The EMBO Journal Vol. 21 No. 20 pp. 5498–5507, 2002

Hmo1, an HMG-box protein, belongs to the yeast ribosomal DNA transcription system

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Hmo1 is one of seven HMG-box proteins of *Saccharomyces cerevisiae*. Null mutants have a limited effect on growth. Hmo1 overexpression suppresses *rpa49-Δ* mutants lacking Rpa49, a non-essential but conserved subunit of RNA polymerase I corresponding to the animal RNA polymerase I factor PAF53. This overexpression strongly increases *de novo* rRNA synthesis. *rpa49-Δ hmo1-Δ* double mutants are lethal, and this lethality is bypassed when RNA polymerase II synthesizes rRNA. Hmo1 co-localizes with Fob1, a known rDNA-binding protein, defining a narrow territory adjacent to the nucleoplasm that could delineate the rDNA nucleolar domain. These data identify Hmo1 as a genuine RNA polymerase I factor acting synergistically with Rpa49. As an HMG-box protein, Hmo1 is remotely related to animal UBF factors. *hmo1-Δ* and *rpa49-Δ* are lethal with *top3-Δ* DNA topoisomerase (type I) mutants and are suppressed in mutants lacking the Sgs1 DNA helicase. They are not affected by *top1-Δ* defective in Top1, the other eukaryotic type I topoisomerase. Conversely, *rpa34-Δ* mutants lacking Rpa34, a non-essential subunit associated with Rpa49, are lethal in *top1-Δ* but not in *top3-Δ*.

Keywords: nucleolus/RNA polymerase I/SGS1/topoisomerases/UBF

Introduction

Ribosomal RNAs (25S, 18S, 5.8S and 5S rRNA) are by far the most abundant RNA species of living cells. They are synthesized by RNA polymerase I (Pol I) for the three largest rRNAs and by RNA polymerase III (Pol III) for the 5S rRNA. Pol III also determines the synthesis of all tRNAs and of at least three non-translated RNAs (the U6 snRNA, the RNA component of RNase P and the 7S RNA required for co-translational secretion) in all eukaryotes examined so far including *Saccharomyces cerevisiae* (Paule and White, 2000; Briand *et al*., 2001b). RNA polymerase II (Pol II) produces all mRNAs and also makes a large number of non-translated RNA species.

To initiate transcription, eukaryotic Pols first have to recognize promoter-bound complexes organized around the TATA box-binding protein TBP. Pol II pre-initiation complexes contain TBP and the Pol II-specific factor TFIIB, whilst Pol III complexes contain TBP and the heterodimeric factor TFIIIB, where subunit Brf1 is akin to TFIIB. In both cases, the pre-initiation complexes are widely conserved among eukaryotes (Chédin *et al*., 1998; Hampsey, 1998). The Pol I enzyme and two of its associated factors, Rrn3 and TFIIH, are also conserved from yeast to human (Gadal *et al*., 1997; Carles and Riva, 1998; Moorefield *et al*., 2000; Iben *et al*., 2002). However, the yeast and human rDNA promoter-binding complexes are largely unrelated. In *S.cerevisiae*, this complex is made of two components. A three-subunit core factor (Keys *et al*., 1994; Lalo *et al*., 1996) directly binds the core promoter domain and is strictly essential for transcription. An upstream activating factor (UAF) containing histone H3 and H4 (Keener *et al*., 1997) and four other polypeptides (Keys *et al*., 1996) strongly enhances the binding of the core factor to the promoter via TBP.

In human cells, the Pol I pre-initiation complex is made of the SL1/TIF-IB factor (Comai *et al*., 1992; Eberhard *et al*., 1993) that combines TBP and three associated TAFs ($TAFI_{48}$, $TAFI_{63}$ and $TAFI_{110}$). It also contains the important but auxiliary factor UBF (Jantzen *et al*., 1990; Smith *et al*., 1993; Moss and Stefanovsky, 2002 and references therein). UBF is a homodimeric protein bearing six HMG domains that interacts with rDNA in a sequence-independent way. Neither SLI nor UBF have sequence similarity with the yeast UAF or core factor. We show here that the yeast HMG-box protein Hmo1 (Lu *et al*., 1996a) also belongs to the rRNA transcription apparatus and may thus be functionally equivalent to UBF, which adds to the mounting evidence that Pol I transcription systems are conserved from yeast to human.

Results

General organization of Hmo1

In the present study, we found that *rpa49-Δ* mutants lacking the conserved Rpa49/PAF53 Pol I subunit (Liljelund *et al*., 1992; Hanada *et al*., 1996) are suppressed *in vivo* by the overexpression of Hmo1 (Figure 1A). Hmo1, one of the seven HMG-box proteins encoded by the genome of *S.cerevisiae*, was first identified by its co-purification with an unidentified DNA helicase (Lu *et al*., 1996a). This protein has strong chromatin-binding properties (Freeman *et al*., 2000; Mitsouras *et al*., 2002) but its biological function so far was unknown. The original null mutants (Lu *et al*., 1996a) were viable, with a strong growth defect. The *hmo1-Δ* mutants of this study were prepared in a somewhat different genetic background (YPH500; Sikorski and Hieter, 1989). As shown Figure 1B and C, they have a <2-fold effect on growth rate between 25 and 37°C. *hmo1* mutants were also isolated by their synthetic lethal effect on *fpr1-Δ* mutants lacking a non-essential prolyl isomerase (Dolinski and Heitman, 1999).

