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Genetic Exploration of Interactive Domains in RNA Polymerase II Subunits

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The two large subunits of RNA polymerase II, RPB1 and RPB2, contain regions of extensive homology to the two large subunits of *Escherichia coli* RNA polymerase. These homologous regions may represent separate protein domains with unique functions. We investigated whether suppressor genetics could provide evidence for interactions between specific segments of RPB1 and RPB2 in *Saccharomyces cerevisiae*. A plasmid shuffle method was used to screen thoroughly for mutations in RPB2 that suppress a temperature-sensitive mutation, *rpb1-1*, which is located in region H of RPB1. All six RPB2 mutations that suppress *rpb1-1* were clustered in region I of RPB2. The location of these mutations and the observation that they were allele specific for suppression of *rpb1-1* suggests an interaction between region H of RPB1 and region I of RPB2. A similar experiment was done to isolate and map mutations in RPB1 that suppress a temperature-sensitive mutation, *rpb2-2*, which occurs in region I of RPB2. These suppressor mutations were not clustered in a particular region. Thus, fine structure suppressor genetics can provide evidence for interactions between specific segments of two proteins, but the results of this type of analysis can depend on the conditional mutation to be suppressed.

Yeast RNA polymerase II is composed of 10 polypeptide subunits whose molecular masses range from 190 to 10 kilodaltons (20). The genes that encode RPB1 and RPB2, the two largest subunits, have been cloned and sequenced (2, 21). RPB1 and RPB2 show extensive sequence similarity to the *Escherichia coli* RNA polymerase subunits β′ and β, respectively. The areas of most extensive sequence similarity between the procaryotic and eucaryotic subunits have been termed homology regions (9). RPB1 has eight regions of homology, regions A through H (2), and RPB2 has nine, regions A through I (21). The presence of these regions of conserved amino acids has led to the suggestion that they define separate protein domains, and it is possible that each of these domains has a specific function that is conserved among RNA polymerases (18).

Multiple functions have been attributed to the two large subunits of procaryotic and eucaryotic RNA polymerases. The *E. coli* RNA polymerase has been most extensively studied in this regard (4, 23). The two large subunits, β and β′, interact in the multisubunit enzyme, possibly at multiple sites. The β′ subunit appears to be largely responsible for DNA binding. The β subunit has been implicated in binding substrates and products and contains at least a portion of the catalytic site for RNA synthesis. β and β′ each bind one atom of Zn^{2+} (12, 22). Sequence conservation among procaryotic and eucaryotic RNA polymerases suggests that the two large subunits of eucaryotic RNA polymerases bind to one another and have functions similar to their procaryotic counterparts.

As a step toward better understanding the functions of the two large subunits of eucaryotic RNA polymerase II, conditional lethal mutations (cold sensitive and temperature sensitive) in both *RPB1* and *RPB2* have been isolated, characterized, and mapped (18). Many of these mutations occur in homology regions, and almost all affect amino acid residues that are invariant among the eucaryotic RNA polymerase II large subunits that have been sequenced thus far. A subset of these conditional mutations produce inositol auxotrophy (18), apparently by affecting the ability to induce *INO1* gene expression in the absence of inositol (19). Most of the mutations that produce inositol auxotrophy occur near the carboxy termini of RPB1 and RPB2.

Suppression of conditional mutations is a genetic means of identifying genes whose protein products interact with the product of a mutated gene (6). Suppression genetics has been used to reveal protein-protein interactions in the large multisubunit complexes of bacterial ribosomes (14) and eucaryotic flagella (10). It has also led to the isolation of new conditional mutations in P22 bacteriophage genes that encode structural components of the phage and permitted an analysis of the path of assembly of these components (8). The α-tubulin gene of *Aspergillus nidulans* was isolated by suppression of a conditional β-tubulin mutation (13). An actin-associating protein was isolated by suppression of a conditional mutation in actin (1). Suppressors of a *Salmonella typhimurium dnaC* temperature-sensitive mutation that occur in *dnaB* were isolated by introducing mutagenized copies of *dnaB* into cells with bacteriophage (11). In all these cases, the defect in a mutant protein is compensated to some degree by the suppressing mutation, probably owing to changes in the affinity of interaction between the two proteins of interest.

To investigate whether suppressor analysis could be extended to provide evidence for interactions between specific domains of proteins already known to associate, we used RPB1 and RPB2 as models. An efficient plasmid shuffle scheme was used to isolate and characterize *RPB2* mutations that suppress a temperature-sensitive mutation in region H of RPB1. We found that multiple independent suppressors of the *RPB1* mutation cluster in region I of RPB2 and are allele specific, suggesting an interaction between these two carboxy-terminal regions of RPB1 and RPB2.

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MATERIALS AND METHODS

Yeast strains and media. Yeast strains are shown in Table 1. Yeast RNA polymerase nomenclature and media are described by Nonet et al. (15). The *rpb1Δ196::HIS3* and *rpb1Δ187::HIS3* alleles are described by Nonet et al. (16); *rpb1-1* is described by Nonet et al. (15); *rpb1-5*, *rpb1-13*, *rpb1-15*, *rpb1-17*, *rpb1-18*, and *rpb1-19* are described by Scafe et al. (18); *rpb2-2* and *rpb2Δ297::HIS3* are described by Scafe et al. (19).

