Supporting Information

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SI Text

Plasmids. pcDNA3.1-Flag-TIF-IA has been described (1). To generate GST-TIF-IA, the complete reading frame for TIF-IA was amplified by PCR and inserted into pGEX-4T1 (Amersham Pharmacia Biotech). Point mutants were constructed by overlap extension PCR, and the amplified DNA was verified by sequence analysis. The primer sequences are available upon request. pMrWT encompasses murine rDNA sequences from −324 to +292, $pHrP_2$ contains human rDNA sequences from −411 to +378. The reporter plasmid $pHrP_2$-BH contains a 5′-terminal human rDNA fragment (from −411 to +378) fused to a 3′-terminal rDNA region including two "Sal box" terminator elements. Expression plasmids for HA-tagged AMPK and the dominant-negative AMPK/DN mutants were gifts from Dr. K. L. Guan (San Diego, CA). GST and GST fusion proteins were expressed in *E. coli* BL21(DE3) codon plus and purified using Glutathione-Sepharose 4B (Amersham Pharmacia Biotech) according to the manufacturer's instructions.

Antibodies. Antibodies against human UBF, TIF-IA, Pol I, and TAF_Is have been described (2–5). Monoclonal antibodies against TIF-IA phosphorylated at Ser-635 were generated using the synthetic phosphopeptide FDTHFRSPpSSVG (amino acids 627–638 of human TIF-IA). Antibodies against FUrd, the Flag epitope (M2), and anti-Flag M2-agarose were purchased from Sigma, and anti-AMPK and anti-phospho-AMPK were obtained from Cell Signaling. Anti-HA antibody was a generous gift from E. Kremmer (Munich). For immobilization of GFP-TIF-IA, GFP-Trap agarose beads were obtained from Chromotek.

Expression and Purification of AMPK. Expression and purification of AMPK was done essentially as described in ref. 6. Briefly, HEK293T cells were transfected with expression plasmids for the αI subunit of AMPK or the kinase inactive mutant AMPK/DN (6). Before harvesting, cells were either treated for 30 min with 50 mM deoxy-glucose or cultured for 6 h in glucose-free DMEM. Cells were lysed in buffer A [50 mM Tris·HCl (pH 7.5), 50 mM NaF, 5 mM sodium pyrophosphate, 1 mM EDTA, 1 mM DTT, 10% glycerol, protease inhibitor mix (Complete, Roche), and 1% Triton X-100], and lysates were cleared by ultracentrifugation. AMPK was precipitated with anti-HA antibodies coupled to Protein G-agarose beads. After several washes in buffer A and buffer N [25 mM HEPES (pH 8.0), 0.5 mM EDTA, and 0.025% β-mercaptoethanol], AMPK was eluted from the beads using buffer AM-300 [20 mM Tris·HCl (pH 7.9), 200 mM KCl, 5 mM $MgCl_2$, 0.2 mM EDTA, and 10% glycerol] containing 0.1% Triton X-100 and 400 μg/mL HA-peptide. Eluted wild-type and mutant AMPK were subjected to Western blot analysis to compare relative amounts.

AMPK Assay. Activity of immunopurified AMPK was determined in a peptide-based kinase assay using the SAMS peptide (HMR-SAMSGLHLVKRR) (ENZO) as a substrate, essentially as described in ref. 7.

1. Zhao J, Yuan X, Frodin M, Grummt I (2003) ERK-dependent phosphorylation of the transcription initiation factor TIF-IA is required for RNA polymerase I transcription and cell growth. *Mol Cell* 11:405–413.
2. Heix J, et al. (1998) Mitotic silencing of human rRNA synthesis: Inactivation of the promoter selectivity factor SL1 by cdc2/cyclin B-mediated phosphorylation. *EMBO J* 17:7373–7381.
3. Seither P, Iben S, Grummt I (1998) Mammalian RNA polymerase I exists as a holoenzyme with associated basal transcription factors. *J Mol Biol* 275:43–53.
4. Bodem J, et al. (2000) TIF-IA, the factor mediating growth-dependent control of ribosomal RNA synthesis, is the mammalian homolog of yeast Rrn3p. *EMBO Rep* 1:171–175.
5. Voit R, et al. (1992) The nucleolar transcription factor mUBF is phosphorylated by casein kinase II in the C-terminal hyperacidic tail which is essential for transactivation. *EMBO J* 11:2211–2218.
6. Inoki K, Zhu T, Guan KL (2003) TSC2 mediates cellular energy response to control cell growth and survival. *Cell* 115:577–590.
7. Davies SP, Carling D, Hardie DG (1989) Tissue distribution of the AMP-activated protein kinase, and lack of activation by cyclic-AMP-dependent protein kinase, studied using a specific and sensitive peptide assay. *Eur J Biochem* 186:123–128.

