In contrast to peptides bound to class I molecules, which are predominantly nonamers[1-4], class II peptides are larger and display a high degree of heterogeneity both in length and the site of terminal truncation, implying that the mechanisms of processing for class I and class II peptides are substantially different. Furthermore, it may suggest that class II processing is a stochastic event and that a DR allele may bind peptides of different lengths from a complex random mixture. The heterogeneity observed may only be due to protection of bound peptides from further degradation. Thus, class II molecules would play an active role in antigen processing (as previously proposed[35]) by protecting the bound peptides from complete degradation. Alternatively, the predominance of 15-amino-acid peptides bound to DR1 (as detected by both the MALD-MS and the yields of sequenced peptides) could be the result of trimming of bound peptides. In any event, the absence of peptides shorter than 13 and longer than 25 residues suggests that there are length constraints intrinsic to either the mechanism of peptide binding or antigen processing. In fact, a 15-residue peptide in an extended conformation can fit in an open cleft, but a longer peptide would have to extend beyond the cleft in such a conformation.

The predominance of peptides bound to DR1 that are derived from endogenously synthesized proteins, and particularly MHC-related proteins, may result from the evolution of a mechanism for presentation of self peptides in connection with the generation of self tolerance. The plethora of self peptides also has potentially important implications for the generation of autoimmune diseases linked to particular alleles of class II molecules, resulting from a breakdown of self tolerance.

Note added in proof: Another report describing naturally processed peptides extracted from murine class II molecules has been published[38]. □

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The structure of ribosomal protein S5 reveals sites of interaction with 16S rRNA

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UNDERSTANDING the process whereby the ribosome translates the genetic code into protein molecules will ultimately require high-resolution structural information, and we report here the first crystal structure of a protein from the small ribosomal subunit. This protein, S5, has a molecular mass of 17,500 and is highly conserved in all lifeforms[1-4]. The molecule contains two distinct α/β domains that have structural similarities to several other proteins that are components of ribonucleoprotein complexes. Mutations in S5 result in several phenotypes which suggest that S5 may have a role in translational fidelity and translocation. These include ribosome ambiguity or *ram*[5], reversion from streptomycin dependence[6] and resistance to spectinomycin[6]. Also, a cold-sensitive, spectinomycin-resistant mutant of S5 has been identified which is defective in initiation[7]. Here we show that these mutations map to two distinct regions of the molecule which seem to be sites of interaction with ribosomal RNA. A structure/function analysis of the molecule reveals discrepancies with current models[8,9] of the 30S subunit.

The ribosomal protein S5 can be crystallized from the thermophilic organism *Bacillus stearothermophilus*[10]. The crystals are in the trigonal space group $P3_221$ with cell dimensions $a = b = 59.3$ Å, $c = 109.8$ Å. We have cloned and overexpressed the gene for *B. stearothermophilus* S5 to provide sufficient material for structural analysis[11]. The *B. stearothermophilus* protein is 55% homologous to its *Escherichia coli* counterpart[1,2] and its function is almost certainly identical in the two organisms. We were able to determine the S5 structure with a single gold ($KAu(CN)_2$) derivative[12] by exploiting the variable wavelength properties of synchrotron radiation (see Fig. 1; details will be presented elsewhere).

S5 has an α/β structure, and the polypeptide chain is folded into two distinct domains to create a somewhat elongated molecule (Fig. 2). There appears to be no flexibility between the two halves of the molecule, which are tightly associated by means of an extensive interdomain hydrophobic core. The N-terminal half consists of three contiguous β-strands arranged as an anti-parallel β-pleated sheet, and a 4-turn α-helix that lies on the sheet surface. The C-terminal half is similar in that it also contains a 3-strand antiparallel sheet, but the strands are not contiguous and there are two α-helices on one surface. The N and C termini are both disordered. The notation for the secondary structure elements and connecting loops, and their locations within the primary structure are shown in Fig. 3. A feature of the fold is that the two halves associate such that the empty surface of the β-sheet in domain 1 packs against the two α-helices of domain 2. This produces two unusual features. First, one surface of the β-sheet in domain 2 is totally exposed, and second, helices $\alpha 2$ and $\alpha 3$ are packed between two β-sheets.

