LETTERS

Transcription of mammalian messenger RNAs by a nuclear RNA polymerase of mitochondrial origin

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Transcription of eukaryotic genes is performed by three nuclear RNA polymerases, of which RNA polymerase II is thought to be solely responsible for the synthesis of messenger RNAs[1]. Here we show that transcription of some mRNAs in humans and rodents is mediated by a previously unknown single-polypeptide nuclear RNA polymerase (spRNAP-IV). spRNAP-IV is expressed from an alternative transcript of the mitochondrial RNA polymerase gene (*POLRMT*). The spRNAP-IV lacks 262 amino-terminal amino acids of mitochondrial RNA polymerase, including the mitochondrial-targeting signal, and localizes to the nucleus. Transcription by spRNAP-IV is resistant to the RNA polymease II inhibitor α-amanitin but is sensitive to short interfering RNA specific for the *POLRMT* gene. The promoters for spRNAP-IV differ substantially from those used by RNA polymerase II, do not respond to transcriptional enhancers and contain a common functional sequence motif.

Replication and expression of the eukaryotic mitochondrial genome fully depends on the nuclear genome: during evolution, most of the genes derived from the DNA of the proto-mitochondrial endosymbiont and required for mitochondrial function were translocated to the nucleus, such that most components of the mitochondrial proteome are imported into the mitochondria[2,3]. The mitochondrial RNA polymerase (mtRNAP) is a homologue of to the bacteriophage single-polypeptide RNA polymerases and displaced the typical, multisubunit bacterial RNA polymerase during the evolution of the endosymbiont[4,5]. The mtRNAP is encoded in the spliced 3.8-kilo-base transcript[6] from the nuclear gene *POLRMT*. The human mtRNAP precursor has an N-terminal mitochondria-targeting peptide[6], which is cleaved from the protein during mitochondrial import[7]. The N-terminal structure of mtRNAP is supported by 19 available expressed sequence tags (ESTs); however, three ESTs correspond to an alternative structure, with a longer exon 1 including the proximal 224 base pairs (bp) of intron 1. The open reading frame (ORF) encoded by the alternative transcript is truncated at the N terminus and would specify a protein lacking the mitochondria-targeting peptide.

To verify the existence and to explore potential functions of an alternative, possibly non-mitochondrial product of the *POLRMT* gene, we designed primers to show variations within the 5′ region of the *POLRMT* transcripts. By sequencing polymerase chain reaction with reverse transcription (RT–PCR) products produced with these primers, we found that a fraction of the transcripts in human, mouse and rat cell lines correspond to the splice variant retaining intron 1 sequences (see Supplementary Fig. 1a, b), confirming that the transcripts specifying an N-terminally truncated mtRNAP are produced in both primates and rodents.

To determine whether the shorter polypeptide lacking the mitochondrial targeting peptide is indeed synthesized, we analysed proteins that react with antibodies to a carboxy-terminal peptide of mtRNAP (ref. 8). Confocal microscopy revealed both mitochondrial and nuclear localization of immunoreactive proteins in HeLa cells (Fig. 1a). On western blots, total cell extracts showed two protein bands of ~135 kDa and ~110 kDa. In isolated mitochondria there was a single band of ~135 kDa, whereas nuclear extracts contained only the ~110 kDa band (Fig. 1b). Total extracts from the mitochondrial DNA-depleted ρ0 HeLa cells[9] (see Supplementary Fig. 2a) contained reduced amounts of the mitochondrial 135 kDa form, whereas the nuclear 110 kDa protein was unaffected (Fig. 1b). Inhibition of the *POLRMT* gene by RNA interference (RNAi) resulted in abrogation of both nuclear and mitochondrial immunostaining (see Supplementary Fig. 2b) and almost complete disappearance of the two protein forms (Fig. 1b) and both transcripts from the *POLRMT* gene (see Supplementary Fig. 1a). By contrast, RNAi against the proximal portion of intron 1 caused abrogation of nuclear immunostaining concomitant with the disappearance of the nuclear 110 kDa band, with no changes in mitochondria (Fig. 1d; see also Supplementary Fig. 2b).

