Nonbridging phosphate oxygens in 16S rRNA important for 30S subunit assembly and association with the 50S ribosomal subunit

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ABSTRACT

Ribosomes are composed of RNA and protein molecules that associate together to form a supramolecular machine responsible for protein biosynthesis. Detailed information about the structure of the ribosome has come from the recent X-ray crystal structures of the ribosome and the ribosomal subunits. However, the molecular interactions between the rRNAs and the r-proteins that occur during the intermediate steps of ribosome assembly are poorly understood. Here we describe a modification-interference approach to identify nonbridging phosphate oxygens within 16S rRNA that are important for the *in vitro* assembly of the *Escherichia coli* 30S small ribosomal subunit and for its association with the 50S large ribosomal subunit. The 30S small subunit was reconstituted from phosphorothioate-substituted 16S rRNA and small subunit proteins. Active 30S subunits were selected by their ability to bind to the 50S large subunit and form 70S ribosomes. Analysis of the selected population shows that phosphate oxygens at specific positions in the 16S rRNA are important for either subunit assembly or for binding to the 50S subunit. The X-ray crystallographic structures of the 30S subunit suggest that some of these phosphate oxygens participate in r-protein binding, coordination of metal ions, or for the formation of intersubunit bridges in the mature 30S subunit. Interestingly, however, several of the phosphate oxygens identified in this study do not participate in any interaction in the mature 30S subunit, suggesting that they play a role in the early steps of the 30S subunit assembly.

Keywords: 16S rRNA; 30S subunit; assembly; phosphorothioate; ribosome

INTRODUCTION

The ribosome is a macromolecular complex made up of two unequally sized subunits. In *Escherichia coli*, the 50S large subunit consists of a 23S rRNA molecule (2904 nt), a 5S rRNA molecule (120 nt), and more than 30 proteins. The 30S small subunit consists of a 16S rRNA (1542 nt) and 21 proteins. The X-ray crystal structures of the ribosomal subunits show that the rRNAs, which form the core of the subunits, are stabilized by RNA–RNA interactions in addition to RNA–protein interactions (Ban et al. 2000; Schluzenzen et al. 2000; Wimberly et al. 2000). Specific bases, ribose 2'-hydroxyl groups, and nonbridging phosphate oxygens within the rRNAs form an array of intra- and inter-

molecular interactions that stabilize the structure of the fully assembled ribosome. However, the molecular mechanism of how the rRNAs associate with the ribosomal proteins to form a supramolecular assembly is poorly understood. *In vivo*, ribosome assembly occurs cotranscriptionally and is orchestrated by assembly factors (Maki et al. 2002, 2003; Alix and Nierhaus 2003; Charollais et al. 2003). Remarkably, both the 30S (Traub and Nomura 1968) and the 50S subunits (Nierhaus and Dohme 1974) assemble *in vitro* from their constituent rRNAs and ribosomal proteins in the absence of assembly factors. These *in vitro* reconstituted 30S and 50S subunits are active in translation.

Ribosome assembly is a dynamic process involving the interaction of more than 50 proteins with three large RNA molecules that must ultimately fold into a compact particle (for review, see Williamson 2003). Early assembly mapping experiments of the 30S subunit from *E. coli* showed that this complex process involves an ordered pathway with the cooperative interaction of small subunit proteins with the 16S rRNA (Held et al. 1974). Based on assembling mapping

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experiments, the ribosomal proteins are classified as primary, secondary, and tertiary binding proteins. Primary binding proteins bind directly to 16S rRNA in vitro, although binding may be cooperative in vivo. Secondary binding proteins interact with 16S rRNA only after one or more primary binding proteins have associated with the 16S rRNA to form a ribonucleoprotein particle (RNP). Tertiary binding proteins require prior binding of at least one secondary binding protein before they can associate with the 16S rRNA. Interestingly, the three major domains in 16S rRNA can assemble independently to form ribonucleoprotein particles (Weitzmann et al. 1993; Samaha et al. 1994; Agalarov et al. 1998). Kinetic analysis of some of these RNPs has revealed the dynamics of 30S assembly (Recht and Williamson 2001). More recently, the assembly of the 30S subunit was examined using molecular mechanics (Stagg et al. 2003). These studies revealed that the primary binding proteins bind to more ordered sites in the 16S rRNA and help to organize the binding sites for proteins that bind later in the assembly pathway. However, very little is known about the contribution of various functional groups in 16S rRNA in the 30S assembly pathway. Chemical modification of the bases in 16S rRNA inhibits in vitro assembly of the 30S subunit (Nomura et al. 1968; Asai et al. 1999) and subunit association (Herr et al. 1979), indicating that they play a role in 30S assembly and subunit association. Similarly, the accessibility of the 16S rRNA backbone to ethylnitrosourea (ENU) in free 16S rRNA, in the 30S subunit, and in the 70S ribosome showed a distinct protection pattern consistent with the idea that functional groups in the RNA backbone are also important for assembly (Baudin et al. 1989). Importantly, heterologous 16S rRNA can be reconstituted in vivo with ribosomal proteins from different species (Nomura et al. 1968; Asai et al. 1999). This indicates that, in addition to the universally conserved bases in 16S rRNA, backbone interactions may contribute to the binding of small subunit proteins and for subunit association.

Previously, the accessibility of *Rp*-phosphate oxygens in domain I of 23S rRNA within the 50S subunit was mapped using phosphorothioate-substituted *Thermus aquaticus* 23S rRNA transcripts (Maivali et al. 2002). Several phosphate oxygens were protected within the 50S subunit, indicating shielding by the ribosomal large subunit proteins. However, they were unable to identify any phosphate oxygens in domain I of 23S rRNA that are important for 50S assembly. In this study, we have used a similar modification-interference approach to identify nonbridging phosphate oxygens within the *E. coli* 16S rRNA backbone that are important for the in vitro assembly of the 30S subunit and for association with the 50S large subunit. First, we show that phosphorothioate-substituted 16S rRNA reconstitutes in vitro to form 30S subunits. Second, these 30S subunits associate with native 50S subunits to form 70S ribosomes. Analysis of the 70S ribosome reveals that several nonbridging phosphate oxygens within the 16S rRNA participate either in the assembly

of the 30S subunit or for subunit association or may be involved in both events. Finally, X-ray crystal structures of the 30S subunit and the 70S ribosome were used to deduce possible roles for these nonbridging phosphate oxygens in 16S rRNA for 30S assembly and for subunit association.