S5 sequences are now known from 13 prokaryotic, eukaryotic and archaebacterial organisms. The conservation of the S5 tertiary structure throughout evolution is evident from Fig. 3. The only major differences occur at the N and C termini, and in loop 3 which connects the two domains. The structurally important residues of S5 are particularly well conserved. These include hydrophobic amino acids at the core, and glycines and alanines

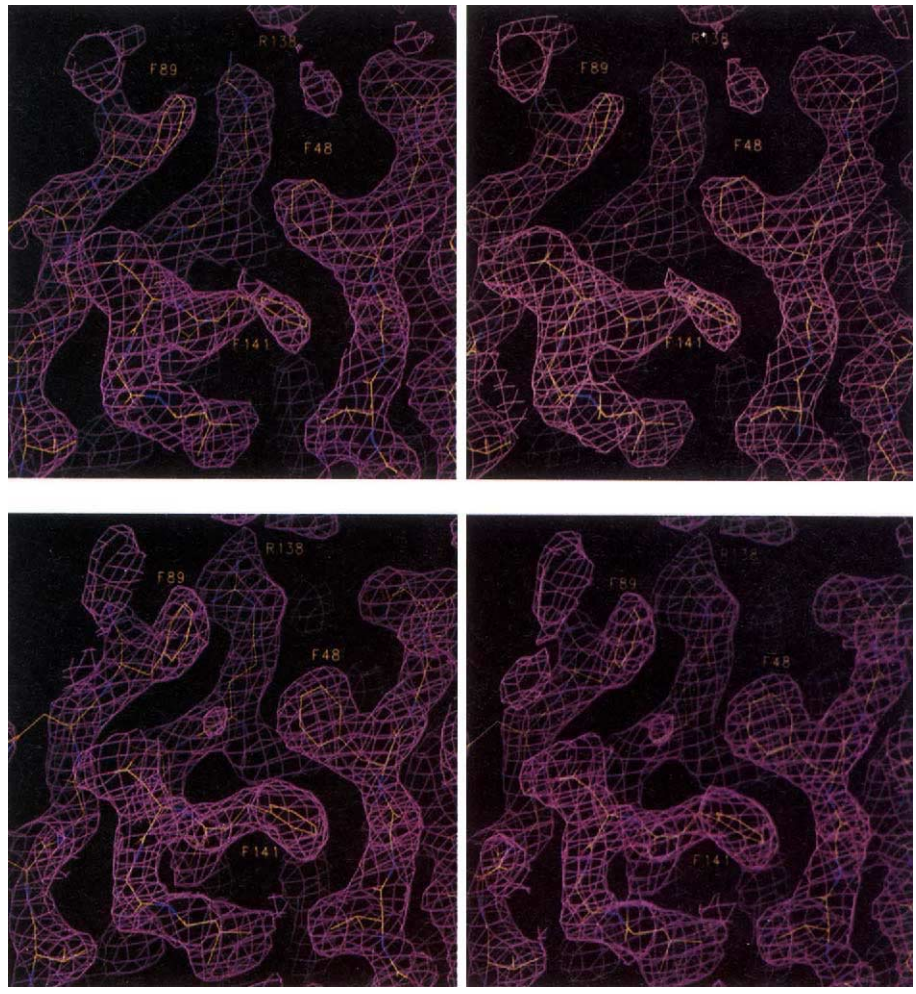


FIG. 1 Stereo electron density maps of S5 showing a cluster of 3 of the 4 phenylalanine residues in the molecule. The maps are contoured at the 1-standard-deviation level. *a*, The initial map that was used to trace the polypeptide chain and to build the molecule. Data were collected on beamline X12C of the National Synchrotron Light Source (Brookhaven National Laboratory) using an Enraf-Nonius FAST area detector[30]. Native data were collected by a rotation about the a^* axis, and 14,806 reflections were measured to give 5,829 unique reflections to 2.7 Å resolution with a merging *R*-factor of 5.1%. Derivative ($KAu(CN)_2$) data were collected about the *a* axis, which allowed the simultaneous collection of anomalous pairs with considerable redundancy. A total of 28,369 reflections were merged to give 6,020 unique reflections to 2.7 Å, with a merging *R*-factor of 7.3%. The X-ray wavelength (1.04 Å) was chosen to maximize the anomalous signal from the gold atoms. The final heavy-atom refinement values for R_{Cullis} and R_{Kraut} to 2.7 Å were 0.47 and 0.12 respectively, and the combined mean figure of merit for isomorphous and anomalous data was 0.77. The map was then solvent-flattened assuming a solvent volume fraction of 0.5 (less than the estimated 0.6 to avoid truncation of density). This improved the mean figure of merit to 0.89; the chain could be continuously traced, with the exception of three residues (28–30) that form part of a loop and