the Connectivity Map immediately suggests that sirolimus should be tested in a clinical trial of ALL patients with dexamethasone resistance. Sirolimus is already FDA approved as an immunosuppressant and is well tolerated in children, and the clinical prognosis of dexamethasone-resistant ALL is poor (37–40). This example demonstrates that the Connectivity Map is one approach to the rapid identification of new potential uses for existing drugs.

Discussion

The value of a Connectivity Map depends on many open questions. How many distinct cellular pathways and states actually exist? How many cell types must be studied to provide sufficient diversity? How many perturbagens (small molecules, inhibitory RNAs, open reading frames) would need to be characterized to provide substantial coverage? How many concentrations, time points, and replicates would be required to provide reliable data? What analytical tools will be needed to interpret the data and determine precise estimates of statistical significance and false-positive rates? And, most important, what will be the biomedical value of the data? Only empirical evidence will resolve these issues. Although only a first step, our results are encouraging. They show that genomic signatures can be used to recognize drugs with common mechanisms of action (HDAC inhibitors and estrogen receptor modulators), discover unknown mechanisms of actions (gedunin as an HSP90 inhibitor), and identify potential new therapeutics (the ability of sirolimus to overcome dexamethasone resistance in ALL). Our findings also reveal that signatures are often conserved across diverse cell types and settings (the signature of dexamethasone resistance was defined in bone-marrow samples but searched against profiles from the MCF7 breast cancer line). At the same time, the results demonstrate the limitations of using only a few cell lines (the signature of estradiol was not detected in cells that lack estrogen receptors) or only a few concentrations (chlorpromazine was not recognized as a phenothiazine at 1 μM). It is also likely that our methodologies can still be refined. Indeed, alternative signature-based pattern-matching methods have been developed [e.g., (41)]. In addition, the interpretation of results depends on the ability to confidently call connections. More rigorous methods for the estimation of statistical significance are therefore probably also required, especially as the size of the database grows. But overall, the basic features of our approach appear to work well. We have, therefore, created a Web-based tool (www.broad.mit.edu/cmap) to allow researchers to perform their own Connectivity Map analyses with user-defined signatures in real time.

On the basis of the results of this pilot study, we propose that a sensible next step would be the generation of an expanded Connectivity Map as a community resource project in the spirit of other genomic efforts. An initial goal might be to profile all FDA-approved drugs and inhibitory RNAs targeting a large collection of genes in perhaps 10 diverse cell lines. Further goals would depend on the utility of the data. Ultimately, it will be interesting to explore whether it is possible to create a truly comprehensive catalog that begins to saturate all possible cellular states. In the meanwhile, even an incomplete Connectivity Map will likely accelerate progress in characterizing new chemical entities, finding new uses for existing drugs, and understanding the molecular mechanisms of disease.

References and Notes

15. J. Fraser et al., Cancer Res. 64, 1522 (2004).
21. H. Hieronymus et al., Cancer Cell, in press.
36. G. Wei et al., Cancer Cell, in press.
41. G. Natsoulis et al., Genome Res. 15, 724 (2005).
42. We thank S. Schreiber, E. Scollnick, D. Althuler, B. Wagner, B. Ebert, N. Tolliday, M. Brown, W. Wong, and members of the Broad Cancer and Chemical Biology Program. This work was supported in part by grants from the National Cancer Institute, Howard Hughes Medical Institute, and The Paul G. Allen Family Foundation.

Supporting Online Material

www.sciencemag.org/cgi/content/full/313/5795/1929/DC1
Materials and Methods
Figs. S1 to S5
Table S1
Signatures S1 to S11
References
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Structure of the 70S Ribosome Complexed with mRNA and tRNA

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The crystal structure of the bacterial 70S ribosome refined to 2.8 angstrom resolution reveals atomic details of its interactions with messenger RNA (mRNA) and transfer RNA (tRNA). A metal ion stabilizes a kink in the mRNA that demarcates the boundary between A and P sites, which is potentially important to prevent slippage of mRNA. Metal ions also stabilize the intersubunit interface. The interactions of E-site tRNA with the 5OS subunit have both similarities and differences compared to those in the archaeal ribosome. The structure also rationalizes much biochemical and genetic data on translation.