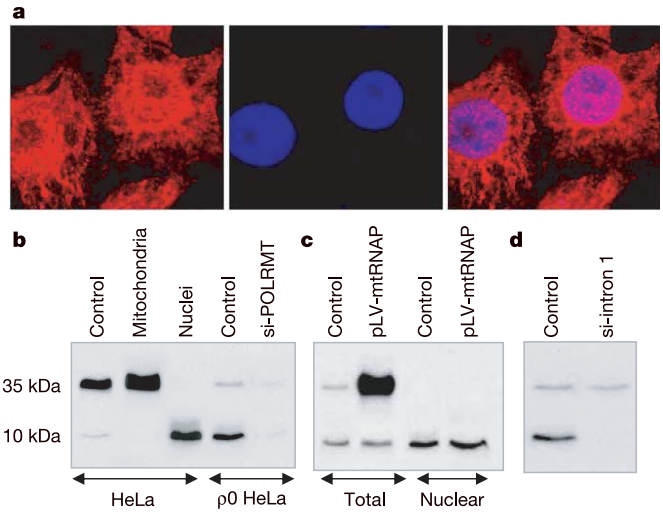

Figure 1 | The *POLRMT* gene is a source of mitochondrial and nuclear protein products. a, Confocal microscopy of HeLa cells stained with antibodies to *POLRMT* peptide and TRITC anti-rabbit IgG counterstained for nuclei with Hoehst 33258. **b**, Two *POLRMT* gene products detected by western blotting in HeLa (three left lanes) and ρ0 HeLa cells (two right lanes) with antibodies to *POLRMT* peptide. Shown are total cell extracts (lanes 1, 4, 5), mitochondrial (lane 2) and nuclear (lane 3) fractions, and sample from cells expressing siRNA to exons 3 and 17 of human *POLRMT* gene (lane 5). **c**, Western blot showing the expression of the mtRNAP-encoding ORF in ρ0 HeLa cells. **d**, Western blot with antibodies to the *POLRMT* peptide showing the expression of siRNA targeting *POLRMT* intron 1 in ρ0 HeLa cells.

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Overexpression of the complementary DNA encoding the full-length mtRNAP (ref. 6) caused a substantial increase in the mitochondrial 135-kDa band but no increase in the 110-kDa nuclear form (Fig. 1c; see also Supplementary Fig. 2b), suggesting that the latter protein does not originate from the mtRNAP mRNA neither through internal initiation of translation nor processing of the 135 kDa precursor. We then overexpressed the predicted alternative ORF and, separately, the mtRNAP ORF in ρ0 HeLa cells, both tagged with a C-terminal Flag epitope. Immunostaining showed exclusively mitochondrial localization of the overexpressed mtRNAP and exclusively nuclear localization of the protein encoded by the alternative ORF (Fig. 2a). The electrophoretic mobility of the overexpressed Flag-tagged proteins corresponded precisely to the two endogenous bands as shown with POLRMT-specific antibodies (Fig. 2b), although the size of the nuclear protein determined by electrophoresis (~110 kDa) was much smaller than the size of the alternative ORF product predicted from the mRNA sequence (~130 kDa). Attaching an N-terminal Flag to the alternative ORF resulted in a protein that did not react with anti-Flag antibodies but reacted with antibodies to mtRNAP (Fig. 2c), suggesting that the first ATG was not used for the translation initiation. Indeed, the product of an N-terminally truncated alternative ORF fragment starting at the first initiation codon conserved between mouse and human (ATG-6) was identical in size to the endogenous nuclear protein, whereas the fragment starting at ATG-8 produced a smaller protein (see Supplementary Figs 3 and 4). Thus, the nuclear form of the protein seems to start at ATG-6 of the ORF and correspond to the 968 C-terminal amino acids of mtRNAP, which include the entire catalytic domain (see Supplementary Fig. 3).

Our findings suggest that the *POLRMT* gene specifies two single-polypeptide polymerases, one (mtRNAP) targeted to the mitochondria, and the other (spRNAP-IV) having a putative function in the nucleus. Conceivably, spRNAP-IV could be the fourth nuclear RNA polymerase of animal cells, although alternative functions of this protein, other than direct involvement in transcription, can not be ruled out. To determine whether or not spRNAP-IV is important for cell viability, we inhibited the expression of *POLRMT* gene by siRNA expression. This resulted in a substantial growth retardation and cell death, which was reversed by co-expression of recombinant spRNAP-IV (Fig. 2d). Therefore, spRNAP-IV seems to have a distinct role in cell physiology.

In an attempt to identify genes that could be transcribed by the putative spRNAP-IV, we hypothesized that transcription of these genes would be resistant to α-amanitin, a specific inhibitor of RNA polymerase II (RNAP-II). Hybridization of a total RNA probe from HeLa cells treated with α-amanitin with an Affymetrix microarray U133A showed ~70 upregulated (twofold or greater) transcripts. Of the 15 transcripts tested by RT–PCR, four showed lack of sensitivity to or even stimulation by α-amanitin (see Supplementary Fig. 5a, b). A similar pattern was observed for the orthologous transcripts in mouse 3T3 cells (Supplementary Fig. 5c). In northern hybridization, the α-amanitin-resistant transcripts encoding the zinc-finger BTB domain-containing protein 1 (*ZBTB1*), prenylcysteine oxidase (MGC3265) and aldehyde dehydrogenase 8A1 (*ALDH12*) genes showed dose-dependent stimulation by α-amanitin, whereas control transcripts from the $p21^{WAF1/CIP1}$ (*CDKN1A*) and *GAPDH* genes were sensitive to α-amanitin (Fig. 3a; see also Supplementary Fig. 6). When RNAP-I or RNAP-II was inhibited by RNAi, gradual decrease and disappearance of transcripts encoding each of these polymerases was observed by day 4, which correlated with substantial stimulation of *ZBTB1*, MGC3265 and *ALDH12* transcripts (Fig. 3b). Inhibition of the largest subunit of RNAP-III with siRNA oligonucleotides also resulted in slightly increased levels of all three transcripts (see Supplementary Fig. 6b). Thus, these genes apparently are not transcribed by known nuclear RNA polymerases. By contrast, inhibition of *POLRMT* transcripts in ρ0 HeLa cells by RNAi was accompanied by a dramatic decrease in transcripts from the α-amanitin-resistant genes (Fig. 3c), which was reversed by overexpression of the spRNAP-IV