Plasmids. The plasmids pRP112 and pRP114 are *RPB1 CEN* plasmids containing *URA3* (pRP112) or *LEU2* (pRP114) (16). The plasmids pRP212 and pRP214 are *RPB2 CEN* plasmids containing *URA3* (pRP212) or *LEU2* (pRP214) (19). The plasmids pRP1-510L, pRP1-511L, pRP1-512L, pRP1-513L, and pRP1-514L are identical to pRP114 but contain the *RPB1* alleles *rpb1-510*, *rpb1-511*, *rpb1-512*, *rpb1-513*, and *rpb1-514*, respectively. The plasmids pRP2-510L, pRP2-511L, pRP2-512L, pRP2-513L, pRP2-514L, pRP2-516L, and pRP2-517L are identical to pRP214 but contain the *RPB2* alleles *rpb2-510*, *rpb2-511*, *rbp2-512*, *rpb2-513*, *rpb2-514*, *rpb2-516*, and *rpb2-517*. The plasmids pRP1-5U, pRP1-13U, pRP1-15U, pRP1-17U, pRP1-18U, and pRP1-19U are identical to pRP112 but contain the *RPB1* alleles *rpb1-5*, *rpb1-13*, *rpb1-15*, *rpb1-17*, *rpb1-18*, and *rpb1-19*, respectively.

Isolation of suppressors. The yeast strain Z324, which contains the temperature-sensitive mutation *rpb1-1* and a deletion of the chromosomal *RPB2* gene complemented by a wild-type *RPB2* gene on pRP212, was transformed with pRP214 DNA that had been mutagenized with hydroxylamine as described by Nonet et al. (15). *LEU2* transformants were replica plated to medium containing 5-fluoroorotic acid (5-FOA) to select against the *URA3*-containing pRP212 plasmid (3). Transformants were also replica plated to −Ura −Leu medium to select for cells containing both pRP212 and pRP214 to score the dominance or recessiveness of the suppressor alleles. After the 5-FOA selection, the transformants were replica plated to −Leu medium and incubated at 24°C and the restrictive temperature for *rpb1-1* (34°C). Transformants that grew at the restrictive temperature were single colony purified and reassayed for growth at 34°C by spot testing. To determine whether suppression was plasmid linked, we subjected the clones that exhibited ample growth to the rapid DNA isolation protocol of Hoffman and Winston (7). Plasmids containing putative suppressors of *rpb1-1* were introduced into yeast strain Z324 and reassayed for suppression by the plasmid shuffle scheme. Six *RPB2* suppressors of *rpb1-1* were isolated, creating yeast strains Z328 to Z339. The wild-type *RPB2* control strains are Z326 and Z327.

RPB1 suppressors of *rpb2-2* were isolated in a similar fashion. Yeast strain Z325 was transformed with hydroxylamine-mutagenized pRP114 DNA, and the plasmid shuffle scheme was used to isolate *RPB1*-linked suppressors. Transformants were screened at the restrictive temperature for *rpb2-2* (36°C). Five *RPB1* suppressors of *rpb2-2* were isolated, creating yeast strains Z343 to Z351. Wild-type *RPB1* controls for these strains are Z341 and Z342.

Sequence analysis of suppressor alleles. The nucleotide sequences of the *RPB1* and *RPB2* alleles were determined by primer extension (5) with oligonucleotide primers that span the two genes. The sequences were compared with the wild-type *RPB1* and *RPB2* sequences to determine the nucleotide position and the amino acid change for each mutation (2, 18, 21).

Phenotype of suppressor alleles in wild-type background. Each of the *RPB2* alleles that suppress the phenotype of *rpb1-1* was transformed into the yeast strain Z24, followed by the removal of the wild-type allele of *RPB2* by 5-FOA selection. The resultant cells were examined for conditional phenotypes by spot testing on YPD plates and incubating at 11, 15, 24, 30, 36, and 38°C. A similar experiment was done with the *RPB1* alleles that suppress *rpb2-2* by introducing them into the yeast strain Z26, removing the wild-type allele of *RPB1*, and studying the growth phenotypes of the resultant cells at various temperatures.

Determining allele specificity of *RPB2* suppressors of *rpb1-1*. To investigate the allele specificity of the *RPB2* alleles that suppress *rpb1-1*, we integrated six *RPB1* temperature-sensitive alleles previously isolated on plasmids into the chromosome. Yeast strain Z322 was transformed independently with the plasmids pRP1-5U, pRP1-13U, pRP1-15U, pRP1-17U, pRP1-18U, and pRP1-19U, which contain the temperature-sensitive alleles *rpb1-5*, *rpb1-13*, *rpb1-15*, *rpb1-17*, *rpb1-18*, and *rpb1-19*, respectively (18). Outgrowth of these transformants under non-*LEU2* selective conditions was used to permit loss of pRP114, which carries the wild-type allele of *RPB1*. The resultant Ura^+ Leu^- strains were patched to 5-FOA plates to select simultaneously for the loss of *URA3* and the replacement of the *rpb1Δ196::HIS3* allele in the chromosome with the plasmid-borne *RPB1* alleles. His^- Ura^- temperature-sensitive papillants were isolated from these plates, and the integration of each *RPB1* allele was confirmed by Southern analysis. These strains were each mated to yeast strain Z323, diploids were selected and sporulated, and tetrads were dissected. Segregants containing an *RPB1* temperature-sensitive allele as well as an *rpb2Δ297::HIS3* allele complemented by pRP212 were isolated. The cells were separately transformed with the plasmids pRP214, pRP2-510L, and pRP2-513L and were plated on 5-FOA-containing medium to select against the pRP212 plasmids. The resultant strains are called Z354 to Z371. The ability of the *RPB2* alleles *rpb2-510* and *rpb2-513* to suppress *rpb1-5*, *-13*, *-15*, *-17*, *-18*, and *-19* was determined by spot testing strains Z354 to Z371 on YPD plates and incubating the plates at 24, 36, and 38°C.

