Halobacterial S9 Operon Contains Two Genes Encoding Proteins Homologous to Subunits Shared by Eukaryotic RNA Polymerases I, II, and III

KEITH MCKUNE AND NANCY A. WOYCHIK*

Roche Institute of Molecular Biology, Nutley, New Jersey 07110

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One key component of the eukaryotic transcriptional apparatus is the multisubunit enzyme RNA polymerase II. We have discovered that two of the subunits shared by the three nuclear RNA polymerases in the yeast *Saccharomyces cerevisiae*, RPB6 and RPB10, have counterparts among the *Archaea*.

Synthesis of mRNA in eukaryotes typically requires RNA polymerase II as well as several gene-specific and general transcription factors. Since many of the players in this process are becoming better defined genetically and biochemically, the conserved nature of these proteins is becoming increasingly apparent.

A major component of the eukaryotic transcriptional apparatus is the multisubunit enzyme RNA polymerase II. *Saccharomyces cerevisiae* RNA polymerase II has been extensively studied. The yeast enzyme consists of 12 subunits, RPB1 to RPB12, whose genes have been cloned, sequenced, and characterized elsewhere (1, 4, 9, 12, 17–23). Five of the 12 total *S. cerevisiae* subunits, RPB5, RPB6, RPB8, RPB10, and RPB12 (A10 α), are referred to as the common subunits since they are identical in RNA polymerases I, II, and III. Several eukaryotic RNA polymerase II subunit genes from other organisms have been isolated and sequenced as well. In general, the level of similarity between yeast RNA polymerase II subunits and their respective eukaryotic counterparts is about 60 to 70%. A comparable level of sequence similarity is seen among eukaryotic RNA polymerase II general transcription factors.

Eukaryotic RNA polymerase II subunit sequences have revealed that not only are subunits similar from one species to the next, but some are also closely related to prokaryotic RNA polymerase subunits (24). The two largest RNA polymerase II subunits are clearly related to β' and β . In addition, the yeast RPB3 subunit is related to the bacterial RNA polymerase α subunit. Therefore, the RPB1, RPB2, and RPB3 subunits are sometimes referred to as the eukaryotic core. *S. cerevisiae* RNA polymerase II does not appear to have a subunit that is closely related to any members in the bacterial σ subunit family. One subunit, RPB4, has an acidic region similar to a segment of the bacterial σ^{70} subunit (22). Since RPB4 and other eukaryotic transcription factors have regions related to conserved subdomains of σ^{70} , σ -like functions appear to be dispersed among several different eukaryotic proteins (7).

Archaeobacterial DNA-dependent RNA polymerase has a subunit architecture and complexity comparable to those of eukaryotic RNA polymerase II. In addition, subunit genes which have been isolated and sequenced revealed that the proteins they encode are more related to eukaryotic RNA polymerase subunits than to those in eubacteria. Archaeobacterial RNA polymerase subunits with similarities to RPB1, RPB2, RPB3, and RPB5 have been identified elsewhere (8, 11,

15, 16). While there are regions of strong similarity between these four archaeobacterial subunits and their yeast relatives, their overall sequence similarity (defined as the sum of identical amino acids and those scoring +1 or greater on the Dayhoff mutation datum matrix [5]) is ~40 to 45%, lower than the ~60 to 70% similarity seen between yeast *S. cerevisiae* subunits and their counterparts in other eukaryotes.

The α and S9 operon in *Haloarcula marismortui*. Two archaeobacterial operons containing genes encoding general components required for transcription and translation have been previously sequenced (10, 16). The general organization of the two adjacent operons, α and S9, is diagrammed in Fig. 1. The identity of HmaRp α was inferred by its relatedness to the yeast subunit RPB3 and other known eukaryotic homologs (16). Consistent with previous comparisons of *S. cerevisiae* subunits with their archaeobacterial counterparts (8, 15), HmaRp α was more closely related to the eukaryotic α -like RPB3 subunit than to the *Escherichia coli* subunit α (16). Interestingly, using TFASTA (14) and NCBI BLAST data base search programs to identify proteins related to yeast *S. cerevisiae* RNA polymerase II subunits, we have discovered that the H9 operon directly adjacent to the α operon has two small open reading frames which encode proteins highly related to eukaryotic common subunits, i.e., those which are components of RNA polymerases I, II, and III (20).

OrfMMV encodes the yeast RPB10 counterpart. OrfMMV encodes a 66-amino-acid protein that is similar in length and sequence (45% identical) to yeast *S. cerevisiae* RPB10. Another 22% of the residues are related, scoring +1 or greater on the Dayoff mutation matrix (5) (Fig. 2A). Alignment of the archaeobacterial subunit with the other known RPB10 homolog, from vaccinia virus (2), revealed two important cysteines containing stretches identical in all three species (Fig. 2B). The first region, C-X-X-C-G, was previously implicated in zinc binding (23). Later studies have shown that this subunit binds zinc in vitro, presumably at this site (4). The second conserved region, C-C-R, suggests a critical role for these three amino acids, possibly in zinc binding as well.