are partially disordered. Data were processed by the program MADNES, phase and map calculations were performed by the package PHASES[31], and the molecule was built using the program O (ref. 32). *b*, $2F_o-F_c$ map of the same region after refinement of the initial model with X-PLOR[33]. Using all data between 6 Å and 2.8 Å with no water molecules added, the *R* factor is currently 22% with a deviation from ideality of 0.017 Å in bond lengths and 3.74° in bond angles.

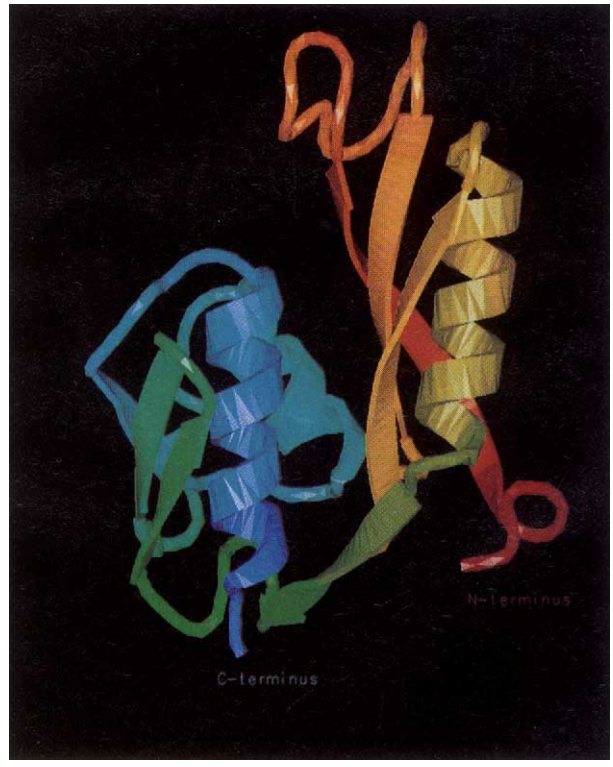


FIG. 2 Ribbon diagram of S5 produced by the program O (ref. 32). The secondary structure elements are colour-ramped red to blue, N terminus to C terminus, to show the path of the polypeptide chain. The molecule has an α/β structure (see Fig. 3 for the secondary structure notation), and is folded into two distinct domains, each containing a 3-strand, antiparallel β-pleated sheet. The N-terminal domain (right) contains one α-helix, and the C-terminal domain (left), two α-helices; the domains associate such that the latter are sandwiched between the two β-sheets. This arrangement exposes one surface of the C-terminal β-sheet which is populated by several conserved hydrophobic residues. Note the close approach of the third β-strand and the α-helix (on the nearside of the molecule) in the N-terminal domain, and the resulting gap between the helix and the first β-strand (on the backside of the molecule). This feature necessitates alanine and glycine residues along the connecting surfaces of the first pair, and results in the exposure of the hydrophobic core between the second pair. The residues at the N and C termini (3 and 19 respectively) are disordered. The molecule is rather flat and elongated, with approximate dimensions 45 Å × 40 Å × 25 Å.

in positions where the fold juxtaposes regions of the polypeptide chain. Notable among the latter is the close approach between almost the entire lengths of β3 and α1 (Fig. 2).