RESULTS

Isolation of supressors of *rpb1-1* in *RPB2*. Figure 1 shows the position of the *rpb1-1* mutation relative to the eight regions of sequence similarity between the yeast RNA polymerase II subunit RPB1 and the *E. coli* RNA polymerase subunit β′. The *rpb1-1* allele is a well-characterized temperature-sensitive mutation that produces a rapid shutoff of mRNA synthesis when cells are shifted to the nonpermissive temperature (15). The *rpb1-1* mutation alters a highly conserved amino acid in region H (17, 18).

A plasmid shuffle scheme was used to systematically screen for mutations in *RPB2* that suppress the temperature-sensitive phenotype of *rpb1-1* (Fig. 2). This method allowed us to introduce a mutagenized *CEN* plasmid copy of *RPB2* into a strain containing *rpb1-1*, to remove the wild-type copy of *RPB2*, and to rapidly score resultant clones for plasmid-linked suppression.

A screen of 17,500 yeast transformants yielded six suppressors of *rpb1-1* that were linked to the *RPB2 CEN* plasmid (see Materials and Methods for details). These *RPB2* mutations were given the allele numbers *rpb2-510*, *rpb2-511*, *rpb2-513*, *rpb2-514*, *rpb2-516*, and *rpb2-517*. All six suppressors exhibited a similar ability to suppress *rpb1-1*. Figure 3 shows the suppression phenotype of *rpb2-510* and

