The nucleotides in domain I of 18 S rRNA that are important for the binding of the essential yeast ribosomal protein YS11 are mainly in a kink-turn motif and the terminal loop of helix 11 (H11). In the atomic structure of the Thermus thermophiles 30 S subunit, 16 amino acids in S17, the homolog of YS11, are within hydrogen bonding distance of nucleotides in 16 S rRNA. The homologous or analogous 16 amino acids in YS11 were replaced with alanine; none of the substitutions slowed the growth of yeast cells. The most severe effects were caused by mutations R103A, N106A, K133A, T134A, and K151A. The T. thermophiles analogs of Arg103, Asn106, Thr134, and Lys151 contact nucleotides in the kink-turn motif of 16 S rRNA, whereas Lys151 contacts nucleotides in the terminal loop of H11. These contacts are predominantly with backbone phosphate and sugar oxygens in regions that deviate from A-form geometry, suggesting that YS11 recognizes the shape of its rRNA-binding site rather than reading the sequence of nucleotides. The effect of the mutations on the binding of YS11 to domain I fragment of 18 S rRNA accorded, in general, with their effect on growth. Mutations of seven YS11 amino acids (Ser77, Met80, Arg88, Tyr97, Pro130, Ser132, and Arg136) whose homologs or analogs in S17 are within hydrogen bonding distance of nucleotides in 16 S rRNA did not affect binding. Apparently, proximities alone do not define either the amino acids or the nucleotides that are important for recognition.

Ribosomes translate genetic information carried in mRNAs into proteins. The chemistry of protein synthesis is based in the rRNAs, whereas the ribosomal proteins facilitate the folding of the rRNAs during biogenesis and stabilize the optimal conformation of the mature particle (1, 2). In accord with this premise, mutations in ribosomal proteins that affect protein synthesis are most often in rRNA-binding sites (3), hence the importance of a description of the nature of the interaction of nucleotides in rRNA and of amino acids in ribosomal proteins. In addition, an analysis of the interactions may help to uncover general principles that govern the association of the proteins and the rRNA and to define the details of ribosome assembly. Of particular importance are the primary binding ribosomal proteins, those that are defined by their ability to bind individually and stoichiometrically to rRNA in vitro reconstitution of the subunits; these proteins condense the rRNA and create binding sites for the remainder of the ribosomal proteins (4).

There is extensive information on rRNA-binding sites for prokaryotic ribosomal proteins (5–9), deriving most definitively from catalogs of the proximities of amino acids to nucleotides in the atomic structures of the Haloarcula marismortui 50 S (10) and Thermus thermophiles 30 S (11) ribosomal subunits. Less is known of the relation of the structure of eukaryotic ribosomal proteins to their function, particularly to their binding to rRNA and to their role in the assembly of ribosomes (12–19). There has been progress recently in this undertaking, facilitated by the application of genetics to the analysis; by the development of in vitro assays for the binding of the proteins to rRNA; and most significant, by the identification of the rRNA-binding sites of homologous prokaryotic ribosomal proteins revealed in the atomic structure of ribosomal subunits.

We have sought to determine the chemistry of the interaction of the yeast Saccharomyces cerevisiae ribosomal protein YS11 with nucleotides in 18 S rRNA (20). YS11 was selected because its homolog in Escherichia coli, S17 (EcS17), is a primary binding protein (21). YS11 is essential, and homologs are found in ribosomes of all species, including T. thermophiles (22). What is known of this family of proteins is inferred mainly from studies in bacteria: EcS17 is on the surface of the small subunit that abuts the large subunit (23), is essential for assembly of the particle (24), and has been implicated in the maintenance of translational accuracy (25, 26).

In the crystal structure of T. thermophiles 30 S ribosomal subunits, ribosomal protein S17 (TsS17), the homolog of YS11, makes extensive contacts with the highly conserved irregular helix 11 (H11; nucleotides 240–286) in domain I (nucleotides 1–560) of 16 S rRNA. A subset of nucleotides in H11 forms a kink-turn motif designated KT-11 (27). This helix-internal loop-helix has an unusual conformation caused by a kink in the phosphodiester backbone of one strand that bends the helix axis by 120° (27). KT-11 is one of two conserved kink-turn motifs in the 30 S subunit (28); both interact with ribosomal proteins (27).

