Structural insights into methyltransferase KsgA function in 30S ribosomal subunit biogenesis*

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*Running title: Structure of KsgA bound to the 30S ribosomal subunit

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Background: The RNA methyltransferase KsgA is required for small ribosomal subunit maturation.

Results: Cryo-electron microscopy reveals the structure of the near mature ribosomal subunit in complex with KsgA.

Conclusion: We suggest that KsgA controls conformational changes in the ribosomal RNA required for final subunit maturation.

Significance: Understanding the remodeling and processing of ribosomal RNA by protein factors required for ribosome biogenesis.

SUMMARY

The assembly of the ribosomal subunits is facilitated by ribosome biogenesis factors. The universally conserved methyltransferase KsgA modifies two adjacent adenosine residues in the 3' terminal helix 45 of the 16S ribosomal RNA (rRNA). KsgA recognizes its substrate adenosine residues only in the context of a near mature 30S subunit and is required for the efficient processing of the rRNA termini during ribosome biogenesis. Here we present the cryo-EM structure of KsgA bound to a non-methylated 30S ribosomal subunit. The structure reveals that KsgA binds to the 30S platform with the catalytic N-terminal domain interacting with substrate adenosine residues in helix 45 and the C-terminal domain making extensive contacts to helix 27 and helix 24. KsgA excludes the penultimate rRNA helix 44 from adopting its position in the mature 30S subunit, blocking the formation of the decoding site and subunit joining. We suggest that the activation of methyltransferase activity and subsequent dissociation of KsgA control conformational changes in helix 44 required for final rRNA processing and translation initiation.

The assembly of the small bacterial ribosomal subunit from its components, a large rRNA molecule and 21 proteins, is facilitated and controlled by ribosome biogenesis factors (1,2). The 16S rRNA forms three domains, with a 5' domain forming the body of the 30S, a central domain arranging into the platform, and a 3' domain. The 3' domain can be divided into a major domain that forms the head and a 3' terminal minor domain that forms helix 45 and helix 44, which stretches down from the head across the long axis of the body (3).

Although the 30S ribosomal subunit can be in vitro assembled from its isolated components (4), ribosome assembly in vivo is significantly faster and requires additional protein factors (5,6). The in vivo ribosome assembly process is initiated by the transcription of a large precursor RNA containing all ribosomal RNA molecules, which is subsequently cleaved by several RNases to its mature length (7,8). In addition, the rRNA is modified at certain nucleotides (7). Whereas these modifications differ significantly when comparing ribosomes from different domains of life, the methylation of
two adjacent adenosines at bases A1518 and A1519 in the small ribosomal subunit is universally conserved (9). The methylation of these adenosine residues is catalyzed by the KsgA methyltransferase in E. coli (10,11). KsgA methylates the loop nucleotides A1518 and A1519 of helix 45 located near the 3' end of the 16S rRNA. The absence of the methylations in strains deficient in methyltransferase activity confers resistance to the antibiotic kasugamycin (10). In the eukaryotic 40S ribosomal subunit the corresponding adenosines bases are methylated by the homologous eukaryotic enzyme Dim1 (12), which has been visualized as part of a multi maturation factor complex on the 40S subunit (13).

The deletion of KsgA results in a cold sensitive phenotype, implicating KsgA in the control of certain 30S ribosomal subunit assembly steps (14). In the KsgA deletion strain a 17S rRNA precursor accumulates, which is similar to the 17S rRNA species observed for the deletion or mutations of other ribosome biogenesis factors (14). These factors include the GTPases Era and RsgA, as well as the small assembly proteins RbfA and RimM, all of which are involved in late assembly steps (15). Overexpression of a mutant KsgA deficient in methyltransferase activity resulted in a large proportion of the 30S subunits being bound to KsgA in an immature state suggesting that the dissociation of KsgA following methylation precedes the end-processing of the 17S rRNA (14).

KsgA only modifies the rRNA in the context of a nearly mature 30S subunit. KsgA requires the presence of all (S6, S8, S11, S15, S18) but one (S21) ribosomal proteins that collectively form the 30S platform and three ribosomal proteins that are part of the 30S body (16,17). Although KsgA has been reported to bind to a fragment of 16S rRNA including the modified nucleotides, it does not modify naked 16S rRNA (10,16,18). More specifically, KsgA requires the 30S subunit to be in a translationally inactive conformation, which can be obtained in vitro by magnesium depletion (17). The interaction sites of KsgA on the 30S subunit were mapped around the decoding site and the 30S platform using hydroxyl radical footprinting (19).