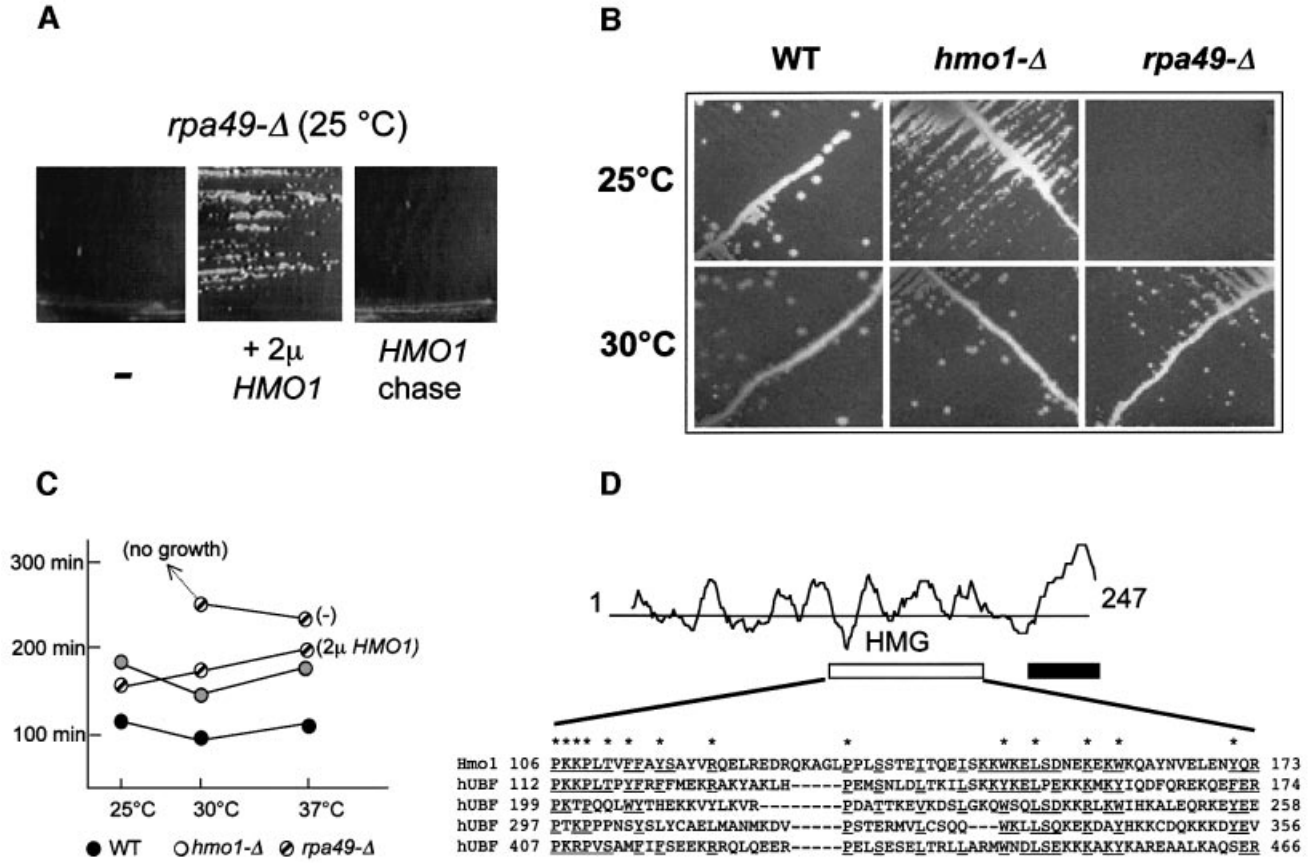

Fig. 1. General properties of Hmo1. (**A**) Suppression of *rpa49-Δ::TRP1* by *HMO1*. Strain 49-ΔA (*rpa49-Δ*) was transformed with pFL44L without an insert (–), and with the multicopy plasmid pD10-ΔSph bearing *HMO1* (2μ *HMO1*). A control strain was obtained by chasing pD10-ΔSph on FOA medium (*HMO1* chase). Individual clones were selected and re-isolated on uracil omission plates, streaked on YPD and incubated for 4 days at 25°C. (**B**) Growth pattern of null mutants. Strains YPH499 (WT), SL13-1A (*hmo1-Δ*) and 49-ΔA (*rpa49-Δ*) were streaked on YPD and incubated for 4 days at 25 and 30°C. These three strains are isogenic and their complete genotype is given in Table I. (**C**) Doubling times of mutants. Strains YPH499 (WT), SL16-2C (*hmo1-Δ*) and 49-ΔA (*rpa49-Δ*) were grown in liquid YPD at the indicated temperatures. Doubling times were determined by nephelometry using a Hatch nephelometer. 49-ΔA (*rpa49-Δ*) was tested with or without the pD10-ΔSph plasmid bearing *HMO1* (2μ *HMO1*). (**D**) Properties of the amino acid sequence of Hmo1. The hydrophilicity pattern of Hmo1 and the localization of its HMG (white box) and basic (black box) domains are indicated. The HMG domain is compared with the first four N-terminal domains of human UBF. Identical or strongly similar amino acids (F = W = Y, E = D, N = Q, E = Q, I = L) are underlined. Asterisks denote the general HMG consensus.

Hmo1 contains a single HMG domain matching the minimal consensus proposed for HMG domains and closely related to four of the six domains of the UBF Pol I factor associated with human Pol I (Figure 1D). The existence of a second HMG domain (Lu *et al*., 1996a) is not supported by our sequence alignments. A basic C-terminal extension (amino acids 210–247) can be deleted with no detectable phenotype (Lu *et al*., 1996a). The N-terminal half of the protein (positions 1–105) lacks homology to any known eukaryotic gene product. Indeed, Hmo1 has only been identified so far in yeasts closely related to *S.cerevisiae* such as *Saccharomyces kluyveri* (Neuvéglise *et al*., 2000).

HMO1 specifically suppresses rpa49-Δ mutants lacking the Pol I subunit Rpa49/PAF53

rpa49-Δ mutants grow slowly at 30°C and are lethal at 25°C (see Figure 1B and C). We exploited the latter property to isolate dosage-dependent suppressors of *rpa49-Δ* (strain 49-ΔA), using a genomic library borne on the *URA3* multicopy vector pFL44L (Stettler *et al*., 1993). This yielded four different *RPA49* clones and one extragenic suppressor clone containing *HMO1*. Subclones bearing HMO1 only restored a doubling of 180 min in *rpa49-Δ* cells, not very different from the 125 min of an isogenic wild-type (Figure 1C). Suppression was due to a gene dosage effect since a low-copy plasmid (pASD10-HMO1) hardly improves growth (data not shown). No other suppressor of *rpa49-Δ* was isolated in a second screen, using less stringent conditions. A reciprocal multicopy suppression effect of *RPA49* on the slow growth of *hmo1-Δ* mutants was not observed.

Three other conditional Pol I mutants (*rpa190-G728D*, *rpa12-Δ* and *rpa43-24*) have been examined for dosage-dependent suppression. They do not respond to *HMO1* but each of them is suppressed specifically by the over-expression of one particular protein of the Pol I transcription system. *rpa190-G728D* responds to Rpb6, a common subunit shared by Pol I, II and III (Briand *et al*., 2001a), and *rpa12-Δ* is suppressed by Rpa190, the largest subunit of Pol I (Nogi *et al*., 1993). *rpa43-24* is suppressed by an increased gene dosage in the Pol I-specific initiation factor Rrn3 (Peyroche *et al*., 2000). Thus, *rpa49-Δ*, *rpa12-Δ*, *rpa190-G728D* and *rpa43-24* each respond to a different suppressor, consistent with the fact that they affect distinct aspects of Pol I activity.