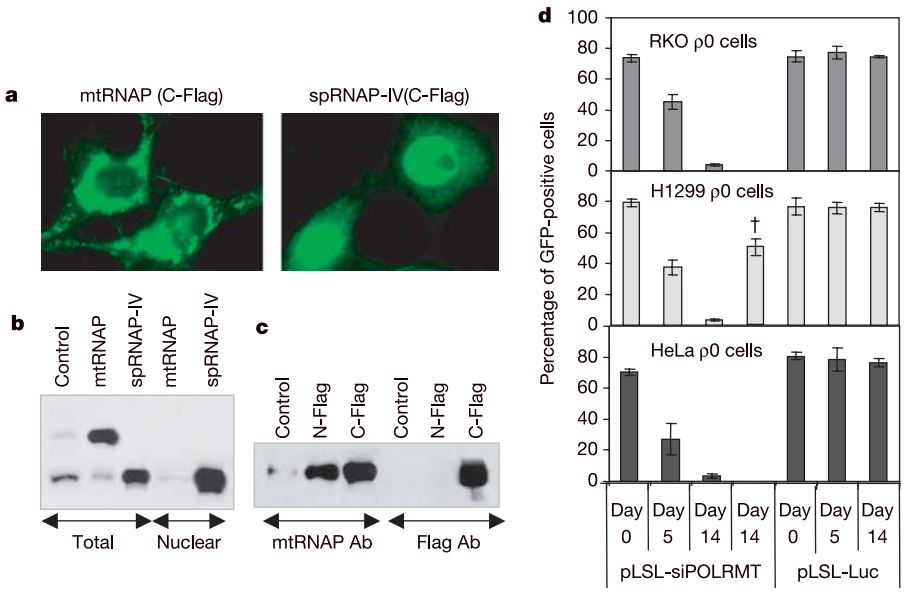

Figure 2 | Two distinct *POLRMT*-specific polypeptides are encoded by alternative transcripts. **a**, Immunostaining with anti-Flag antibodies of HeLa cells transduced with Flag-tagged ORF for mtRNAP (left panel) or Flag-tagged alternative ORF encoding spRNAP-IV (right panel). **b**, Western blot analysis of proteins reactive with antibodies to a *POLRMT* peptide after overexpression of mtRNAP ORF (lanes 2 and 4) or spRNAP-IV ORF (lanes 3 and 5). Total cell extracts (lanes 2 and 3) or nuclei (lanes 4 and 5) are shown. **c**, Western blot analysis of HeLa cells transduced with spRNAP-IV Flag-tagged at the N- or C terminus and stained with antibodies to *POLRMT* (left) or to Flag (M2). **d**, Expression of POLRMT siRNA affects cell growth. Specified ρ0 cell lines were infected with lentivirus vector LSLG co-expressing GFP and siRNA targeting *POLRMT* exon 3 (or exon 17, which produced a similar result), or GFP and siRNA targeting luciferase. The infected cells were mixed with uninfected cells, and the proportion of fluorescent cells was monitored by FACS. A control sample of H1299 ρ0 HeLa cells was co-infected with lentivirus vector expressing spRNAP-IV ORF (indicated by a dagger). Data represent the means of two experiments with standard deviation (s.d.).

ORF (Fig. 3d). These results indicate that the truncated nuclear protein product of the *POLRMT* gene participates in the production of certain mRNAs.

To examine the repertoire of transcripts affected by spRNAP-IV, we monitored changes in the expression pattern in ρ0 HeLa cells after 48 h expression of siRNA targeting the *POLRMT* gene using hybridization to a cDNA microarray. Of ~20,000 transcripts that gave a positive signal, ~1,000 transcripts were substantially inhibited (twofold or greater), whereas ~300 transcripts showed comparable levels of upregulation (see Supplementary Table 1). Certainly, some of the changes might be attributed to secondary effects of inhibition of the true spRNAP-IV-regulated genes. Also, cooperation between spRNAP-IV and RNAP-II in the expression of certain genes cannot be ruled out. Of 17 randomly selected transcripts that showed down-regulation on the microarray, nine demonstrated an α-amanitin-resistant/inducible expression when assayed by RT–PCR (data not shown), suggesting that the number of mammalian spRNAP-IV-dependent genes might be in the hundreds. Upregulation of some of these transcripts by α-amanitin was confirmed by northern analysis in three different ρ0 HeLa cell lines (see Supplementary Fig. 7).