The crystal structures of E. coli KsgA and its eukaryotic and archaeal homologue Dim1 have been determined (20-23). The crystal structures share a high degree of structural similarity and can be divided into a C-terminal domain and an N-terminal catalytic domain. The N-terminal catalytic domain shares sequence and structural similarity to the adenine DNA methyltransferase (20). Furthermore, two structures of the Aquifex aeolicus KsgA in complex with an RNA fragments of helix 45 were solved (24,25). These structures show catalytically inactive complexes and are inconsistent with structural model based on footprinting data (19). Other structural data on this system include the X-ray crystal structure of a 30S ribosomal subunit lacking the methylations on A1518 and A1519 (26). However, this structure was obtained under high magnesium conditions under which the subunit can not be modified by KsgA.

To investigate the complex substrate specificity of KsgA we determined the structure of KsgA in complex with a substrate 30S subunit by cryo electron microscopy. The structure reveals that KsgA recognizes the 30S in a particular, translationally inactive, conformation. The dissociation of KsgA is required for formation of the translationally active 30S subunit conformation. We suggest that a similar conformational change occurs in the last steps of the small ribosomal subunit biogenesis.

**EXPERIMENTAL PROCEDURES**

**Preparation of the KsgA-30S complex**

KsgA was expressed and purified as described previously (27). For complex formation 30S ribosomal subunits from a ksg^R strain (19) were isolated essentially as described (28). The purified submethylated 30S subunits were resuspended in buffer K (40 mM KCl, 4mM MgCl2, 20 mM HEPES/KOH pH 7.6, 6 mM 2-mercaptoethanol) and stored at -80 °C (19). For complex formation 100 nM 30S subunits and a 20 fold molar excess of KsgA were incubated in buffer K for 37 °C for 10 min and placed on ice for at 10 min.

**Preparation of control 30S subunits**

For the control, translationally inactive 30S subunits were purified from a ksgA deletion strain JW0050-3 obtained from the E. coli Genetic Resource Center at Yale University (29) similarly
as for the KsgA-30Si complex. For the reactivation of the inactivated subunits, the magnesium concentration was increased to 20 mM and the sample was incubated at 40 °C for 20 min (28).

**Electron microscopy grid preparation and image acquisition**

5 µl of the KsgA-30Si complex or the control 30Si ribosomal subunit samples were applied to a holey carbon copper grid (Quantifoil, Jena, Germany) and plunged into liquid ethane (30). The grids were image in a Tecnai F20 electron microscope (FEI, Hillsboro, OR) operated at 200 kV at a magnification of 82,000 fold. Images were acquired on an Ultrascan 4000 CCD camera (Gatan, Pleasanton, CA) under low dose conditions at 2-4 micron defocus. Carbon foil holes showing sufficiently thin ice were selected manually and image series of 2 times 2 images were collected semi-automatically in each hole using a SerialEM script (31). In addition to controlling the spot scan the script monitors specimen drift and sets the desired defocus. The exact defocus of each image was subsequently determined using the Ctffind3 program (32). Single particle images were windowed out semi-automatically with Boxer (33).

**Image processing and 3D reconstruction**

For the single particle datasets ctf correction was applied using Spider (34). The images were computationally coarsened to a pixel size of 4.47 Å on the object scale. Initial 3D reconstructions were obtained using the Image5 software with a down filtered 30S subunit as initial reference, which was obtained from 70S structure (35,36). Final rounds of refinement were done using the Spider software with images computationally coarsened to a pixel size of 3.07 Å (34). In the last rounds of refinement amplitude correction was applied to the reconstruction by Fourier filtering using B factors (37). For the reconstruction of the KsgA-30Si complex the initial dataset was split into more homogeneous subpopulations using a bootstrap 3D variance analysis and classification approach (38,39). The aligned single particles were sorted by projection direction and from each projection direction particles were chosen randomly with replacement. From each random sample of particles a 3D structure was calculated. 3D MSA of the resulting 3D structures gave eigenvolumes corresponding to the main structural differences in the particle population using the Imagic5 software (35). Based on the second eigenvolume the single particle image set was split into more homogeneous subgroups. The second eigenvolume was projected in each projection direction and factorial coordinates were calculated for each single particle image (40). The single particle images were sorted according to the factorial coordinates and 3D structures were calculated. The resulting structures were refined in a multi particle refinement approach in Spider (41). The number of particles used and the resolution of the final reconstructions are shown in table S1. Structural figures were prepared with Chimera (42). The surface charge distribution of KsgA was calculated using DelPhi (43).