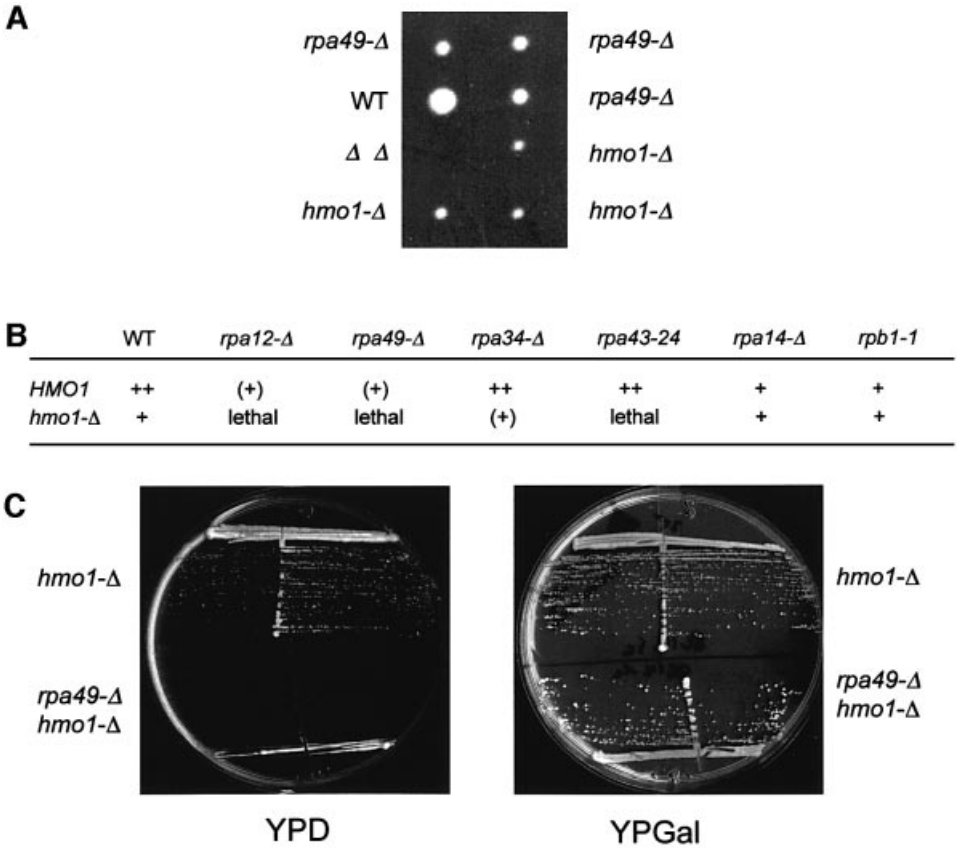

Fig. 2. Synthetic lethality between *hmo1-Δ* and RNA polymerase I mutants. (**A**) Synthetic lethality of *hmo1-Δ rpa49-Δ* double mutants. Strain SL6-10A (*hmo1-Δ*) was crossed to A49ΔA (*rpa49-Δ*). The resulting diploid (SL8) was submitted to tetrad analysis (20 asci). Replica plating on appropriate omission media identified the genotype of individual segregants. The growth pattern of one tetratype and one parental ditype ascus is shown after 6 days on YPD at 30°C (the position of the non-growing double-mutant spore is denoted *ΔΔ*). (**B**) Effect of *hmo1-Δ* on other Pol I mutants. Strain SL13-1A (*hmo1-Δ*) was crossed to T4-1D (*rpa34-Δ*) and GPY11-24 (*rpa43-24*). SL6-10b (*hmo1-Δ*) was crossed to SL9-6B (*rpa12-Δ*), D191-7C (*rpa49-Δ*), RY260 (*rpb1-1*) and D360-1A (*rpa14-Δ*). Genetic crosses were as described above, with at least 10 tetrads analysed in each case. The complete genotypes of the parental strains are given in Table I. Non-lethal growth patterns were assessed at 30°C and are indicated as follows: ++, +, (+): no, slight or intermediate growth defect, respectively. (**C**) Suppression of *rpa49-Δ hmo1-Δ* lethality by Pol II-dependent transcription. OG14-1a with plasmid pNOY103 (*rpa49-Δ*) and SL13-1a (*hmo1-Δ*) were crossed to give the SL14 diploid strain. Spores were germinated and grown on YPGal. In contrast to the SL8 cross described above, viable *rpa49-Δ hmo1-Δ* double mutants were obtained but invariably harboured the pNOY103 plasmid. The growth patterns of SL14-6A (*hmo1-Δ*) and of the SL14-5C (*rpa49-Δ hmo1-Δ*) double mutant, both harbouring pNOY103, are shown on YPD (repression) and YPGal (induction), after 4 days at 30°C. The complete genotype of these strains is given in Table I.

Hmo1 is synthetic lethal with several Poll-defective mutants

hmo1-Δrpa49-Δ double mutants are lethal (Figure 2A). In contrast, viable double mutants (e.g. SL11-1B, see Table I) are recovered readily in the presence of a complementing plasmid bearing either *RPA49* (pSLA49 = 2μ *URA3 RPA49*) or *HMO1* (pASD10 = *CEN6 ADE2 HMO1*). In both cases, the spontaneous loss of the *RPA49* or *HMO1* complementing plasmid can be monitored easily by the formation of 5-fluoro-orotic acid (FOA)-resistant colonies (loss of *URA3*) or of red sectors on YPD plates (loss of *ADE2*). Unlike their single mutant parents, *hmo1-Δ* and *rpa49-Δ* double mutants were unable to lose these plasmids, further demonstrating that the combination of these two mutations is lethal.

As shown in Figure 2B, lethality with *hmo1-Δ* extends to at least two other Pol I-defective mutants (*rpa12-*Δ and *rpa43-24*) but not to the *rpa14-Δ* and *rpa34-Δ* mutants of the non-essential subunits Rpa14 and Rpa34. Moreover, *hmo1-Δ* is viable when combined with the temperature-sensitive Pol II mutant *rpb1-1* (Nonet *et al.*, 1987). The synthetic lethality of *hmo1-Δ* with *rpa12-Δ* is consistent with the fact that Rpa49 dissociates from the immuno-purified *rpa12-Δ* mutant enzyme (Van Mullem *et al.*, 2002a). The lethality of *rpa43-24 hmo1-Δ* is consistent with recent data showing that Rpa43 and Rpa49 have synergic effects *in vivo* (S.Labarre and P.Thuriaux, unpublished results). The fact that *hmo1-Δ* exacerbates the growth defect of several Pol I mutants supports the idea that Hmo1 contributes to the Pol I-dependent synthesis of rRNA (see below). The suppression effect associated with its overexpression, however, seems specific for *rpa49-Δ*. It implies that the absence of Rpa49 is somehow compensated by a mere increase in the cellular concentration in Hmo1, therefore suggesting that both proteins affect the same aspect of Pol I-dependent transcription.

Hmo1 stimulates rRNA synthesis in rpa49-Δ cells

Figure 3 compares isogenic *hmo1-Δ*, *rpa49-Δ* (with or without *HMO1* overexpression), *rpb9-Δ* and wild-type cells for their *de novo* synthesis of rRNAs and tRNAs by [^{3}H]uracil pulse labelling. The steady-state levels of

