Biochemical and Biophysical Research Communications 379 (2009) 489–493

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Positive modulation of RNA polymerase III transcription by ribosomal proteins

Giorgio Dieci [a,*], Roberta Ruotolo [a], Priscilla Braglia [a,1], Christophe Carles [b], Andrea Carpentieri [c], Angela Amoresano [c], Simone Ottonello [a,*]

[a] Dipartimento di Biochimica e Biologia Molecolare, Università degli Studi di Parma, Viale G.P. Usberti 23/A, 43100 Parma, Italy
[b] Commissariat à l'Énergie Atomique, Institut de Biologie et de Technologies de Saclay, SBIGeM, F-91191 Gif sur Yvette Cedex, France
[c] Dipartimento di Chimica Organica e Biochimica, Università di Napoli Federico II, Via Cinthia 4, 80126 Napoli, Italy

ARTICLE INFO

Article history:
Received 11 December 2008
Available online 29 December 2008

Keywords:
RNA polymerase III
tRNA
Ribosomal proteins
Saccharomyces cerevisiae
TFIIIE

ABSTRACT

A yeast nuclear fraction of unknown composition, named TFIIIE, was reported previously to enhance transcription of tRNA and 5S rRNA genes *in vitro*. We show that TFIIIE activity co-purifies with a specific subset of ribosomal proteins (RPs) which, as revealed by chromatin immunoprecipitation analysis, generally interact with tRNA and 5S rRNA genes, but not with a Pol II-specific promoter. Only Rpl6Ap and Rpl6Bp, among the tested RPs, were found associated to a TATA-containing $tRNA^{Ile}$(TAT) gene. The *RPL6A* gene also emerged as a strong multicopy suppressor of a conditional mutation in the basal transcription factor TFIIIC, while *RPL26A* and *RPL14A* behaved as weak suppressors. The data delineate a novel extra-ribosomal role for one or a few RPs which, by influencing 5S rRNA and tRNA synthesis, could play a key role in the coordinate regulation of the different sub-pathways required for ribosome biogenesis and functionality.

A number of accessory factors, in addition to the core transcription factors, are required for efficient gene transcription by the three eukaryotic RNA polymerases (Pol). In the case of the Pol III system, specialized in the synthesis of tRNA, 5S rRNA and other untranslated RNAs [1], basal transcription requires, in addition to Pol III, the multisubunit factor TFIIIC (specifically binding to internal control regions) and the transcription initiation factor TFIIIB, which in *Saccharomyces cerevisiae* consists of Brf1p, Bdp1p and the TATA box binding protein [2]. 5S rRNA gene transcription further requires the gene-specific factor TFIIIA. Several factors have been reported to stimulate or inhibit basal transcription by the core Pol III machinery in different eukaryotes. In budding yeast, a relatively small set of modulators has been described: casein kinase II [3]; Maf1p, a repressor regulated by nutritional and stress signalling pathways [4]; Nhp6p, an HMG-like protein required for efficient and faithful transcription of the U6 snRNA and some tRNA genes [5–8]; and TFIIIE, a stimulatory activity of as yet unknown polypeptide composition, originally identified by biochemical fractionation of yeast nuclear extracts [9,10].

Studies in the last five years have revealed further levels of complexity in the mechanisms of Pol III transcription regulation, which can fully be appreciated only by considering Pol III transcription within its nuclear context. In particular, the yeast TFIIIB component Bdp1p was found to interact with the tRNA-processing factor RNase P [11], which in humans has been reported to act as a Pol III transcription factor [12], thus linking tRNA gene transcription and processing. In yeast, active tRNA genes have been shown to be preferentially localized in the nucleolus, where 5S rRNA gene transcription by Pol III also takes place, thus suggesting the existence of a functional relationship between nuclear organization and Pol III transcription regulation [13]. Adding to the *in vivo* complexity and potential regulatory cross-talks of the Pol III system, we show here that the transcriptional stimulatory activity of TFIIIE is associated with a specific subset of ribosomal proteins.

Materials and methods

Purification of TFIIIE. Yeast nuclear extracts were prepared and fractionated up to the Sephacryl S-300 HR step as described [9]. Pooled active fractions from five Sephacryl S-300 runs (460 ml, 5 mg of protein) were loaded onto a 40-ml Heparin–Ultrogel column (BioSepra) equilibrated in buffer C (25 mM Tris–HCl pH 8.0, 20% glycerol, 0.2 mM EDTA, 0.5 mM benzamidine, 5 mM β-mercaptoethanol) containing 0.45 M NaCl. After extensive washing with buffer C-0.45 (1 column volume) and buffer C-0.6 (6 volumes), a 0.6–1.2 M NaCl gradient in buffer C was applied (6 volumes). Active fractions (eluted between 0.7 and 0.9 M NaCl) were pooled, dialyzed against buffer C-0 to a final NaCl concentration of 0.13 M, and loaded onto a Q-Sepharose column equilibrated in buffer C-0.13. TFIIIE activity was recovered in the flow-through and it was concentrated on a Heparin-Ultrogel column to a final

* Corresponding authors. Fax: +39 0521 905151.
E-mail addresses: giorgio.dieci@unipr.it (G. Dieci), simone.ottonello@unipr.it (S. Ottonello).
[1] Present address: Sir William Dunn School of Pathology, Oxford OX1 3RE, UK.