TABLE 1. Yeast strains

Strain	Alias	Genotype
Z24	N162	*MATα ura3-52 leu2-3,112 his3-Δ200 rpb2Δ297::HIS3* pRP212[*URA3 CEN RPB2*]
Z26	N247	*MATα ura3-52 leu2-3,112 his3-Δ200 rpb1Δ187::HIS3* pRP112[*URA3 CEN RPB1*]
Z96		*MATα ura3-52 leu2-3,112 his3-Δ200 rpb2Δ297::HIS3* pRP214[*LEU2 CEN RPB2*]
Z322	N118	*MATa ura3-52 leu2-3,112 his3-Δ200 tyr1-501 ade2-101 rpb1Δ196::HIS3* pRP114[*LEU2 CEN RPB1*]
Z323	N232	*MATα ura3-52 leu2-3,112 his3-Δ200 lys2-Δ201 trp1Δ1 ade2 rpb2Δ297::HIS3* pRP212[*URA3 CEN RPB2*]
Z324	CM190	*MATα rpb1-1 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3* pRP212[*URA3 CEN RPB2*]
Z325	CM200	*MATα rpb2-2 ura3-52 leu2-3,112 his3-Δ200 rpb1Δ187::HIS3* pRP112[*URA3 CEN RPB1*]
Z326	CM209	*MATα rpb1-1 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3* pRP212[*URA3 CEN RPB2*] pRP214[*LEU2 CEN RPB2*]
Z327	CM210	*MATα rpb1-1 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3* pRP214[*LEU2 CEN RPB2*]
Z328	CM201	*MATα rpb1-1 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3* pRP212[*URA3 CEN RPB2*] pRP2-510L[*LEU2 CEN rpb2-510*]
Z329	CM202	*MATα rpb1-1 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3* pRP2-510L[*LEU2 CEN rpb2-510*]
Z330	CM203	*MATα rpb1-1 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3 pRP212*[*URA3 CEN RPB2*] pRP2-511L[*LEU2 CEN rpb2-511*]
Z331	CM204	*MATα rpb1-1 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3* pRP2-511L[*LEU2 CEN rpb2-511*]
Z332	CM207	*MATα rpb1-1 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3* pRP212[*URA3 CEN RPB2*] pRP2-513L[*LEU2 CEN rpb2-513*]
Z333	CM208	*MATα rpb1-1 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3* pRP2-513L[*LEU2 CEN rpb2-513*]
Z334	CM211	*MATα rpb1-1 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3* pRP212[*URA3 CEN RPB2*] pRP2-514L[*LEU2 CEN rpb2-514*]
Z335	CM212	*MATα rpb1-1 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3* pRP2-514L[*LEU2 CEN rpb2-514*]
Z336	CM215	*MATα rpb1-1 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3* pRP212[*URA3 CEN RPB2*] pRP2-516L[*LEU2 CEN rpb2-516*]
Z337	CM216	*MATα rpb1-1 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3* pRP2-516L[*LEU2 CEN rpb2-516*]
Z338	CM217	*MATα rpb1-1 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3* pRP212[*URA3 CEN RPB2*] pRP2-517L[*LEU2 CEN rpb2-517*]
Z339	CM218	*MATα rpb1-1 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3* pRP2-517L[*LEU2 CEN rpb2-517*]
Z340	CM233	*MATα rpb2-2 ura3-52 leu2-3,112 his3-Δ200 rpb1Δ187::HIS3* pRP112[*URA3 CEN RPB1*] pRP114[*LEU2 CEN RPB1*]
Z341	CM234	*MATα rpb2-2 ura3-52 leu2-3,112 his3-Δ200rpb1Δ187::HIS3* pRP114[*LEU2 CEN RPB1*]
Z342	CM235	*MATα rpb2-2 ura3-52 leu2-3,112 his3-Δ200 rpb1Δ187::HIS3* pRP112[*URA3 CEN RPB1*] pRP1-510L[*LEU2 CEN rpb1-510*]
Z343	CM236	*MATα rpb2-2 ura3-52 leu2-3,112 his3-Δ200rpb1Δ187::HIS3* pRP1-510L[*LEU2 CEN rpb1-510*]
Z344	CM237	*MATα rpb2-2 ura3-52 leu2-3,112 his3-Δ200 rpb1Δ187::HIS3* pRP112[*URA3 CEN RPB1*] pRP1-511L[*LEU2 CEN rpb1-511*]
Z345	CM238	*MATα rpb2-2 ura3-52 leu2-3,112 his3-Δ200rpb1Δ187::HIS3* pRP1-511L[*LEU2 CEN rpb1-511*]
Z346	CM239	*MATα rpb2-2 ura3-52 leu2-3,112 his3-Δ200 rpb1Δ187::HIS3* pRP112[*URA3 CEN RPB1*] pRP1-512L[*LEU2 CEN rpb1-512*]
Z347	CM240	*MATα rpb2-2 ura3-52 leu2-3,112 his3-Δ200rpb1Δ187::HIS3* pRP1-512L[*LEU2 CEN rpb1-512*]
Z348	CM241	*MATα rpb2-2 ura3-52 leu2-3,112 his3-Δ200 rpb1Δ187::HIS3* pRP112[*URA3 CEN RPB1*] pRP1-513L[*LEU2 CEN rpb1-513*]
Z349	CM242	*MATα rpb2-2 ura3-52 leu2-3,112 his3-Δ200rpb1Δ187::HIS3* pRP1-513L[*LEU2 CEN rpb1-513*]
Z350	CM243	*MATα rpb2-2 ura3-52 leu2-3,112 his3-Δ200 rpb1Δ187::HIS3* pRP112[*URA3 CEN RPB1*] pRP1-514L[*LEU2 CEN rpb1-514*]
Z351	CM244	*MATα rpb2-2 ura3-52 leu2-3,112 his3-Δ200rpb1Δ187::HIS3* pRP1-514L[*LEU2 CEN rpb1-514*]
Z352	CM225	*MATα ura3-52 leu2-3,112 his3-Δ200 rpb2Δ297::HIS3* pRP2-510L[*LEU2 CEN rpb2-510*]
Z353	CM227	*MATα ura3-52 leu2-3,112 his3-Δ200 rpb2Δ297::HIS3* pRP2-513L[*LEU2 CEN rpb2-513*]
Z354	CM278	*MATa rpb1-5 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3* pRP214[*LEU2 CEN RPB2*]
Z355	CM279	*MATa rpb1-5 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3* pRP2-510L[*LEU2 CEN rpb2-510*]
Z356	CM280	*MATa rpb1-5 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3* pRP2-513L[*LEU2 CEN rpb2-513*]
Z357	CM281	*MATα rpb1-13 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3* pRP214[*LEU2 CEN RPB2*]
Z358	CM282	*MATα rpb1-13 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3* pRP2-510L[*LEU2 CEN rpb2-510*]
Z359	CM283	*MATα rpb1-13 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3* pRP2-513L[*LEU2 CEN rpb2-513*]
Z360	CM284	*MATα rpb1-15 ura3-52 leu2-3,112 his3-Δ200 ade2 tyr1-501 rpb2Δ297::HIS3* pRP214[*LEU2 CEN RPB2*]
Z361	CM285	*MATα rpb1-15 ura3-52 leu2-3,112 his3-Δ200 ade2 tyr1-501 rpb2Δ297::HIS3* pRP2-510L[*LEU2 CEN rpb2-510*]
Z362	CM286	*MATα rpb1-15 ura3-52 leu2-3,112 his3-Δ200 ade2 tyr1-501 rpb2Δ297::HIS3* pRP2-513L[*LEU2 CEN rpb2-513*]
Z363	CM287	*MATα rpb1-17 ura3-52 leu2-3,112 his3-Δ200 ade2 tyr1-501 rpb2Δ297::HIS3* pRP214[*LEU2 CEN RPB2*]
Z364	CM288	*MATα rpb1-17 ura3-52 leu2-3,112 his3-Δ200 ade2 tyr1-501 rpb2Δ297::HIS3* pRP2-510l[*LEU2 CEN rpb2-510*]
Z365	CM289	*MATα rpb1-17 ura3-52 leu2-3,112 his3-Δ200 ade2 tyr1-501 rpb2Δ297::HIS3* pRP2-513L[*LEU2 CEN rpb2-513*]
Z366	CM290	*MATα rpb1-18 ura3-52 leu2-3,112 his3-Δ200 ade2 tyr1-501 rpb2Δ297::HIS3* pRP214[*LEU2 CEN RPB2*]
Z367	CM291	*MATα rpb1-18 ura3-52 leu2-3,112 his3-Δ200 ade2 tyr1-501 rpb2Δ297::HIS3* pRP2-510L[*LEU2 CEN rpb2-510*]
Z368	CM292	*MATα rpb1-18 ura3-52 leu2-3,112 his3-Δ200 ade2 tyr1-501 rpb2Δ297::HIS3* pRP2-513L[*LEU2 CEN rpb2-513*]
Z369	CM293	*MATα rpb1-19 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3* pRP214[*LEU2 CEN RPB2*]
Z370	CM294	*MATα rpb1-19 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3* pRP2-510L[*LEU2 CEN rpb2-510*]
Z371	CM295	*MATα rpb1-19 ura3-52 leu2-3,112 his3-Δ200 ade2 rpb2Δ297::HIS3* pRP2-513L[*LEU2 CEN rpb2-513*]