RESULTS

Reconstitution of 30S subunit with phosphorothioate-modified 16S rRNA

The reconstitution procedure of Ofengand and coworkers was used to assemble the 30S ribosomal subunit from purified total proteins of the 30S subunit (TP30) and in vitro transcribed 16S rRNA (Krzyszosiak et al. 1987). Incorporation of *Rp*-phosphorothioate substitutions in 16S rRNA transcripts was accomplished during in vitro T7 RNA polymerase transcription by adding a low level of a single (α -S) phosphorothioate nucleotide triphosphate. In order to reduce the possibility of thiophilic metal ion contamination that may interfere with our final analysis, diethyl dithiocarbamate (DTC) was included in the reconstitution buffers. DTC chelates and precipitates thiophilic metal ions but with very little affinity for Mg^{2+} (Derrick et al. 2000). Control experiments showed that the presence of DTC in the reconstitution buffers have no adverse effects on 30S assembly. The reconstituted 30S subunits were purified on sucrose density gradients (Fig. 1). Fractionation of the gradients and absorbance measurement at 254 nm show peaks corresponding to the free 16S rRNA and the reconstituted 30S subunit (Fig. 1B). Since the free 16S rRNA migrates close to the 30S subunit, it can potentially contaminate the 30S fraction. Therefore, after the 30S reconstitution procedure, a cDNA primer complementary to 906–923 of 16S rRNA was added to the reaction mixture and treated with RNase H to cleave the free 16S rRNA into two fragments of ~620 nt (3'-fragment) and 906 nt (5'-fragment). The cleaved 16S rRNA fragments sedimented as two distinct peaks away from the 30S subunits, thereby minimizing the possibility of free 16S rRNAs contaminating the 30S subunit (Fig. 1C). Control experiments showed that the 16S rRNA in the 30S subunit is not cleaved by RNase H. In order to further improve the separation between the free 16S rRNA and the 30S subunits, the concentration of $MgCl_2$ in the sucrose gradient was lowered from 15 mM to 7.5 mM (Fig. 1D). These modifications resulted in better separation between the free 16S rRNA and the 30S subunit.

Subunit association experiments were performed to verify that the 30S peak represents authentic reconstituted 30S subunits. The 30S subunits reconstituted with phosphorothioate-modified 16S rRNA associate with the native 50S large subunits to form 70S ribosomes (Fig. 1I). Furthermore, these 70S ribosomes are active in tRNA binding (Table 1). The protein composition of the reconstituted 30S subunit was analyzed by two-dimensional gel electro-

FIGURE 1. Sucrose gradient profile of in vitro reconstituted 30S subunits. (A) Separation of phosphorothioate-substituted 16S rRNA transcripts and native 30S subunits as control markers on sucrose gradient. (B) Separation of 30S subunits reconstituted using 16S rRNA transcripts substituted randomly with adenosine phosphorothioate at 2.5% incorporation level. (C) Same as B but treated with RNase H to cleave free 16S rRNAs. (D) Same as C but separated on sucrose gradient containing 7.5 mM $MgCl_2$. (E–H) Separation of native 30S subunits, 50S subunits, 70S ribosomes, and 30S subunits reconstituted in vitro with phosphorothioated 16S rRNA, respectively, as a control marker on sucrose gradients. (I) Association of in vitro reconstituted 30S subunits with native 50S subunits to form 70S ribosomes. The 30S subunits were reconstituted using phosphorothioated 16S rRNA. (16S) 16S rRNA; (30S) 30S small ribosomal subunit; (5'F) 16S rRNA fragment corresponding from 1–906 (906 nucleotides); (3'F) 16S rRNA fragment corresponding from 923–1542 (620 nucleotides); (50S) 50S large ribosomal subunit; (70S) 70S ribosome. The shaded areas indicate the positions of 30S, 50S, and 70S on the sucrose gradients. The Y-axis is absorbance at 254 nm and the X-axis is fractions from the top to the bottom of the sucrose gradient. A–D are 10%–30% (w/v) linear sucrose gradients, and E–I are 10%–40% (w/v) linear sucrose gradients. All gradients contain 15 mM $MgCl_2$, except D as indicated.

phoresis. Reconstituted 30S subunit contains the same complement of small subunit ribosomal proteins as the native 30S subunit (Fig. 2), indicating that ribosomal proteins associate with phosphorothioate-substituted 16S rRNA to form the 30S subunit. Next, the 16S rRNA extracted from the reconstituted 30S subunits were analyzed for the presence of phosphorothioate by iodine cleavage followed by primer extension. Primer extension analysis showed reverse transcriptase stops corresponding to the cleavage pattern expected from the incorporation of a specific phosphorothioate-substituted nucleotide in 16S rRNA (data not shown). These results demonstrate that 30S subunits can be reconstituted with phosphorothioated 16S rRNA.

Nonbridging phosphate oxygens in 16S rRNA that are important for 30S reconstitution

In order to identify nonbridging phosphate oxygens in 16S rRNA that are important for 30S reconstitution, the level of phosphorothioate substitution in the 16S rRNA transcripts before reconstitution (total population) was compared to the level in 16S rRNA extracted from reconstituted 30S subunits (selected population). Sites of phosphorothioate substitution in 16S rRNA can be determined at nucleotide resolution by iodine cleavage and primer extension analysis. Phosphorothioate substitution at positions in 16S rRNA that are important for 30S reconstitution will be underrepresented in the selected 30S subunit and result in lower-intensity reverse transcriptase stop bands relative to the total population. Surprisingly, primer extension analysis showed no significant difference in band intensity between the selected and the total population. This suggests that even though attempts were made to remove free 16S rRNA from the 30S fraction, the problem persists and trace amounts of partially assembled 30S subunits are present as contaminants in the 30S fraction, making it difficult to directly identify sites that are important for 30S reconstitution. To confirm this hypothesis, we treated 16S rRNA transcripts, native 30S subunits, and reconstituted 30S subunits that were purified by sucrose gradient centrifugation with base-specific chemical probes and compared the protection pattern by primer extension analysis (Fig. 3). Chemical probing reveals the higher-order structure of the 16S rRNA in the 30S subunit (Moazed et al. 1986). Chemical probing showed that the protection pattern of 16S rRNA in the reconstituted 30S subunit is similar to that in the native 30S subunit; however, the level of protection is incomplete. Especially, regions in the 16S rRNA that interact with the tertiary binding proteins show incomplete protection compared to regions that bind the primary and secondary binding proteins (Powers et al. 1988; Stern et al. 1988a,c; Svensson et al. 1988). This further indicates that the reconstituted 30S subunits purified by a single sucrose gradient centrifugation are a mixture of fully and partially assembled 30S particles.