Table I. Yeast strains

Strain	Genotype	Source
49-ΔA	*MATa rpa49-Δ::TRP1 ura3-52 his3-Δ200 trp1Δ-1 lys2-801 ade2-1*	Liljelund *et al*. (1992)
D191-7C	*MATα rpa49-Δ::TRP1 ura3-52 his3-Δ200 trp1 leu2-Δ1 lys2-801 ade2-1*	This work
SL6-10B	*MATα ade2-1 ura3-52 lys2-801 trp1-Δ63 his3-Δ200 leu2-Δ1 hmo1-Δ::URA3*	This work
SL16-2C	*MATα ade2-1 ura3-52 lys2-801 his3-Δ200 leu2-Δ1 hmo1-Δ::HIS3*	This work
SL13-1A	*MATa ade2-1 ura3-52 lys2-801 trp1-Δ63 his3-Δ200 leu2-Δ1 hmo1-Δ::HIS3*	This work
SL6-10A	*MATα ade2-1 ura3-52 lys2-801 his3-Δ200 leu2-Δ1 hmo1-Δ::URA3*	This work
BMA64-1A	*MATa leu2-3 112 his3-11,15 trp1Δ2 CAN1-100 ade2-1 ura3-52*	Galy *et al*. (2000)
BMA64-1B	*MATα leu2-3 112 his3-11,15 trp1Δ2 CAN1-100 ade2-1 ura3-52*	Galy *et al*. (2000)
BMA-Hmo1–GFP	*MATa leu2-3 112 his3-11,15 trp1Δ2 CAN1-100 ade2-1 ura3-52 HMO1::GFP-TRP1*	This work
BMA-Fob1–GFP	*MATa leu2-3 112 his3-11,15 trp1Δ2 CAN1-100 ade2-1 ura3-52 FOB1::GFP-TRP1*	This work
BMA-Hmo1–YFP	*MATa leu2-3 112 his3-11,15 trp1Δ2 CAN1-100 ade2-1 ura3-52 HMO1::YFP-HIS3MX6*	This work
BMA-Fob1–CFP	*MATα leu2-3 112 his3-11,15 trp1-Δ2 CAN1-100 ade2-1 ura3-52 FOB1::CFP-TRP1*	This work
D432-6D	*MATa rpa190-G728D trp1 leu2 lys2-801 ura3-52*	This work
SL9-6B	*MATα ade2-1 lys2-801 ura3-52 trp1-Δ63 his3-Δ200 leu2-Δ1 rpa12-Δ::LEU2*	Gadal *et al*. (1997)
RY260	*MATa ura3-52 rpb1-1*	Nonet *et al*. (1987)
GPY11-24	*MATa ade2-1 lys2-801 ura3-52 trp1-Δ63 his3-Δ200 leu2-Δ1 rpa43-Δ::LEU2 (CEN TRP1 rpa43-24)*	Peyroche *et al*. (2000)
SL11-1B	*MATα ade2-1 ura3-52 lys2-801 trp1 his3-Δ200 leu2-Δ1 hmo1Δ::URA3 rpa49-Δ::TRP1* (pASD10 = *CEN6 ADE2 HMO1*)	This work (SL6-10A × 49-ΔA, transformed with pASD10)
SL14-5C	*MATa leu2-D1 hmo1-D::HIS3 rpa49-D::TRP1 ura3-52 his3-Δ200 trp1 lys2-801 ade2-1* (pNOY103 = *pGAL7::rDNA ADE3 URA3* 2μ)	This work (SL13-1A × OG14-1A)
SL14-6A	*MATa leu2-D1 rpa49-D::TRP1 hmo1-D::HIS3 ura352 his3-Δ200 trp1 lys2-801 ade2-1* (pNOY103 = *pGAL7::rDNA ADE3 URA3* 2μ)	This work (SL13-1A × OG14-1A)
OG14-1A	*MATa rpa49-D::TRP1 ura3-52 his3-Δ200 trp1Δ-1 lys2-801 ade2-1 leu2-D1* (pNOY103 = *pGAL7::rDNA ADE3 URA3* 2μ)	This work (see Gadal *et al*., 1997)
SL7-4A	*MATα rpa34-Δ::HIS3 ura3 ade2-1 lys2-801 his3-Δ200 trp1-Δ lhmo1-Δ::URA3 leu2*	This work (T4-1C × SL6-10B)
T4-1C	*MATα rpa34-Δ::HIS3 ura3-52 ade2-1 lys2-801 his3-Δ200 trp1-Δ1*	Gadal *et al*. (1997)
D313-3D	*MATα his3 trp1 ura3-1 ade2-1 lys2-801 top3-4Δ::URA3 sgs1-Δ::TRP1*	D64-10B × YPH52 (Sikorski and Hieter, 1989)
D64-10B	*MATa CAN1-100 his3-11,15 leu2-3 112 trp1-1 ura3-1 ade2-1 top3-Δ::URA3 sgs1-Δ::TRP1*	Gangloff *et al*. (1994)
A14-U	*MATa ade2-1 ura3-52 lys2-801 trp1-Δ63 rpa14::URA3 his3-Δ200 leu2-Δ1*	Smid *et al*. (1995)
D308-6B	*MATα his3 trp1 ura3-1 ade2-1 leu2-3 112 top3-Δ::URA3*	This work
D211-3B	*MATα ade2-1 his3-11,15 leu2-3 112 trp1-1 ura3-1 top3-Δ::HIS3 lys2*	This work
D360-1A	*MATα ade2-1 lys2-801 ura3-52 trp1-Δ63 his3-Δ200 leu2-Δ1 rpa14-Δ::HIS3*	This work
D360-1A	*MATα ade2-1 lys2-801 ura3-52 trp1-Δ63 his3-Δ200 leu2-Δ1 rpa14-Δ::HIS3*	This work
D386-9B	*MATa lys2-Δ201 ura3-52 trp1-Δ63 his3-Δ200 rpb9-Δ::HIS3*	Van Mullem *et al*. (2002b)

rRNAs and tRNAs were estimated by ethidium bromide staining. *rpb9-Δ* has a partial Pol II defect, due to the inactivation of the non-essential Pol II subunit Rpb9 (Woychik *et al*., 1991), and serves here as a slow-growing control. Since rRNAs contribute ~80% of the total RNA in wild-type cells (Waldron and Lacroute, 1975), partial Pol I defects are already apparent when comparing steady-state rRNA and tRNA content. As shown in Figure 3A, the wild-type and *rpb9-Δ* controls have a similar rRNA/tRNA ratio. *Hmo1-Δ* and, even more so, *rpa49-Δ* are characterized by a shortage of rRNA compared with the tRNA level.

To measure the *de novo* synthesis of rRNA, cells were exposed to a 20 min pulse with [^{3}H]uracil. At 30°C, *rpa49-Δ* has an ~5-fold effect on Pol I-dependent synthesis of rRNA (e.g. 5.8S rRNA) relative to Pol III transcripts such as 5S rRNA and tRNAs. The presence of *HMO1* largely reverses this effect (Figure 3B and C), confirming that the gene dosage of *HMO1* is important for Pol I-dependent transcription in cells lacking the Rpa49 subunit of Pol I. *hmo1-Δ* cells also partly compromise the *de novo* synthesis of rRNA, with no detectable accumulation of pre-rRNA intermediates. Along with their low steady-state level in rRNA, this is consistent with a partial Pol I defect. However, these data should be considered with some caution, since slowing down growth also has some effect on rRNA synthesis (Waldron and Lacroute, 1975), as can be seen in our *rpb9-Δ* control (Figure 3B and C).

rpa49-Δ hmo1-Δ is rescued by the Pol II-dependent synthesis of rRNA

Nogi *et al*. (1991) have shown that Pol I-specific growth defects are bypassed by high copy number plasmids where rDNA transcription is under the control of a Pol II promoter such as the *GAL7* promoter. We thus repeated the *hmo1-Δ* × *rpa49-Δ* crosses mentioned above in diploid cells harbouring pNOY103, a *URA3* pGAL7::rDNA vector. Tetrad analysis was done on YPGal medium at 25°C. Under these conditions, viable *hmo1-Δ rpa49-Δ* double mutants were readily recovered (e.g. SL14-5C, Table I). They invariably harboured pNOY103 and were unable to lose it in the presence of FOA. Moreover, they failed to grow when turning off the GAL7 promoter on glucose-containing medium (Figure 2C), demonstrating that the synthetic lethality of *hmo1-Δ rpa49-Δ* is due solely to a Pol I-dependent defect.

