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Deletion of Swm2p selectively impairs trimethylation of snRNAs by trimethylguanosine synthase (Tgs1p)

Kum-Loong Boon *, Martin Košˇ **

Biochemistry Center (BZH), University of Heidelberg, Im Neuenheimer Feld 328, 69120 Heidelberg, Germany

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ABSTRACT

The 5′ cap trimethylation of small nuclear (snRNAs) and several nucleolar RNAs (snoRNAs) by trimethylguanosine synthase 1 (Tgs1p) is required for efficient pre-mRNA splicing. The previously uncharacterised protein Swm2p interacted with Tgs1p in yeast two-hybrid screens. In the present study we show that Swm2p interacts with the N-terminus of Tgs1p and its deletion impairs pre-mRNA splicing and pre-rRNA processing. The trimethylation of spliceosomal snRNAs and the U3 snoRNA, but not other snoRNAs, was abolished in the absence of Swm2p, indicating that Swm2p is required for a substrate-specific activity of Tgs1p.

Structured summary:
MINT-7949608: *p53* (uniprotkb:P02340) *physically interacts* (MI:0915) with *large T-antigen* (uniprotkb:P03070) by *two-hybrid* (MI:0018)
MINT-7949574: *swm2* (uniprotkb:P40342) *physically interacts* (MI:0915) with *swm2* (uniprotkb:P40342) by *pull down* (MI:0096)
MINT-7949556: *swm2* (uniprotkb:P40342) *physically interacts* (MI:0915) with *TGS1* (uniprotkb:Q12052) by *pull down* (MI:0096)
MINT-7949587: *swm2* (uniprotkb:P40342) *physically interacts* (MI:0915) with *tgs1* (uniprotkb:Q12052) by *two-hybrid* (MI:0018)
MINT-7949641: *nop1* (uniprotkb:P15646) *colocalizes* (MI:0403) with *TGS1* (uniprotkb:Q12052) by *fluorescence microscopy* (MI:0416)
MINT-7949627: *swm2* (uniprotkb:P40342) and *nop1* (uniprotkb:P15646) *colocalize* (MI:0403) by *fluorescence microscopy* (MI:0416)
MINT-7949540: *swm2* (uniprotkb:P40342) *physically interacts* (MI:0915) with *TGS1* (uniprotkb:Q12052) by *tandem affinity purification* (MI:0676)

1. Introduction

Maturation of many cellular RNAs requires small ribonucleoproteins, such as small nuclear RNPs (snRNPs) and small nucleolar RNPs (snoRNPs involved in pre-mRNA splicing and ribosomal RNA processing respectively). Most spliceosomal snRNAs (U1, U2, U4 and U5) and a subset of snoRNAs (e.g. U3, snR10) are synthesized by RNA polymerase II and their m^7G cap is trimethylated to a trimethylguanosine (m_3G) cap by trimethylguanosine synthase 1 [1–3]. The trimethylation of the snRNAs caps is required for their nuclear localisation and for efficient pre-mRNA splicing. Besides its function in trimethylation of snRNA or snoRNA (further referred to as sn(o)RNAs) caps, the nucleolar-localised Tgs1p is also required for intact nucleolar morphology and ribosome synthesis in yeast [4]. Although *TGS1* is not essential for yeast viability, its deletion leads to defects in splicing and ribosomal RNA processing [1,4]. Recently, a number of proteins involved in snRNP function, spliceosome assembly and RNA processing has been found to genetically interact with *TGS1* [5,6]. The large scale yeast two-hybrid screens have identified yeast Swm2p (synthetic with Mud2/*YNR004W*) as an interacting partner of Tgs1p [7,8]. Yeast *SWM2* is an uncharacterized gene encoding a non-essential 17 kDa protein with no known domain or homology to other proteins. Deletion of *SWM2* causes similar genetic interaction profile to *tgs1Δ* [6], and is synthetic lethal with the deletion of *MUD2* gene, which appears to be a functional partner of Tgs1p [9].

* Corresponding author.
** Corresponding author.
E-mail addresses: kum-loong.boon@bzh.uni-heidelberg.de (K.-L. Boon), martin.kos@bzh.uni-heidelberg.de (M. Košˇ).