Hydrogen bonds, non-polar contacts, and electrostatic interactions between amino acids and nucleotides can be derived from proximities in the atomic structures of the ribosomal subunits, but their importance to the structure and function of the ribosome and to the economy of the cell can come only from genetic and biochemical experiments (29). What is needed to define the subset of contacts that have functional relevance is best determined from the phenotype of mutations of nucleotides in RNA and of amino acids in ribosomal proteins.

The primary binding site of YS11 is in domain I of 18 S rRNA, and the nucleotides important for recognition are located in the KT-11 motif and in the terminal loop of H11 (20). Guided by the three-dimensional structure of TsS17 and 16 S rRNA in the T. thermophiles 30 S ribosomal subunit, we used reverse genetics and biochemical assays to determine the amino acids in YS11 that are important for the recognition of nucleotides in RNA.
EXPERIMENTAL PROCEDURES

Yeast and Bacterial Strains and Plasmids—Haploid S. cerevisiae strain LSF325 is a derivative of LSF327 in which the chromosomal ribosomal protein gene YS11-A was disrupted and replaced with a UR3 gene; haploid strain LSF326 is a derivative of LSF327 in which the chromosomal ribosomal protein gene YS11-B was disrupted and replaced with a LEU2 gene (22). For the genotypes of these and related strains, see Table 1. Plasmid pLY204 has the YS11-A gene between the BamHI and SalI sites in a CEN vector with a TRP1 selectable marker (22); this plasmid was used for complementation. Plasmid pMALc2-YS11-B has the YS11-B open reading frame fused to the open reading frame of a maltose-binding protein (MBP) between the BamHI and SalI sites in the pMALc2 vector. This plasmid was used for the expression of recombinant wild-type and mutant YS11 proteins. E. coli DH5α cells (Stratagene), the hosts for the plasmids, were grown in LB medium supplemented with kanamycin (50 mg/ml) and/or ampicillin (50 mg/ml).

Site-directed Mutagenesis of Ribosomal Protein YS11 Genes—Mutagenesis at specific sites in plasmids pMALc2-YS11-B and pLY204 (carrying YS11-A) was performed using the QuikChange II XL kit (Stratagene). A diploid strain from a cross of LSF325 and LSF326 was transformed with pLY204 (obtained from DH5α cells with the relevant mutations, and the effect on yeast viability and growth was assessed following sporulation and tetrad dissection.

Growth of Yeast Strains—Yeast cells were grown either in YPD liquid medium (1% (w/v) yeast extract, 2% (w/v) Bacto-peptone, and 2% (w/v) glucose) or in Ura⁻′/Leu⁺′/Trp⁻′ selective medium (0.67% Bacto-yeast nitrogen base without amino acids in 2% glucose to which 20 mg/liter adenine was added). The cultures were incubated with agitation at 30 °C, and the absorbance at 600 nm was determined at 60-min intervals. The doubling time was calculated from the growth curve, i.e., the increase in absorbance as a function of time.

Yeast Transformation, Sporulation, and Tetrad Dissection—Transformation of yeast cells was performed as described (18). A diploid strain produced by a cross of LSF325 and LSF326 was transformed with either wild-type or mutant pLY204 plasmids, and the effect on yeast viability and growth was assessed following sporulation and tetrad analysis. The viability of the spores was determined by replica plating on Ura⁻′/Leu⁺′/Trp⁻′ selective medium; incubation was carried out at 30 °C for 2–10 days. Successful complementation of the ys11 null mutant (ys11-a::URA3 ys11-1-h::LEU2) with pLY204 produced Ura⁺′ Leu⁺′ Trp⁺ spores, which were viable when grown in Ura⁻′/Leu⁺′/Trp⁻′ selective medium. The viable cells were grown in YPD medium, and the doubling time was determined as described above.

Preparation of Plasmids Encoding YS11 and Expression and Purification of the MBP-YS11 Fusion Protein—A cDNA encoding ribosomal protein YS11 was generated by PCR from a yeast cDNA library; MBP-YS11 was expressed in E. coli BL21(DE3) cells transformed with pMALc2-YS11-B and purified as described (20).

Preparation of Domain I of Yeast 18 S rRNA—Domain I of 18 S rRNA (nucleotides 1–610) was amplified PCR from plasmid pNOY102, which has an S. cerevisiae rRNA operon; the DNA fragment was then inserted into plasmid vector pBluescript adjacent to a T7 RNA polymerase promoter. Internally radiolabeled domain I RNA was synthesized by runoff transcription in vitro with T7 RNA polymerase and purified (20).