**RESULTS**

We studied the complex of KsgA with 30S ribosomal subunits lacking the methylations at A1518 and A1519 by electron microscopy. Mature 30S ribosomal subunits were isolated and brought into the inactive conformation recognized by KsgA, hereafter named 30Si, by magnesium depletion as described previously (19,28). Initial reconstructions of the KsgA-30Si complex could not be refined to high resolution (Supplemental figure 1), indicating significant conformational heterogeneity of the 30Si subunit. The reconstruction showed no significant density for helix 44 of the 16S rRNA. To investigate the structural heterogeneity and refine the reconstructions to higher resolution, we employed a bootstrap variance analysis and 3D classification technique (38,39). Based on the variance analysis the dataset can then be split into subgroups of particles (40) and from these subgroups 3D reconstructions showing the main conformational states can be reconstructed. These reconstructions can then be refined in a multi particle alignment approach (41) to refine conformational substates to higher resolution. Using this approach, four subgroups of particles showing different positions of the head relative to the body of the 30Si subunit were obtained (Supplemental figure 2). Further refinement of these four subgroups significantly improved the resolution of the group 1 and 4.
reconstructions (Fig. 1, supplemental figure 3). The resolution of these reconstructions is 12.3 Å and 13.5 Å for classes 1 and 4, respectively (0.5 criterion) (Supplemental table 1). In addition, we obtained the structure of the 30Si ribosomal subunit in the absence of KsgA at 15.5 Å resolution (0.5 criterion) (Supplemental table 1). The structures were interpreted by fitting the crystal structure of the E. coli small ribosomal subunit (44) into the densities (Fig. 1). As the relative orientations of the head and body vary for the KsgA-30Si reconstructions, we independently fitted the head and body as rigid bodies. In class 1 of the KsgA-30Si complexes, the head density is very weak and could not be fitted with the crystal structure (Supplemental figure 3, class 1). Helix 44 is not visible in any of the 30Si reconstructions (Fig. 1a, b), indicating that this RNA helix is flexibly linked to the 30Si structure. In addition, we obtained a structure of a reactivated 30Si ribosomal subunit mimicking the formation of the mature 30S subunit after KsgA mediated methylation (Fig. 1c). We reactivated the 30Si ribosomal subunit by incubation in a high magnesium buffer at 42°C. In the 3D reconstruction we observe a well defined density for helix 44 in a conformation similar to the crystal structure of translationally active 30S subunits, confirming the structural integrity of our 30Si preparation (3,26). Thus, the absence of a helix 44 density in the reconstructions of the inactive 30Si ribosomal subunit is due to a conformational change of helix 44 upon inactivation.

Comparing the structure of the inactive apo-30Si to the structure of the KsgA-30Si complex revealed extra density at the platform of the 30Si in the KsgA complex (Fig. 1a, b). Using the fitted 30Si structure, we separated this extra density from the 30Si subunit structure (Fig. 1b). The density has an elongated shape and is slightly bent with a broader part near the decoding site and a narrower tail. These features allow unambiguous fitting of the crystal structure of KsgA into the density (correlation coefficient 0.87) (20). The location of KsgA on the intersubunit side of 30Si ribosomal subunit would prevent subunit joining and 70S formation by a steric clash with H69 of the large ribosomal subunit.

KsgA is bound to the platform at helices 24, 27, and 45 (Fig. 2a-c). The catalytic N-terminal domain of KsgA contacts the loop of helix 45, which brings A1518 and A1519 close to the active site of KsgA. The substrate adenosine residues A1518 and A1519 are located at the entry of the negatively charged catalytic center of KsgA, which binds the methyl donor S-adenosyl-L-methionine (SAM). The substrate adenosines would have to shift by about 7 Å in a flipped out conformation to reach the active site of KsgA, possibly accompanied by a slight conformational rearrangement of the helix 45 loop (Fig. 2c). Such substrate binding mode can be compared to the structure of DNA methyltransferases in complex with their substrate. The DNA methyltransferase M.TaqI in complex with its substrate DNA shows a flipped out base reaching into the catalytic center of M.TaqI (45) (Fig. 2d).