Nucleolar localization of Hmo1

C-terminal fusions of GFP to Hmo1 and to Fob1, a proven rDNA-binding protein, were expressed from their chromosomal loci [strains BMA-Hmo1–green fluorescent protein (GFP) and BMA-Fob1–GFP, see Table I]. Their localization was compared with that of a DsRed–Nop1 fusion protein, where Nop1 is a part of the nucleolar dense fibrillar component (Léger-Silvestre *et al*., 1999), and with DNA staining (Hoechst 33352), which preferentially

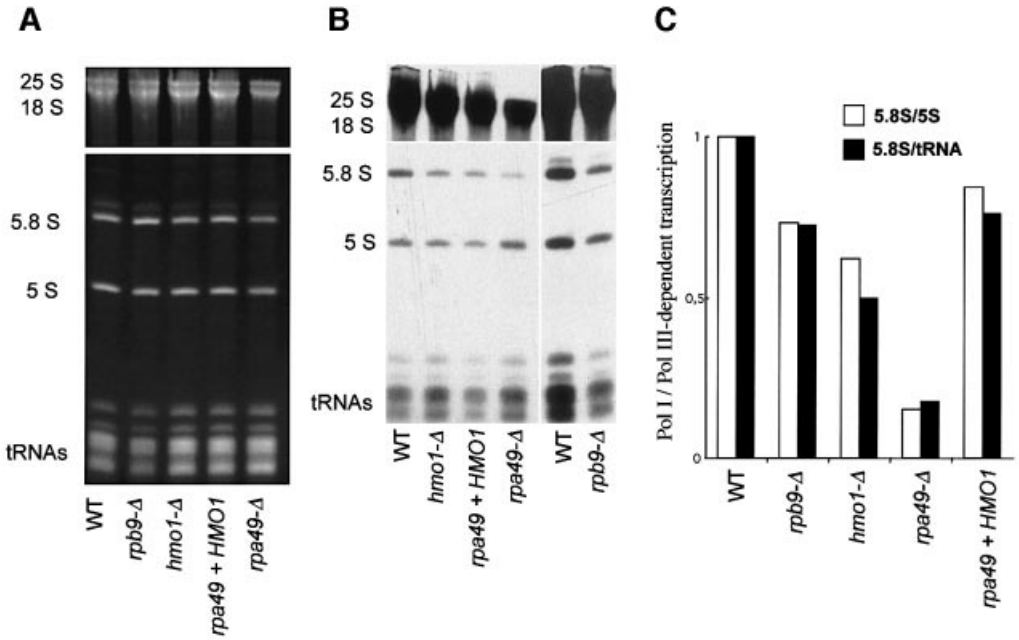

Fig. 3. Effect of *hmo1-Δ* and *rpa49-Δ* on rRNA. (**A**) Steady-state level of rRNA and tRNA. Strains YPH499 (WT), D386-9B (*rpb9-Δ*), SL16-2C (*hmo1-Δ*) and 49-ΔA (*rpa49-Δ*) with or without the pD10-ΔSph plasmid bearing *HMO1* (2μ *HMO1*) were grown on YPD at 30°C and harvested in mid-log phase (OD_{600} = 0.5). Total rRNA was extracted in hot phenol (Hermann-Le Denmat *et al*., 1994) and quantified by spectrophotometry at 260 and 280 nm. A 10 μg aliquot of total RNA was separated by gel electrophoresis on polyacrylamide and revealed by ethidium bromide staining. (**B**) *In vivo* labelling of rRNA and tRNA. The same strains as above were transformed with the *URA3* centromeric plasmid YCp50 to complement their *ura3-52* mutation. The resulting transformants were grown in uracil omission medium and exposed to 150 μCi of [^{3}H]uracil for 20 min at 30°C (Hermann-Le Denmat *et al*., 1994). Total RNA was extracted and separated as described above. [^{3}H]Uracil incorporation was revealed by overnight autoradiography (see Material and methods). The last two lanes correspond to a 4 day exposure. (**C**) Ratio of Pol I (5.8S) and Pol III (5S, tRNA) −signals. Non-saturated autoradiograms were scanned as described in Materials and methods. The two ratios (5.8S/5S) and (5.8S/tRNA) were normalized relatively to the wild-type control.

reveals the nucleoplasmic DNA with nucleolar staining exclusion. As shown in Figure 4A, Fob1 stains a structure located at the nucleolar–nucleoplasmic interface (compare with DNA and Nop1 staining). Hmo1 has a very similar localization pattern. Fob1 is a *bona fide* rDNA-binding protein (Defossez *et al*., 1999) and we are therefore inclined to believe that this staining pattern defines the localization of rDNA in living cells. In a second experiment (Figure 4B), Fob1 and Hmo1 were tagged with different GFP spectral variants, cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP), in a diploid strain prepared by crossing strains BMA-Fob1–CFP and BMA-Hmo1–YFP (Table I). Staining the resulting diploid entirely confirmed the co-localization of these two proteins, supporting our conclusion that Hmo1 is an rDNA-binding component of the yeast Pol I transcription system.

hmo1-Δ and rpa49-Δ are synthetic lethal with top3-Δ but not with top1-Δ mutants

Like other eukaryotes, yeast has two unrelated type I topoisomerases, Top1 and Top3. Their separate or simultaneous inactivation has little (Top1) or moderate (Top3) effect on growth (Thrash *et al*., 1985; Gangloff *et al*., 1994). *top1-Δ* mutants strongly affect the topology of rDNA and increase mitotic recombination within the rDNA cluster (Christman *et al*., 1993), but have little or no effect on rRNA formation (Thrash *et al*., 1985). *top3-Δ* mutants also strongly increase mitotic recombination at the rDNA repeat (Gangloff *et al*., 1994). They grow poorly above 35°C, but their effect on rRNA transcription had not been examined. We show here that *top3-Δ* mutants have a wild-type steady-state level of tRNA and rRNA at 35°C (Figure 5A). Hence, their adverse effect at high temperature probably is not due to a preferential defect in rRNA synthesis.

As shown in Figure 5B and C, *top3-Δ* interferes with a subset of Pol I mutants, namely *hmo1-Δ*, *rpa49-Δ* and the conditional *rpa43-24* mutant. *rpa12-Δ*, *rpa34-Δ* and *rpa14-Δ* are not or slightly (*rpa12-Δ*) impaired by *top3-Δ*. Moreover, this effect is specific for the type I topoisomerase considered, since *hmo1-Δ*, *rpa49-Δ* and *rpa43-24* are not affected by *top1-Δ* mutants. Conversely, *rpa34-Δ* is nearly synthetic lethal with *top1-Δ* (Gadal *et al*., 1997). *In vitro*, human Top1 stimulates Pol II-dependent transcription (Kretzschmar *et al*., 1993) in a way that does not depend on catalytic activity (Shykind *et al*., 1997). In contrast, the Pol I-specific effect associated with the lethality of *rpa34-Δ top1-Δ* strictly depends on the catalytic activity of Top1, since it is not reversed by a plasmid bearing the *top1-Y727W* mutant at the catalytic tyrosine (data not shown).