A major breakthrough for our mechanistic understanding of translation was achieved some years ago when high-resolution structures of the 50S and 30S ribosomal subunits were solved (1, 2). Progress has also been made in obtaining structural data on the whole ribosome. The subunit structures were used to facilitate interpretation of maps at 5.5 Å resolution of the whole 70S ribosome complexed with mRNA and tRNA (3). More recently, the structure of the Escherichia coli ribosome was solved at 3.5 Å resolution (4). At the same resolution, the structure of the human ribosome was solved (5). Our structures provide a comprehensive view of the interactions of tRNA at both sites of the 70S complex. The tRNA distribution is consistent with the current view of E-site recognition and also reveals a possible role for the K147 residue of the E site that increases the affinity of the E-site tRNA. The mRNA binding domain of the 70S structure is in a different conformation from that of the E-site tRNA.

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time, electron cryomicroscopy (cryo-EM) studies have yielded increasingly detailed structures of various functional states of the ribosome (3).

Important as these results are, current structural data on whole ribosomes have limitations. At resolutions lower than 3.5 to 4 A, it is possible to model known structures into maps but difficult to interpret previously unknown regions in molecular terms. For example, in the 5.5 A crystal structure of the 70S ribosome, an attempt was made to interpret the L1 and L7/L12 stalks, which consist of both proteins and RNA (3). These interpretations were at variance with high-resolution structures of the components determined later (6, 7). Typical cryo-EM maps of the ribosome have even lower resolution and thus suffer the same limitations. The 3.5 A resolution crystal structure of the empty *Thermus thermophilus* ribosome (4) allowed many molecular details to be seen directly. However, this structure lacks direct information about the interactions of the ribosome with its mRNA and tRNA ligands.

We report here the structure of a pretranslocation state of the *Thermus thermophilus* ribosome at 2.8 A resolution, which has allowed us to build an accurate model that reveals the structures of tRNA and mRNA in situ and the molecular details of their interaction with the ribosome.

**Crystallization and structure determination**

A pretranslocation complex of the ribosome was formed by confusing it with mRNA, deacylated initiator tRNA{	extsuperscript{Met}} in the P site, and aminoacyl tRNA{	extsuperscript{Phe}} in the A site (8). The antibiotic paromomycin, which is known both to increase the affinity of A-site tRNA and to bind the ribosome, was made to interpret the L1 and L7/L12 stalks, and previous 70S ribosomal subunits. All ligands were removed from the search model before its use. This solution was used as a starting point to build the structure and refine it to 2.8 A resolution. The mRNA, tRNA, and antibiotic ligands were only included in the final rounds of refinement, so that they could be built into unbiased difference Fourier maps (8). A summary of crystallographic data and refinement statistics is given (table S1).

After refinement, the tRNA and mRNA ligands, as well as differences from the input model, could clearly be seen in difference Fourier maps (Fig. 1). It was easily possible to distinguish between purines and pyrimidines, and well-ordered side chains of proteins were clearly visible. It was also possible to see a large number of metal ions.

The current structure consists of nearly the entire 70S ribosome with its tRNA and mRNA ligands (Fig. S1). The L7/L12 stalk, along with its base consisting of the L10, L11, and L6 regions as well as the elbow and acceptor arm of A-site tRNA, was poorly ordered or disordered. The E site was occupied with a noncognate tRNA.

In the following description of the structure, we focus mainly on those details that have not been seen directly in previous work. These include details of the interactions of tRNA and mRNA with the ribosome and the participation of proteins and metal ions, especially in intersubunit bridges. For the sake of conciseness, we refer to the ribosomal subunits as 30S and 50S, and to the whole ribosome as 70S.

**Overall structure.** The two molecules in the asymmetric unit are nearly identical, in contrast to the two ribosomes in the asymmetric unit of the *E. coli* 70S crystals (4). Surprisingly, even mobile regions such as the L1 stalk of the 50S are in similar conformations. Thus, binding of mRNA and tRNA confers conformational homogeneity to the ribosome.

The structure of the 50S is very similar to that of both 50S molecules in the asymmetric unit of the *E. coli* 70S crystals (4). The only significant difference is that the regions surrounding the E site are further apart in the empty ribosome but move toward each other to bind E-site tRNA (Fig. S2). The most notable movement is that of the L1 stalk. Differences in the L1 stalk conformation have also been observed between empty 50S and previous 70S structures containing E-site tRNA (1, 3, 10).