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.12.097

concentration of ~1.4 mg/ml. For high-resolution fractionation, 60 μg of purified TFIIIE were loaded onto a Superdex-75-SMART column (GE Healthcare; 25 mM Tris–HCl, 0.2 mM EDTA, 10% glycerol, 0.2 M NaCl), from which 48 fractions (40 μl each) were collected, and analyzed for transcriptional activity and polypeptide composition. *In vitro* transcription assays (30 min at 22 °C) were carried out as described [7] using a plasmid DNA (*Leu-45*) bearing an internally shortened version of the $tRNA^{Leu}$(CAA) gene [16] as template. A standard TFIIIE-complementing reaction mixture contained: 50 ng of affinity-purified TFIIIC, 40 ng of recombinant (r) TBP, 50 ng of rBrf1p, 40 ng of rBdp1p and 50 ng of Pol III. *In vitro* synthesized RNAs were electrophoretically separated on 8 M urea, 6% polyacrylamide gels and visualized by either autoradiography or phosphorimaging using a Personal Imager FX (Bio-Rad). Quantification of radioactive RNA bands was carried out by scintillation counting of excised gel slices and/or by phosphorimage analysis using the Quantity One software (Bio-Rad).

Sequencing and mass spectrometric analysis of TFIIIE-associated polypeptides. Following SDS–PAGE of Q-Sepharose-purified TFIIIE and a 5 min Coomassie Blue staining, gel slices corresponding to bands 1/2, 3, 5, 6/7 and 8 (see Fig. 1C) were excised and trypsin-digested. Tryptic peptides were first separated by reverse-phase HPLC on an Aquapore RP-300 column (0–60% acetonitrile gradient in 25 mM ammonium acetate, pH 6.5), followed by a second round of fractionation as above, but in the presence of 0.1% trifluoroacetic acid. Eluted peptides were sequenced by automatic Edman degradation (see Table 1 for diagnostic peptide sequences). A larger set of TFIIIE-associated polypeptide bands was subjected to mass spectrometric (MS) analysis. Following reduction/carboxymethylation and trypsin digestion under standard conditions, peptides were extracted with 10 mM ammonium bicarbonate and 1% formic acid in 50% acetonitrile (ACN), and desalted with ZipTip pipettes (Millipore). MALDI-TOF spectra were recorded with an Applied Biosystems Voyager DE-STR spectrometer using an analyte/matrix mixture (10 mg/ml α-cyano-hydroxycinnamic acid in 70% ACN, 0.1% citric acid) dried at room temperature and external peptide standards. Data expressed as monoisotopic masses were used to search protein databases (MASCOT software; Matrix Science).

Multicopy suppression analysis. DNA fragments harbouring the coding sequences of the *RPS4A*, *RPS5*, *RPL6A*, *RPL7A*, *RPS8A*, *RPS9A*, *RPL13A*, *RPL14A*, *RPS24A*, *RPS25A*, *RPL26A* and *RPL36A* genes, together with upstream and downstream control regions, were PCR-amplified from yeast genomic DNA (see Supplementary Table S1 for primer sequences). Sequence-verified amplicons cloned into the multicopy vector pFL44L [14] were used for transformation of the *tfc3-G349E* mutant strain [15]. Yeast transformants precultured to an OD_{600} of 1.0 and serially diluted were spotted (5 μl) onto selective plates and examined for growth at either 30 °C or 37 °C for 2 to 3 days. Cells transformed with the empty pFL44L vector or with pFL44L constructs bearing the *TFC3*, *BRF1* or *BDP1* genes served as control for these experiments [10].

Chromatin immunoprecipitation analysis. Yeast strains expressing TAP-tagged versions of the Rps8A, Rpl6A, Rpl6B, Rpl14A, Rpl26A, Rpl36A, Rpb3 and Rap1 proteins were from the Yeast TAP Fusion Collection (Open Biosystems; Huntsville, AL, USA). Control experiments were performed with the untagged BY4741 strain. Immunoprecipitation of formaldehyde cross-linked chromatin (ChIP) was performed as described [7], using 1/150th of the total immunopurified DNA and 0.005% of the "input control" DNA for PCR reactions (see Supplementary Table S1 for primer sequences). PCR products were resolved on 6% polyacrylamide gels and visualized and quantified by phosphorimaging. After normalization for amplification efficiency, specific target gene enrichment in immunoprecipitates was calculated as the ratio of the abundance of each gene-specific product with respect to the abundance of an amplicon derived from a nontranscribed region of chromosome V.

