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Rpa43 and its partners in the yeast RNA polymerase I transcription complex

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ABSTRACT

An Rpa43/Rpa14 stalk protrudes from RNA polymerase I (RNAPI), with homology to Rpb7/Rpb4 (RNAPII), Rpc25/Rpc17 (RNAPIII) and RpoE/RpoF (archaea). In fungi and vertebrates, Rpa43 contains hydrophilic domains forming about half of its size, but these domains lack in *Schizosaccharomyces pombe* and most other eukaryote lineages. In *Saccharomyces cerevisiae*, they can be lost with little or no growth effect, as shown by deletion mapping and by domain swapping with fission yeast, but genetically interact with *rpa12Δ*, *rpa34Δ* or *rpa49Δ*, lacking non-essential subunits important for transcript elongation. Two-hybrid data and other genetic evidence suggest that Rpa43 directly bind Spt5, an RNAPI elongation factor also acting in RNAPII-dependent transcription, and may also interact with the nucleosomal chaperone Spt6.

Structured summary of protein interactions:
RPA43 physically interacts with **SPT6** by two hybrid (View interaction)
HMO1 and **SPT6** colocalize by fluorescence microscopy (View interaction)
RPA43 physically interacts with **RPA14** by two hybrid (View interaction)
RPA43 physically interacts with **SPT5** by two hybrid (View interaction)
HMO1 and **SPT5** colocalize by fluorescence microscopy (View interaction)

1. Introduction

RNA polymerase I (RNAPI), one of the three nuclear transcription enzymes present in all eukaryotes, mobilises ~70% of the total transcriptional capacity to produce a single transcript matured into the 18S, 5.8S and 25S ribosomal RNAs [1]. In budding and fission yeasts, RNAPI contains a 10-subunit core conserved in RNAPII, RNAP III and archaeal RNAPs [2,3], an Rpa34/Rpa49 dimer related to the TFIIF factor of RNAPII [4–6]. Finally, a Rpa43/Rpa14 'stalk' (Figs. 1 and 2) is homologous to Rpb7/Rpb4 (RNAPII), Rpc25/Rpc17 (RNAPIII) and RpoE/RpoF (archaea) but absent in bacteria [3,7–9]. Its biological role is unclear. In RNAPI, transcription starts when Rpa43 binds the Rrn3/TIF-1A pre-initiation factor [10–13]. In RNAPIII, *rpc25* mutations also interfere with initiation and Rpc17 possibly binds the Brf1 initiation factor [14,15]. In RNAPII, Rpb7/Rpb4 may form a dissociable initiation factor [16], but recent data suggest that Rpb4 and Rpb7 are required throughout the transcription cycle and that Rpb4 mediates the co-transcriptional processing of mRNAs [17–19]. Moreover, Rpb4/7 may shuttle between the nucleus and the cytoplasm [20], unlike its RNAPI, RNAPIII and archaeal counterparts which are stably associated to their core RNAP [9,21,22].

The fission yeast Rpa43 is a short protein (173 amino acids) limited to a conserved domain related to Rpb7/Rpc25/RpoE, with two additional RNAPI-specific motifs. In *Saccharomyces cerevisiae*, *Homo sapiens* and many other species, Rpa43 is a much larger protein with bulky hydrophilic domains [22]. We show here that these domains, although non-essential, become critical in the absence of non-essential RNAPI subunits involved in transcript elongation (Rpa12, Rpa34, Rpa49). Spt5, an elongation factor known to be an RNAPI partner [23], binds Rpa43 in a two-hybrid test, suggesting a direct interaction with the stalk domain. Finally, two-hybrid and genetic data relate Rpa43 to the Spt6 histone chaperone [24].

2. Materials and methods

Yeast strains and sequence alignments are detailed in Supplementary data S1 and S2. Fluorescence microscopy was done in a W303 background, but other strains derive from the *rpa43-Δ::LEU2/RPA43* diploid D101 [22], after transformation with YCp43-12 (*URA3 CEN RPA43*) and appropriate genetic back-crosses.