Several approaches were tried to further purify fully reconstituted 30S subunits. First, we collected reconstituted 30S subunits from only the late part of the sucrose gradients

TABLE 1. tRNA-binding activity

Particle	Percent activity
Native 70S	100
Reconstituted 70S (16S rRNA transcript)	23
Reconstituted 70S (phosphorothioated 16S rRNA transcript)	24

100% activity of native 70S corresponds to 0.8 pmol of tRNA bound per pmol of 70S.

FIGURE 2. Analysis of 30S subunit proteins by two-dimensional gel electrophoresis. (A) Protein composition of native 30S subunits. (B) Protein composition of 30S subunits reconstituted in vitro using unmodified 16S rRNA transcripts. (C) Protein composition of 30S subunits reconstituted in vitro using phosphorothioate-modified 16S rRNA transcripts. Labels S1–S20 correspond to the 30S small subunit proteins.

(away from the 16S rRNA fractions) and repurified the 30S subunits on a second sucrose gradient. A second approach that we tried was to use tRNA binding to select the fully reconstituted 30S subunits. Reconstituted 30S subunits were purified on sucrose gradients, programmed with the T4 gene32 mRNA fragment, and allowed to bind 3'-biotinylated tRNA^{Val1} in the P-site. The complex was irradiated with UV light to form a site-specific cross-link between the tRNA^{Val1} and 16S rRNA in the 30S subunit (Ofengand et al. 1979; Prince et al. 1982). The 16S rRNA cross-linked to 3'-biotin-tRNA^{Val1} was extracted and purified by binding to magnetic streptavidin beads (von Ahlsen and Noller 1995). The third approach that we tried was to purify reconstituted

30S particles on sucrose gradients followed by purification on a 3.5% native polyacrylamide gel (Dahlberg et al. 1969). The band corresponding to the 30S subunit was excised and the 16S rRNA was extracted by passive elution. The 16S rRNA was cleaved with iodine and analyzed by primer extension analysis. However, no difference in band intensity was observed between the selected and total population, indicating that it is difficult to directly select fully assembled 30S subunits using these approaches.

To overcome this problem, we indirectly selected fully assembled 30S subunits by their ability to bind 50S subunits and form 70S ribosomes. More importantly, the 70S ribosomes are well separated from the free 16S rRNA and the heterogeneous 30S peak in a sucrose gradient (Fig. 1I). The 30S subunits reconstituted with phosphorothioate-modified 16S rRNA were mixed with native 50S subunits to form 70S ribosomes. The 70S ribosomes were purified on sucrose gradients, and the level of phosphorothioate substitution at each position in 16S rRNA (selected population) was compared to the level in the 16S rRNA transcript before reconstitution (total population). A decreased level of phosphorothioate modification was observed at several positions within 16S rRNA that was isolated from the 70S ribosome (Fig. 4). At positions U37, A1171, G1270, and G1272 the effects were subtle but consistently observed in independent experiments (data not shown).

The 5'-domain

The 5'-domain of 16S rRNA constitutes the body of the 30S subunit (helix 1–18) (Fig. 5). Small subunit proteins S4, S5, S12, S16, S17, and S20 bind to this region of the 16S rRNA (Mizushima and Nomura 1970; Held et al. 1973; Brodersen et al. 2002). In the 5'-domain of 16S rRNA, protection was observed at Rp nonbridging phosphate oxygen 5' to U37, G76, C95, C110, G198, G213, G220, U224, G230, G232, A270, and C308. Position U37 is located at the junction of five helices (3, 4, 16–18). Positions G76 and C95 in helix 6 form a feature called the “spur” that is solvent exposed in the mature 30S subunit.

The central domain

The central domain of 16S rRNA constitutes the platform of the 30S subunit (helix 19–27). Protection was observed at positions G606, U619, A622, G626, G627, C739, C754, C770, and G838. Small subunit proteins S6, S8, S11, S15, S18, and S21 bind to the central domain of 16S rRNA (Mizushima and Nomura 1970; Held et al. 1973; Brodersen et al. 2002). In addition, the central domain forms several intersubunit bridges with the 50S large ribosomal subunit (Yusupov et al. 2001; Gao et al. 2003). The phosphate oxygen at C770 in helix 24 of 16S rRNA is a part of the intersubunit bridge B2c and may play a direct role in subunit association (Yusupov et al. 2001; Gao et al. 2003).

FIGURE 3. Chemical probing of reconstituted 30S subunit. Higher-order structure of 16S rRNA transcripts (16), native 30S subunits (30), and reconstituted 30S subunits containing phosphorothioate-substituted 16S rRNA (30*) were probed using kethoxal. (Lanes A,G) Dideoxy sequencing lanes; (–) indicates no treatment with kethoxal; and (+) indicates treatment with kethoxal. The filled arrowheads indicate positions in 16S rRNA protected by the ribosomal proteins in native and reconstituted 30S subunits relative to 16S rRNA transcript. Shown are protection by primary binding proteins (S4, S8, S17, and S20), secondary binding proteins (S6, S11, S12, S16, and S18), and tertiary binding proteins (S2 and S21) (Powers et al. 1988; Stern et al. 1988a,c; Svensson et al. 1988). Sites of enhanced reactivity observed in native and reconstituted 30S subunits relative to 16S rRNA transcripts are indicated by the unfilled arrowheads.

The 3'-domain

In the 3' major domain of 16S rRNA (helix 28–45), Rp phosphate oxygens at positions A1152, G1164, A1171, G1175, G1270, A1271, and G1272 are important for 30S assembly and subunit association (Fig. 5). Proteins S2, S3, S7, S9, S10, S13, S14, and S19 interact with the 3' major domain of 16S rRNA to form the “head” of the 30S subunit (Mizushima and Nomura 1970; Held et al. 1973; Brodersen et al. 2002). Surprisingly, we do not observe any protections in helices 44 and 45, which make up the 3' minor domain

of 16S rRNA. Helices 44 and 45 are located at the subunit interface and form four intersubunit bridges (B2a, B3, B5, and B6) with the 50S large ribosomal subunit (Yusupov et al. 2001; Gao et al. 2003). These intersubunit bridges, however, may not involve contacts with the phosphate oxygens in helix 44 of 16S rRNA. Ribosomal protein S20 contacts the bottom tip of helix 44, but these interactions may not be essential for 30S assembly and subunit association (Brodersen et al. 2002).