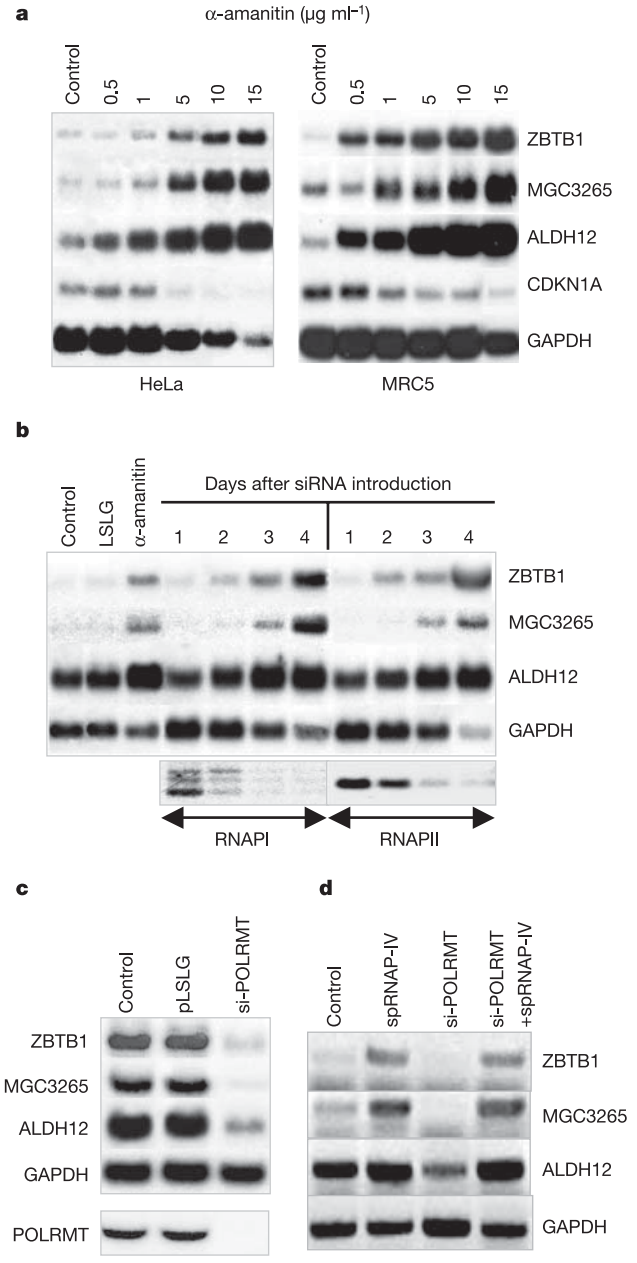

Figure 3 | A nuclear protein product of the *POLRMT* gene participates in the expression of the *ZBTB1*, MGC3265 and *ALDH12* genes. a, Northern blot showing the dose-dependent stimulation of *ZBTB1*, MGC3265 and *ALDH12* transcripts in HeLa and MRC5 cells treated for 14 h with α-amanitin. **b**, Northern blot analysis showing time-dependent stimulation of the transcripts following infection of HeLa cells with lentiviral vector LSLG expressing siRNAs targeting RNAP I or RNAP II. Inhibition of RNAP I and II mRNAs is shown on the lower panels. **c**, Northern blot showing the inhibition of the transcripts 4 days after infection of ρ0 HeLa cells with LSLG lentivirus expressing siRNA targeting *POLRMT*. **d**, Northern blot showing the stimulation of the same transcripts after infection with a lentiviral vector expressing spRNAP-IV ORF (RT–PCR).

To analyse promoters used for initiation of transcription by spRNAP-IV, we amplified and cloned the upstream regions of *ALDH12* and *ZBTB1* genes into a reporter plasmid. When they were transfected into HeLa cells, both putative spRNAP-IV promoters supported luciferase expression with activities comparable to that of the SV40 early promoter (Fig. 4a). The expression of luciferase driven by the *ALDH12* and *ZBTB1* promoters was resistant to α-amanitin, in contrast to the α-amanitin-sensitive expression from the SV40 promoter (Fig. 4a). Inhibition of *POLRMT* by RNAi 24 h before the transfection of reporter constructs led to a decreased activity of the *ALDH12* promoter but did not affect the SV40 promoter (Fig. 4b). To determine whether spRNAP-IV binds to the *ALDH12* promoter, formaldehyde-fixed extracts of HeLa cells transfected with an empty vector or with the constructs bearing *ALDH12* and SV40 promoters were precipitated with antibodies to RNAP-II or *POLRMT*, and the number of ampicillin-resistant colonies indicating the efficiency of immunoprecipitation was determined by transformation of *Escherichia coli*. For the *ALDH12*-promoter-containing plasmid, substantial enrichment was observed with the *POLRMT* antibodies; a tenfold lower enrichment was seen with antibodies to RNAP-II, suggesting that spRNAP-IV preferentially binds to this construct. The reciprocal enrichment was observed with the SV40 promoter construct (Fig. 4c).