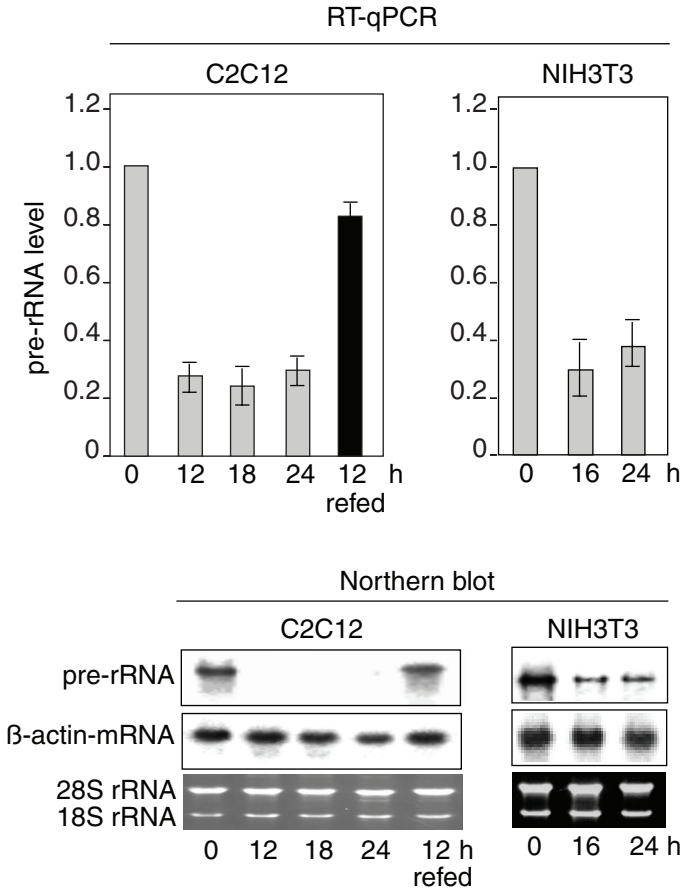

Fig. S1. Glucose restriction or AICAR treatment inhibits Pol I transcription in mouse C2C12 and NIH 3T3 cells. C2C12 (*Upper Left*) or NIH 3T3 cells (*Upper Right*) were cultured in glucose-free DMEM (gray bars) for the indicated times (0–24 h) or recultured in glucose-rich DMEM for 12 h after 12 h of glucose deprivation (black bar, refed). Pre-rRNA levels were determined by RT-qPCR and normalized to the amounts of β-actin mRNA. Data are from three independent experiments; error bars, the mean $\pm$ SD. (*Lower*) The Northern blots show the level of pre-rRNA (*Top*) and β-actin mRNA (*Middle*) upon glucose deprivation. (*Bottom*) Agarose gel stained with ethidium bromide.