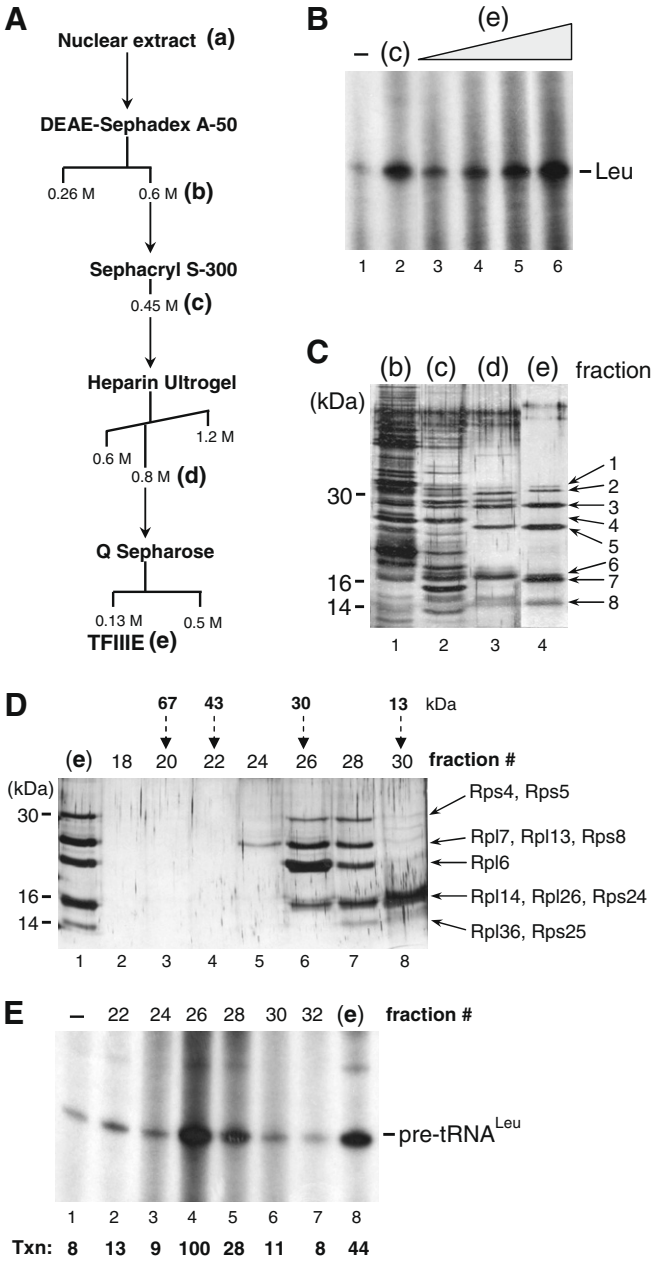

Fig. 1. TFIIIE purification. (A) Flow-chart of the chromatographic fractionation. (B) *In vitro* transcription analysis. Purified TFIIIE (fraction (e)) was added to the complementing system in the presence of a $tDNA^{Leu}$ (CAA) template (8, 20, 40 and 200 ng in lanes 3–6, respectively). Lane 2, reaction supplemented with 1 μg of partially purified TFIIIE (fraction (c)); lane1, TFIIIE-unsupplemented reaction. (C) 2.5 μg of fraction (b), 1 μg of fraction (c), 0.5 μg of fraction (d) and 0.5 μg of fraction (e) were subjected to SDS–PAGE followed by silver staining. The numbers on the right indicate the bands whose polypeptide composition was determined (see Table 1). The migration positions of molecular weight markers are indicated on the left (carbonic anhydrase, 30 kDa; myoglobin, 16 kDa; lysozyme, 14 kDa). (D) Fractions obtained from Superdex-75 chromatography (5 μl each) were subjected to SDS–PAGE/silver staining. Fraction numbers are reported on top of lanes 2–8, with the elution positions and molecular masses of Superdex-75 calibration proteins indicated above; the starting sample is shown in lane 1. The migration positions of SDS–PAGE markers and the names of the r-proteins corresponding to each band are indicated on the left- and on the right-hand side, respectively. (E) Transcriptional stimulatory activity of the indicated TFIIIE fractions. Lane 1, unsupplemented reaction; lane 8, reaction supplemented with the starting TFIIIE sample (e). The transcriptional output of each reaction, relative to the output (arbitrarily set to 100) obtained with fraction #26, is reported below each lane (*Txn*).