycerides (Table 1), and blood glucose, whether fed, fasting, or fast-refeeding, was slightly but significantly higher in LIMP2 KO mice (Fig. 4F). At this time, serum ALT and AST levels were not elevated in the LIMP2 KO mice (Table 1), nor was their liver histology (H&E stain, Fig. S3) visibly different from WT littermates.

Discussion
The primary finding reported here is that elimination of the IGF2 mRNA-binding protein 2/IMP2 from adult mouse liver is accompanied by increased intrahepatic triglyceride deposition in mice fed a high-fat diet. This outcome was unexpected because transgenic overexpression of IMP2 in hepatocytes is accompanied by increased triglyceride deposition (11), and global elimination of IMP2 confers strong protection against fatty liver in mice fed a high-fat diet (8). We have no direct information concerning the mechanisms by which transgenic IMP2 overexpression causes hepatic steatosis; however, a plausible inference is that transgenic overexpression of IMP2 in hepatocytes modifies the abundance of client RNAs, including IGF2 mRNA (13) and perhaps including some mRNAs not recruited by the normally low levels of IMP2 in adult hepatocytes. Although the latter might be considered an "artifact" of IMP2 overexpression, the cohort of IMP2 RNA clients does vary in a tissue-specific manner, determined both by the abundance of IMP2 and of its candidate client RNAs. Regarding the protective effect of global IMP2 deficiency on liver triglyceride deposition in mice fed a HFD, the present...
results indicate strongly that this protection is entirely due to the lesser mass of white adipose tissue in Imp2-null mice and is probably offset slightly because of the diminished fatty acid oxidation of the IMP2-deficient liver. A caveat, however, is that we have not characterized lipid metabolism in hepatocytes derived from mice with germline Imp2 inactivation. It remains conceivable, therefore, that loss of IMP2 in endoderm or liver bud precursors might reprogram hepatocyte triglyceride metabolism in a manner distinct from that engendered by deletion using albumin-cre.

Regarding the mechanism by which IMP2 deficiency in the adult liver causes increased triglyceride deposition, we show that the mRNAs encoding PPARα, PPARγ, CPT-1A, FGF21, and SCD1 are IMP2 clients, and elimination of IMP2 was accompanied by substantially reduced levels of PPARα, PPARγ, and CPT1A mRNAs because of a 2-fold or more increased rate of mRNA turnover; the corresponding polypeptide levels were comparably reduced. Notably, expression of the Cpt1A gene was strongly up-regulated by PPARα, so that the somewhat greater reduction of CPT-1A mRNA consequent to Imp2 inactivation may reflect the loss of PPARα-stimulated Cpt1A transcription as well (24). Direct measurement of [1-14C]palmitate oxidation by intact hepatocytes and liver mitochondria demonstrated a modest reduction by those isolated from IMP2-deficient liver. In contrast, the mRNAs of all lipogenic genes examined (including SCD1) were unaltered in IMP2-deficient liver. This is somewhat surprising given the substantial drop in PPARγ in LIMP2 KO liver, which is generally considered to drive lipogenesis (25).

In summary, IMP2 binds and stabilizes the mRNAs encoding the critical regulators of hepatic fatty acid oxidation, PPARα and CPT-1A; loss of IMP2 diminishes the abundance of those mRNAs, resulting in reduced mitochondrial fatty acid oxidation. Over time, mice with hepatic IMP2 deficiency fed a high-fat diet show a modest, progressive accumulation of hepatic triglycerides beyond that of HFD-fed controls, ultimately reflected in elevated circulating triglycerides and mildly elevated blood glucose. Notably, however, evidence of liver damage is lacking, at least at 6 months age. The proclivity to increased hepatic triglyceride deposition caused by hepatocyte-selective IMP2 deficiency is entirely masked in mice with global IMP2 deficiency by their marked reduction in white adipose mass. For hepatocyte IMP2 deficiency to contribute to type 2 diabetes risk, the intronic SNPs in the human IMP2 gene associated with type 2 diabetes would have to cause a profound hepatocyte-selective decrease in IMP2 expression, an outcome that appears highly unlikely. Moreover, recent evidence indicates that the diabetogenic risk conferred by the intronic SNPs is attributable to a reduction of IMP2 abundance in β cells (26).