A comparison of the upstream sequences of the *ALDH12*, *ZBTB1* and MGC3265 genes revealed two shared motifs (see Supplementary Fig. 8); counterparts to these motifs were also identified in several additional genes putatively transcribed by spRNAP-IV, including *ULK1* (data not shown). Both motifs were conserved among the orthologous genes from other mammals (see Supplementary Fig. 8). The putative spRNAP-IV promoter motifs showed no detectable similarity to the sequences of RNAP I–III promoters[10,11], those of the known transcription-factor-binding sites[12], the sequences of mtDNA that are involved in mtRNAP-directed transcription, or the promoters of T-odd bacteriophage genes transcribed by single-polypeptide phage polymerases homologous to mtRNAP. Deletion of the sequences spanning these motifs from the *ALDH12*, *ZBTB1* and MGC3265 promoters abrogated α-amanitin-resistant expression of luciferase, suggesting that the motifs are, indeed, functional elements of spRNAP-IV promoters (see Supplementary Fig. 9).

Transcription initiated by RNAP-II can be substantially activated by transcription factors that bind to transcriptional enhancers[1]. We tested whether the activity of promoters of spRNAP-IV-controlled genes could be stimulated by placing a strong cytomegalovirus (CMV) enhancer close to *ALDH12*, SV40 and mitochondrial DNA H-strand (mtHs) promoters in a luciferase reporter plasmid. On transfection into HeLa cells, the CMV enhancer stimulated α-amanitin-sensitive transcription from the SV40 promoter by more than tenfold. By contrast, the *ALDH12* promoter fragment did not respond to the enhancer at all, and its basal activity remained responsive to stimulation by α-amanitin. Similarly, in HeLa cells producing the artificial transcription factor tTA[13], the activity of the *ALDH12* promoter did not change when coupled with the tetracycline-responsive element (see Supplementary Fig. 10), confirming that RNAP-II enhancers do not affect transcription by spRNAP-IV. The mtHs promoter was inactive in this system, suggesting that the