Figure 5D summarizes the genetic interactions relating null mutants of Hmo1, Top1, Top3 and the Pol I-specific subunits Rpa49 and Rpa34 (Gadal *et al*., 1997). Hmo1/Rpa49/Top3 and Top1/Rpa34 clearly define two groups of null mutants. Crosses within a group invariably yield lethal double mutants. Crosses between groups produce double mutants comparable with the slowest-growing single mutant. In addition, null mutants of the Sgs1 DNA

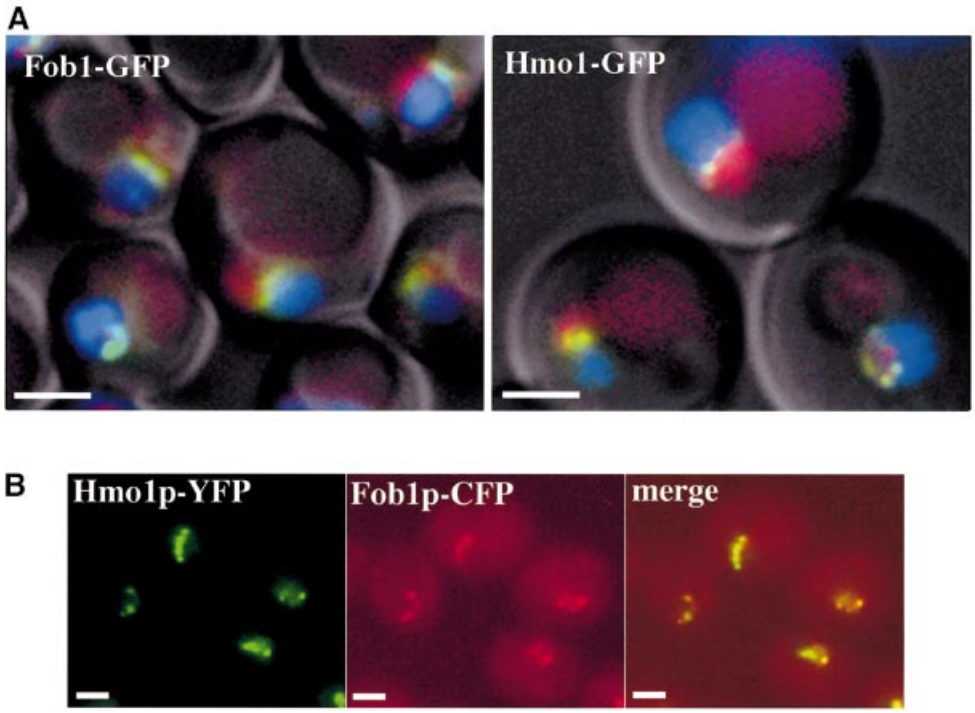

Fig. 4. *In vivo* localization of Hmo1–GFP and Fob1–GFP fusion proteins. (**A**) Fob1–GFP and Hmo1–GFP are concentrated at the interface between the nucleoplasm and the nucleolus. Strains BMA-Fob1–GFP and BMA-Hmo1–GFP bearing the pUN100-DsRed-NOP1 plasmid (Gadal *et al*., 2001) were grown at 30°C to mid-log phase (OD_{600} = 0.5) in YPD. Cells were washed with water. Nucleoplasm and nucleolus were visualized using DNA staining and Nop1 staining, respectively. GFP (green), DsRed (red) and Hoechst 33352 (blue) signals were monitored by fluorescence microscopy as described in Materials and methods. Cells were examined by Nomarski imaging. (**B**) Fob1 and Hmo1 co-localize. Fob1 and Hmo1 were tagged with different GFP spectral variants, CFP and YFP, and visualized in a diploid strain prepared by crossing strains BMA-Fob1–CFP and BMA-HMO1–YFP as described in Materials and methods (scale bars correspond to 2 μm).

helicase discriminate between the two topoisomerases since *sgs1-Δ* suppresses the adverse effects of *top3-Δ* (Gangloff *et al*., 1994) and its lethality with *hmo1-Δ* and *rpa49-Δ* (data not shown), but are synthetic lethal with *top1-Δ* (Lu *et al*., 1996b). *sgs1-Δ* has many pleiotropic properties including a strong accumulation of the 3μ rDNA episome (Sinclair and Guarente, 1997). The latter phenotype tentatively suggests that the Pol I-specific effects associated with the loss of Hmo1, Rpa49 or Top3 may not concern the episomal form of rDNA.

Discussion

Pol I-specific subunits and transcription factors that are specialized in yeast rRNA synthesis were discovered by a genetic screen based on the Pol II-dependent rescue of lethal Pol I mutants (Nogi *et al*., 1991). However, factors like Hmo1 that only lead to a partial growth defect would be overlooked by this approach. Hmo1 was first identified by its co-purification with an unidentified DNA helicase (Lu *et al*., 1996a). Apart from their slow growth rate, *hmo1-Δ* null mutants may alter plasmid stability (Lu *et al*., 1996a) and are synthetic lethal with *fpr1-Δ* mutants lacking a non-essential prolyl isomerase (Dolinski and Heitman, 1999).

Overexpressing Hmo1 strongly suppresses the cold-sensitive defect of *rpa49-Δ* mutants lacking the conserved Pol I-specific Rpa49/PAF53 subunit. Null mutants are lethal in *rpa49-Δ* cells. This defect is rescued by Pol II-dependent transcription of rDNA. It extends to other Pol I mutants, in a mutant-specific way. Finally, Hmo1 co-localizes with Fob1, a *bona fide* rDNA-binding protein (Defossez *et al*., 1999). By fluorescence microscopy, Hmo1 and Fob1 were shown to delineate a narrow segment located at the nucleolar–nucleoplasmic interface, that we propose to be the rDNA localization domain in living cells. These data strongly suggest that Hmo1 is a nucleolar factor specifically associated with rDNA, that acts synergistically with the conserved Rpa49/PAF53 subunit of Pol I during rDNA transcription.

Hmo1 is only found in *S.cerevisiae* and closely related yeast such as *S.kluyveri*, where the *HMO1* gene is immediately near the rDNA cluster (Neuvéglise *et al*., 2000). No homologue protein is encoded by the *Schizosaccharomyces pombe* genome (Wood *et al*., 2002). This poor conservation may be a general property of HMG-box proteins since *S.cerevisiae* and *S.pombe* each encode seven HMG-box proteins that are largely unrelated to each other, except for Nhp6A and Nhp6B, two nearly identical proteins that control the Pol III-dependent transcription of U6 splicing snRNA (Kruppa *et al*., 2001; Lopez *et al*., 2001) in *S.cerevisiae* and are closely related to an *S.pombe* gene product. This poor conservation is not incompatible with a conserved function, as the Abf2 HMG-box protein of *S.cerevisiae* may be functionally equivalent to the human mitochondrial transcription factor A (Parisi *et al*., 1993). It is therefore tempting to speculate that Hmo1 is functionally related to the animal UBF factor acting in Pol I-dependent transcription (Jantzen *et al*., 1990; Moss and Stefanovsky, 2002). UBF