The C-terminal domain of KsgA binds to the ribosome exclusively by contacting rRNA, specifically 16S rRNA helices 27 and 24. The electrostatic surface potential of KsgA shows a large positively charged region, on the C-terminal domain and in the cleft formed by the N and C-terminal domains facing the ribosome, in contact with the 30S subunit (Fig. 2b). The positive patch on the C-terminal domain involves the highly conserved residues 221-223 and 248 of KsgA (20). These residues contact helix 27 and helix 24 where the GCAA tetraloop of helix 24 forms a tertiary interaction with the base of helix 27. Mutation of R248 to alanine or the mutation of the sequence R221RK223 to AAA results in a more than 100 fold reduction of binding affinity as assayed in fluorescence polarization experiments (Supplemental Fig 4, Supplemental Methods). In methylation assays the R248A variant retained wild type activity (Supplemental Fig 5a, Supplemental Methods). The triple mutant showed a significantly reduced activity and produced significantly more mono-methyladenosine over di-methyladenosine, which indicates that this mutant releases intermediate products due to its reduced affinity for the 30S subunit (Supplemental Fig. 5b-c, Supplemental Methods).

When KsgA is bound to 30Si, it blocks the tertiary interaction between helix 45 and helix 44, which is important for the formation of the decoding site in the mature subunit (Fig. 3).
DISCUSSION

The inactive conformation of the 30S mimics ribosome biogenesis intermediates

The methylation of 30S ribosomal subunits by KsgA is studied conveniently in vitro by isolating non-methylated 30S subunits and bringing them into a methylation competent 30Si conformation by depletion of magnesium ions (17). Using this system it is possible to dissect the requirements for the methylation reaction. Our reconstruction of the 30Si shows that this conformation of the 30S subunit shares similarity with intermediates of in vitro ribosome assembly and in vivo pre-ribosomal subunits. The most prominent conformational change compared to the mature 30S subunit is the disorder of helix 44 as evidenced by the lack of density for this rRNA element. Chemical modification data on the 30Si subunit indicated that the base pairing in the upper part of helix 44 and the neighboring helix 28 is disrupted (Supplemental figure 6) (28). Loss of rRNA structure in this region probably destabilizes the entire helix 44 as the lower parts of the helix make less extensive contacts to the 30S subunit. A rearrangement of helix 44 has been suggested based on a structural analysis of in vivo purified pre-30S particles (46) and a similar rearrangement has been shown in a recent structure of a eukaryotic pre-40S subunit (13). In both cases the upper part of helix 44 is bent similarly away from the small subunit platform.

Interestingly, some structures of the 3D classification procedure that showed no well defined density for the 30S head resemble ribosome assembly intermediates of in vitro ribosome assembly (47). These structures correspond to assembly intermediates lacking mainly proteins of the 30S head, giving structural support for the proposed 5’ to 3’ directionality of the assembly process (48,49). The magnesium depletion appears to destabilize the 30S head relative to the 30S body and platform, leading to an apparently reverse process of weakening 3’ domain interactions relative to 5’ interactions.

Conserved C-terminal residues of KsgA provide an important binding interface

KsgA is bound to the platform of the 30Si subunit formed by the central domain of the 16S rRNA including three RNA helices h45, h24 and h27 (Fig. 2), revealing the basis for the complex substrate specificity of KsgA. The tertiary interaction of the RNA helices 24, 27 and 45 in the 30Si subunit is probably important for the binding of KsgA since KsgA does not methylate RNA fragments or 16S rRNA in isolation (10). This suggests that for KsgA activity the 30S platform has to adopt a near mature conformation but a translationally inactive conformation of helix 44 is required.

The cluster of charged residues in the C-terminal domain of KsgA that binds to the junction formed by helix 24 and helix 27 is highly conserved, indicating that the recognition of this site by KsgA is an important additional structural determinant of its function (Fig. 2b) (20). A KsgA variant in which one of these positively charged amino acids, R248, is mutated to alanine can not suppress the cold sensitive phenotype of an Era (E200K) strain (50). Nevertheless, the KsgA R248A variant retains its methyltransferase activity, indicating that binding of KsgA is important for Era function and 30S maturation irrespective of the factor’s catalytic activity.