Preparation of Truncated YS11 Protein Variants—We constructed derivatives of YS11 that lacked the N-terminal 65 residues (designated YS11ΔN65), the eight residues in the C terminus (YS11ΔC8), or both ends of the protein (YS11ΔN65ΔC8). The coding sequences for these truncated peptides were amplified by PCR from pMALc2-YS11-B. The BamHI and SalI restriction sites were incorporated into either the 5′- or 3′-end of each fragment to allow swapping with the wild-type YS11 coding sequence at the identical sites in pMALc2-YS11-B. E. coli DH5α cells were transformed with these plasmids and screened for clones containing plasmids with the desired coding sequences. The resulting recombinant plasmids (pMALc2-YS11ΔN65, pMALc2-YS11ΔC8, and pMALc2-YS11ΔN65ΔC8) were used to transform E. coli BL21(DE3) cells, and the truncated proteins were expressed and purified. The identity of the truncated YS11 variants was confirmed from the molecular weight determined by mass spectroscopy. The fold of the mutant proteins was assessed by circular dichroism spectroscopy.

Assay of Binding of YS11 to a Domain I Fragment of 18 S rRNA—Increasing concentrations of wild-type or mutant MBP-YS11 fusion protein (up to 4 μM) were mixed with a radioactive domain I fragment of 18 S rRNA (1 nM) and incubated at room temperature for 10 min. The binding reaction (10 μl) was performed in 20 mM HEPES (pH 7.9), 30 mM NaCl, 100 mM KCl, 2 mM MgCl₂, 1 mM dithiothreitol, 4% (v/v) glycerol, 0.1% (v/v) Nonidet P-40, and 0.2 unit/μl RNasin. The reaction mixture also contained 120 nM tRNA and 0.05 μg/μl acetylated bovine serum albumin to reduce nonspecific binding of rRNA to protein. The reaction mixture was spotted in a single drop onto a nitrocellulose filter (13 cm²; Millipore Corp.) that had been soaked in the binding buffer. As many as 12 samples from a single experiment were placed on a filter, assuring the exposure of all to uniform filtration conditions. The binding reaction was stopped by filtration with gentle suction. The nitrocellulose filters (0.45 μm) were washed with 40 ml of the binding buffer and dried at room temperature. The binding of MBP-YS11 to rRNA was quantified with a Storm 860 PhosphoImager (Amersham Biosciences). The binding data were processed with ImageQuant software (Amer sham Biosciences) and fitted to two-component binding isotherms using KaleidaGraph Version 3.0 software (Synergy Software). The Kd and S.E. were obtained from a least square fit of the binding data. The average difference in replicate experiments in which the Kd was determined was ±11%. The amount of background RNA retention was determined by filtration in the absence of MBP-YS11 and was subtracted from the experimental values. Samples containing MBP and RNA but not YS11 were filtered to determine the level of nonspecific binding. An aliquot of each reaction mixture was spotted onto a filter to measure the input of radioactive RNA. The Kd for the binding of mutant YS11 to RNA and the value relative to that for the binding to wild-type YS11 were determined in the same experiment.

RESULTS AND DISCUSSION

Preparation of YS11 Mutants—Because YS11 binds predominantly to domain I of yeast 18 S rRNA (20), it was likely that the amino acids important for the interaction would be among those in close proximity to that domain. There are 16 amino acids in YS11 whose homologs or analogs in T. thermophilus (Tt S17) are within hydrogen bonding distance (3.4 Å) of nucleotides in domain I (11). Each of the 16 residues was replaced with alanine, and the effect of the mutations on cell viability and growth on the binding of mutant YS11 to a domain I fragment of 18 S rRNA was evaluated.