DISCUSSION

The 30S ribosomal subunit assembles in vitro using 16S rRNA from one species and the ribosomal proteins from a different species (Nomura et al. 1968). Even more remarkable is the fact that heterologous 16S rRNA can be reconstituted in vivo with ribosomal proteins from a species that diverged more than 350 million years ago (Niebel et al. 1987; Asai et al. 1999). Thus, sequence-independent contacts with the 16S rRNA backbone are likely to dominate reconstitution and subunit association. Consistent with these results, the 16S rRNA backbone is shielded from chemical probes in 30S subunits and 70S ribosomes (Baudin et al. 1989; Merryman et al. 1999). X-Ray crystal structures of the 30S subunit show that the backbone of 16S rRNA forms numerous intramolecular and intermolecular interactions with the small subunit ribosomal proteins (Brodersen et al. 2002). It is, however, difficult to determine the relative importance of specific backbone contacts for 30S subunit assembly and subunit association from the crystal structures. Furthermore, it is possible that

either additional or even different contacts are required during the intermediate states of 30S assembly that are no longer present in the fully assembled 30S subunit. Indeed, the 16S rRNA undergoes significant conformational changes during assembly into the 30S subunit (Hochkeppel and Craven 1977; Moazed et al. 1986; Powers et al. 1993; Holmes and Culver 2004).

Previously, Nierhaus and coworkers identified nonbridging phosphate oxygens within 5S rRNA that are important for incorporation into the 50S large subunit (Shpanchenko et al. 1998). More recently, the bases in 23S rRNA that are

FIGURE 4. Primer extension analysis of phosphorothioate-substituted 16S rRNA. (Lanes G,A,U,C) Dideoxy sequencing lanes; (lane K) primer extension analysis of phosphorothioate-substituted 16S rRNA extracted from the 70S ribosomes in the absence of iodine treatment; (lane T) primer extension analysis of phosphorothioate-substituted 16S rRNA transcript before reconstitution (total population); (lane S) primer extension analysis of phosphorothioate-substituted 16S rRNA extracted from 30S subunits that associated with the 50S subunits to form 70S ribosomes (selected population). 16S rRNA samples T and S were cleaved with iodine before primer extension analysis in order to identify the positions in 16S rRNA where phosphorothioate substitution causes inhibition of 30S assembly or subunit association. Arrows indicate positions in 16S rRNA where phosphorothioate substitution causes inhibition of 30S assembly or subunit association.

important for ribosomal subunit association were identified using a modification-interference approach (Maivali and Remme 2004). In addition, the accessibility of nonbridging phosphate oxygens in domain I of 23S rRNA within the 50S

subunit was mapped using the phosphorothioate approach (Maivali et al. 2002). However, this study did not identify any nonbridging phosphate oxygens in domain I of 23S rRNA that are important for 50S assembly. Here, we used a similar modification-interference approach to identify nonbridging phosphate oxygens within 16S rRNA that are important for 30S assembly and subunit association. Our results demonstrate that phosphorothioate-modified 16S rRNA assemble into functional 30S subunits and 70S ribosomes. This makes it feasible to identify nonbridging phosphate oxygens (Rp-isomer) that are important for 30S subunit assembly and for the association with the large ribosomal subunit. Furthermore, this method will permit the identification of other functional groups in 16S rRNA that are important for 30S subunit assembly and subunit association. These studies will provide a framework for understanding the molecular mechanism of ribosome assembly.

Phosphate oxygens important for subunit association

The large and small ribosomal subunits associate with each other by forming several intersubunit bridges. These intersubunit bridges are composed of rRNA and proteins from both the subunits. The 70S crystal structure shows that 16S rRNA helices 14, 20, 23, 24, 27, 44, and 45 form intersubunit bridges (Yusupov et al. 2001). Helix 24, which forms bridge B2c, involves interactions between the minor groove backbone of nucleotides (nt) 770–771 in 16S rRNA with the backbone of nt 1832–1833 in 23S rRNA (Fig. 5). Bridge B2c is one of the most conserved intersubunit bridges and may play an important role in subunit association. Consistent with this view, our results show that an Rp-phosphorothioate substitution at C770 in 16S rRNA is not tolerated because it may inhibit subunit association (Fig. 4K). In contrast, we do not observe a requirement for nonbridging phosphate oxygens within helix 44 of 16S rRNA. Helix 44 is the long, penultimate helix that spans the body of the 30S subunit at the subunit interface (Fig. 5). Helix 44 constitutes the intersubunit bridges B2a, B3, B5, and B6 that play an important role in subunit association (Yusupov et al. 2001). However, our results suggest that these bridges do not involve contact between the phosphate oxygens in 16S rRNA and the large ribosomal subunit.

Phosphate oxygens that interact with ribosomal proteins

The X-ray crystal structures of the 30S subunit show that the phosphate oxygens at U37, G230, G626, G627, C739, C754, and A1152 in 16S rRNA interact with ribosomal proteins (Wimberly et al. 2000). These phosphate oxygens may facilitate the binding of specific ribosomal proteins in the assembly pathway and stabilize the mature 30S structure. Residue U37 is present in helix 3 of 16S rRNA, which forms a five-way junction with helices 4, 16, 17, and 18 (Fig. 5).

FIGURE 5. Rp-phosphate oxygens important for 30S assembly and subunit association. (A) Secondary structure of 16S rRNA showing the positions of the important phosphate oxygens. (B) Three-dimensional structure of the *T. thermophilus* 30S subunit showing the positions of the important phosphate oxygens. The important phosphate oxygens are indicated by the black circles in A and by the magenta spheres in B. The 16S rRNA helices are numbered in bold on the secondary structure. The three-dimensional structure of the 30S subunit with 16S rRNA (cyan tube) and the proteins (yellow ribbon) facing the 50S subunit was created using ViewerLite (Accelrys) and rendered with PovRay (<http://www.povray.org>).