The eukaryotic KsgA homolog Dim1 has been localized on the platform of the 40S small ribosomal subunit in a recent cryo-EM reconstruction (13). The conformation of Dim1 on the small subunits appears to be similar, consistent with the results of methylation assays showing that Dim1 can complement for KsgA in the methylation of A1518 and A1519 (9).

Comparison to recent models of KsgA binding to the 30S

In agreement with our structure RNA helices h45, h24 and h27 show the highest level of protection in hydroxyl radical footprinting (19). However, the structural model derived from these chemical modification data showed KsgA interacting with its N- and C-terminal domains with helix 44, which is not consistent with the structure presented here.

Based on the co-crystal structure of an RNA duplex-KsgA complex, a model of KsgA interacting with helix 45 was suggested that is inconsistent with the EM structure (24). The interactions involve the same positively charged surface of the protein, but the RNA binding mode differs significantly (Supplemental figure 7). In the crystal structure of the ternary complex of
KsgA and Era bound to a helix 45 containing RNA fragment the modified adenosines are bound by the C-terminal domain and not the catalytic N-terminal domain (25). These structures do not correspond to catalytically active complexes and might be an artifact of unspecific RNA binding by KsgA.

The position of KsgA in the network of ribosome biogenesis factors

Absence of several 30S biogenesis factors leads to 17S rRNA processing defects (1). These factors include the GTPases Era and RsgA, as well as the small assembly proteins RbfA and RimM, which are bound at the junction of the 3 domains of the 30S subunit. The binding of these factors covers important functional sites where mRNA and tRNAs bind to the 30S subunit, rendering the subunit incompetent for translation initiation (6). In addition, these factors coordinate the end processing of the small subunit rRNA. It remains to be established whether these factors act concomitantly or sequentially during 30S subunit maturation.

Superimposing the coordinates of Era-30S complex onto the KsgA-30Si complex shows that KsgA would not interact directly with Era bound between the head and the platform of the 30S subunit, where it interacts with the 3’ end of the 16S rRNA (51) (Fig. 4). As both biogenesis factors interact with the 3’ end of the rRNA, the suppression of the phenotype of an Era E200K point mutation by KsgA overexpression (52) might be exerted by changes in the conformation of the rRNA. A cold sensitive phenotype and defect in 17S rRNA processing is also observed for the overexpression of KsgA, indicating that KsgA stabilizes an intermediate of subunit assembly (14). Therefore, the stabilization of the translationally inactive subunit conformation by KsgA might allow for a conformational change to happen at the 3’ terminus required for processing by Era.

Whereas simultaneous binding of KsgA and Era is plausible, the conformation of the 30S subunit in complex with RbfA as observed in a recent cryo-EM reconstruction, is inconsistent with simultaneous binding of KsgA and RbfA (53). The RbfA complex structure showed helix 45 rotated out of the 30S platform and helix 44 is displaced. As RNA fragments are not methylated by KsgA, it is unlikely that helix 45 in the RbfA-30S complex can be methylated before it is brought into position within the mature 30S platform structure. This argues that RbfA has to act before KsgA in the cascade of biogenesis factors interacting with the pre-30S subunit. Furthermore, a recent structure of RsgA, which is the release factor of RbfA, on the 30S subunit shows RsgA in a conformation that would sterically exclude KsgA (54,55). This suggests that RsgA removes RbfA before KsgA can bind to the immature 30S subunit.

A model for KsgA mediated control of ribosome biogenesis

The release of KsgA is required for helix 44 to assume its native position in the 30S subunit and for 17S rRNA processing. It has been suggested that the methylation immediately precedes the dissociation of KsgA. Variants of KsgA inactive in methylation function remain bound to 17S rRNA precursors (14) and methylation has been shown to decrease the affinity of KsgA for the small subunit (11). These findings suggest that KsgA may serve as a checkpoint during 30S subunit maturation by remaining bound until the methylation reaction takes place (14).

The C-terminal domain in itself probably provides sufficient affinity for KsgA to bind to the 30S subunit platform and position the catalytic domain optimally for the methylation of helix 45, which is being remodeled through action of Era, RbfA and RsgA and other biogenesis factors. This observation is further supported by the biochemical experiments where it was shown that mutations in the catalytic interface of the enzyme do not significantly affect its affinity for the 30Si subunit and still allow the consecutive methylation of both adenosine residues without dissociation of KsgA (56).