Ribosomal protein YS11 has 156 residues, whereas Tt S17 has 105. An alignment of the amino acid sequences of Tt S17 and YS11 revealed that the latter has 65 additional residues at the N terminus and 15 fewer at the C terminus (Fig. 1). In 83 amino acid comparisons, there are 33 identities (40%) and 15 conservative changes or 58% similarity. In an alignment of Tt S17 and Ec S17 (data not shown), there is 48% identity and 58% similarity. In a simultaneous alignment of the amino acid sequences of Tt S17, YS11, and Ec S17, the C-terminal 23 residues of Tt S17 (positions 83–105) are missing in Ec S17, and 15 of the 23 residues
The residues of YS11 that were mutated to alanine had a growth phenotype. The residues in 18 S and 16 S rRNAs in the two species. Conservation of the sequence of amino acids in YS11 and results obtained in our experiments. This assumption is favored by the ribosomal subunits can be used to interpret the biochemical and genetic changes (K/R, I/V/L, and F/Y) are indicated by dots. Alignment of the amino acid sequences of TS17 and S. cerevisiae YS11. The nucleotides in yeast 18 S rRNA important for YS11 recognition and are missing in YS11 (data not shown). Nonetheless, data from biochemical experiments indicate that E. coli, despite lacking the C-terminal residues, interacts predominantly with H11 in domain I rRNA (5–9, 23), as do TS17 and YS11 (11, 20). This indicates that the additional C-terminal residues in TS17 do not make physiologically significant contacts with H11 and indicates perhaps why they are not conserved in YS11 and E. coli. It appears that the amino acid residues that are important for recognition of and for binding to domain I are largely, if not exclusively, among the N-terminal 83 residues of TS17. Not surprisingly, this is the region of the greatest identity to amino acids in YS11 and TS17, and this is where most of the residues that were mutated are concentrated.

**Effect of Mutations in YS11 on Cell Growth**—Ribosomal protein YS11 is essential for cell viability (22). The lethal phenotype of a strain in which both copies of the YS11 gene are disrupted can be complemented successfully with a copy of either a YS11-A or YS11-B gene. Because most ribosomal YS11 is the product of transcription of the YS11-A gene (22), it was chosen for complementation. The YS11-A gene was replaced with URA3, and the YS11-B gene with LEU2; the resulting strains, LSF325 and LSF326, respectively, were crossed (Table 1). The diploid strain was transformed with a YS11-A gene in the pLFY204 vector carrying a TRP+ selectable marker and sporulated. The YS11-A gene has been mapped to chromosome IV, and YS11-B to chromosome II (22); therefore, the genes segregate independently during meiosis. Thus, when transformation is with pLFY204, one-quarter of the spores of the diploid strain are expected to grow on Ura+/Leu+/Trp+ selective plates.

The nucleotides in yeast 18 S rRNA important for YS11 recognition were determined previously (20). What we assessed here is the contribution of amino acids in YS11 to the interaction with rRNA. We made a strong assumption that the structure of TS17 in _T. thermophilus_ 30 S ribosomal subunits can be used to interpret the biochemical and genetic results obtained in our experiments. This assumption is favored by the conservation of the sequence of amino acids in YS11 and TS17 and that of nucleotides in 18 S and 16 S rRNAs in the two species.

Sixteen amino acids whose homologs or analogs in TS17 are in close proximity to nucleotides in domain I rRNA were replaced with alanine, and the rescue of the null strain by plasmids harboring a single mutation was assessed (Table 2). Of the 16 single substitutions, one was lethal (N106A), and eight had a slow growth phenotype (R82A, R103A, K105A, R129A, K133A, T134A, N138A, and K151A). Mutations of seven residues (Ser77, Met80, Arg86, Tyr97, Pro130, Ser132, and Arg136) did not affect growth despite their homologs or analogs in _T. thermophilus_ being within 3.4 Å of nucleotides in domain I.

### TABLE 1

| Strain | Genotype |
|--------|----------|
| LSF327 | MA/ade2-leu1-12-trp1-1-ura3-1 |
| LSF325 | MA/ade2-leu1-12-trp1-1-ura3-1::URA3 |
| LSF326 | MA/ade2-leu1-12-trp1-1-ura3-1::URA3 |
| LSF306 | MA/ade2-leu1-12-trp1-1-ura3-1::URA3 |
| JD031 | MA/ade2-leu1-12-trp1-1-ura3-1::URA3 |
| JD033 | MA/ade2-leu1-12-trp1-1-ura3-1::URA3 |
| JD034 | MA/ade2-leu1-12-trp1-1-ura3-1::URA3 |
| JD035 | MA/ade2-leu1-12-trp1-1-ura3-1::URA3 |
| JD036 | MA/ade2-leu1-12-trp1-1-ura3-1::URA3 |
| JD037 | MA/ade2-leu1-12-trp1-1-ura3-1::URA3 |
| JD038 | MA/ade2-leu1-12-trp1-1-ura3-1::URA3 |
| JD039 | MA/ade2-leu1-12-trp1-1-ura3-1::URA3 |
| JD040 | MA/ade2-leu1-12-trp1-1-ura3-1::URA3 |
| JD041 | MA/ade2-leu1-12-trp1-1-ura3-1::URA3 |
| JD042 | MA/ade2-leu1-12-trp1-1-ura3-1::URA3 |
| JD043 | MA/ade2-leu1-12-trp1-1-ura3-1::URA3 |
| JD044 | MA/ade2-leu1-12-trp1-1-ura3-1::URA3 |
| JD045 | MA/ade2-leu1-12-trp1-1-ura3-1::URA3 |
| JD046 | MA/ade2-leu1-12-trp1-1-ura3-1::URA3 |
| JD047 | MA/ade2-leu1-12-trp1-1-ura3-1::URA3 |
Binding of YS11 to 18 S rRNA