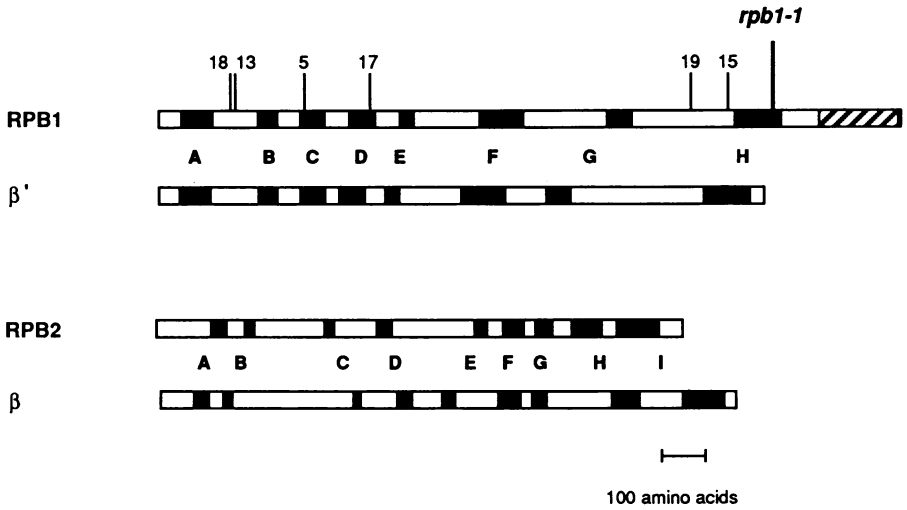
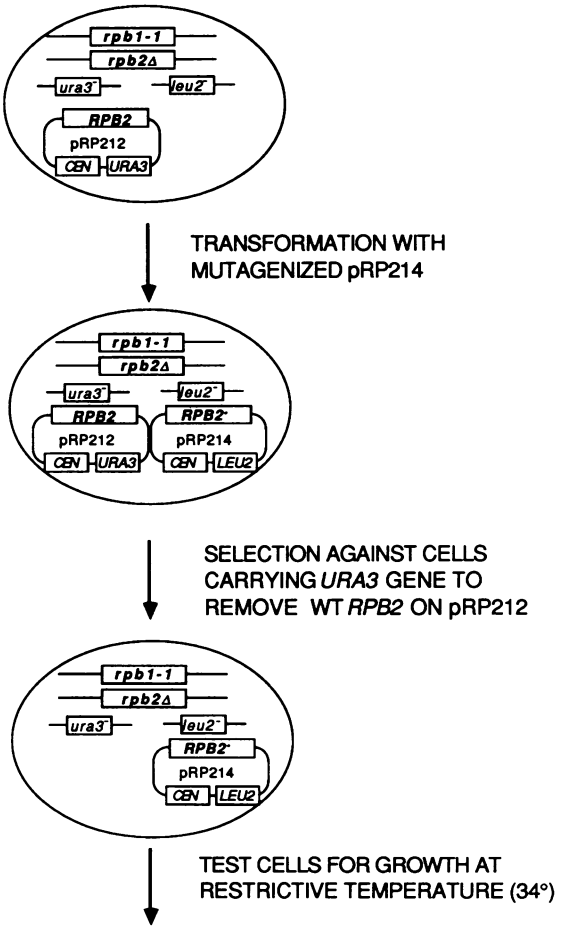


FIG. 1. Schematic diagram of the two largest subunits of RNA polymerase II. The eight regions of substantial homology between RPB1 protein and the β' subunit of *E. coli* RNA polymerase (2) and the nine regions conserved between RPB2 and the β subunit of *E. coli* RNA polymerase (21) are shown as black boxes in the diagram. The box with diagonal lines represents the heptapeptide repeat domain (2). The locations of the *rpb1-1* mutation and the *rpb1-5*, *rpb1-13*, *rpb1-15*, *rpb1-17*, *rpb1-18*, and *rpb1-19* mutations are indicated.



SUPPRESSORS OF *rpb1-1* IN *RPB2*

FIG. 2. Isolation of suppressors of *rpb1-1* in *RPB2* by using a plasmid shuffle. Asterisk indicates mutagenized DNA. Details are described in Materials and Methods. WT, Wild type.

rpb2-513. Cells containing the *rpb1-1* mutation were capable of growth at 24°C but not at 34°C unless they contained either *rpb2-510* or *rpb2-513*. This effect was observed on both rich medium (YPD) and on synthetic dropout (−Leu) medium.

To investigate whether the suppressors were dominant or recessive, we tested cells containing both a suppressor allele of *RPB2* on a *LEU2* plasmid and a wild-type copy of *RPB2* on a *URA3* plasmid for growth at the restrictive temperature. These cells were scored on −Leu −Ura medium (Fig. 3). The supressor alleles *rpb2-510* and *rpb2-513* exhibited a semidominant phenotype. They were capable of suppressing the temperature-sensitive phenotype of *rpb1-1* to some degree even in the presence of the wild-type copy of *RPB2*.