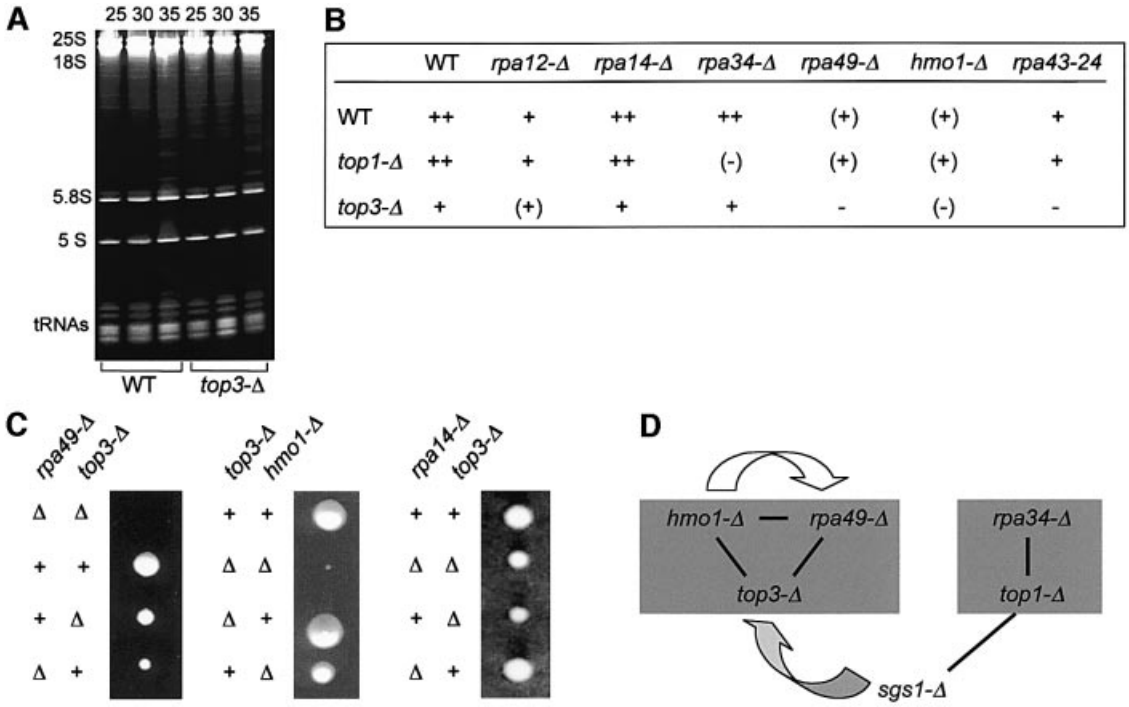

Fig. 5. Synthetic lethality and extragenic suppression between RNA polymerase I, type 1 topoisomerases and Hmo1 mutants. (**A**) Steady-state levels in rRNA and tRNA. Strains YPH499 (WT) and D211-3B (*top3-Δ*) were grown on YPD. Growth temperatures (°C) are given above each lane. Cells were harvested in mid-log phase (OD_{600} = 0.5). Note that *top3-Δ* has a severe growth defect at 35°C (Gangloff *et al*., 1994). Total RNA was extracted in hot phenol (Hermann-Le Denmat *et al*., 1994) and quantified by spectrophotometry at 260 and 280 nm. For each lane, 10 μg of total RNA were separated by polyacrylamide gel electrophoresis and revealed by ethidium bromide staining. (**B**) Synthetic lethality pattern. D308-6B (*top3-Δ*) was crossed to strains SL13-1A (*hmo1-Δ*), T4-1D (*rpa34-Δ*), 49-ΔA (*rpa49-Δ*) and GPY11-24 (*rpa43-24*). Strain D211-3B (*top3-Δ*) was used in the genetic cross to A14::URA3 (*rpa14-Δ*). At least 10 tetrads were analysed in each cross. Since *top3-Δ* mutants tend to accumulate *sgs1* suppressors (Gangloff *et al*., 1994), the parental *top3-Δ SGS1*$^+$ mutants were re-isolated freshly before each cross by meiotic out-crossing from *top3-Δ sgs1-Δ* double mutants. Growth patterns are as follows ++, +, (+): growth ranging from wild-type to intermediate level after 5 days at 30°C. –, (–): no or barely detectable growth, respectively. (**C**) Tetratype asci corresponding to *top3-Δ* crossed with *rpa49-Δ*, *hmo1-Δ* and *rpa14-Δ*. The wild-type (++), double mutant (*ΔΔ*) and parental single mutant genotype are indicated on the left of each panel. Spores were germinated on YPD and incubated at 30°C for 5 days. (**D**) Genetic interactions between Hmo1, Rpa49, Top3, Sgs1, Rpa34 and Top1. The two shaded grey boxes correspond to epistatic null mutants. Epistasis is defined here by the fact that double mutants between these two groups are viable (with a growth pattern similar to that of the slowest growing parent), whilst double mutants within each group are lethal or barely grow. Dark lines denote synthetic lethality. The white arrow indicates the dosage-dependent suppression of *rpa49-Δ* by *HMO1*. The grey arrow stands for the extragenic suppression of *top3-Δ* by *sgs1-Δ*. Data based on Gangloff *et al*. (1994), Lu *et al*. (1996a), Gadal *et al*. (1997) and this study.

is homodimeric and contains six HMG domains, but nothing is known of the number of Hmo1 molecules associated with rDNA.

UBF is present in mammals and amphibians (*Xenopus laevis*) but is probably restricted to vertebrates. Like Hmo1, UBF is important but partly dispensable for rDNA transcription (Smith *et al*., 1993). Its overexpression strongly increases rDNA transcription in human cardiomyocytes, in a way that is reminiscent of the dosage-dependent suppression phenotype found in the present work (Hannan *et al*., 1996). HMG domains have strong DNA-bending properties, and the corresponding proteins may act as architectural factors favouring the recruitment and co-operative interaction of transcription factors (Thomas and Travers, 2001; Mitsouras *et al*., 2002). Animal UBFs, for example, could form an rDNA 'enhanceosome' facilitating the recycling of Pol I between closely associated rDNA repeats (Moss and Stefanovsky, 2002). Likewise, our genetic suppression data suggest that Hmo1 alters the accessibility of individual rDNA to the Pol I transcription complex, in a way that compensates for the lack of Rpa49/PAF53. These data are based on the bypass of null mutants and thus need not imply a physical interaction between Hmo1 and Rpa49 itself. In animal cells, such an interaction is actually suggested by protein pull-down and far-western blotting data between UBF and PAF53 (Hanada *et al*., 1996).

The bending induced by HMG proteins may also strongly alter the topology of DNA. In the case of Hmo1, this would be consistent with its tight association with a DNA helicase (Lu *et al*., 1996a). Topological constraints on rDNA could explain the curious pattern of synthetic lethality that relates type 1A topoisomerases (Top1 and Top3) to Hmo1 and to non-essential subunits of Pol I. Briefly, cells lacking either Hmo1 or Rpa49 are lethal in a *top3-Δ* context but are not affected detectably by *top1-Δ*, whilst the converse pattern is observed on *rpa34-Δ* (Gadal *et al*., 1997). Another player in the game is Sgs1, a DNA helicase that can be inactivated genetically with no major effect on growth (Gangloff *et al*., 1994), but strongly interferes with genetic recombination (Gangloff *et al*., 2000). *sgs1-Δ* suppresses the adverse effects of *top3-Δ* (Gangloff *et al*., 1994), including its lethality with *hmo1-Δ* and *rpa49-Δ*, but is synthetic lethal with *top1-Δ* (Lu *et al*., 1996b). *sgs1-Δ* cells accumulate rDNA in its 3μ episomal form (Sinclair and Guarente, 1997). A simple

way of explaining the genetic interactions just mentioned could be that episomal transcription does not require Hmo1, Rpa49 or Top3, but is strictly dependent on Top1.