**TABLE 2**

**Doubling times of S. cerevisiae strains with mutations in a plasmid-borne copy of the YS11-A gene**

| YS11          | Doubling time in YPD medium (min ± S.E.) |
|---------------|-----------------------------------------|
| Wild-type     | 106 ± 4                                  |
| Mutations of amino acids that contact nucleotides in KT-11 |
| YS11-S77A     | 105 ± 7                                  |
| YS11-R103A    | 304 ± 16                                 |
| YS11-N106A    | Lethal                                  |
| YS11-T134A    | 211 ± 10                                 |
| YS11-K151A    | 243 ± 9                                  |
| Mutations of amino acids that contact nucleotides in the lower stem-loop of H11 |
| YS11-M80A     | 102 ± 5                                  |
| YS11-R82A     | 173 ± 8                                  |
| YS11-R129A    | 195 ± 8                                  |
| YS11-P130A    | 111 ± 4                                  |
| YS11-S132A    | 109 ± 7                                  |
| YS11-K133A    | 353 ± 19                                 |
| Mutations of amino acids that contact nucleotides outside of H11 |
| YS11-R88A     | 104 ± 3                                  |
| YS11-Y97A     | 105 ± 4                                  |
| YS11-K105A    | 166 ± 9                                  |
| YS11-R136A    | 103 ± 3                                  |
| YS11-N138A    | 176 ± 6                                  |

* The S.E. was calculated from values in replicate experiments.

**CONTRIBUTION OF AMINO ACIDS IN YS11 TO BINDING TO 18 S rRNA**—Next, we sought to determine whether an effect on the binding to rRNA could account for the growth phenotype of mutations in YS11. A fragment of *S. cerevisiae* 18 S rRNA corresponding to domain I was cloned and transcribed *in vitro* with T7 RNA polymerase. The domain I rRNA migrated in a single band on native polyacrylamide gels. The average $K_d$ for eukaryotic ribosomal proteins is 11; hence, most, including YS11, are insoluble in the buffers (pH 7.0–8.0) used in experiments dedicated to the measurement of the binding of ribosomal proteins to rRNA. For this reason, YS11 was expressed in *E. coli* as a fusion with MBP. MBP does not bind to rRNA (20); however, it has the advantage that it renders the MBP-YS11 fusion protein soluble and makes the binding experiments feasible.

The affinity of recombinant wild-type and mutant MBP-YS11 fusion proteins for domain I rRNA was determined in a filter binding assay (Fig. 2). In this assay (20), the concentration of radioactive RNA (nanomolar) was lower than that of the protein (micromolar), so the amount of complex formed was small compared with the amount of protein added. Under these conditions, the binding of wild-type MBP-YS11 to domain I had an equilibrium dissociation constant ($K_d$) of 0.13 μM, and ~50% of the input of domain I rRNA was in complexes with MBP-YS11 (Fig. 2). We interpret the plateau of the binding isotherm, when ~55% of the input rRNA is in complexes with YS11, as an indication that only that amount of the domain I fragment of 18 S rRNA is correctly folded. Given the size of the RNA fragment (610 nucleotides), the efficiency of the refolding can be considered to be good.

Mutations in YS11 that increased the doubling time of yeast cells (R82A, K105A, R129A, T134A, N138A, and K151A) (Table 2) reduced the binding of YS11 to domain I rRNA (Fig. 2 and Table 3). T134A and K151A increased the mutant $K_d$/wild-type $K_d$ ratio by 4.9- and 4.8-fold, respectively ($K_c$ calculated from the binding isotherms in Fig. 2); the other mutations increased the ratio by 1.7–2.8-fold. The R103A, N106A, and K133A mutations abolished binding (Fig. 2). A $K_d$ could not be calculated (Table 3); hence, it is unlikely that there is any physiologically relevant binding of these YS11 mutants to rRNA.