Location of suppressors of *rpb1-1* within *RPB2*. The DNA sequences of the six *RPB2* suppressor alleles (*rpb2-510*,

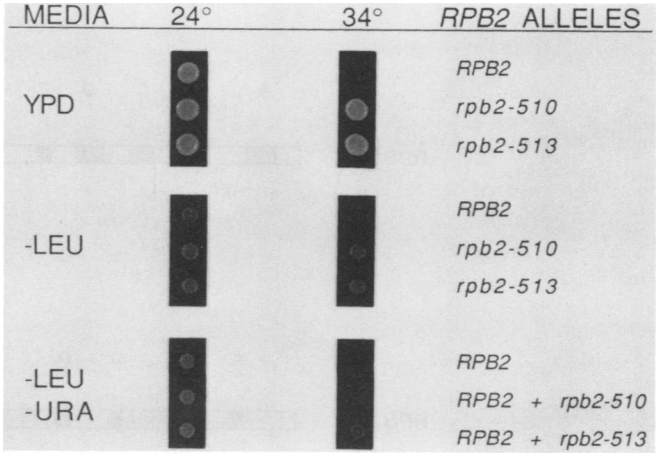


FIG. 3. Ability of *rpb2-510* and *rpb2-513* mutations to suppress the *rpb1-1* mutation at 34°C. Yeast strains Z327 (*RPB2*), Z329 (*rpb2-510*), and Z333 (*rpb2-513*) were spot tested on YPD and −Leu plates and incubated for 2 days at 24 and 34°C. Yeast strains Z326 (*RPB2*), Z328 (*RPB2* and *rpb2-510*), and Z332 (*RPB2* and *rpb2-513*) were spot tested on −Leu −Ura plates and incubated for 2 days at 24 and 34°C.

TABLE 2. Nucleotide and amino acid changes of suppressor alleles[a]

Allele	Nucleotide	Amino acid
rpb1-510	3497 A to C	Glu to Ala
rpb1-511, -512	1287 C to T	Arg to Cys
rpb1-513	4542 T to G	Phe to Leu
rpb1-514	4658 C to T	Ser to Leu
rpb2-510, -511, -516, -517	4242 C to T	Ser to Leu
rpb2-513, -514	4181 G to A	Asp to Asn

[a] Nucleotides are numbered according to Allison et al. (2) for RPB1 and Sweetser et al. (21) for RPB2.

rpb2-511, rpb2-513, rpb2-514, rpb2-516, and *rpb2-517*) were obtained with a set of oligonucleotide primers for the gene. The single nucleotide change observed for each allele and the amino acid change caused by each mutation are summarized in Table 2. The data show that the six independently isolated suppressors actually represent only two different mutations. The positions of these two mutations are shown schematically in Fig. 4. The amino acid changes that result in suppression were both in homology region I of *RPB2* and were 20 amino acids apart. Because each of these mutations was isolated multiple times from a screen of 17,500 cells transformed with a mutagenized plasmid library, and because the same pool of mutagenized DNA was used previously to obtain a dozen different *RPB2* conditional mutations in a screen of only 3,000 transformants (19), it appears that only a limited number of hydroxylamine-mediated mutations in *RPB2* can suppress *rpb1-1*.

Allele specificity of *RPB2* suppressors of *rpb1-1*. If the region of the RPB2 subunit containing the suppressing mutations interacts with the region in RPB1 containing the *rpb1-1* mutation, the *RPB2* mutations that suppress *rpb1-1* are unlikely to suppress a broad spectrum of other *RPB1* mutations. The ability of *rpb2-510* and *rpb2-513* to suppress six temperature-sensitive mutations which span the *RPB1* gene (*rpb1-5, rpb1-13, rpb1-15, rpb1-17, rpb1-18,* and *rpb1-19* [Fig. 1]) was investigated. *rpb2-510* and *rpb2-513* were allele-specific suppressors (Fig. 5). With one exception, neither of the two suppressors was able to suppress the growth defects of any of the six *RPB1* mutants at 36 or 38°C. The exception was that *rpb2-513* could suppress the temperature-sensitive defect of *rpb1-5* to some degree. Together, the repeated isolation of the two *RPB2* suppressors of *rpb1-1*, their adjacent location in region I, and their allele specificity are all consistent with the possibility that a portion of *RPB1* region H interacts with RPB2 region I.

Isolation of suppressors of *rpb2-2* in *RPB1*. The *RPB2* suppressors of *rpb1-1* mapped on either side of the temperature-sensitive mutation *rpb2-2* (19). This observation led us to investigate *RPB1* suppressors of *rpb2-2*. The *rpb2-2* mutation is a tight temperature-sensitive mutation with a restrictive temperature of 36°C. A plasmid shuffle scheme similar to that shown in Fig. 2 was used to isolate suppressors of *rpb2-2* in *RPB1* (see Materials and Methods for details).

A screen of 38,000 transformants yielded five *RPB1* plasmid-linked suppressors of *rpb2-2*. These *RPB1* mutations were given the allele numbers *rpb1-510, rpb1-511, rpb1-512, rpb1-513,* and *rpb1-514*. The ability of each allele to suppress *rpb2-2* mutants at 36°C is shown in Fig. 6 (*rpb1-512* is omitted because it is identical to *rpb1-511*). All cells were capable of growth at 24°C. At 36°C, only those cells containing *rpb1-510, rpb1-511, rpb1-513,* or *rpb1-514* were capable of growth. The ability of each allele to suppress *rpb2-2* varied slightly. The *rpb1-511* allele had a fairly strong dominant phenotype, while the others had only slightly dominant or semi-dominant phenotypes.