The phosphate oxygen at U37 is protected from ethylnitrosourea (ENU) in the 30S subunit (Baudin et al. 1989). Small subunit protein S12 interacts with helix 3 in 16S rRNA. Residues Arg117, Lys123, and Lys124 of S12 are in the vicinity of the phosphate oxygen at U37 (Fig. 6A). The phosphate oxygen of G230 potentially interacts with Arg26 and Ile33 of S16 (Fig. 6E). The phosphate oxygen at G626, which is present in helix 21 of 16S rRNA, interacts with S16. Residues Arg18, Lys35, and Tyr38 of S16 are within 5 Å of G626 (Fig. 7A). Similarly, the phosphate oxygen of G627 is close to Tyr38 of S16 (Fig. 7B). Proteins S6 and S15 bind near nt C739 of 16S rRNA. Residue Arg2, Glu69 of S6, and Pro2 of S15 are closest to the nonbridging phosphate oxygen of C739 (Fig. 7C).

In the X-ray crystal structure of an RNP complex, the phosphate oxygen at C754 forms a hydrogen bond with the exocyclic 2-amino group of G587 in 16S rRNA (Agalarov et al. 2000). However, in the 30S crystal structure, Arg72 of S15 is within hydrogen-bonding distance of the phosphate oxygen at C754 (Fig. 7D; Wimberly et al. 2000). This suggests that the phosphate oxygen at C754 may play a dual role, first interacting with G587 to facilitate assembly of an intermediate, followed by interaction with S15 in the mature 30S subunit. Nucleotide A1152 present in helix 39 of 16S rRNA interacts with proteins S9 and S10. Residues His68 and Arg70 of S10 are closest to the phosphate oxygen at A1152 (Fig. 7E).

Phosphate oxygens that interact with metal ions

Assembly of the 30S subunit is strictly dependent on the presence of monovalent and divalent metal ions (Traub and

FIGURE 6. Interactions in the body of the 30S subunit with the important Rp-phosphate oxygens. The 30S subunit residues that are within a radius of 5 Å from the respective Rp-phosphate oxygens (magenta spheres) are displayed. The yellow sphere indicates metal ions. The small subunit proteins shown are S12 (red) and S16 (green).

FIGURE 7. Interactions in the platform and head of the 30S subunit with the important Rp-phosphate oxygens. The 30S subunit residues that are within a radius of 5 Å from the respective Rp-phosphate oxygens (magenta spheres) are displayed. The small subunit proteins shown are S6 (brown), S10 (light blue), S15 (blue), and S16 (green).

Nomura 1969). Metal ions stabilize the folded structure by inner-sphere or outer-sphere coordination with the non-bridging phosphate oxygens of the RNA. Replacement of the nonbridging phosphate oxygens with sulfur potentially disrupts the coordination of metal ions, preventing 30S subunit assembly. Positions C110 and G232 in the body of the 30S subunit are close to metal ions in the 30S X-ray crystal structure (Fig. 6B,F; Wimberly et al. 2000). The phosphate oxygens at these two positions may play a direct role in coordinating metal ions that are important for 30S assembly.

Phosphate oxygens that may play a role during the early steps of 30S subunit assembly

Surprisingly, several of the nonbridging phosphate oxygens identified in this study are not involved in RNA–RNA or RNA–protein interaction in the mature 30S subunit. Although the precise role for these phosphate oxygens is not known, it is likely that they play a role in the early steps in the 30S assembly pathway. In vitro reconstitution of the 30S subunit proceeds by forming two distinct reconstitution intermediates (RI and RI*). It is known that RI undergoes a conformational change at elevated temperature to form RI* (Traub and Nomura 1969; Holmes and Culver 2004). This conformational change is essential for the association

of tertiary binding proteins with the RNP to form the mature 30S subunit. Some of the phosphate oxygens may stabilize the structure of the intermediates during assembly by interacting with functional groups in rRNA, r-proteins, or by coordinating divalent metal ions. These interactions are then disrupted when the assembly intermediates undergo conformational changes to form the mature 30S subunit. For example, G838 in helix 26 of 16S rRNA undergoes conformational changes as a result of RI-to-RI* transition (Holmes and Culver 2004) and when the 30S subunit associates with the 50S large subunit (Baudin et al. 1989). Also, positions G1170, A1271, and G1272 undergo conformational changes when the r-proteins associate with 16S rRNA to form the 30S subunit (Moazed et al. 1986). Thus, the phosphate oxygens in these regions of the 16S rRNA may play a dynamic role in the early steps of 30S assembly. Another formal possibility is that phosphorothioate modifications at certain positions in the 16S rRNA cause indirect effects that inhibit assembly or subunit association.

The assembly of this megadalton RNA–protein complex represents a fascinating and challenging biological puzzle. An actively growing *E. coli* cell takes ~10 to 20 min to synthesize a single ribosome (Mangiarotti et al. 1968). Our study shows that the RNA backbone interactions play an important role in ribosome assembly. Functional groups in the 16S rRNA backbone may accelerate proper folding of the RNA by facilitating intramolecular interactions, through coordination of divalent metal ions that stabilize the RNA tertiary structure, or by providing binding specificity for the ribosomal proteins. Mapping the various functional groups within rRNAs that are involved in ribosome assembly is essential for understanding this complex process.

MATERIALS AND METHODS

Plasmid

Plasmid pUC11816S, which contains the entire 16S rRNA gene of *E. coli*, was used as the template for in vitro transcription with T7 RNA polymerase (Newcomb and Noller 1999). The nt A1 and A2 are mutated to G to improve the efficiency of in vitro transcription. The plasmid contains the T7 promoter site at the 5'-end and a restriction site for BsaI at the 3'-end.

Reagents

Buffer A contains 20 mM NH_4^+ -HEPES (pH 7.5), 20 mM MgCl_2 , 500 mM NH_4Cl , 0.01% Nikkol, 4 mM 2-mercaptoethanol, and 1 mM diethyl dithiocarbamate (DTC); buffer B contains 20 mM NH_4^+ -HEPES (pH 7.5), 15 mM MgCl_2 , 100 mM NH_4Cl , 0.002% Nikkol, and 1 mM DTC; buffer C contains 20 mM NH_4^+ -HEPES (pH 7.5), 20 mM MgCl_2 , and 100 mM NH_4Cl .