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In this study, we report a detailed characterisation of Swm2p and its functions in RNA cap trimethylation and ribosome biogenesis. Deletion of *SWM2* impairs pre-mRNA splicing and ribosome biogenesis, exhibiting a similar phenotype to *tgs1Δ*. However, only trimethylation of snRNAs and the U3 snoRNA, but not other snoRNAs tested, is affected in the *swm2Δ* strain. Therefore, Swm2p is specifically required for trimethylation of spliceosomal snRNAs and the U3 snoRNA by Tgs1p.

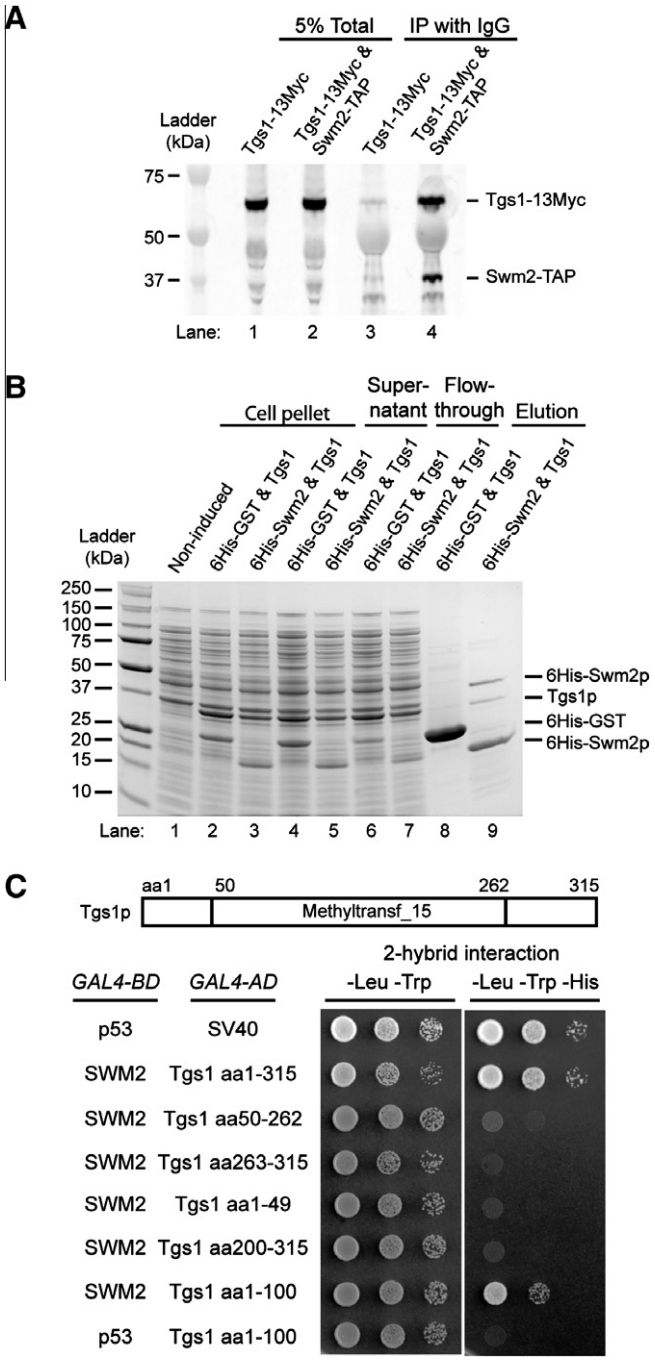

Fig. 1. Swm2p interacts with N-terminus of Tgs1p. (A) Immunoprecipitation (IP) of Tgs1p-13Myc from protein extracts derived from yeast. Extracts were incubated with IgG–sepharose and precipitated proteins were analysed by Western blotting, probing for both c-Myc and protein-A. Lanes 1 and 2 show 5% of the input extract used for each IP. Note that the Swm2-TAP protein in the whole cell lysate is very poorly recognised by IgG in the blot. (B) Copurification of recombinant Tgs1p with 6His-GST (lane 8), or 6His-Swm2p (lane 9) directly from *E. coli* extracts. The protein identities in the lane 9 were confirmed by mass spectrometry. (C) Swm2p interacts with N-terminus of Tgs1p. Yeast two-hybrid plasmids expressing Swm2p fused to GAL4-BD (GAL4 DNA binding domain) and either full-length or truncated Tgs1p fused to GAL4-AD (GAL4 activation domain) were cotransformed into the PJ69-4A reporter strain. Transformants were spotted in 10-fold serial dilutions on SDC-Trp-Leu and SDC-Trp-Leu-His plates. Positive yeast two-hybrid interaction allows growth on SDC-Trp-Leu-His plates. Plates were incubated for 3 days at 30 °C. The combination of p53 and SV40 large T-antigen served as a positive control.

2. Materials and methods

2.1. Yeast strains and plasmids