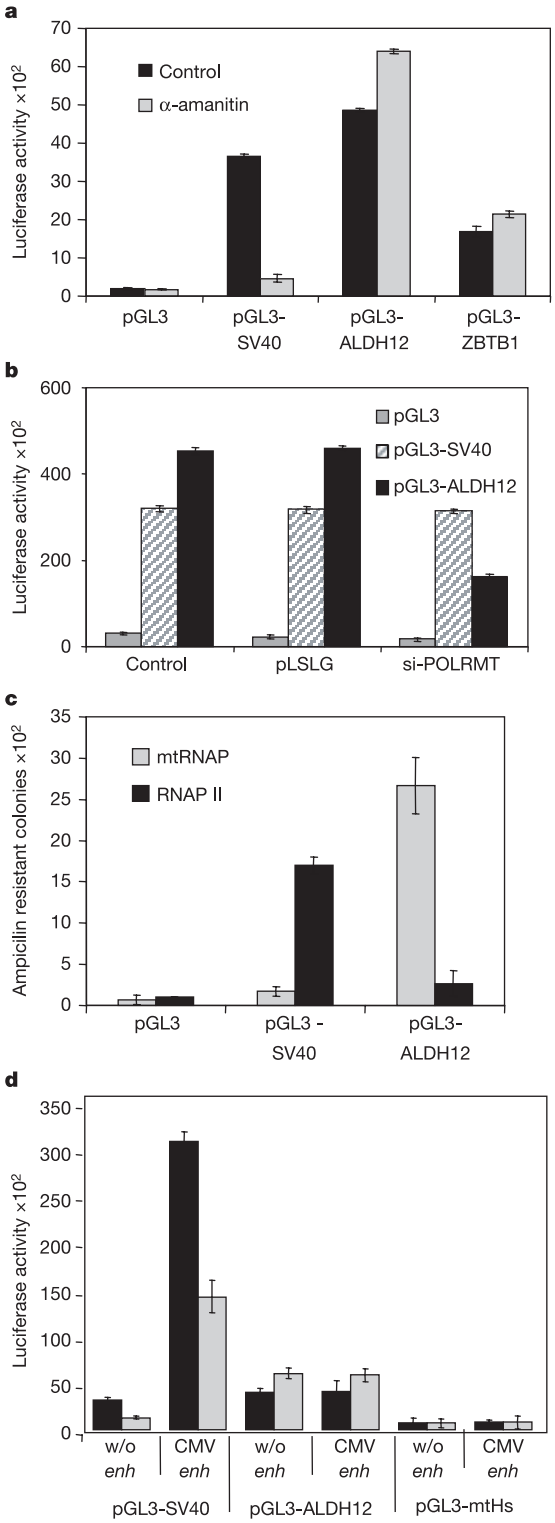

Figure 4 | Transcription from the *ALDH12* and *ZBTB1* promoters depends on spRNAP-IV. a, Luciferase assay measuring pGL3-luc constructs containing *ALDH12* (−482 to −12) and *ZBTB1* (−330 to +12) promoters that were transfected into HeLa cells (48 h). The cultures were treated with α-amanitin for 12 h. Data represent the means of two experiments with s.d. **b**, Suppression of *ALDH12* promoter by siRNA to *POLRMT*. Twenty-four hours after infection of HeLa cells with siRNA-expressing LSLG constructs the cells were transfected with pGL3-ALDH12 (−482 to −12 upstream fragment) or control plasmids and assayed for luciferase activity over 48 h. Data represent the means of two experiments with s.d. **c**, Chromatin immunoprecipitation of promoter-containing plasmids transfected into HeLa cells with antibodies to RNAP II or *POLRMT*. The plasmids contained either *ALDH12* (−290 to −12) or SV40 promoters. Four experiments were averaged. Error bars indicate s.d. **d**, CMV-enhancer (−600 to −112, CMV enh) does not cooperate with *ALDH12* promoter (−290 to −12). Forty-eight hours after transfection of the promoter-containing pGL3-luc constructs (*ALDH12*, mtH-strand promoter and SV40 early promoter) the cultures were treated with α-amanitin for 12 h and assayed for luciferase. Data represent the means of two experiments with s.d.

regulation of spRNAP-IV transcription in the nucleus is distinct from that of the mtRNAP in the mitochondria (Fig. 4c).

Identification of spRNAP-IV as a second polymerase transcribing mRNAs in mammals raises a wealth of questions for future investigation. In particular, it remains to be determined at what stage of eukaryotic evolution was a single-polypeptide RNA polymerase recruited for a nuclear role, what is the repertoire of genes transcribed by spRNAP-IV, how is the spRNAP-IV-dependent transcription regulated, how RNAP-II and spRNAP-IV divide and/or share their tasks in the transcription of mRNAs, and whether spRNAP-IV participates in gene silencing, similarly to the recently identified structurally unrelated RNAP IV of *Arabidopsis*[14,15]. A further important question is what are the transcription factors that affect spRNAP-IV function? The fact that spRNAP-IV promoters share no sequence similarity with mtDNA and that three known transcription factors (mtTFA, mtTFB1 and mtTFB2) that cooperate with mtRNAP seem to localize exclusively to the mitochondria[16–18] suggest that spRNAP-IV might use a distinct set of factors for transcription of nuclear genes.

METHODS

Cell culture. Human carcinoma cell lines HeLa, A431, H1299 and RKO, normal human lung fibroblasts MRC5, mouse fibroblasts 3T3 and rat fibroblasts Rat1 were cultured in Dulbecco's modified Eagle medium (DMEM), supplemented with 10% fetal bovine serum (HyClone) and penicillin/streptomycin. Mitochondrial DNA-deficient ρ0 HeLa were obtained, subcloned and cultured as described[19]. The absence of mtDNA was confirmed by PCR. The mitochondria in control and ρ0 cells were stained with MitoTracker Red 589 (Invitrogen).

Northern analysis. Radioactive probes were prepared from DNA inserts of appropriate I.M.A.G.E. Consortium clones: *RNAP I* large subunit, I.M.A.G.E: 4413694; *RNAP II* large subunit, I.M.A.G.E: 4359716; *POLRMT*, I.M.A.G.E. 6572256; *GAPDH*, MGC:2031, I.M.A.G.E. 3543589; *p21 (CDKN1A)*, MGC:97077, I.M.A.G.E:7262289; *ALDH12*, I.M.A.G.E. 4998434; MGC3265, I.M.A.G.E: 3506623; *ZBTB1*, MGC:60335, I.M.A.G.E: 6141266.

RT–PCR amplification. Total RNA that was isolated by Trizol reagent was converted to cDNA using SuperScript II reverse transcriptase (Invitrogen). PCR amplifications (typically 20–25 cycles) were performed with primers specified in the figure legends.

Recombinant constructs. Inhibition of expression of different genes was achieved by infection with recombinant lentiviral vector constructs (pLSLG) expressing hairpin siRNAs controlled by H1 RNA gene promoter as previously described[20]. The following 19–21-bp regions corresponding to appropriate mRNAs were present in the hairpin transcripts: *RNAP I* large subunit, 5′-GTTTACAAGCCACTGAATCGC-3′ and 5′-GAAACCAGCTTCCAGTTTCTG-3′; *RNAP II* large subunit, 5′-GAACTATTCTCCAACCAGTCC-3′ and 5′-GATACACACCACAGTCTCCAA-3′; exon 3 of *POLRMT* gene, 5′-CAAAGATACTGGAGAAGGATA-3′; exon 17 of *POLRMT* gene, 5′-CAACACACGTAAGCAGAAGAA-3′; intron 1 of *POLRMT* gene, 5′-GGCAAAGAAGGTAACACAA-3′. For the expression of recombinant mtRNAP cDNA[6] (a gift from V. Tiranti) or truncated forms of mtRNAP we used modified lentiviral expression vector pLV (ref. 21) (from I. Verma). Preparation of high-titre lentiviral stocks and infection of target cell cultures were performed as described[21].