Preparation of 16S transcript in vitro

In vitro transcript was generated using T7 RNA polymerase from BsaI-linearized plasmid. The transcription reaction was carried

out for 3 h in buffer containing 40 mM Tris-HCl (pH 7.9), 24 mM MgCl_2 , 10 mM DTT, 4 mM spermidine, 0.05% Triton X-100, 4 mM each NTP, and 0.1 mM [α -S]NTP (Sp isomer). The reaction mixture was ethanol-precipitated. Template DNA was removed by DNase I treatment, and the 16S rRNA was purified by successive extraction with phenol/chloroform and by chromatography on a sephacryl S-200 column eluted with 2.5 M NH_4OAc . Samples were precipitated at -80°C for 30 min with 2.5 vol of alcohol. Precipitated RNA was recovered by centrifugation for 20 min at 4°C at 10,000 rpm, resuspended in H_2O , and frozen in aliquots. For phosphorothioate-containing transcript, each phosphorothioate NTP was incorporated at a level of 2.5% during the transcription reaction. This results in the incorporation of eight phosphorothioates per 16S rRNA on the average. Preliminary studies showed that this level of incorporation within 16S rRNA resulted in good signal over noise in the primer extension analysis.

In vitro reconstitution of 30S subunit

In vitro reconstitution was performed essentially as described by Ofengand and coworkers (Krzyzosiak et al. 1987) with slight modification. The activity of each new batch of TP30 was tested by titration experiments, which showed that 2–4 molar equivalent of TP30 over the 16S rRNA results in a maximum yield of reconstituted 30S subunits. Addition of more TP30 did not increase the yield of 30S subunits or change our results. Typically, 156 pmol of 16S rRNA and 2 molar equivalent of TP30 was combined in buffer A containing DTC and heated sequentially for 15 min at each temperature 40°C , 43°C , 46°C , 48°C , and 50°C . Then 1 pmol of primer complementary to the 906–923 region of 16S rRNA was added and incubated at 50°C for another 10 min, followed by the addition of 1 μL of RNase H (2 units/ μL ; Invitrogen), and the reaction was incubated at 37°C for 30 min. The mixture was quickly cooled to 4°C and loaded onto a 10%–30% (w/v) linear sucrose gradient in buffer B containing 7.5 mM MgCl_2 . We used a 10%–30% sucrose gradient for purifying the reconstituted 30S subunits because it better separates the 16S rRNA fragments from the 30S peak.

Subunit association

For subunit association the RNase H cleavage step was omitted and the 30S reconstituted particles were incubated with excess 50S subunits (50 pmol) in buffer B at 37°C for 30 min. Samples were layered on 11 mL of 10%–40% (w/v) linear sucrose gradient prepared in buffer B without DTC. Gradients were spun in an SW 41 rotor at 32,000 rpm for 15.5 h at 4°C . The peak sedimenting with 70S was isolated, and sucrose was removed by centrifugation at 4°C at 2000 rpm in centricon-100 by three to four sequential washes with buffer D.

Iodine cleavage and primer extension

The phosphorothioate-substituted 16S rRNA from the total and selected population was cleaved using iodine to identify the location of individual phosphorothioate substitutions within the 16S rRNA (Schnitzer and von Ahsen 1997). About 40 μg of purified 16S rRNA was incubated in 80% formamide and 2 mM iodine

(final concentration) in 100 μL of total reaction volume for 2 min at room temperature. Reactions were stopped by adding 50 μL of 7.5 M NH_4OAc followed by ethanol precipitation to recover the cleaved 16S rRNA. The rRNAs were resuspended in water and stored at -80°C . Aliquots of 16S rRNA were analyzed by primer extension (Stern et al. 1988b).

tRNA-binding activity

70S ribosomes reconstituted from phosphorothioate-modified 16S rRNA transcripts or from unmodified 16S rRNA transcripts were assayed for tRNA-binding activity using [^{32}P]-labeled tRNA^{Phe}. Reconstituted ribosomes were purified on sucrose gradients and recovered by ethanol precipitation (Chapman and Noller 1977). [α - ^{32}P]UTP was incorporated at internal positions within tRNA^{Phe} during transcription. Then 10 pmol of 70S ribosome was programmed with 20 pmol of gene32 mRNA fragments in binding buffer (80 mM potassium cacodylate at pH 7.5, 10 mM MgCl_2 , 150 mM NH_4Cl). Then 15 pmol of [^{32}P]-labeled tRNA^{Phe} was added to the ribosome mix and incubated at 37°C for 30 min. The reaction mixture was diluted in 1 mL of binding buffer and passed through nitrocellulose filter circles (0.45 microns; HAWP, Millipore). The filters were washed with 4 mL of binding buffer and dried, and the radioactivity was measured by scintillation counting.

Protein analysis

Total protein from sucrose-gradient-purified reconstituted 30S subunits or native 30S subunits (equivalent amounts based on absorbance of rRNAs at 260 nm) was isolated by acetic acid extraction as described (Nierhaus 1990) and suspended in 10 μL of loading buffer containing 8 M urea, 1% 2-mercaptoethanol, and 10 mM Bis-tris acetate (pH 4.1), and analyzed by two-dimensional gel electrophoresis (Geyl et al. 1981). The proteins were visualized by silver staining using a silver stain kit and the manufacturer's recommended procedure (BioRad).

Chemical probing

Kethoxal modification of 16S rRNA transcript, native 30S subunits, and reconstituted 30S subunits containing phosphorothioate 16S rRNA was carried out following the standard method (Stern et al. 1988b). The 16S rRNA transcript was incubated under 30S subunit reconstitution conditions before kethoxal modification. Typically 50 pmol of each sample in 25 μL of binding buffer was incubated with 2 μL of kethoxal (37 mg/mL in 20% [v/v] ethanol) at 37°C for 10 min. Reactions were stopped by adding 12.5 μL of 0.5 M K-borate (pH 7), 12.5 μL of 3 M sodium acetate (pH 6), and 300 μL of ethanol. rRNAs were extracted with phenol and precipitated with ethanol. The rRNAs were resuspended in 25 mM K-borate (pH 7) and analyzed by primer extension (Stern et al. 1988b).