In contrast, the 30S subunit of the 70S complex reported here is more similar to the closed 30S form described previously, as a consequence of A-site tRNA binding (11), than to either of the two 30S molecules of the empty *E. coli* ribosome (4), which are both more open to varying degrees (Fig. S3). A comparison of the rmmid and median distance of equivalent phosphorus atoms for the subunits from various 70S structures is shown in table S2.

An *E. coli* numbering for *Thermus* ribosomal RNA (rRNA) based on structural alignment with the *E. coli* ribosome (4) is used throughout. We also used the standard Brimacombe helix numbering for rRNA, with a lowercase h prefix for 16S RNA and an uppercase H for 23S RNA.

**Interactions with tRNA and mRNA.** In the hybrid states model, tRNAs move first relative to the 50S subunit and then with respect to the 30S subunit during translocation (12). Thus

![Fig. 1. Representative electron density in the 70S structure. (A) Fo – Fc map showing unbiased density of the acceptor end of P-site tRNA (not included in the refinement), with refined 3Fo – 2Fc density for 23S RNA. Base pairs between C74 and C75 with G2252 and G2251 of 23S RNA are clearly visible. (B) Simulated annealing omit map of the large subunit protein L34 showing the visibility of well-ordered side chains. (C) A Mg ion shown in unbiased difference Fourier maps that is coordinated to the nonbridging phosphate oxygens at the 45° mRNA kink between the A and P site as well as the nonbridging phosphate oxygens of nucleotides 1400 and 1401 of h44 of 16S RNA. All RNA is shown in final 3Fo – 2Fc density. The Mg ions are shown in green. This and all other figures were made with Pymol (47).](downloaded_from www.sciencemag.org on August 15, 2007)
The P site. Except during initiation, the tRNA has already been selected by the time it is translocated to the P site, and codon-anticodon interaction is not monitored. Instead, the P site has evolved mainly to hold the tRNA tightly in position to maintain the reading frame and for peptidyl transfer. The entire P-site tRNA$^{\text{Phe}}$ was visible in an unbiased difference Fourier map, a portion of which is shown (Fig. 1A). As seen in the 5.5 Å structure (3), the P-site tRNA makes extensive contacts with both ribosomal subunits, with 2481 Å$^2$ or 19% of its surface area buried in interactions with the ribosome or mRNA (Fig. 2A). The distinguishing features of initiator tRNA were clearly seen. These include the absence of a Watson-Crick base pair at C1-A72, a purine-pyrimidine base pair, 11-24, and three consecutive GC base pairs in the anticodon stem (14, 15).

Interactions of P-site tRNA with mRNA and the 30S subunit. The backbone of the mRNA codon in the P site interacts with C1402, C1403, and U1498 of 16S RNA. In addition, we saw a metal ion that bridged C1401 and G1402 to the codon. G1401 was shown in modification interference experiments to be important for codon-dependent P-site tRNA binding (16). The wobble base pair is held in place by stacking interactions from two sides; C1400 stacks with the base pair and G966 against the ribose of C34 (Fig. 2B). Recent mutagenesis on the P site showed that mutation of C1400 to a purine reduced translation at least 12-fold (17), presumably because a purine would clash with G966 in the current structure and the distortion required to accommodate it would lower the affinity of P-site tRNA.

Interestingly, A1339 makes a suboptimal A-minor interaction, with the distance between the N1 of A1339 and the 2'-OH of G29 too long for a hydrogen bond (4 Å; marked with an “x” in Fig. 3A). It is possible that during initiation, small conformational changes in the head induced by the binding of initiation factor IF3 bring the head into an optimal orientation to inspect the initiator tRNA.

A1339 and G1338 in the head of the 30S interacts with the ASL of P-site tRNA on one side, while nucleotide 790 at the tip of the 30S platform contacts the ASL on the other side, thus preventing its movement into the E site (Fig. 3B). During translocation of tRNA from the P to the E site, these two elements would have to move apart, presumably by a movement of the head (4, 21). Thus, G1338 and A1339 may not only confer additional stability to initiator tRNAs but may also act as a switch during both initiation and translocation.