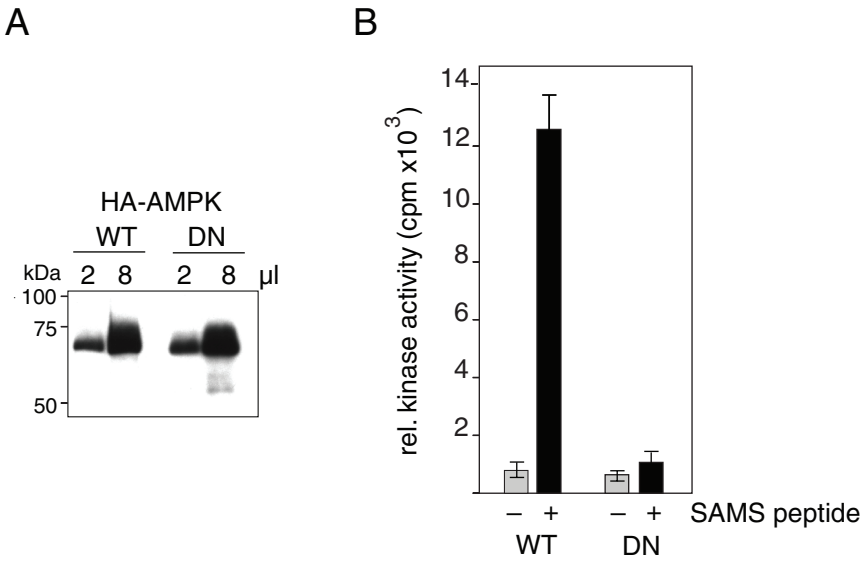

Fig. S2. Immunoprecipitation of HA-AMPK and determination of kinase activity. (*A*) HA-AMPK wild-type (WT) or the dominant-negative mutant HA-AMPK/DN (DN) were expressed in HEK293T cells, immunoprecipitated using HA-antibodies, and eluted from the beads with HA peptide. Two- and eight-microliter eluates were analyzed on immunoblots using α-HA antibody. (*B*) Kinase activity assay. Two microliters of HA-AMPK/WT or HA-AMPK/DN were tested in a peptide-based kinase assay using the AMPK substrate-peptide SAMS (black bar). Control reactions were performed in the absence of the SAMS peptide (gray bar). The graphs indicate the incorporation of ^{32}P into the peptide and are derived from three different experiments. Error bars, the mean $\pm$ SD.

A

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                      635
human        ...DTHFRSPSSSVGSPPVLY...

mouse        ...DTHFQSPSSSVPSPPVLY...
chicken      ...NSNTRSPVSSIGSPSDCH...
Xenopus      ...DSHLRSPSS-VGSPPAAF...
Zebra fish   ...ESHLQSPRS-VGSPPISF...
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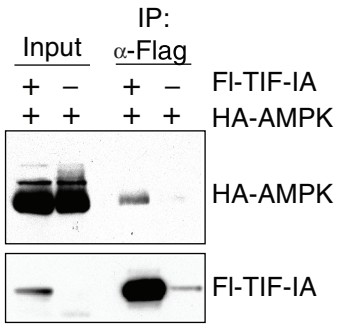
B



C

Spot	Tryptic phosphopeptide	phospho-serine
c	RSP**pS**SSVGSPPVLYMQPSPL	pS635
d	RSPSSSVGSPPVLYMQP**pS**PL	pS649 (WT)
d′	RSP**A**SSVGSPPVLYMQP**pS**PL	pS649 (S635A)
f	R**pS**PSSSVGSPPVLYMQP**pS**PL	pS633+pS649
g	KGEVPQNDTVIGITPSSFDTHFR**pS**PSSSVGSPPVLYMQP**pS**PL*	pS633+pS649

* partial tryptic cleavage

D

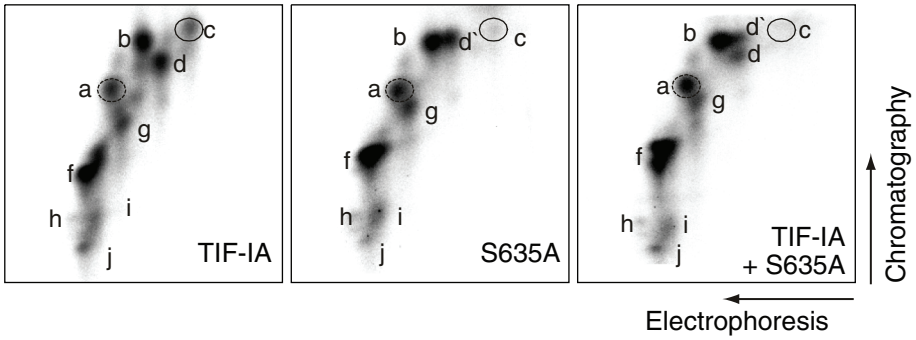


Fig. S3. TIF-IA is phosphorylated by AMPK at Ser-635 that is conserved among vertebrates. (*A*) The predicted AMPK site Ser-635 in human TIF-IA is conserved across vertebrates. Alignment of the C-terminal sequence of TIF-IA from different vertebrates. The serine residues identified as AMPK target sites by group-based phosphosite prediction (GPS 2.1) are shown in red. (*B*) TIF-IA interacts with AMPK. HEK293T cells expressing HA-AMPK and Flag-TIF-IA (Fl-TIF-IA) were incubated with 50 mM 2-deoxyglucose for 30 min before immunoprecipitation with α-Flag antibodies. Coprecipitated AMPK was detected on Western blots using anti-HA antibodies. (*C*) Phosphorylation of serine residues 633, 635, and 649 in the C-terminal part of TIF-IA generates four phosphopeptides with different mobilities in two-dimensional tryptic maps. The phosphorylation state of phosphopeptides c, d, f, and g from wild-type TIF-IA and d′ from TIF-IAS635A is indicated. (*D*) Substitution of serine 635 by alanine in TIF-IA changes the migration of the tryptic phosphopeptide d in two-dimensional phosphopeptide mapping. Fl-TIF-IA or Fl-TIF-IAS635A expressed in HEK293T cells were metabolically labeled with [^{32}P]-orthophosphate, immunoprecipitated, and subjected to tryptic phosphopeptide mapping (*Left* and *Middle*). (*Right*) Tryptic phosphopeptides from wild-type and mutant TIF-IA were mixed, resulting in two spots for peptide d (d and d′).