Chromatin immunoprecipitation. pGL3-luc (Promega) constructs containing the 278-bp upstream region of human *ALDH12* gene (−290 to −12) or the 202-bp SV40 early promoter were lipofected into HeLa cells. The cells were fixed with 1% formaldehyde 24 h thereafter and the prepared cell extracts[22] were incubated overnight with antibodies to either mtRNAP (1:200) or RNAP II (N-2, sc-899, Santa Cruz Biotech) and Protein G Agarose beads. The beads were washed six times with 20 mM Tris-HCl, 200 mM NaCl, 0.5% Triton-X100, 0.05% deoxycholate, 0.1% NP-40, 1 mM phenylmethylsulphonyl fluoride (PMSF)

containing 250 mM LiCl, and precipitates were eluted with 1% SDS and 200 mM NaCl. The samples were heated at 65 °C for 6 h, extracted with phenol/chloroform, ethanol-precipitated and transfected as five independent parallel samples into competent *DH5α E. coli*. The number of ampicillin-resistant colonies representing immunoprecipitated plasmids was counted.

Microarray hybridizations. The microarray hybridizations were performed using total RNA from HeLa or ρ0 HeLa cells treated with 10 μg ml^{-1} α-amanitin for either 12 h or 48 h after introduction of siRNA-expressing lentivirus vector. The U133A or U133 2.0 Plus human cDNA microarrays were used at the Gene Expression Array Core Facility (Case Western Reserve University) according to Affymetrix protocols.

Western analysis. Samples of total protein, mitochondrial and nuclear fractions were isolated as described[23], heated at 95 °C, separated in 4–12% SDS polyacrylamide electrophoresis (SDS–PAGE), transferred to nitrocellulose membranes and probed with antibodies raised to a peptide (vnlepsdvpqdvy) of human mtRNAP (gift from G. Shadel) or anti-Flag antibodies M2 (Sigma-Aldrich). The filters were developed with peroxidase-labelled anti-rabbit polyclonal antibodies and ECL-Plus Western detection reagents (Amersham Biosciences).

Immunostaining. Cells were fixed with 4% formaldehyde in phosphate-buffered saline (PBS) for 20 min, washed and incubated overnight at 4 °C with anti-mtRNAP or anti-Flag M2 antibodies (1:200) in blocking buffer[23]. TRITC-labelled anti-rabbit or FITC-labelled anti-mouse (FITC) antibodies (Jackson Immune Research) were used for immunostaining.

Luciferase assays for promoter activity. Upstream regions of *ALDH12*, *ZBTB1* and MGC3265 genes and mitochondrial DNA H-strand promoter (mtHs) were cloned into pGL3-luc reporter vector (Promega). The plasmids were transfected into HeLa or ρ0 HeLa cells using Lipofectamine reagent (Invitrogen), along with a plasmid expressing β-galactosidase for normalization. The activity of luciferase was measured in cell extracts with the Luciferase assay system (Promega) using a fluorimetric plate reader.

Computational methods. Amino acid sequence alignments were constructed using the MACAW program[24]. The search for nucleotide motifs in promoter regions and statistical assessment of the detected motifs was performed using the ParaMEME[25], Gibbs sampling[26], CONSENSUS[27] and MULTIPROFILER[28] methods. Search for known transcription-factor binding sites was performed using the MATCH program[29]. The conservation of the promoter sequences in orthologous mammalian genes was determined using the BLAT server[30].