Two protein tails from the 30S extend into the P site (Fig. 2A) (22). Lys127 of S9 interacts with the phosphate oxygens of P-site tRNA positions 33 and 34, and the backbone of S13 at residue 118 comes close to the phosphate oxygens of P-site tRNA position 29.
In the structure of the 30S, the spur of a symmetry-related molecule interacted with the 3’ end of the 16S RNA, mimicking a P-site codon-anticodon interaction (22). Because this interaction formed noncanonical pyrimidine-pyrimidine base pairs at all three positions, it was not clear how accurately the structure represented the details of true tRNA-codon interactions. The present structure shows that despite the noncanonical base pairs, the spur in the 30S is indeed an excellent mimic of the ASL part of the tRNA. The main differences are that G966 packs optimally against the ribose of anticodon nucleotide 34 and that the upper part of the ASL in the spur of the 30S was displaced by 4 Å relative to the P-site tRNA in the 70S structure.

The P-site tRNA was distorted relative to the isolated crystal structure of yeast tRNA \(^{\text{Phe}}\) (fig. S4) (23, 24). A deformation of the anticodon stem was caused by opposing interactions with the head of the 30S subunit and H69 of the 50S subunit, resulting in an opening up of the major groove around the 26:44 base pair. Interestingly, this means that if constraints on the tRNA were released in the 50S, e.g., after peptidyl transfer, relaxation of the deformation would have the effect of driving the tRNA toward the E site (fig. S4).

**Interactions at the PTC.** The acceptor end of P-site tRNA interacts with the PTC in the 50S subunit in a manner similar to that observed for oligonucleotide mimics of tRNA soaked into the Haloarcula 50S subunit [e.g., (25, 26)], in contrast to the suggestion that the orientation of tRNA in the PTC is determined by remote interactions (27). Bases C74 and C75 form Watson-Crick base pairs with G2252 and G2251, while the terminal A76 stacks with 75 and 74 and forms an A-minor interaction with the A2450-C2063 base pair (fig. 4). The 2’-OH of A76 is in hydrogen-bonding distance of both the N3 and 2’-OH of A2451, showing its importance in stabilizing the conformation of P-site tRNA. The 2’-OH of A76 and nucleotide A2451 are known to be important for peptidyl transferase activity and for substrate stabilization (28, 29).

The PTC itself was in a very similar conformation to those reported in studies of the Haloarcula 50S with various ligands as well as to the PTC of the Deinococcus radiodurans 50S subunit (10). The A and P loops superimposed closely, with the P loop accommodating an extra nucleotide in the Haloarcula structure while closely maintaining the positions of the other bases. Nucleotides known to be mobile in the 50S subunit (28, 29), such as A2062 at the entrance to the nascent peptide channel and A2602, which lies between the A and P sites, also appeared to be mobile in our structure, because they had significantly weaker density. The conformation of the PTC was also similar to that of the empty E. coli 70S structure (4). Although concerted differences in the conformation of the PTC between the E. coli and Haloarcula 50S structures were reported (4), those differences are small and comparable in magnitude to the difference between the current structure and the E. coli structure or to differences between apo- and ligand-bound Haloarcula structures (rmsd ~ 0.7 Å).

In E. coli, the N-terminal tail of L27 can be cross-linked to A76 of P-site tRNA (30), and deletion of as few as the first three residues can significantly reduce peptidyl transferase activity (31). These observations suggest that the N terminus of L27 is close to or at the PTC. In the 70S structure, L27 has the fold of the crystal structure of the isolated protein (32) rather than that reported in the Deinococcus 50S subunit structure (10). It is well defined from residue 10 onward, but very weak density is also visible for residues 1 to 9. The density is consistent with a position of the N terminus close enough to interact with A76 of P-site tRNA, where it could additionally stabilize the P-site substrate and thereby enhance peptidyl transferase activity. This is in contrast to the Haloarcula 50S structure, in which Asp\(^{111}\) of L10e, adjacent to a disordered loop, is 11.5 Å away (26). We also find that the closest ordered metal ion is 8.5 Å away from the 3’-OH of A76, in agreement with studies on the Haloarcula 50S structure reporting the absence of metal ions in the immediate vicinity of the PTC (26).