**Materials and methods**

**Animal studies**

Generation of floxed-IMP2 mice is described in Ref. 10. The albumin-cre mice were purchased from The Jackson Laboratory (stock no. 003574). Hemizygous Alb-Cre transgenic mice were crossed with mice homozygous for a floxed Imp2 allele (Imp2ff), the latter backcrossed onto the C57Bl/6j background for 12 generations. F1 pups with the Alb-Cre<sup>+/−</sup>/Imp2ff+ haplotype were crossed again with Imp2ff mice, generating F2 pups with four different genotypes: Alb-Cre<sup>+/−</sup>/Imp2ff (hereafter called homozygous LIMP2 KO), Imp2ff (referred to as WT), Alb-Cre<sup>+/−</sup>/Imp2ff+, and Imp2ff+; the latter two genotypes were not used in this study. All animal procedures were approved by the Institutional Animal Care and Use Committee of Massachusetts General Hospital. Mice were maintained on the C57Bl/6j background in a specific pathogen–free facility with a 12:12 h light-dark cycle. After weaning at 4 weeks, they were fed irradiated chow (Prolab 5P75 Isopro 3000, 5% crude fat, PMI Nutrition International) or a high-fat diet (D12492i, 60 kcal percent fat; Research Diets Inc.). Additional aspects of animal care were as described in Ref. 10.

**Glucose and insulin tolerance tests**

Mice were fasted overnight (16 h). Twenty percent D-glucose (Sigma) (1 g/kg of body weight) was administered by intraperitoneal injection. 0, 20, 40, 60, and 120 min after administration, blood was collected by tail vein bleeding. Glucose levels were measured with a One Touch Ultra AlphaTrak2 glucometer (Zoetis, Parsippany, NJ). For insulin tolerance tests, mice fed the high-fat diet were fasted for 5 h. Human insulin (Eli Lilly, 0.75 units/kg) was injected intraperitoneally. Blood was drawn from the tail vein 0, 20, 40, 60, and 90 min after injection, and glucose levels were measured as above.

**Serum analyses**

Blood was collected into EDTA-coated tubes (Sarstedt, Newton, NC). Serum was separated by centrifugation at 4 °C, frozen in liquid nitrogen, and assayed by the Vanderbilt University Mouse Metabolic Phenotyping Center.

**Liver lipid analyses**

Livers were collected, weighed, and snap-frozen in liquid nitrogen. Liver triglyceride content was measured by the Vanderbilt Hormone Assay and Analytical Service Core.

**Real-time PCR**

Total liver RNA was extracted directly using the Qiagen RNase kit, and 1 μg was used for complementary DNA preparation with random hexamer primers using Super Script III reverse transcriptase (Invitrogen). Steady-state mRNA expression was measured by quantitative real-time PCR using SYBR Green Master Mix (Bio-Rad) with a CFX96 real-time PCR system (Bio-Rad). Primer sequences for real-time PCRs are listed in Table S1.

**IMP2-associated RNAs**

Livers were extracted using a tissue homogenizer (Qiagen) for 10 min in ice-cold lysis buffer (140 mM KCl, 1.5 mM MgCl₂, 20 mM Tris-HCl (pH 7.4), 0.5% Nonidet P-40, 0.5 mM DTT, 1 unit/μl RNase inhibitor, and one complete EDTA-free protease inhibitor mixture tablet). The lysates were centrifuged for 10 min at 14,000 rpm, and the supernatant was transferred to a fresh 1.5-ml tube. Total protein was measured by Bradford
assay, and 5 mg of cytoplasmic lysate protein was subjected to immunoprecipitation as in Ref. 8. Lysates were incubated with 500 μl of protein A magnetic Dynabeads precoated with IMP2 antibody or nonimmune IgG and incubated for 6 h at 4°C with rotation. Dynabeads were washed extensively with lysis buffer five times and digested with DNase I and protease K. RNA was extracted with phenol/chloroform and precipitated with ethanol. Real-time PCR was performed to examine RNAs associated with cytoplasmic IMP2.