Location of *rpb2-2* suppressors within *RPB1*. The five *RPB1* mutations that suppress *rpb2-2* were mapped at the nucleotide level by DNA sequence analysis. Table 2 summarizes the nucleotide and amino acid changes caused by the mutations. The data revealed that *rpb1-511* and *rpb1-512* were identical mutations. The positions of these suppressors are shown in Fig. 4. These *RPB1* mutations did not cluster at one location. Two of the suppressors flanked the position of the *rpb1-1* mutation, and the other suppressing mutations occurred in RPB1 region C (*rpb1-511, rpb1-512*) and in region G (*rpb1-510*). The position of the *rpb2-2* suppressors cannot be

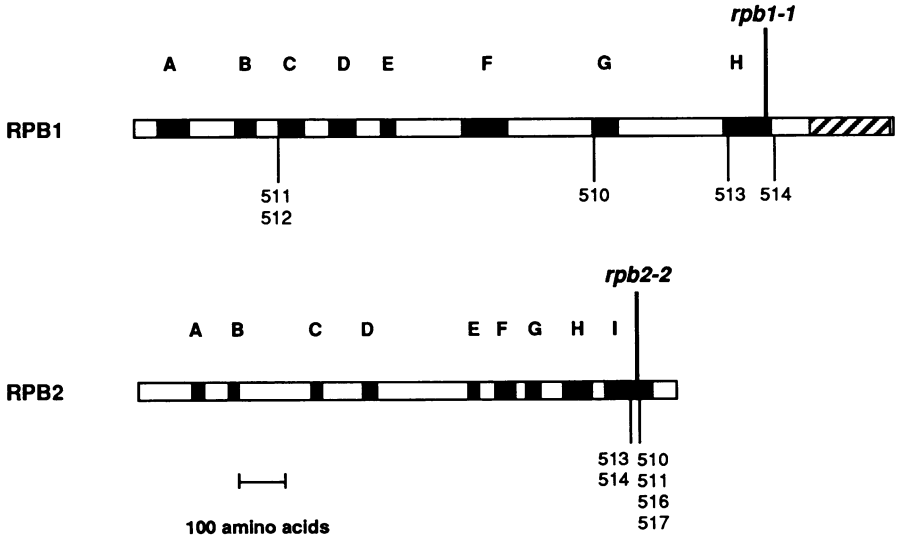


FIG. 4. Location of mutations in *RPB1* and *RPB2*. Locations of the temperature-sensitive alleles *rpb1-1* and *rpb2-2*, the *RPB2* alleles that suppress *rpb1-1* (*rpb2-510, -511, -513, -514, -516, -517*), and the *RPB1* alleles that suppress *rpb2-2* (*rpb1-510, -511, -512, -513, -514*) are indicated.

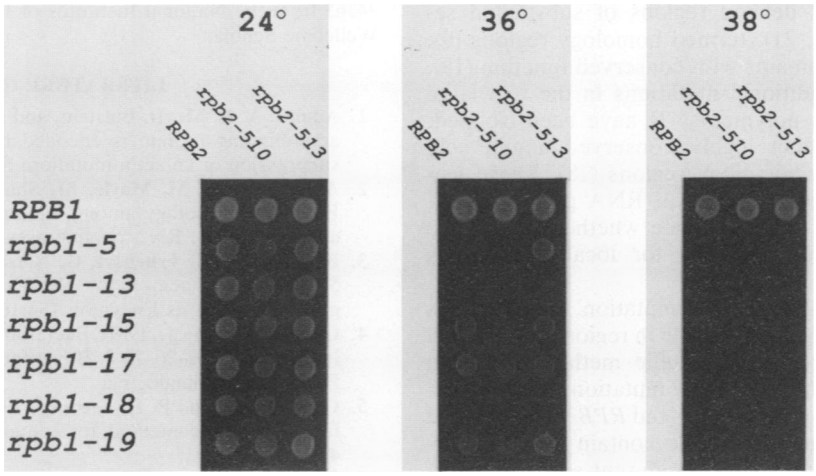


FIG. 5. Allele specificity of *rpb2-510* and *rpb2-513* suppression. *RPB1* strains Z96, Z352, and Z353, *rpb1-5* strains Z354 to Z356, *rpb1-13* strains Z357 to Z359, *rpb1-15* strains Z360 to Z362, *rpb1-17* strains Z363 to Z365, *rpb1-18* strains Z366 to Z368, and *rpb1-19* strains Z369 to Z371 were spot tested to YPD plates and incubated at 24, 36, and 38°C for 2 days. Cells containing the *rpb1-5*, *rpb1-13*, and *rpb1-18* mutations have a restrictive temperature of 36°C, and those containing the three other *RPB1* mutations have a restrictive temperature of 38°C.

interpreted in terms of a single domain-domain interaction. This could reflect multiple interactions and/or suppression via indirect mechanisms.

The *RPB1* and *RPB2* suppressor alleles have no conditional phenotypes in a wild-type strain background. The plasmid shuffle method was used to determine whether the *RPB1* and *RPB2* suppressor alleles had any conditional phenotypes when introduced into a wild-type strain background (see Materials and Methods for details). Cells containing any one of the mutations *rpb2-510*, *rpb2-511*, *rpb2-513*, *rpb2-514*, *rpb2-516*, and *rpb2-517* and a wild-type copy of *RPB1* were capable of growth at all temperatures tested (12, 15, 24, 30, 36, 38°C). A similar experiment with the *RPB1* suppressor alleles showed that none of these alleles limited cell growth over the same range of temperatures.