Apart from the ASL and CCA ends, other parts of the P-site tRNA makes extensive interactions with the 50S subunit (Fig. 2A). As seen earlier at 5.5 Å (3), H69 of 23S RNA makes direct minor groove interactions with the D helix (nucleotides 11 and 12) and also with the adjacent nucleotides 24 and 25 in the anticodon stem. At the elbow, protein L5 forms two hydrogen bonds with C56, and L16 is close to G53 but too far to make an interaction in the current structure. Also, we saw five Mg ions coordinated within the tRNA and a sixth bringing together the nonbridging phosphate oxygens of tRNA nucleotides 75 and 76 and 23S RNA nucleotide 2602.

**The E site.** During translation, the ribosomal E site is occupied by deacylated tRNA that has been translocated from the P site. The nature and role of the E site remains controversial. E-site tRNA was postulated to bind both 30S and 50S subunits and to make interactions with the codon of mRNA (33). However, footprinting and other
biochemical data questioned the existence of an E site on the 30S (34, 35). The 5.5 Å structure of the Thermus ribosome unambiguously established the presence of an E site in both the 30S and 50S subunits (3). However, at that resolution it was unclear to what extent codon-anticodon base pairing occurred.

We saw density for E-site tRNA, and the absence of the insertion at nucleotide 17 that was present in initiator tRNAfMet as well as other features identify it as deacylated tRNAPhe. Both the anticodon and CCA ends of tRNA are well defined, whereas some regions around the elbow region are poorly ordered.

**The 30S E site.** We did not observe any codon-anticodon interactions in the E site. In fact, A35, the middle anticodon base, was closer to G693 of 16S RNA than to the E-site codon. The tRNA here is noncognate, but it would not be possible to make codon-anticodon base pairs even with a cognate codon unless the mRNA or tRNA were to move significantly relative to their present locations (Fig. 5A). Whereas A and P site tRNAs made extensive interactions with 16S RNA, the 30S E site is primarily made of proteins S7 and S11, as seen earlier (3, 22), explaining the absence of footprints to 16S RNA (34).

**The 50S E site.** In the 50S, the acceptor end of E-site tRNA interacts with residues at the base of H82, whereas bases at the ends of the T and D loops interact with the L1 stalk, thus stabilizing the stalk in a closed conformation. A number of tRNA mutations and modifications that affect translocation are thought to have altered interactions with the 50S E site, presumably during the formation of the P/E hybrid state. The interactions of tRNA with the 50S E site were seen in sufficient detail to analyze these data (Fig. 5B).

The base and 2'-OH of A76 have been shown to dramatically affect E-site tRNA binding and translocation (36, 37). The base of A76 intercalates between G2421 and A2422 of 23S RNA and makes hydrogen bonds with the universally conserved C2394, as seen recently in the E site of the Haloarcula 50S subunit, by using a minihelix representing the acceptor arm of tRNA (38). The 3'-OH of A76 is surrounded by 23S RNA elements, and the site could not accommodate an amino acid on the tRNA, explaining the requirement for deacylated tRNA.

However, the interactions of E-site tRNA in the bacterial 70S structure also show striking differences from those in the archaeal Haloarcula 50S subunit. The orientations of the acceptor arms were different, possibly because the minihelix used in the Haloarcula structure lacked the D and T loops that make interactions with the L1 stalk. Moreover, we find that C75 stacks directly on C74, whereas in the archeal structure the base of C75 was splayed out from the tRNA in the opposite direction from A76, where it was stabilized by interactions with L44e (38). This protein is not present in bacterial ribosomes, and in any case such a conformation would be precluded by clashes with U2431 and A2432 and by the presence of protein L28 (previously identified as L31).
The common mode of binding of A76 suggests that the existence of the E site predates the divergence of bacteria from archaea. However, differences in the conformation of 70S and the identities of the proteins present suggest that the E site has subsequently evolved differently in bacteria and other kingdoms.

**General features of mRNA.** The sharp kink between the A- and P-site codons (3) clearly delineates the border between these two sites and presumably is important for defining the reading frame and preventing slippage of the mRNA. In the 70S structure, this kink is stabilized by a Mg ion, which makes interactions with the phosphate oxygens of the third P-site nucleotide and the first A-site nucleotide, allowing them to come closer together (Fig. 1C). Interactions of the Mg ion with phosphate oxygens of nucleotides 1401 and 1402 of 16S RNA further fix the frame of mRNA with respect to the 30S subunit.