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Plasmids were prepared from PCR-amplified DNA, re-sequenced to discard spurious mutations and cloned in suitable vectors by DNA ligation or by the Gateway® technique, as previously described [4]. Hybrid *S. cerevisiae* and *Schizosaccharomyces pombe* constructs were based on overlap PCR (Pfu DNA polymerase).

Exponentially grown cells were stained for 5 min with Hoechst 33352 (5 ng/μl) and examined with a Leica DMRXA fluorescence microscope. Fluorescent signals were collected with single band pass filters. Images were acquired with a Hamamatsu C4742-95 cooled CCD camera, controlled by the Openlab® software and processed using Adobe Photoshop®. A two-hybrid screening [25] with a RPA43::$Gal4_{BD}$ bait identified fifty-three clones (out of ~5.10^6 transformants), corresponding to 14 distinct fusions determined by the DNA sequence of their insertion ends.

3. Results

3.1. Complementation between the Rpa43 subunits of S. cerevisiae and S. pombe

Rpa43 is widely conserved among eukaryotes (Fig. 1, Supplementary data S2), to the exception of Trypanosomes [26]. This conservation corresponds to positions 47–149 in *S. cerevisiae*, homologous to the Rpb7/Rpc25/RpoE hinge domains in other non-bacterial RNAPs, with two additional RNAPI-specific motifs (positions 153–169 and 215–235). In *S. cerevisiae*, hydrophilic and structurally disorganised domains corresponding to positions 1–46, 170–214 and 235–326, respectively exist in nearly all fungal or vertebrate Rpa43 but are largely absent in other lineages, including fission yeast where Rpa43 is a short protein of 173 amino acids limited to the hinge and RNAPI-specific motifs.

In *S. cerevisiae*, the conserved Rpa43 hinge is intertwined with Rpa14 [5] and its RNAPIspecific motifs form the outer part of the stalk (Fig. 2). In budding yeasts, Rpa43 also harbour hydrophilic and structurally disorganised domains that are internal (positions 170–214) or form variable N- and C-tails (1–46 and 235–326).

A similar conservation holds for Rrn3/TIF-1A and the Rpa49 subunit (Table 1). Thus, plants encode Rpa43-, Rpa49- and Rrn3-like proteins, although it has been argued that *Arabidopsis thaliana* may use a TFIIB-like factor (instead of Rrn3) to recruit RNAPI [27]. Nevertheless, the Rpa14 stalk subunit is only detected in budding and fission yeasts, and the Rpa34 partner of Rpa49 only exists in Unikonts (fungi, animals, amoebozoa), whilst the Rrn6 and Rrn7 components of the RNAPI pre-initiation complex are distantly related to the animal SL1/TIF-IB complex [15,16].

The fission yeast Rpa43 fails to complement *rpa43Δ* in *S. cerevisiae*, but *rpa43-chim1* and *rpa43-chim2* hybrids are viable (Fig. 3). However, they do not grow in an *rpa14Δ* background, fail to interact with Rpa14 in two-hybrid tests and are lethal in deletions lacking the non-essential Rpa12, Rpa34 or Rpa49 RNAPI subunits, which can be simultaneously inactivated without preventing growth [28]. Unlike *rpa12Δ* and *rpa49Δ*, these *rpa43* mutations presented a wild-type response to 6-azauracil and mycophenolate, which deplete the nucleotide triphosphate pool. Hybrids further encroaching on the Rpa43N-end (*rpa43-chim4, rpa43-chim7*) were lethal. Compared to *rpa43-chim1/chim2*, they lack a short insert ($_{88}$KILDADPLSKEDTS$_{101}$), characteristic of budding yeasts and filamentous fungi, which presumably accounts for their non-complementation.