It was not unexpected that there was, in general, a correspondence between the deleterious effect of a mutation in YS11 on growth and binding to 18 S rRNA. The strongest effects were caused by mutations of amino acids that altered the interaction of YS11 with nucleotides either in KT-11 or in the terminal loop of H11, results that are consistent with and complement an earlier determination of the identity elements in 18 S rRNA (20). Of the 16 amino acids in YS11 that were mutated, atoms in the side chains of nine (Arg82, Arg103, Lys105, Asn106, Arg129, Lys133, Thr134, Asn138, and Lys151) were within 3.4 Å of nucleotides outside of H11 but in domain I of 18 S rRNA.

The contacts presumed to have been altered by mutations in YS11 and to have affected binding to rRNA are predominantly between amino acid side chains and nucleotide phosphate oxygens or sugar oxygens in regions where the conformation deviates from A-form geometry (Fig. 3 and Table 4). That the main amino acid contacts are not with...
nucleotide bases suggests that YS11 recognizes the shape of its rRNA-binding site rather than reading the nucleotide sequence.

**Effect of Mutations of Amino Acids in YS11 in Proximity to Nucleotides in KT-11—**Among the nine amino acids whose replacement with alanine altered the binding of YS11 to domain I of 18 S rRNA, four (Arg103, Asn106, Thr134, and Lys133) contact nucleotides in KT-11 (Table 2). The side chain of Arg103 in KT-11, the homolog of Arg103 in YS11, contacts nucleotide C280 (C281 in yeast 18 S rRNA) in a putative position of the homologous yeast YS11 structure of S. cerevisiae. In T. thermophilus, the structure of KT-11 that is mediated by a lysine (Lys41) in TtYS17 are mediated by an asparagine (Asn106) in YS11.

The amino acid in TtYS17 that aligns with Thr134 in YS11 (Fig. 1), Arg256, contacts nucleotides G277 (yeast G347) and C277 (yeast U346) in the KT-11 stem as well as nucleotide G254 (yeast G325) at the junction of KT-11 with the lower part of H11 (Fig. 3). Presumably, the T134A mutation affected growth and RNA binding (Fig. 2A and Table 3) as a result of these contacts being compromised, a conclusion reinforced by the observation that mutations of nucleotides G347, U346, and, to a lesser extent, G325 in yeast lead to a decrease in the binding of YS11 to an H11 oligoribonucleotide (20). It is a surprise that a threonine in YS11 can apparently replace an arginine in contacting nucleotides in KT-11.

The homolog of Lys133 in TtYS17 (Lys100) contacts nucleotide G257 (yeast A316) in the canonical stem in the KT-11 motif (Fig. 3); O-1 and O-2 of the phosphate of G257 make van der Waals contacts with the amino acid side chain of Lys100 (Table 4). The K131A mutation caused a decrease in the binding of YS11 to rRNA (Fig. 2A and Table 3) presumably because these contacts were absent. Indeed, the 316A→U mutation in a yeast H11 oligoribonucleotide decreases the binding of YS11 (20).

The S77A mutation in YS11 affected neither cell growth (Table 2) nor binding to domain I rRNA (data not shown) despite the observation that its homolog in TtYS17 (Ser12) interacted with nucleotide G257 (yeast G347) in KT-11 (Fig. 3). In contrast to the lack of a phenotype of the S77A mutation, a G47 mutation in 18 S rRNA decreases RNA-protein formation by >10-fold (20). We note, however, that in the T. thermophilus 30 S subunit, G276 interacts not only with Ser12, but also with Arg48 (yeast Thr134) (Table 4); the latter interaction appears to be the one important for recognition because the T134A mutation reduced the binding of YS11 to RNA (cf. above and Fig. 2A and Table 3). The resolution of these apparently conflicting results is that the decrease in the binding of YS11 to 18 S rRNA caused by mutation of G276 was the result of the loss of a contact with Thr134 rather than with Ser77.