Apart from the codons at the tRNA binding sites, we saw two additional nucleotides beyond the E site. The rest of the mRNA appeared disordered, just as in the 5.5 A 70S crystal structure (3), although Yusupova et al. could see its extended path, including the Shine-Dalgarno interaction at the 5′ end, by using a low resolution difference Fourier map between ribosomes containing and lacking mRNA (39).

**Overview of proteins.** Whereas the 30S proteins were little changed from the 30S structure of the same species (2), all of the 50S proteins in the structure had to be rebuilt, some of them ab initio (8) (table S3). We report here some significant differences from previous work.

A region of the 50S subunit was originally interpreted as corresponding to protein L31 (10), and this interpretation was subsequently propagated into low-resolution structures of the 70S ribosome as well as that of the higher-resolution E. coli ribosome structure (4). However, this region of the electron density showed an additional α helix that could be accounted for by the L31 sequence. Moreover, it is a region where cross-links have been observed to ribosomal protein L28 (40), whereas no biochemical data connected it to L31. We could satisfactorily build the L28 sequence into the electron density.

At the same time, unexplained density was found for a protein adjacent to protein L5 (fig. S5). We suggest that this is protein L31 on the basis of reports that it formed a cross-linked dimer with L5 (41). This location of L31 would also be consistent with the ease with which it dissociates from the ribosome (42). Helical density from L31 packs against an α helix of L5 and the intersubunit surface α helix of S13.

We saw no evidence for L36, although the structure of this protein from Thermus has been solved in isolation (43). Interestingly, the pocket where L36 was seen in the Deinococcus 50S (10) was also empty in the Haloarcula 50S (1). It is possible that the protein was lost during purification, but, given the nature of the pocket and the highly charged nature of L36, we consider this unlikely. An alternative hypothesis is that the protein is not a true ribosomal protein, but this idea would be difficult to reconcile with its presence in the Deinococcus 50S structure (10).

**Intersubunit bridges.** During translation, ribosomal subunits need to associate during initiation and dissociate during recycling after termination. However, they also need to move relative to each other, especially during translocation (21). Because translation is a highly specific and intricate process, the association of ribosomal subunits, as well as the changes in their interaction during relative movement, must be both highly specific as well as dynamic. It has also been known for almost 50 years that ribosomal subunits from all species studied can reversibly associate and dissociate in vitro as a function of Mg concentration, suggesting the universal importance of divalent ions in intersubunit contacts (44).

The interactions between subunits occurs through a number of bridges, first seen and named as B1 to B6 in cryo-EM maps of the ribosome (45). In particular, the long penultimate h44 of 16S RNA extended from the interdomain junction of the 30S to the bottom of the subunit and made a number of intersubunit contacts (Fig. 6A). These bridges are essential for subunit association, but some of them also need to be formed and broken during the translation process. The 5.5 A structure of the ribosome described many of these interactions in greater detail, especially between components of tRNA. The various bridges differ in character, probably reflecting their nature as static or dynamic contacts. We saw not only the RNA elements but also the side chains of proteins, as well as ions that are involved in the formation of bridges (table S4). Apart from being essential to our understanding of intersubunit interactions, these details help rationalize recent biochemical and genetic data as well as the role of metal ions in subunit association. We describe some examples of bridges: an induced conformational change in a bridge as a result of tRNA binding, a bridge that is entirely mediated by a metal ion, and a bridge in which both metal ions and protein side chains contribute to bridge formation.

In bridge B2a, H69 of 23S RNA (3, 4) extends across the interface to interact with h44 of 16S RNA. The loop of H69 was disordered in the Haloarcula 50S (1) and formed a compact structure in Deinococcus 50S (10). However, comparison of our structure to the E. coli 70S structure shows that A1913 of H69 flips to insert into a tight pocket formed by the backbone of h44 and A-site tRNA, forming a hydrogen bond between its N1 to the 2′-OH of A37 of A-site tRNA (Fig. 6B). The base is then oriented toward the bases of A1492 and A1493 that flip out during decoding to interact with tRNA and mRNA (13). A Mg ion bridges the ribose O4′ of A1913 of H69 with a phosphate oxygen of position 38 of A-site tRNA, and a second Mg ion bridges the ribose 2′-OH with the nonbridging phosphate oxygens of 16S 1493 and 1494. The result of this rather tight interaction in combination with the h44 movement to monitor decoding is that the entire H69 is shifted slightly toward the 30S subunit relative to the empty E. coli structure. This conformational change may offer one route for signaling correct 30S decoding to the 50S guanosine triphosphatase center before tRNA accommodation.