3.2. The Rpa43 N- and C-ends interact with Rpa49

A deletion removing the last two-thirds of Rpa43 (*rpa43-12,65*) was wild-type in every respect and *rpa43-chim2*, which lacks the internal and C-terminal hydrophilic domains of Rpa43, has a temperature-sensitive defect also observed in *rpa43-14*, a frame-shift mutation removing most of the C-end [7]. The C-terminal extension of Rpa43 is therefore essentially neutral, despite its wide occurrence in fungi and vertebrates (Fig. 3). Yet, its full-deletion (*rpa43-12,35*), which also presented no detectable growth defect, was synthetic-lethal with *rpa14Δ*, *rpa12Δ*, *rpa34Δ* or *rpa49Δ*, but not with *top1Δ* or *hmo1Δ*, lacking the corresponding RNAPI co-factors [29]. Lethality was achieved by a slightly larger deletion (*rpa43-12,16*) removing the $_{215}$GHWVDSNGEPIGKLRFTVRN$_{235}$ RNAPI-specific motif (conserved positions are underlined), which is thus strictly required for growth. Conversely, N-terminal deletions (*rpa43-35,326*, *rpa43-35,281*) had a wild-type growth, except for their dependency on Rpa14. They were not synthetic-lethal with *rpa12Δ*, *rpa34Δ* or *rpa49Δ* but suppressed the cold-sensitive defect of *rpa49Δ* [4]. Taken together, these data point to a close functional interaction between the stalk and Rpa34/Rpa49 domains of RNAPI.

3.3. Spt5 and Spt6 are two-hybrid partners of Rpa43 and genetically interact with that subunit

Using a $Gal4_{BD}$::Rpa43 bait, we screened a $Gal4_{AD}$ library of randomly sheared yeast genomic fragments (~700 bp), to detect

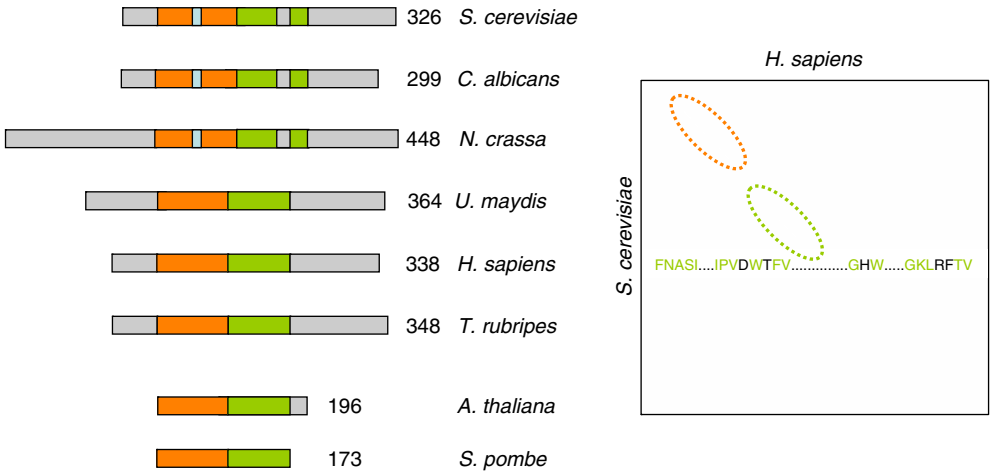

Fig. 1. Rpa43 conservation. The Rpa43 of fungi (*S. cerevisiae*, *Candida albicans, Neurosopora crassa*, *Ustilago maydis*) and vertebrates (*H. sapiens*, *Takifugu rupribes*) were aligned against the much shorter Rpa43 of *S. pombe* and *A. thaliana* (Supplementary data S2). The hinge, RNAPI-specific and hydrophilic domains are in orange, green and grey respectively. A short domain specific of budding yeasts and filamentous Ascomycetes is shown in blue. A dot matrix illustrates the homology of the *S. cerevisiae* and human subunits.