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1. Lewin, B. *Genes VIII* 597–626 (Pearson Prentice Hall, New Jersey, 2004).
2. Kurland, C. G. & Andersson, S. G. Origin and evolution of the mitochondrial proteome. *Microbiol. Mol. Biol. Rev.* **64,** 786–820 (2000).
3. Gabaldon, T. & Huynen, M. A. Reconstruction of the proto-mitochondrial metabolism. *Science* **301,** 609 (2003).
4. Masters, B. S., Stohl, L. L. & Clayton, D. A. Yeast mitochondrial RNA polymerase is homologous to those encoded by bacteriophages T3 and T7. *Cell* **51,** 89–99 (1987).
5. Gray, M. W. & Lang, B. F. Transcription in chloroplasts and mitochondria: a tale of two polymerases. *Trends Microbiol.* **6,** 1–3 (1998).
6. Tiranti, V. *et al.* Identification of the gene encoding the human mitochondrial RNA polymerase (h-mtRPOL) by cyberscreening of the Expressed Sequence Tags database. *Hum. Mol. Genet.* **6,** 615–625 (1997).
7. Taroni, F. & Rosenberg, L. E. The precursor of the biotin-binding subunit of mammalian propionyl-CoA carboxylase can be translocated into mitochondria as apo- or holoprotein. *J. Biol. Chem.* **266,** 13267–13271 (1991).
8. Seidel-Rogol, B. L. & Shadel, G. S. Modulation of mitochondrial transcription in response to mtDNA depletion and repletion in HeLa cells. *Nucleic Acids Res.* **30,** 1929–1934 (2002).
9. King, M. P. & Attardi, G. Injection of mitochondria into human cells leads to a rapid replacement of the endogenous mitochondrial DNA. *Cell* **52,** 811–819 (1988).
10. Smale, S. T. & Kadonaga, J. T. The RNA polymerase II core promoter. *Annu. Rev. Biochem.* **72,** 449–479 (2003).
11. Paule, M. R. & White, R. J. Survey and summary: transcription by RNA polymerases I and III. *Nucleic Acids Res.* **28,** 1283–1298 (2000).
12. Matys, V. *et al.* TRANSFAC: transcriptional regulation, from patterns to profiles. *Nucleic Acids Res.* **31,** 374–378 (2003).
13. Gossen, M. & Bujard, H. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc. Natl Acad. Sci. USA* **89,** 5547–5551 (1992).
14. Onodera, Y. *et al.* Plant nuclear RNA polymerase IV mediates siRNA and DNA methylation-dependent heterochromatin formation. *Cell* **120,** 613–622 (2005).
15. Herr, A. J., Jensen, M. B., Dalmay, T. & Baulcombe, D. C. RNA polymerase IV directs silencing of endogenous DNA. *Science* **308,** 118–120 (2005).
16. Rantanen, A., Jansson, M., Oldfors, A. & Larsson, N. G. Downregulation of Tfam and mtDNA copy number during mammalian spermatogenesis. *Mamm. Genome* **12,** 787–792 (2001).
17. Falkenberg, M. *et al.* Mitochondrial transcription factors B1 and B2 activate transcription of human mtDNA. *Nature Genet.* **31,** 289–294 (2002).
18. McCulloch, V. & Shadel, G. S. Human mitochondrial transcription factor B1 interacts with the C-terminal activation region of h-mtTFA and stimulates transcription independently of its RNA methyltransferase activity. *Mol. Cell. Biol.* **23,** 5816–5824 (2003).
19. King, M. P. & Attardi, G. Isolation of human cell lines lacking mitochondrial DNA. *Methods Enzymol.* **264,** 304–313 (1996).
20. Budanov, A. V., Sablina, A. A., Feinstein, E., Koonin, E. V. & Chumakov, P. M. Regeneration of peroxiredoxins by p53-regulated sestrins, homologs of bacterial AhpD. *Science* **304,** 596–600 (2004).
21. Pfeifer, A., Ikawa, M., Dayn, Y. & Verma, I. M. Transgenesis by lentiviral vectors: lack of gene silencing in mammalian embryonic stem cells and preimplantation embryos. *Proc. Natl Acad. Sci. USA* **99,** 2140–2145 (2002).
22. Kaeser, M. D. & Iggo, R. D. Chromatin immunoprecipitation analysis fails to support the latency model for regulation of p53 DNA binding activity *in vivo*. *Proc. Natl Acad. Sci. USA* **99,** 95–100 (2002).
23. Harlow, E. & Lane, D. *Using Antibodies: A Laboratory Manual* 276–301; 103–148 (Cold Spring Harbor Laboratory Press, New York, 1999).
24. Schuler, G. D., Altschul, S. F. & Lipman, D. J. A workbench for multiple alignment construction and analysis. *Proteins* **9,** 180–190 (1991).
25. Grundy, W. N., Bailey, T. L. & Elkan, C. P. ParaMEME: a parallel implementation and a web interface for a DNA and protein motif discovery tool. *Comput. Appl. Biosci.* **12,** 303–310 (1996).
26. Lawrence, C. E. *et al.* Detecting subtle sequence signals: a Gibbs sampling strategy for multiple alignment. *Science* **262,** 208–214 (1993).
27. Hertz, G. Z. & Stormo, G. D. Identifying DNA and protein patterns with statistically significant alignments of multiple sequences. *Bioinformatics* **15,** 563–577 (1999).
28. Keich, U. & Pevzner, P. A. Subtle motifs: defining the limits of motif finding algorithms. *Bioinformatics* **18,** 1382–1390 (2002).
29. Kel, A. E. *et al.* MATCH: A tool for searching transcription factor binding sites in DNA sequences. *Nucleic Acids Res.* **31,** 3576–3579 (2003).
30. Kent, W. J. BLAT–the BLAST-like alignment tool. *Genome Res.* **12,** 656–664 (2002).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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