Twelve of the 14 subunits of yeast Pol I are present in the human enzyme (Gadal *et al*., 1997; Carles and Riva, 1998). Three components of the Pol I transcription system, TBP (Comai *et al*., 1992; Schultz *et al*., 1992), TFIIH (Iben *et al*., 2002) and Rrn3/TIF-IA (Schnapp *et al*., 1990; Yamamoto *et al*., 1996; Moorefield *et al*., 2000) are also conserved from yeast to man. On the other hand, the $TAFI_{48}$, $TAFI_{63}$ and $TAFI_{110}$ subunits of the mammalian SL1/TIF-IB factor (Eberhard *et al*., 1993; Zomerdijk *et al*., 1994) bear no detectable homology to any *S.cerevisiae* protein. Conversely, the Pol I-specific subunits of the yeast core factor and UAF (Rrn5, Rrn6, Rrn9, Rrn10 and Rrn11) are only found in *S.cerevisiae* and closely related species such as *Kluyveromyces lactis* (Bolotin-Fukuhara *et al*., 2000).

This lack of conservation evidently reflects the very fast evolution of the rDNA promoter (Grummt *et al*., 1982). It raises a curious paradox by imposing that a highly conserved enzyme (Pol I) must be able to recognize the highly variable platform defined by the yeast and human rDNA-binding complex. The initiation factor Rrn3/TIF-IA could be part of the answer, as the yeast and human forms can be exchanged *in vivo* despite a moderate sequence conservation (Moorefield *et al*., 2000). On the other hand, recent results suggest that Rrn3/TIF-IA may not determine Pol I recruitment, as initially proposed (Peyroche *et al*., 2000; Miller *et al*., 2001), but could instead be involved in a later step of Pol I initiation (Aprikian *et al*., 2001). Interestingly, Hmo1 stimulates the *in vitro* binding of a viral Pol II activator (the Rta factor of the Epstein–Barr virus) to its target gene by the simple virtue of its DNA-bending effect, apparently without any specific protein–protein interaction (Mitsouras *et al*., 2002). This supports the idea that Hmo1 and UBF could help in organizing yeast and animal rDNA-binding complexes into a similar target structure for Pol I-dependent transcription.

Materials and methods

Yeast media and genetic techniques were described previously (Stettler *et al*., 1993; Hermann-Le Denmat *et al*., 1994). Cells were grown on rich medium YPD or YPGal (with glucose or galactose as the main carbon source), and tested for auxotrophy on omission media derived from the synthetic complete medium (SC). *URA3* plasmids were counter-selected on FOA medium (SC with 0.1% FOA). Dosage-dependent suppressors of *rpa49-Δ* were selected from a genomic library prepared in the multicopy vector pFL44L (2μ *URA3*), with a total number of clones (~50 000) corresponding roughly to five yeast genomes (Stettler *et al*., 1993). This yielded four plasmids with different *RPA49* inserts and a fifth plasmid (pFL44-HMO1) with a 7 kbp insert bearing several genes including *HMO1*. The latter was the only gene retained in pD10-ΔSph, a suppressor plasmid obtained by deleting the 6.5 kbp *Sph*I fragment of pFL44-HMO1. Eliminating pD10-ΔSph or pFL44-HMO1 by selection on FOA restored the initial mutant growth defect.

Plasmid pASD10-HMO1 contains the *Sac*I–*Sph*I fragment of pD10-ΔSph (with *HMO1*) cloned into the *ADE2* centromeric vector pASZ11 (Stotz and Linder, 1990). pSLA49 (2μ *URA3 RPA49*) was constructed by cloning a 3.8 kbp *RPA49* insert (from plasmid pFL44S-49; Liljelund *et al*., 1992) between the *Sal*I and *Sac*I sites of pFL44L. pUN100-DsRed-NOP1 was described previously (Gadal *et al*., 2001). pFA6-YFP-TRP1, pFA6-CFP-TRP1, pFA6-YFP-HIS3MX6 and pFA6-CFP-HIS3MX6 are derivatives of pFA6a-GFP(S65T)-HIS3MX6 and pFA6-GFP-TRP1 (Longtine *et al*., 1998) where the *Pac*I–*Asc*I fragment, bearing the GFP-coding sequence, was PCR exchanged with the corresponding eYFP and eCFP spectral variants of GFP, using vectors pECFP-C1 and pEYFP-C1 from Clontech.

Yeast strains are listed in Table I. *hmo1-Δ::HIS3*, *hmo1-Δ::URA*3 and *hmo1Δ::TRP1* mutants were constructed in a YPH99 × YPH500 diploid (Sikorski and Hieter, 1989) by integrative transformation with a *Sma*I–*Sph*I fragment where *HMO1* has been interrupted by inserting the appropriate *HIS3*, *URA3* or *TRP1* cassette between the intragenic *Hpa*I and *Kpn*I sites. *rpa14-Δ::HIS3* was constructed by direct *in situ* deletion in the wild-type strain YPH499 (Baudin *et al*., 1993). The genetic structure of the diploid transformants was checked by genomic hybridization, and the corresponding haploid segregants were obtained by tetrad analysis. Strains BMA-*HMO1::GFP*, BMA-*FOB1::GFP*, BMA-*HMO1::CFP* and BMA-*FOB1::YFP* were constructed in a BMA64-1A or BMA64-1B background by C-terminal fusions (see Table I) as described previously (Longtine *et al*., 1998).

Fluorescence microscopy was done on exponentially grown cells washed in water and stained with Hoechst 33352 (5 ng/μl) for 5 min. Samples were examined using a Leica DMRXA fluorescence microscope. Fluorescent signals were collected with single band pass filters for excitation of DsRed (XF137-2, Omega optical), GFP (GFP, Leica), YFP (XF104, Omega Optical), CFP (XF114-2, Omega optical) and Hoechst 33352 (A, Leica). Images were acquired with a Hamamatsu C4742-95 cooled CCD camera controlled by the Openlab® software (version 2.2.4, Improvision) and processed using Adobe Photoshop® software (version 5, Adobe).

Steady-state levels of rRNA and tRNAs were measured in exponentially growing cells by ethidium bromide staining, and their *de novo* synthesis was monitored after a 20 min pulse of [^{3}H]uracil (Hermann-Le Denmat *et al*., 1994). The signal was quantified by scanning non-saturated autoradiogramms originating from two different assays with ImageQuant (Molecular Dynamic). The following ratios were measured: (18S + 25S) versus tRNAs (Pol I/Pol III), 5.8S versus 5S rRNA (Pol I/Pol III) and 5S versus tRNA (Pol III internal control).

Acknowledgements

We thank Ulf Nehrbass and André Sentenac for their kind support, Serge Gangloff for *top3* and *sgs1* strains, Rolf Sternglanz for the *top1-Y827F* mutant, Michel Werner for useful suggestions in the course of this work, and Frank Feuerbach-Fournier, Ivan Le Masson and Annette Boese for critical reading of the manuscript. O.G. received fellowships from the Institut de Formation Supérieure Bio-Médicale and from Institut Pasteur.

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Received March 22, 2002; revised July 25, 2002; accepted August 21, 2002