The *Saccharomyces cerevisiae* strains, plasmids and oligonucleotides used in this study are listed in the Supplementary Tables 1 and 2. The yeast transformation procedure was performed as described previously [10]. For metabolic depletion of Prp42, cultures of P_{GAL1}-*PRP42* and P_{GAL1}-*PRP42-swm2Δ* were grown in YPG (1% yeast extract, 2% Bacto Peptone, 2% galactose) medium to log phase, spun down, washed with YPD (1% yeast extract, 2% Bacto Peptone, 2% glucose) and resuspended to the original culture volume of YPG. The cultures were harvested at 0, 4 and 8 h after the medium shift. Yeast cultures were maintained at log phase by adding fresh YPD medium.

2.2. Live cell imaging

Cells were grown in SDC-Leu-Ura liquid medium at 30 °C. Fluorescence microscopy was performed using Imager Z1 (Carl Zeiss). Pictures were acquired with AxioCamMRm camera (Carl Zeiss) and software Axio Vision 4.3 (Carl Zeiss). Picture were exported as jpg files and processed in Adobe PhotoShop CS2.

2.3. Protein extract preparation

Preparation of yeast whole cell lysate was performed as described previously [11]. For recombinant protein production, *Escherichia coli* BL21 (DE3) cells (Novagen) were grown at 37 °C to $OD_{600\ nm}$ of 0.7, induced with IPTG (0.75 mM) and incubated for a further 3 h. Cells were pelleted, resuspended in lysis buffer (150 mM NaCl, 50 mM Tris–HCl (pH 7.5), 10 mM imidazole) and

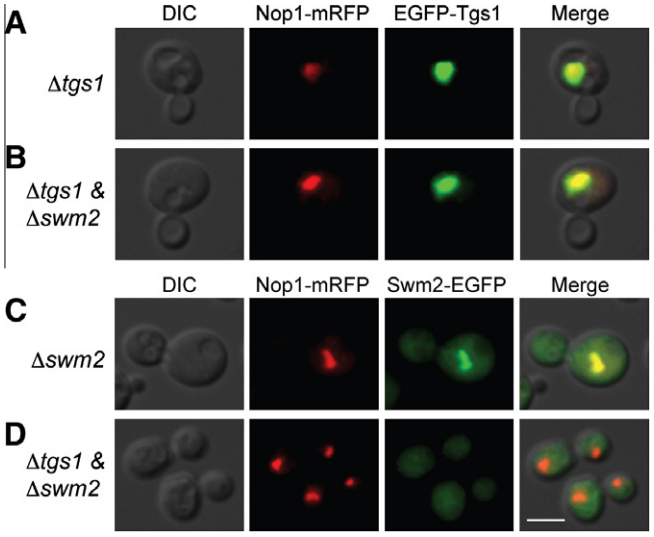

Fig. 2. Nucleolar localisation of Tgs1p and Swm2p. The EGFP-Tgs1 and Swm2-EGFP subcellular localisation was analysed for the colocalisation with a nucleolar marker Nop1-mRFP. Localisation of EGFP-Tgs1p in *Δtgs1* (A) or *Δtgs1–Δswm2* (B) and Swm2-EGFP in *Δswm2* (C) or *Δtgs1–Δswm2* (D) yeast strains. In each panel the proteins being detected by fluorescent microscopy are indicated at the top. DIC: differential interference contrast. Scale bar, 2 μm.