**Effect of Mutations of Amino Acids in YS11 in Proximity to Nucleotides in the Lower Stem-Loop of H11—**Alanine substitutions were made at six amino acids in YS11 (Met30, Arg2, Arg29, Pro30, Ser32, and Lys33) that are presumed, from the structure of TtYS17, to be within hydrogen bonding distance of a nucleotide in the lower stem-loop of

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**TABLE 3**

| Wild-type YS11 | Mutant Kd/ wild-type Kd |
|----------------|-------------------------|
| YS11-R82A      | 0.29 ± 0.05              |
| YS11-R103A     | 0.22 ± 0.02              |
| YS11-K105A     | 0.37 ± 0.08              |
| YS11-K133A     | 0.64 ± 0.12              |
| YS11-T134A     | 0.24 ± 0.03              |
| YS11-K151A     | 0.62 ± 0.14              |

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**FiguRe 3. Structural diagrams.** A, secondary structure of S. cerevisiae H11; B, secondary structure of T. thermophilus H11; C, three-dimensional structure of T. thermophilus H11 and TtYS17 (11) generated with the program Ribbons (31). H11 is shown in blue, and TtYS17 in green and orange. The putative positions of the homologous yeast YS11 amino acids and 18 S rRNA nucleotides are given in parentheses.
H11. However, only three mutations (R82A, R129A, and K133A) affected growth and binding to rRNA (Fig. 2 and Table 2). Arg82 in YS11 aligns with Lys17 in Tt S17 (Fig. 1) and contacts nucleotides G255 and U256 (yeast G326 and U327) at the junction between KT-11 and the lower part of H11 (Fig. 3). Paradoxically, mutation of yeast G326 or U327 does not affect the binding of rRNA to YS11 (20). We note, however, that the side chain of Lys17 in Tt S17 contacts the sugar phosphate backbone of G255 and U256 (Table 4). The amino acid side chain changes of course when alanine is substituted for arginine; however, the backbone around G326 and U327 might not be appreciably altered when their bases are mutated.

Arg129 in YS11 (Arg63 in Tt S17) is assumed, from the structure of S17 in the T. thermophilus 30 S subunit, to interact with U335 (U264 in T. thermophilus) in the terminal loop of H11 (Fig. 3). However, the 335U->A mutation does not affect binding (20), whereas the R129A mutation did. Arg63 in Tt S17 contacts the backbone of U264; and once again, the backbone may not be appreciably altered when the base is mutated. On the other hand, it is the side chain of Arg129 that contacts U335, a contact that must be affected when alanine is substituted for arginine. It is also possible that the R129A mutation affects the structure of YS11 adversely; and in this case, the effect of the R129A mutation is indirect. There may be a lesson to be learned from these two examples, viz. when a contact is between the side chain of an amino acid and the sugar phosphate backbone of a nucleotide, a mutation in the former is more likely to have a phenotype than a mutation in the latter.

Arg129 in YS11 (Arg63 in Tt S17) is assumed, from the structure of S17 in the T. thermophilus 30 S subunit, to interact with U335 (U264 in T. thermophilus) in the terminal loop of H11 (Fig. 3). However, the 335U->A mutation does not affect binding (20), whereas the R129A mutation did. Arg63 in Tt S17 contacts the backbone of U264; and once again, the backbone may not be appreciably altered when the base is mutated. On the other hand, it is the side chain of Arg129 that contacts U335, a contact that must be affected when alanine is substituted for arginine. It is also possible that the R129A mutation affects the structure of YS11 adversely; and in this case, the effect of the R129A mutation is indirect. There may be a lesson to be learned from these two examples, viz. when a contact is between the side chain of an amino acid and the sugar phosphate backbone of a nucleotide, a mutation in the former is more likely to have a phenotype than a mutation in the latter.

The side chain of the Lys133 homolog in Tt S17 (Lys67) contacts the 3'-oxygen of the ribose in nucleotide G254 (yeast G325) (Table 4); this nucleotide is bulged out of the terminal loop of H11 (Fig. 3). Lys67 also contacts nucleotides G254 (yeast G325) and C277 (yeast C338). Mutations

### Table 4

| Amino acids | T. thermophilus 16 S rRNA nucleotide | Atoms in amino acida | Atomic contact | Distance Å |
|-------------|-------------------------------------|----------------------|----------------|-----------|
| Lys17 Arg82 | G255 U256                           | N1·O-3'              | 3.09           |
| Arg38 Arg103 | C280                                | C5·O-1P              | 3.27           |
| Lys40 Lys105 | G236 C237                            | N4·O-1P              | 3.13           |
| Arg52 Arg129 | U264 A130                          | U190 N               | 2.98           |
| Lys67 Lys133 | G254                                | NH4·O-2'             | 3.38           |
| Arg68 Thr134 | G266 C267                            | O·O-1P               | 3.06           |
| Arg72 Asn138 | U190                                | N·O-1P               | 2.97           |
| Lys100 Lys151 | G247                                | NH4·O-2'             | 3.36           |

The side chain atoms are designated by Greek letters; van der Waals contacts are in boldface.