OrfMNA encodes a protein similar to the highly conserved half of RPB6. The second unknown open reading frame, designated OrfMNA (10), encodes only a 57-amino-acid protein. This protein has significant similarity to a portion of the highly conserved carboxy-terminal half of the RPB6 common RNA polymerase subunit in the yeast *S. cerevisiae* (20), human, and hamster RNA polymerases (13) (Fig. 3A). Alignment of the archaeobacterial protein with the *S. cerevisiae* RPB6 subunit sequence reveals that 63% of the residues are identical or

* Corresponding author. Phone: (201) 235-4708. Fax: (201) 235-5848.

FIG. 1. The α and S9 operon in *H. marismortui* (adapted from data presented by Kromer and Arndt [10] and Scholzen and Arndt [16]).

conserved in these two species (Fig. 3B). The level of conservation between the two proteins suggests that OrfMNA encodes the RPB6 counterpart in archaeobacteria. The fact that the archaeobacterial subunit lacks the amino acid residues corresponding to the amino-terminal half of RPB6 suggests that this portion of the subunit may be required for a transcriptional function specific for eukaryotic RNA polymerases I, II, and III.

hmRBP6 or hmRBP10 cannot functionally substitute for their *S. cerevisiae* counterparts. All five of the *S. cerevisiae* common subunits are essential for cell growth and viability. Since human *RPB6* (*hsRBP6*) can function in place of *S. cerevisiae* *RPB6* to support wild-type cell growth (13), we thought that it would be useful to see if subunits from archaeobacteria could also function in *S. cerevisiae*. We used the plasmid shuffle method (3) to see if expression of either archaeobacterial *hmRBP10* or *hmRBP6* could support growth in yeast cells lacking the gene encoding their respective *S. cerevisiae* homologs.

Six oligonucleotides were used to create synthetic *hmRBP6* and *hmRBP10* genes using a two-step PCR method (6). The *hmRBP6* and *hmRBP10* genes were then placed under control of the *RPB6* or *RPB10* promoters, respectively, and expressed in yeast centromere plasmids marked with the *LEU2* gene (pRP640 [*hmRBP6*]; pRP10/14 [*hmRBP10*]). DNAs from both plasmids were sequenced to verify the accuracy of the PCR reaction.

To determine if hmRBP6 can functionally substitute for

RPB6, haploid WY-37 yeast cells (*MATa ura3-52 his3 Δ 200 leu2-3 leu2-112 lys2 Δ 201 ade2 rpb6 Δ 2::HIS3* [pRP628]) were transformed with pRP640. WY-37 cells have a deleted chromosomal copy of *RPB6* replaced with the selectable marker *HIS3* plus a complementing plasmid containing a wild-type copy of *RPB6* on a yeast centromere plasmid marked with *URA3*. His⁺ Leu⁺ Ura⁺ transformants were subsequently placed on medium containing 1 mg of the selective agent 5-fluoroorotic acid per ml to prevent growth of *URA3*⁺ cells. Selection for the loss of the wild-type Ura⁺ RPB6 plasmid (pRP628) enabled us to determine whether the remaining plasmid (containing the *hmRBP6* gene) supported growth at either 24 or 30°C.

Similarly, to test if hmRBP10 can function in *S. cerevisiae*, haploid WY-40 yeast cells (*MATa ura3-52 his3 Δ 200 leu2-3 leu2-112 lys2 Δ 201 ade2 rpb10 Δ 1::HIS3* [pRP10/10]) were transformed with pRP10/14. WY-40 cells have a deleted chromosomal copy of *RPB10* replaced with the selectable marker *HIS3* plus a complementing plasmid containing a wild-type copy of *RPB10* on a yeast centromere plasmid marked with *URA3*. As with the RPB6 experiment, His⁺ Leu⁺ Ura⁺ transformants were transferred to 5-fluoroorotic acid plates to select against the Ura⁺ RPB10 plasmid (pRP10/10) and monitored for growth at the two permissive temperatures mentioned above.

S. cerevisiae cells carrying the *hmRBP6 LEU2* or *hmRBP10 LEU2* plasmids did not grow on 5-fluoroorotic acid plates placed at either 24 and 30°C. The control plasmids (an *RPB6 LEU2* plasmid in WY-37 and an *RPB10 LEU2* plasmid in WY-40) supported yeast cell growth on 5-fluoroorotic acid

FIG. 2. OrfMMV encodes the yeast RPB10 counterpart. Conserved residues are boxed; identical amino acid residues are boxed and in boldface.

FIG. 3. OrfMNA encodes a small protein similar to the highly conserved carboxy-terminal half of yeast RPB6. Conserved residues are boxed; identical amino acid residues are boxed and in boldface.

plates at both 24 and 30°C. The 5' and 3' DNA sequences of the control plasmids were identical to those of the corresponding plasmid containing the archaeobacterial gene. Therefore, the hmRBP6 and hmRBP10 subunits cannot functionally substitute for RPB6 and RPB10 in the yeast transcriptional machinery, at least not at a level which allows for sustained cell growth.

Concluding remarks. We have identified additional similarities between RNA polymerase subunits in eukaryotes and archaeobacteria. Two previously unknown open reading frames in the halobacterial S9 operon encode proteins that are significantly related to the yeast RPB6 and RPB10 subunits. The level of amino acid identity between the eukaryotic and archaeobacterial proteins is over 60%. This strong similarity suggests that the two open reading frames encode RNA polymerase subunits and that these subunits play key roles in transcription because of their high degree of conservation.

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