**Immunoblotting**

Proteins were extracted from the livers of HFD-fed WT male mice 6–8 weeks of age. Livers were homogenized using a tissue homogenizer (Qiagen) in ice-cold buffer (20 mM Tris (pH 7.5), 2.7 mM KCl, 1 mM MgCl2, 1% Triton X-100, 10% (v/v) glycerol, 1 mM EDTA, and 1 mM DTT) supplemented with protease (Thermo Scientific) and phosphatase inhibitor mixture (Millipore). Samples were then centrifuged at 13,000 rpm for 10 min at 4°C, and the supernatants were collected. The protein content of the supernatant was determined using a BCA assay (Thermo Scientific). 50 μg of each sample was loaded for Western blotting. Proteins were resolved on a 4% to 12% gradient SDS BisTris gel (Invitrogen). The IMP2 antibody is described in Ref. 4, and sources of other antibodies are shown in Table S1. All commercial antibodies were used at 1:1000 dilution, with specificity validated by the vendors.

**Fatty acid oxidation by liver mitochondria**

Liver mitochondria were isolated from 6-week-old male mice as described in Ref. 19. The protocol for the fatty acid oxidation assay was adapted from Ref. 20. Imp2ff and Alb-cre+/−/Imp2ff mitochondria were incubated in triplicate for 30 min at 37°C in a reaction mixture with 0.7% FFA-poor BSA, 500 μM palmitate, and 1 μCi [14C]palmitate (21). The reaction mixtures were then transferred to a new tube containing 1 M cold perchloric acid and, in the cap, a paper disc saturated with 1 M NaOH. After closing the cap, the tubes were incubated for 1 h at 37°C. [14C]radioactivity contained in the paper and the acidic fraction was measured by liquid scintillation counting.

**Polysome isolation**

AML12 cells were rinsed with PBS and lysed with ice-cold buffer (140 mM KCl, 1.5 mM MgCl2, 20 mM Tris–HCl, pH 7.4, 0.5% Nonidet P-40, 0.5 mM DTT, 1 μM RNase inhibitor, and one complete EDTA-free protease inhibitor mixture tablet) with 150 mg/ml cycloheximide, 1000 units/ml RNase inhibitor (Roche Diagnostics), and 40 mM vanadyl–ribonucleoside complex (Cayman Chemical). The lysate was centrifuged at 10,000 × g for 10 min at 4°C. The supernatant was applied to a linear 20%–47% sucrose gradient in 20 mM Tris–HCl (pH 8.0), 140 mM KCl, and 5 mM MgCl2, and subjected to centrifugation at 40,000 rpm for 3 h with Beckman SW41 rotors. 1-ml fractions were collected with concomitant measurement of the absorbance at 260 nm. RNAs were recovered from polysome-containing fractions by extraction with an equal volume of phenol–chloroform–isoamyl alcohol and analyzed as in Ref. 4.

**mRNA turnover**

AML12 cells were treated with actinomycin D (1 μM, Sigma). At the indicated times, the total RNAs were extracted, followed...
by DNase digestion and then complementary DNA synthesis. The amount of mRNA was quantified by real-time PCR.

**Author contributions**—L. R., J. A., and N. D. conceptualization; L. R. and N. D. investigation; L. R. methodology; L. R., L. M., J. A., and N. D. writing-review and editing; L. M. and J. A. resources; J. A. and N. D. supervision; J. A. and N. D. writing-original draft; J. A. project administration.

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