The data presented here presents a plausible model for the mechanism of KsgA action during the biogenesis of the small ribosomal subunit: Initially KsgA is bound to the platform with helix 44 in a displaced conformation (Fig. 5a). When a certain, near mature, conformation of the platform and helix 45 is reached, KsgA methylates helix 45 (Fig. 5b). Methylation reduces the affinity of KsgA for the substrate and leads to dissociation. This allows a
conformational rearrangement of helix 44 and final end-processing of the 16S rRNA (Fig 5c). Thereby KsgA could ensure that the final rRNA processing steps are delayed until a properly assembled 30S intermediate has formed.

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FOOTNOTES
The atomic coordinates (code 4ADV) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).
The cryo-electron microscopic maps have been deposited in the 3D-EM database under accession number EMD-2017 and EMD-2019, EMD-2020.
FIGURE LEGENDS

FIGURE 1. KsgA binds to the platform of the 30S ribosomal subunit. A) Cryo-EM reconstruction of the translationally inactive 30Si ribosomal subunit at 15.5 Å resolution. The crystal structure of the 30S subunit from *E. coli* was fitted into the density with head and body as rigid bodies (blue ribbon). Helix 44 of the 16S rRNA is colored magenta. B) Reconstruction of the KsgA-30Si complex at 13.5 Å resolution. The density for the KsgA is colored red. C) Structure of the reactivated 30S ribosomal subunit. The structure shows density for the full helix 44 of the 16S rRNA (magenta).

FIGURE 2. Contact sites of KsgA on the 30Si subunit platform. A) The crystal structure of *E. coli* KsgA (pdb 1QYR, N-terminal domain, red, C-terminal domain, dark red) is fitted into the cryo-EM density (grey mesh). The methyl donor S-adenosyl-L-methionine is shown in yellow (modeled from (26)). KsgA is bound to helix 24 (orange), 27 (blue) and helix 45 (cyan) of the 16S rRNA (pdb 2AVY). The substrate adenosine bases methylated by KsgA are shown in magenta (arrow). B) Rotated view of A. The crystal structure KsgA is shown in surface representation colored by surface charge (calculated with DelPhi). A conserved positively charged surface patch interacts with the 16S rRNA (helix 45, cyan; helix 24, orange). C) Rotated view of A (without the cryo-EM density). Positively charged residues important for 30S binding are shown as spheres (R221RK223, blue; R248, purple). D) Superposition of the core catalytic domain of DNA methyltransferase M.TaqI (pdb 1G38, blue) in complex with substrate DNA (grey) onto KsgA (red, same view as in C). In the M.TaqI structure the substrate adenosine base (green) is flipped out into the catalytic center.

FIGURE 3. KsgA sterically excludes helix 44 from its position in the mature 30S subunit. KsgA (red) bound to the 30S subunit (blue, helix 28 yellow) at helix 45 (cyan) overlaps with helix 44 (orange) as observed in the crystal structure of the 30S subunit used for fitting the cryo-EM density (pdb 2AVY). Inset: overview of the KsgA-30Si structure (30S, yellow; KsgA, red) with the head removed (grey surface capping) in the same orientation.

FIGURE 4. Binding sites of Era and KsgA on the 3’ end of the rRNA. KsgA (red) binds at helix 45 (cyan, A1518 and 1519 magenta) of the 30S subunit (blue). Era (pdb 1X18, green) is bound between the platform and the head contacting helix 28 (yellow) (Sharma et al., 2005). Helix 44 is shown in orange. (The 3’ end of the 16S rRNA is labeled with a star.)

FIGURE 5. Schematic illustration of the conformational rearrangements of the 30S subunit rRNA controlled by KsgA. The rRNA of the 30S subunit is shown colored by domain (5’ domain, 30 body, yellow; central domain, 30S platform, green; 3’ domain, 30S head, blue). The 3’ minor domain rRNA helix 44 and helix 45 undergoing conformational changes are shown schematically in blue. KsgA is shown in red. The methylated bases in helix 45 are indicated in magenta. The binding sites of RbfA, RsgA and Era are indicated by blue and orange circles respectively. The arrow indicates the cleavage of the 17S rRNA following dissociation of KsgA. (for explanation see text)
Figure 1
Figure 4
Structural insights into methyltransferase KsgA function in 30S ribosomal subunit biogenesis
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