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REFERENCES

- Agalarov, S.C., Zheleznyakova, E.N., Selivanova, O.M., Zheleznyaya, L.A., Matvienko, N.I., Vasiliev, V.D., and Spirin, A.S. 1998. In vitro assembly of a ribonucleoprotein particle corresponding to the platform domain of the 30S ribosomal subunit. *Proc. Natl. Acad. Sci.* **95**: 999–1003.
- Agalarov, S.C., Sridhar Prasad, G., Funke, P.M., Stout, C.D., and Williamson, J.R. 2000. Structure of the S15,S6,S18-rRNA complex: Assembly of the 30S ribosome central domain. *Science* **288**: 107–113.
- Alix, J.H. and Nierhaus, K.H. 2003. DnaK-facilitated ribosome assembly in *Escherichia coli* revisited. *RNA* **9**: 787–793.
- Asai, T., Zaporozhets, D., Squires, C., and Squires, C.L. 1999. An *Escherichia coli* strain with all chromosomal rRNA operons inactivated: Complete exchange of rRNA genes between bacteria. *Proc. Natl. Acad. Sci.* **96**: 1971–1976.
- Ban, N., Nissen, P., Hansen, J., Moore, P.B., and Steitz, T.A. 2000. The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. *Science* **289**: 905–920.
- Baudin, F., Mouguel, M., Romby, P., Eyermann, F., Ebel, J.P., Ehresmann, B., and Ehresmann, C. 1989. Probing the phosphates of the *Escherichia coli* ribosomal 16S RNA in its naked form, in the 30S subunit, and in the 70S ribosome. *Biochemistry* **28**: 5847–5855.
- Brodersen, D.E., Clemons Jr., W.M., Carter, A.P., Wimberly, B.T., and Ramakrishnan, V. 2002. Crystal structure of the 30 S ribosomal subunit from *Thermus thermophilus*: Structure of the proteins and their interactions with 16 S RNA. *J. Mol. Biol.* **316**: 725–768.
- Chapman, N.M. and Noller, H.F. 1977. Protection of specific sites in 16 S RNA from chemical modification by association of 30 S and 50 S ribosomes. *J. Mol. Biol.* **109**: 131–149.
- Charollais, J., Pflieger, D., Vinh, J., Dreyfus, M., and Iost, I. 2003. The DEAD-box RNA helicase SrmB is involved in the assembly of 50S ribosomal subunits in *Escherichia coli*. *Mol. Microbiol.* **48**: 1253–1265.
- Dahlberg, A.E., Dingman, C.W., and Peacock, A.C. 1969. Electrophoretic characterization of bacterial polyribosomes in agarose–acrylamide composite gels. *J. Mol. Biol.* **41**: 139–147.
- Derrick, W.B., Greef, C.H., Caruthers, M.H., and Uhlenbeck, O.C. 2000. Hammerhead cleavage of the phosphorodithioate linkage. *Biochemistry* **39**: 4947–4954.
- Gao, H., Sengupta, J., Valle, M., Korostelev, A., Eswar, N., Stagg, S.M., Van Roey, P., Agrawal, R.K., Harvey, S.C., Sali, A., et al. 2003. Study of the structural dynamics of the *E. coli* 70S ribosome using real-space refinement. *Cell* **113**: 789–801.
- Geyl, D., Bock, A., and Isono, K. 1981. An improved method for two-dimensional gel-electrophoresis: Analysis of mutationally altered ribosomal proteins of *Escherichia coli*. *Mol. Gen. Genet.* **181**: 309–312.
- Held, W.A., Mizushima, S., and Nomura, M. 1973. Reconstitution of *Escherichia coli* 30 S ribosomal subunits from purified molecular components. *J. Biol. Chem.* **248**: 5720–5730.
- Held, W.A., Ballou, B., Mizushima, S., and Nomura, M. 1974. Assembly mapping of 30 S ribosomal proteins from *Escherichia coli*. Further studies. *J. Biol. Chem.* **249**: 3103–3111.
- Herr, W., Chapman, N.M., and Noller, H.F. 1979. Mechanism of ribosomal subunit association: Discrimination of specific sites in 16 S RNA essential for association activity. *J. Mol. Biol.* **130**: 433–449.
- Hochkeppel, H.K. and Craven, G.R. 1977. Significant changes in 16 S RNA conformation accompanying assembly of the 30 S ribosome in vitro. *J. Mol. Biol.* **113**: 623–634.
- Holmes, K.L. and Culver, G.M. 2004. Mapping structural differences between 30S ribosomal subunit assembly intermediates. *Nat. Struct. Mol. Biol.* **11**: 176–186.
- Krzyzosiak, W., Denman, R., Nurse, K., Hellmann, W., Boublik, M., Gehrke, C.W., Agris, P.F., and Ofengand, J. 1987. In vitro synthesis of 16S ribosomal RNA containing single base changes and assembly into a functional 30S ribosome. *Biochemistry* **26**: 2353–2364.
- Maivali, U. and Remme, J. 2004. Definition of bases in 23S rRNA essential for ribosomal subunit association. *RNA* **10**: 600–604.
- Maivali, U., Pulk, A., Loogvali, E.L., and Remme, J. 2002. Accessibility of phosphates in domain I of 23 S rRNA in the ribosomal 50 S subunit as detected by R(P) phosphorothioates. *Biochim. Biophys. Acta* **1579**: 1–7.
- Maki, J.A., Schnobrich, D.J., and Culver, G.M. 2002. The DnaK chaperone system facilitates 30S ribosomal subunit assembly. *Mol. Cell* **10**: 129–138.
- Maki, J.A., Southworth, D.R., and Culver, G.M. 2003. Demonstration of the role of the DnaK chaperone system in assembly of 30S ribosomal subunits using a purified in vitro system. *RNA* **9**: 1418–1421.
- Mangiarotti, G., Apirion, D., Schlessinger, D., and Silengo, L. 1968. Biosynthetic precursors of 30S and 50S ribosomal particles in *Escherichia coli*. *Biochemistry* **7**: 456–472.
- Merryman, C., Moazed, D., McWhirter, J., and Noller, H.F. 1999. Nucleotides in 16S rRNA protected by the association of 30S and 50S ribosomal subunits. *J. Mol. Biol.* **285**: 97–105.
- Mizushima, S. and Nomura, M. 1970. Assembly mapping of 30S ribosomal proteins from *E. coli*. *Nature* **226**: 1214.
- Moazed, D., Stern, S., and Noller, H.F. 1986. Rapid chemical probing of conformation in 16S ribosomal RNA and 30S ribosomal subunits using primer extension. *J. Mol. Biol.* **187**: 399–416.
- Newcomb, L.F. and Noller, H.F. 1999. Directed hydroxyl radical probing of 16S ribosomal RNA in 70S ribosomes from internal positions of the RNA. *Biochemistry* **38**: 945–951.
- Niebel, H., Dorsch, M., and Stackebrandt, E. 1987. Cloning and expression in *Escherichia coli* of *Proteus vulgaris* genes for 16S ribosomal RNA. *J. Gen. Microbiol.* **133** (Pt 9): 2401–2409.
- Nierhaus, K. 1990. Reconstitution of ribosomes. In *Ribosomes and protein synthesis: A practical approach* (ed. G. Spedding), pp. 161–189. IRL Press, Oxford, UK.
- Nierhaus, K.H. and Dohme, F. 1974. Total reconstitution of functionally active 50S ribosomal subunits from *Escherichia coli*. *Proc. Natl. Acad. Sci.* **71**: 4713–4717.
- Nomura, M., Traub, P., and Bechmann, H. 1968. Hybrid 30S ribosomal particles reconstituted from components of different bacterial origins. *Nature* **219**: 793–799.
- Ofengand, J., Liou, R., Kohut, J.D., Schwartz, I., and Zimmermann, R.A. 1979. Covalent cross-linking of transfer ribonucleic acid to the ribosomal P site. Mechanism and site of reaction in transfer ribonucleic acid. *Biochemistry* **18**: 4322–4332.
- Powers, T., Stern, S., Changchien, L.M., and Noller, H.F. 1988. Probing the assembly of the 3' major domain of 16 S rRNA. Interactions involving ribosomal proteins S2, S3, S10, S13 and S14. *J. Mol. Biol.* **201**: 697–716.
- Powers, T., Daubresse, G., and Noller, H.F. 1993. Dynamics of in vitro assembly of 16 S rRNA into 30 S ribosomal subunits. *J. Mol. Biol.* **232**: 362–374.
- Prince, J.B., Taylor, B.H., Thurlow, D.L., Ofengand, J., and Zimmermann, R.A. 1982. Covalent crosslinking of tRNA^{Val} to 16S RNA at the ribosomal P site: Identification of crosslinked residues. *Proc. Natl. Acad. Sci.* **79**: 5450–5454.
- Recht, M.I. and Williamson, J.R. 2001. Central domain assembly: Thermodynamics and kinetics of S6 and S18 binding to an S15–RNA complex. *J. Mol. Biol.* **313**: 35–48.
- Samaha, R.R., O'Brien, B., O'Brien, T.W., and Noller, H.F. 1994. Independent in vitro assembly of a ribonucleoprotein particle containing the 3' domain of 16S rRNA. *Proc. Natl. Acad. Sci.* **91**: 7884–7888.
- Schlutzen, F., Tocilj, A., Zarivach, R., Harms, J., Gluehmann, M., Janell, D., Bashan, A., Bartels, H., Agmon, I., Franceschi, F., et al. 2000. Structure of functionally activated small ribosomal subunit at 3.3 angstroms resolution. *Cell* **102**: 615–623.
- Schnitzer, W. and von Ahsen, U. 1997. Identification of specific Rpphosphate oxygens in the tRNA anticodon loop required for ribosomal P-site binding. *Proc. Natl. Acad. Sci.* **94**: 12823–12828.
- Shpanchenko, O.V., Dontsova, O.A., Bogdanov, A.A., and Nierhaus, K.H. 1998. Structure of 5S rRNA within the *Escherichia coli* ribosome: Iodine-induced cleavage patterns of phosphorothioate de-