B2c is a purely Mg-mediated bridge in which ordered metal ions mediate interactions between the backbones of h24 and h27 of 16S RNA and H67 of 23S RNA (Fig. 6C and table S4). The structure rationalizes the observation that phosphothiorate substitution at C770 in h24 of 16S RNA is not tolerated, presumably because it would inhibit Mg-dependent subunit association via nonbridging phosphate oxygens (46). In addition to B2c where they are crucial, metal ions also appear to confer additional stability to B5, B6, and B8 (table S4).

Proteins in the bridges interact with RNA, other proteins, and metal ions. The direct interactions of proteins to RNA in bridges seem to be entirely to the backbone rather than to specific bases. Such interactions may be characteristic of dynamic elements that have to make different contacts in different states of the ribosome. In B5, the only direct interaction between L14 and 16S RNA is between Arg69 of L14 and a nonbridging phosphate oxygen of nucleotide 1423 of 16S (Fig. 6D). Interestingly, a Mg ion is coordinated to the nonbridging phosphate oxygens of 1421 of h44, 1950 and 1951 of H71, and Glu54 of L14 (Fig. 6D). Additionally, L14 and L19 also interacted with two ordered Mg ions in B8 (Fig. 6E).

In B6, the minor grooves of h44 and H66 approached each other but left a 6 A gap as seen in the E. coli structure, where it was suggested that there might be a monolayer of water molecules (4). We observed one ordered solvent molecule that is coordinated by the backbone of nucleotides 1703 and 1704 of H62 and 1429 and 1430 of h44.

In the Thermus 70S structure, there appears to be an additional bridge (not present in E. coli) involving an interaction of G1442a at the bottom of h44 and a nonbridging phosphate oxygen of 23S G2864 of 23S RNA that is mediated by an extended loop of L19. This contact might lend some additional stability to the Thermus ribosome and is the only example of a protein interaction with an RNA base rather than its backbone.

**Conclusions.** The structure of the 70S ribosome describes the detailed interactions of the...
mRNA and tRNA substrates with the ribosome, the interactions between the ribosomal subunits, and the role of metal ions in the structure. The interactions of A-site tRNA in the decoding center and P-site tRNA at the PTC were in good agreement with work done on the 30S and 50S subunits using oligonucleotide mimics of tRNA. We saw no codon-anticodon interactions with the noncognate tRNA in the E site. The interactions of E-site tRNA with the 50S subunit showed both similarities and differences with the previously studied *Haloarcula* E site. The involvement of magnesium ions has long known to be crucial for several aspects of translation, such as subunit association and codon-dependent tRNA binding. We saw metal ions in key positions of critical areas, such the interface between subunits and between the ribosome and tRNA and mRNA. In particular, a magnesium ion stabilized a kink in mRNA at the boundary between the A- and P-site codons, which is of potential importance in preventing slippage during translation. The structure helps to rationalize much detailed biochemical, mutational, genetic, and conservation data and should be useful for the design of future experiments. Moreover, because the A and P sites of the structure are relatively protected from crystal contacts, it is hoped that this crystal form will pave the way for high-resolution structures of functional complexes involving other substrates as well as 50S antibiotics that require an occupied P-site substrate.