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**FIGURE 4.** Binding isotherms for the association of truncated forms of YS11 and a domain I fragment of 18 S rRNA. A radioactive domain I fragment of 18 S rRNA (1 nM) was incubated with increasing concentrations of wild-type or truncated MBP-YS11, and the binding was determined in a filter binding assay.
of yeast G$^{337}$ abolish the binding of YS11 to rRNA, and mutations of G$^{325}$ and C$^{338}$ reduce binding (20). The phenotype of the K133A mutation, slower cell growth and a decrease in the affinity of binding to rRNA (Fig. 2 and Table 3), is surely caused by impairment of the interactions of Lys$^{133}$ with G$^{325}$, G$^{337}$, and C$^{338}$.

Effect of Mutations of Amino Acids in Proximity to Nucleotides in Domain I of 18 S rRNA That Are outside of H11—Five amino acids in this category (Arg$^{66}$, Tyr$^{77}$, Lys$^{105}$, Arg$^{136}$, and Asn$^{138}$) were replaced with alanine. Two of these (K105A and N138A) had a modest effect on growth (Table 2) and on binding to rRNA (Fig. 2C and Table 3). The amino acids in TIS17 (Lys$^{40}$ and Arg$^{57}$) that align with Lys$^{105}$ and Asn$^{138}$ in YS11 are in proximity to nucleotides that are in domain I but outside of H11 (Lys$^{105}$ with nucleotides G$^{236}$ and C$^{237}$ and Asn$^{138}$ with U$^{190}$) (Table 4). As was indicated previously (20), H11 has most of the elements that are critical for YS11 recognition, hence the relatively weak effect of the two mutations on cell growth and RNA binding.

Effect of Deletions at the N and C Termini of YS11 on Binding to Domain I of 18 S rRNA—Derivatives of YS11 lacking the N-terminal 65 residues that are absent in TIS17 (designated YS11ΔN65), the eight residues at the C terminus that show minimal conservation with residues in TIS17 (designated YS11ΔC8), or both ends of the protein (designated YS11ΔN65ΔC8) were constructed, and their binding to domain I of 18 S rRNA was assessed. YS11ΔC8 bound to domain I rRNA with an affinity ($K_d = 0.13 \mu M$) similar to that of the wild-type protein ($K_d = 0.11 \mu M$) despite somewhat fewer RNA-protein complexes having been formed (Fig. 4 and Table 3). However, YS11ΔN65 exhibited a 5.8-fold increase in the $K_d$ (0.64 $\mu M$). We presume that one or more amino acids at the N terminus make a contribution to the free energy of binding. The mutant with a combination of both deletions in YS11 (YS11ΔN65ΔC8) hardly bound to domain I rRNA at all (Fig. 4). Circular dichroism revealed that the secondary structures of YS11ΔC8 and YS11ΔN65 closely resembled that of wild-type YS11. On the other hand, YS11ΔN65ΔC8 lacks $\alpha$-helices and $\beta$-strands; the lack of secondary structure is sufficient to explain why the severely truncated protein did not bind to rRNA.

Mutations of Amino Acids in YS11 That Have No Phenotype Despite Their Proximity to Nucleotides in Domain I of 18 S rRNA—The S77A, M80A, R88A, Y97A, P130A, S132A, and R136A mutations in YS11 did not affect the growth of yeast cells or the binding of the protein to domain I. Binding was assessed at a single concentration of domain I rRNA and of mutant YS11 and did not differ from the binding of wild-type YS11 (data not shown).

The lack of a phenotype for these mutations in YS11 despite the mutated amino acids being within 3.4 Å, i.e., within hydrogen bonding distance, of an atom in an RNA nucleotide is as important as the positive results. It confirms that proximity does not by itself define the subset of bonds that are essential for rRNA-ribosomal protein association. Once again, a diagnosis of the physiologically relevant contacts requires genetics and biochemistry.

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