- rivatives. *RNA* **4**: 1154–1164.
- Stagg, S.M., Mears, J.A., and Harvey, S.C. 2003. A structural model for the assembly of the 30S subunit of the ribosome. *J. Mol. Biol.* **328**: 49–61.
- Stern, S., Changchien, L.M., Craven, G.R., and Noller, H.F. 1988a. Interaction of proteins S16, S17 and S20 with 16 S ribosomal RNA. *J. Mol. Biol.* **200**: 291–299.
- Stern, S., Moazed, D., and Noller, H.F. 1988b. Structural analysis of RNA using chemical and enzymatic probing monitored by primer extension. *Methods Enzymol.* **164**: 481–489.
- Stern, S., Powers, T., Changchien, L.M., and Noller, H.F. 1988c. Interaction of ribosomal proteins S5, S6, S11, S12, S18 and S21 with 16 S rRNA. *J. Mol. Biol.* **201**: 683–695.
- Svensson, P., Changchien, L.M., Craven, G.R., and Noller, H.F. 1988. Interaction of ribosomal proteins, S6, S8, S15 and S18 with the central domain of 16 S ribosomal RNA. *J. Mol. Biol.* **200**: 301–308.
- Traub, P. and Nomura, M. 1968. Structure and function of *E. coli* ribosomes. V. Reconstitution of functionally active 30S ribosomal particles from RNA and proteins. *Proc. Natl. Acad. Sci.* **59**: 777–784.
- . 1969. Structure and function of *Escherichia coli* ribosomes. VI. Mechanism of assembly of 30 s ribosomes studied in vitro. *J. Mol. Biol.* **40**: 391–413.
- von Ahsen, U. and Noller, H.F. 1995. Identification of bases in 16S rRNA essential for tRNA binding at the 30S ribosomal P site. *Science* **267**: 234–237.
- Weitzmann, C.J., Cunningham, P.R., Nurse, K., and Ofengand, J. 1993. Chemical evidence for domain assembly of the *Escherichia coli* 30S ribosome. *FASEB J.* **7**: 177–180.
- Williamson, J.R. 2003. After the ribosome structures: How are the subunits assembled? *RNA* **9**: 165–167.
- Wimberly, B.T., Brodersen, D.E., Clemons Jr., W.M., Morgan-Warren, R.J., Carter, A.P., Vornrhein, C., Hartsch, T., and Ramakrishnan, V. 2000. Structure of the 30S ribosomal subunit. *Nature* **407**: 327–339.
- Yusupov, M.M., Yusupova, G.Z., Baucom, A., Lieberman, K., Earnest, T.N., Cate, J.H.D., and Noller, H.F. 2001. Crystal structure of the ribosome at 5.5 Å resolution. *Science* **292**: 883–896.

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