The structures of three small proteins that are components of ribonucleoprotein complexes have been determined previously. These include two prokaryotic ribosomal proteins L12CTF (ref. 13) and L30 (ref. 14), and the RNA recognition motif A-RRM1 from the (human) U1snRNP[15,16]. All three have similar structures involving helices packed against one side of an anti-parallel β-pleated sheet. The identical secondary structure topologies in

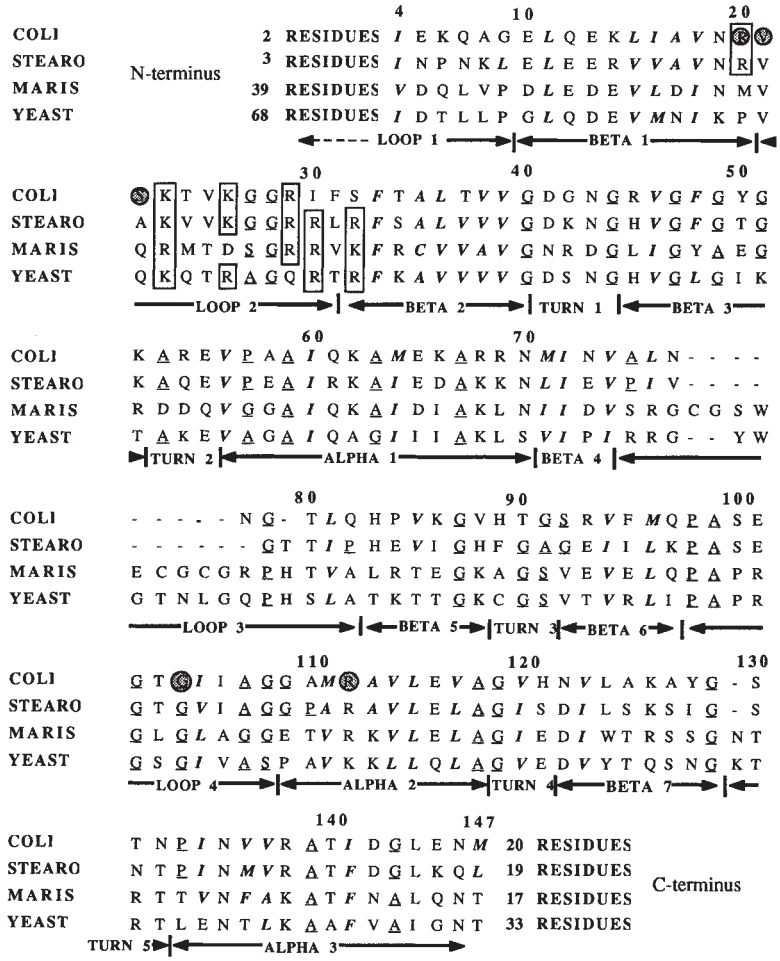
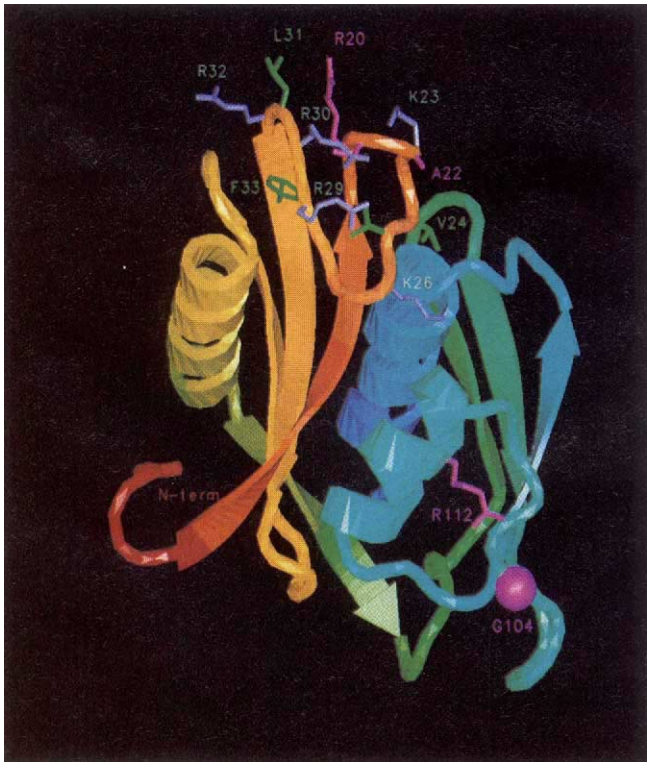