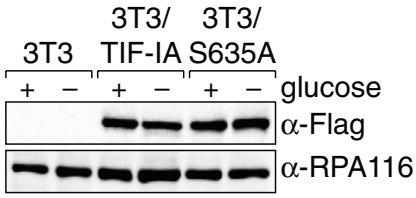


Fig. S4. Expression analysis of Flag-tagged TIF-IA and TIF-IAS635A in retrovirally infected NIH 3T3 cells. NIH 3T3 cells were infected with pBabe-puro based retroviruses coding for Fl-TIF-IA and Fl-TIF-IAS635A (3T3/TIF-IA and 3T3/S635A). In parallel, cells were infected with an empty pBabe-puro virus (3T3). After selection with puromycin, cells were cultured in the presence or absence of glucose for 12 h and expression of Fl-TIF-IA and Fl-TIF-IAS635A was analyzed on Western blots using α-Flag antibodies. As a loading control, blots were reprobed with antibodies against Pol I (RPA116).

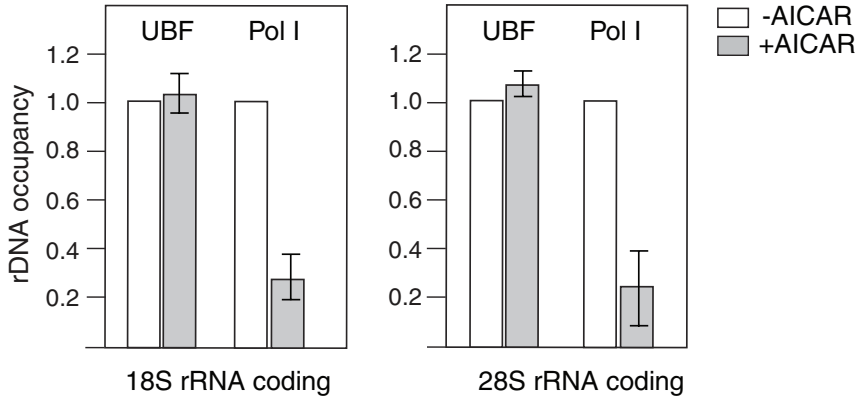


Fig. S5. Activation of AMPK impairs association of Pol I, but not of UBF, with the rRNA coding region. Cross-linked chromatin from HEK293T cells cultured in DMEM in the absence (white bars) or presence of AICAR (0.5 mM, 6 h; gray bars) was immunoprecipitated with antibodies against Pol I (RPA116) and UBF. Precipitated DNA was assayed by qPCR using primers (Table S1) that amplify sequences of the 18S (*Left*) and 28S (*Right*) rRNA coding regions. Amplified DNA was normalized to input DNA. The bar diagrams show rDNA occupancy in AICAR-treated cells relative to untreated cells. Error bars, the mean ± SD from two independent experiments.

Table S1. Sequences (5′ to 3′) of the primers used for PCR amplification of human rDNA in ChIP assays

	Forward primer sequence	Reverse primer sequence
Promoter −42/+32	GACGACAGGTCGCCAGAGGA	GGTATATCTTTCGCTCCGAG
18S rRNA coding region + 3990/+4092	CGACGACCCATTCGAACGTCT	CTCTCCGGAATCGAACCCTGA
28S rRNA coding region + 12855/12875	ACCTGGCGCTAAACCATTCGT	GGGACAAACCCTTGTGTCGAGG

Primer sequences are according to O'Sullivan et al. (1).

1. O'Sullivan AC, Sullivan GJ, McStay B (2002) UBF binding in vivo is not restricted to regulatory sequences within the vertebrate ribosomal DNA repeat. *Mol Cell Biol* 22:657–668.

Table S2. Sequences (5′ to 3′) of the primers used for RT-qPCR in this study

	Forward primer sequence	Reverse primer sequence
Human pre-rRNA	TGTCAGGCGTTCTCGTCTC	AGCACGACGTCACCACATC
Human rDNA reporter transcript	GTGCGTGTCAGGCGTTCTCG	CGTTGTAAAACGACGGCCAGT
Mouse pre-rRNA	CTCTTAGATCGATGTGGTGCTC	GCCCGCTGGCAGAACGAGAAG
Human β-actin mRNA	CGTCACCAACTGGGACGACA	CTTCTCGCGGTTGGCCTTGG
Mouse β-actin mRNA	GGCACCACACCTTCTACAATG	GGGGTGTTGAAGGTCTCAAC