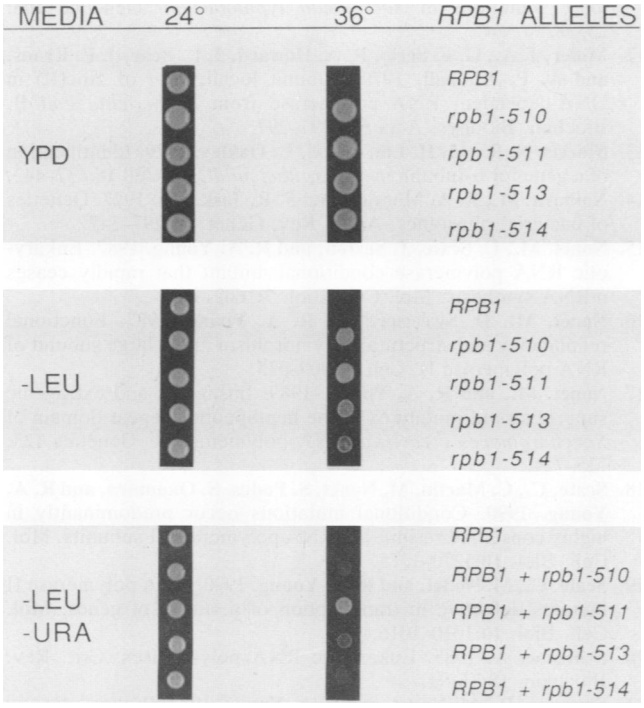


FIG. 6. Ability of *rpb1-510*, *rpb1-511*, *rpb1-513*, and *rpb1-514* to suppress the *rpb2-2* mutation at 36°C. Yeast strains Z341 (*RPB1*), Z343 (*rpb1-510*), Z345 (*rpb1-511*), Z349 (*rpb1-513*), and Z351 (*rpb1-514*) were spot tested on YPD and −Leu plates and incubated for 2 days at 24 and 36°C. Yeast strains Z340 (*RPB1*), Z342 (*RPB1* and *rpb1-510*), Z344 (*RPB1* and *rpb1-511*), Z348 (*RPB1* and *rpb1-513*), and Z350 (*RPB1* and *rpb1-514*) were spot tested on −Leu −Ura plates and incubated at 24 and 36°C.

DISCUSSION

Placing cells containing a particular conditional mutation under conditions restrictive for that mutation selects for secondary mutations (suppressor alleles) that allow growth of the cells. These mutations can be intragenic, occurring within the same gene, or extragenic, occurring in another gene. Extragenic suppressors are particularly useful because they can occur in genes whose protein products directly interact with the mutant protein produced by the conditional allele. The identification of the gene containing the suppressing mutation can lead to new insights about the biology of the gene products involved.

We took analysis of extragenic suppressors one step further by attempting to define not which proteins interact but rather, where two proteins are known to associate, which parts of these proteins interact. One premise underlying these experiments is that suppressing mutations generally act in one of two ways to compensate for the defect caused by the initial mutation. Suppressing mutations can affect amino acid residues that interact directly with residues in the domain affected by the initial mutation, or they can affect residues that compensate indirectly and at a distance. A second premise is that the former class of suppressors will tend to cluster in a limited segment of interacting protein and will frequently be allele specific, while the latter class of suppressors will be more dispersed and may be capable of suppressing a variety of alleles.

RNA polymerase II contains two very large subunits whose multiple functions include DNA and nucleotide triphosphate binding and RNA catalysis. They also associate with one another and with the other components of the enzyme. Sequence analysis of the procaryotic and eucary-

otic large subunits has defined regions of substantial sequence conservation (2, 21), termed homology regions (9), which may represent domains with conserved function (18). A large number of conditional mutations in the two large subunits of yeast RNA polymerase II have been isolated, and many of these affect highly conserved amino acid residues located within homology regions (18). These features make the two large subunits of RNA polymerase II good experimental tools to investigate whether second-site suppressors can provide evidence for local interactions between two proteins.

The temperature-sensitive *rpb1-1* mutation, which affects a highly conserved amino acid residue in region H, was used as a starting point. A plasmid shuffle method permitted screening of a large number of *RPB2* mutations for suppressors of *rpb1-1*, using a well-characterized *RPB2* mutagenized library that was previously shown to contain a wide distribution of mutations (19). The two different suppressors of *rpb1-1* were repeatedly isolated and cluster in region I of RPB2. These data suggest that this region of RPB2 interacts with region H of RPB1.

Characteristic of the two *RPB2* suppressors of *rpb1-1* was their inability to suppress most other temperature-sensitive mutations in *RPB1*. The allele specificity of suppression strengthens the argument for protein-protein interaction between specific domains. An exception to the allele specificity of the *RPB2* suppressors of *rpb1-1* was the ability of the *rpb2-513* allele to suppress the *rpb1-5* mutation, which occurs in region C of RPB1. This result suggests that region C also interacts with region I of RPB2 or that the *rpb1-5* mutation causes some defect that can be suppressed at a distance by the *rpb2-513* mutation.

The *RPB2* suppressors of *rpb1-1* mapped to nucleotide positions on either side of the *rpb2-2* temperature-sensitive mutation (18), motivating us to attempt the reciprocal experiment, that is, to investigate suppressors of *rpb2-2*. Suppressors of *rpb2-2* linked to *RPB1* were isolated, and their positions were mapped within the gene. The four *rpb2-2* suppressor alleles in *RPB1* did not cluster in a single region. However, two of the suppressors flanked the *rpb1-1* mutation in region H, consistent with an interaction between the carboxy-terminal segments of RPB1 and RPB2.

This study indicated that rapid isolation of a large number of suppressor alleles by a plasmid shuffle method can be a useful technique for locating sites of potential protein-protein interaction. The ability to screen large numbers of mutations in a specific gene permits one to isolate multiple suppressors and thus to investigate whether they tend to occur within a particular segment. A limitation of the approach is likely to be in the selection of the conditional mutation to be suppressed. Some amino acid alterations may affect structural or functional attributes of the protein that cannot be compensated directly by mutations in an interacting protein. Moreover, even when direct compensation can occur, mutations that compensate by indirect mechanisms may also be obtained, complicating the analysis. Despite these limitations, the ability to obtain multiple allele-specific suppressors that cluster may provide important insights into protein-protein interactions, especially with large multisubunit proteins that are not yet amenable to crystallography.

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