FIG. 3 Amino-acid sequence comparisons for ribosomal protein S5 (one-letter code). Also shown are the locations of the secondary structure elements deduced from the electron density map. The four sequences include representatives from each of the three principal life forms, two from prokaryotes: *Escherichia coli*[1] (COLI) and *Bacillus stearothermophilus*[2] (STEARO); one from archaebacteria: *Halobacterium marismortui*[3] (MARIS); and one eukaryote: *Saccharomyces cerevisiae*[4] (YEAST). The numbering scheme is based on the *B. stearothermophilus* sequence. Only the region visible in the electron density map is shown; the N and C termini are disordered, of variable length and show little or no homology. The only variable region within the body of the molecule is loop 3 which connects the two domains. This loop is considerably longer in the eukaryotic and archaebacterial molecules. Residues in the hydrophobic core are indicated in bold italics. Underlined residues are glycines, alanines and prolines in structurally restricted regions of the molecule. Circled residues are sites of mutation in *E. coli* that produce spectinomycin resistance (residues 20, 21, 22), and reversion from streptomycin dependence and ribosome ambiguity or *ram* (residues 104, 112). Boxed residues are conserved arginine and lysine residues in loop 2 that are proposed to interact with 16S rRNA.



◀ FIG. 4 Ribbon diagram of S5 produced by the program O (ref. 32) showing the two proposed sites of interaction with 16S rRNA. The secondary structure elements are colour-ramped red to blue, N terminus to C terminus, to show the path of the polypeptide chain. The N-terminal domain is to the left, the C-terminal domain is to the right, and the C-terminal helices are sandwiched between the two β-pleated sheets. The first site of interaction is on the C-terminal domain at the bottom right of the molecule. Two residues, glycine 104 and arginine 112 (magenta), are highly conserved, adjacent, and can be mutated to produce the *ram* and the revertant from streptomycin dependence phenotypes (see Fig. 3 and text). The second site is loop 2 (see Fig. 3 for notation) in the N-terminal domain at the top of the molecule centred on phenylalanine 33 (green). This contains several highly conserved arginine (20, 29, 30 and 32) and lysine (23 and 26) residues (blue), and residues (20, 21 and 22; magenta) that can be mutated to produce the spectinomycin-resistant phenotype (see Fig. 3 and text). Note that for reasons of clarity the side chain of residue 21 is not shown. Also shown are several hydrophobic residues (green) in this loop.

these molecules has led to the suggestion that they evolved from an ancient RNA-binding protein[15,17]. This simple evolutionary scenario is complicated by S5. At first sight it would appear that each domain contains the typical helix–sheet structure and that the protein is a result of gene duplication from a more primitive member of this general family. But closer inspection reveals that the topological arrangements within each domain are not only different from each other but also from the other three proteins.

As mutations within S5 have important effects on translation (see earlier), the molecule represents an excellent probe of the structural basis of ribosome function. The mutations that alter

the ribosome's response to streptomycin and spectinomycin are particularly revealing. Streptomycin acts by reducing translational fidelity, whereas spectinomycin inhibits the translocation of peptidyl transfer RNA from the A site to the P site[18]. Both antibiotics interact directly with 16S rRNA; streptomycin with the base of the 900 stem-loop (helix 27; ref. 19) in the vicinity of nucleotide C912 (refs 18, 20), and spectinomycin with the helix 34 (ref. 19) close to the base-paired nucleotides G1,064-C1,192 (refs 18, 21). The mutations within S5 that relate to these antibiotics are clustered into two distinct regions of the molecule. The first involves residues glycine at position 104 and arginine 112, which are highly conserved and can be mutated to produce the streptomycin revertant and *ram* phenotypes[1] (Fig. 3). These are adjacent on the C-terminal half of the molecule close to the domain interface (Fig. 4). The second region is loop 2 (Fig. 4), which encompasses the amino acids responsible for spectinomycin resistance[1] (Fig. 3). This forms a mini-domain centred on phenylalanine 33 and contains six highly conserved arginine and lysine residues. As conserved arginine and lysine residues are often vital in protein-nucleic acid interactions[22], we propose that the two S5 regions bind 16S rRNA close to the respective antibiotic-binding sites and that mutations directly modulate their mechanism of action.