**Fig. 6.** Examples of the roles of metal ions and proteins in the intersubunit bridges. (A) Overview of the extensive intersubunit bridge interactions that 50S proteins L19 (gray) and L14 (purple) makes with h44 of 16S RNA (orange). L14 is involved in bridge B5 that interacts with 16S h44, L19 is involved with bridge B6 that interacts with 16S RNA h44, and both L14 and L19 are involved in bridge B8 that interacts with h14. The 23S RNA is shown in cyan, 50S proteins in blue, 16S RNA (except for h44) in yellow, 30S proteins in tan, and Mg ions in green. (B) An example of an induced change in a bridge on A-site tRNA binding. In bridge B2a, A1913 of 16S RNA flips out of its loop toward 16S RNA bases A1492 and A1493 to form a hydrogen bond with A37 of A-site tRNA. Two Mg ions (green) also interact with A1913: One is coordinated between its O4′ and nonbridging phosphate oxygen of U38 of A-site tRNA, and another between its 2′-OH and the nonbridging phosphate oxygens of both 1492 and 1493. Paromomycin is shown in red and mRNA in purple. (C) A Mg-mediated bridge. In bridge B2c, a Mg ion (green) mediates the interaction between the RNA backbones of h24 and h27 of 16S RNA and H67 of 23S RNA. The Mg ion is coordinated between the 2′-OH of 770 and O2 of C899 of 16S RNA and the nonbridging phosphate oxygens of 1832 and 1833 of 23S RNA. (D and E) Proteins and ions in bridges. In bridge B5 (D), L14 is involved by either directly interacting with the backbone of 16S RNA or via a Mg ion (green) that also contacts the backbone of both 16S and 23S RNA. Arg49 of L14 interacts directly with the backbone of 16S RNA, and Glu54 is coordinated to a Mg ion that in turn interacts with the backbones of nucleotide 1421 of 16S RNA and 1950 and 1951 of 23S RNA. Bridge B8 (E) consists of interactions of the backbone of h14 of 16S RNA with both L14 and L19 via two Mg ions (green). In the center, a Mg ion is coordinated to the backbone oxygens of both Val115 and Ala318 of L14 and to h14 of 16S RNA. Below and to the left, a Mg ion is coordinated between the side chain of Lys35 of L19 and h14 of 16S RNA. These interactions show the importance of Mg ions in additionally helping to stabilize bridging interactions between the 50S and 30S subunits.
Tunable Quasi–Two-Dimensional Electron Gases in Oxide Heterostructures

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We report on a large electric-field response of quasi–two-dimensional electron gases generated at interfaces in epitaxial heterostructures grown from insulating oxides. These device structures are characterized by doping layers that are spatially separated from high-mobility quasi–two-dimensional electron gases and therefore present an oxide analog to semiconducting high-electron mobility transistors. By applying a gate voltage, the conductivity of the electron gases can be modulated through a quantum phase transition from an insulating to a metallic state.

Complex oxides show a broad spectrum of intrinsic functionalities, such as ferroelectricity, magnetism, superconductivity, and multiferroic behavior [see (1)], which can be used and combined in electronic devices that are based on epitaxially grown heterostructures. Physical properties may arise in such multilayers that are not found in either of their constituents. One example, a conducting quasi–two-dimensional electron gas (q2-DEG) is formed at the interface between the two insulating, dielectric perovskites, LaAlO₃ and SrTiO₃ (2, 3). The electrons at this interface are highly mobile, with values up to 10⁴ cm² V⁻¹ s⁻¹ (4.2 K) having been reported (2–5), and were found to have densities orders of magnitude higher than the densities of two-dimensional electron gases induced at interfaces in heterostructures based on III–V semiconductors. Exploring whether the q2-DEGs can be applied to fabricate high electron mobility transistor (HEMT)–type field effect devices (6), we observed that they can be tuned by altering on the unit cell level the thickness of the LaAlO₃ sheets. For LaAlO₃ layers that are up to 3 unit cells (uc) thick, highly insulating interfaces are obtained. In field-effect transistor configurations that use such interfaces as drain-source (DS) channels, a phase transition to the conducting state is readily achieved by gate fields. Upon change of their carrier densities with applied electric fields, the q2-DEGs react with a pronounced memory effect.

Previous work revealed the existence of metallic electron gases at LaTiO₃–SrTiO₃ (7) and at LaAlO₃–SrTiO₃ interfaces (2, 3). Because electron energy loss measurements of LaTiO₃–SrTiO₃ interfaces showed that the electron gas is confined within a ~2-nm-thick layer (7), the gas is described to be quasi–two-dimensional. Whereas the LaTiO₃–SrTiO₃ interface is doped by transfer of electrons from the LaTiO₃ to the SrTiO₃ (7, 8), the LaAlO₃–SrTiO₃ interface two different mechanisms have been reported to generate the gas: In some heterostructures, it was found (2, 3) that the carriers are induced by the polarity discontinuity of the TiO₂–LaO⁺ stacking.