How well does this scheme agree with current models for the organization of the 30S subunit[8,9]? As regards the interaction with helix 27, the agreement is good. Both S5 and helix 27 are accepted components of the 'proofreading domain' that also contains S4, S12 and the 530 stem-loop (helix 18; ref. 19) and 5′ terminus of 16S rRNA[23]. These components are clustered together in the ribosome[24-27] and are involved in translational fidelity. Mutations in this region of S5 also produce a *ram* phenotype[5], in support of its association with the proof-reading components. The interaction with helix 34 is more problematical. In present models[8,9], these components are some 70 Å apart and communicate by an indirect 'allosteric' process involving S2 (ref. 23). We propose an alternative scheme which is consistent with both the biochemical data and the structure. Proteins S2 and S3 protect identical nucleotides in helix 34 (ref. 27). It has been suggested that these 'polyspecific' effects are not due to a direct association of the proteins with helix 34 but rather to a conformational change in the RNA[27]. We suggest that this S2/S3-dependent change brings about an interaction between helix 34 and S5 which stabilizes the new and functional RNA conformation. Such a scheme would explain why S5 has no apparent effect on helix 34 in the absence of S2 and S3 (ref. 27), and is compatible with the relative locations of S2, S3 and S5 (refs 24, 25). The scheme is also consistent with the 30S assembly map which shows that the addition of S2 and S3 is dependent upon the presence of S5 (ref. 28). This assembly step may involve the binding of S2 to S5, which are adjacent in the particle[24,25]; in fact, a hydrophobic region on S5 between β1 and α1 is ideally positioned to be the contact site as it is close to loop 2 (Fig. 2). Finally, our results are in agreement with another report that strongly supports a revised 30S model in which S5 and helix 34 are neighbours[29]. □

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Convergence of 9-*cis* retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors

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PEROXISOMES are cytoplasmic organelles which are important in mammals in modulation of lipid homeostasis, including the metabolism of long-chain fatty acids and conversion of cholesterol to bile salts (reviewed in refs 1 and 2). Amphipathic carboxylates such as clofibric acid have been used in man as hypolipidaemic agents and in rodents they stimulate the proliferation of peroxisomes. These agents, termed peroxisome proliferators, and all-*trans* retinoic acid activate genes involved in peroxisomal-mediated β-oxidation of fatty acids[1-4]. Here we show that the receptor activated by peroxisome proliferators[5] and the retinoid X receptor-α (ref. 6) form a heterodimer that activates acyl-CoA oxidase gene expression in response to either clofibric acid or the retinoid X receptor-α ligand, 9-*cis* retinoic acid, an all-*trans* retinoic acid metabolite[7,8]; simultaneous exposure to both activators results in a synergistic induction of gene expression. These data demonstrate the coupling of the peroxisome proliferator and retinoid signalling pathways and provide evidence for a physiological role for 9-*cis* retinoic acid in modulating lipid metabolism.

The peroxisome proliferator responsive element (PPRE) located in the rat acyl-CoA oxidase (*AOX*) promoter is composed of two direct AGG(A/T)CA repeats separated by a single nucleotide (Fig. 1*a*)[9-11] and thus conforms with previously described retinoid X response elements[12,13]. Indeed, cotransfection of the retinoid X receptor-α (RXRα) expression plasmid in the presence of 9-*cis* retinoic acid (Fig. 1*b*, 9-*cis* RA, RXR) resulted in activation of a reporter construct containing the *AOX* promoter upstream of the luciferase gene (*AOX-LUC*). As previously shown[9], the *AOX* promoter was also induced by the peroxisome proliferator-activated receptor (PPAR) in the presence of clofibric acid (Fig. 1*b*, clofibric acid, PPAR). Interest-