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Assembly of the 5′ and 3′ Minor Domains of 16S Ribosomal RNA as Monitored by Tethered Probing from Ribosomal Protein S20

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The ribosomal protein (r-protein) S20 is a primary binding protein. As such, it interacts directly and independently with the 5′ domain as well as the 3′ minor domain of 16S ribosomal RNA (rRNA) in minimal particles and the fully assembled 30S subunit. The interactions observed between r-protein S20 and the 5′ domain of 16S rRNA are quite extensive, while those between r-protein S20 and the 3′ minor domain are significantly more limited. In this study, directed hydroxyl radical probing mediated by Fe(II)-derivatized S20 proteins was used to monitor the folding of 16S rRNA during r-protein association and 30S subunit assembly. An analysis of the cleavage patterns in the minimal complexes [16S rRNA and Fe(II)-S20] and the fully assembled 30S subunit containing the same Fe(II)-derivatized proteins shows intriguing similarities and differences. These results suggest that the two domains, 5′ and 3′ minor, are organized relative to S20 at different stages of assembly. The 5′ domain acquires, in a less complex ribonucleoprotein particle than the 3′ minor domain, the same architecture as observed in mature subunits. These results are similar to what would be predicted of subunit assembly by the 5′-to-3′ direction assembly model.

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Introduction

S20 is one of a few small subunit ribosomal proteins (r-proteins) that interacts with two domains of 16S ribosomal RNA (rRNA), the 5′ and 3′ minor domains[1–4] (Fig. 1a and b). R-protein S20 is a primary binding protein and thus can interact directly and independently with 16S rRNA.[1,6,7] Footprinting experiments[1] and the crystal structure of *Escherichia coli* 30S subunits[4] revealed that S20 binds several helices from the 5′ domain and the major helical element (helix 44: the penultimate stem) of the 3′ minor domain of 16S rRNA. The structure of r-protein S20 has not been determined in its free state, but in the assembled 30S subunit, S20 is a three-helix bundle located at the bottom of the body of the small subunit, where it is "sandwiched" between the 5′ domain and the penultimate stem,[4] helix 44 (Fig. 1a and b). In addition, it has been shown that the penultimate stem interacts extensively with the large subunit[4]; thus, appropriate positioning of helix 44 could be critical for accurate and rapid translation. Based on the model of 5′-to-3′ co-transcriptional assembly[8] and on the suggestion that the interaction between r-proteins and 16S rRNA occurs in discrete stages of 30S subunit assembly,[8–10] it is possible that S20 interacts differently with these two domains during the course of 30S subunit assembly.

Recently, directed hydroxyl radical probing was used to explore changes in 16S rRNA elements surrounding the r-protein S15 during 30S subunit assembly.[11,12] Changes in 16S rRNA cleavage patterns generated by Fe(II)-S15 in ribonucleoprotein particles (RNPs) of different complexities were related to conformational changes in 16S rRNA as a

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Abbreviations used: r-protein, ribosomal protein; rRNA, ribosomal RNA; RNP, ribonucleoprotein particle; BABE, 1-(*p*-bromoacetamidobenzyl) ethylenediaminetetraacetate.

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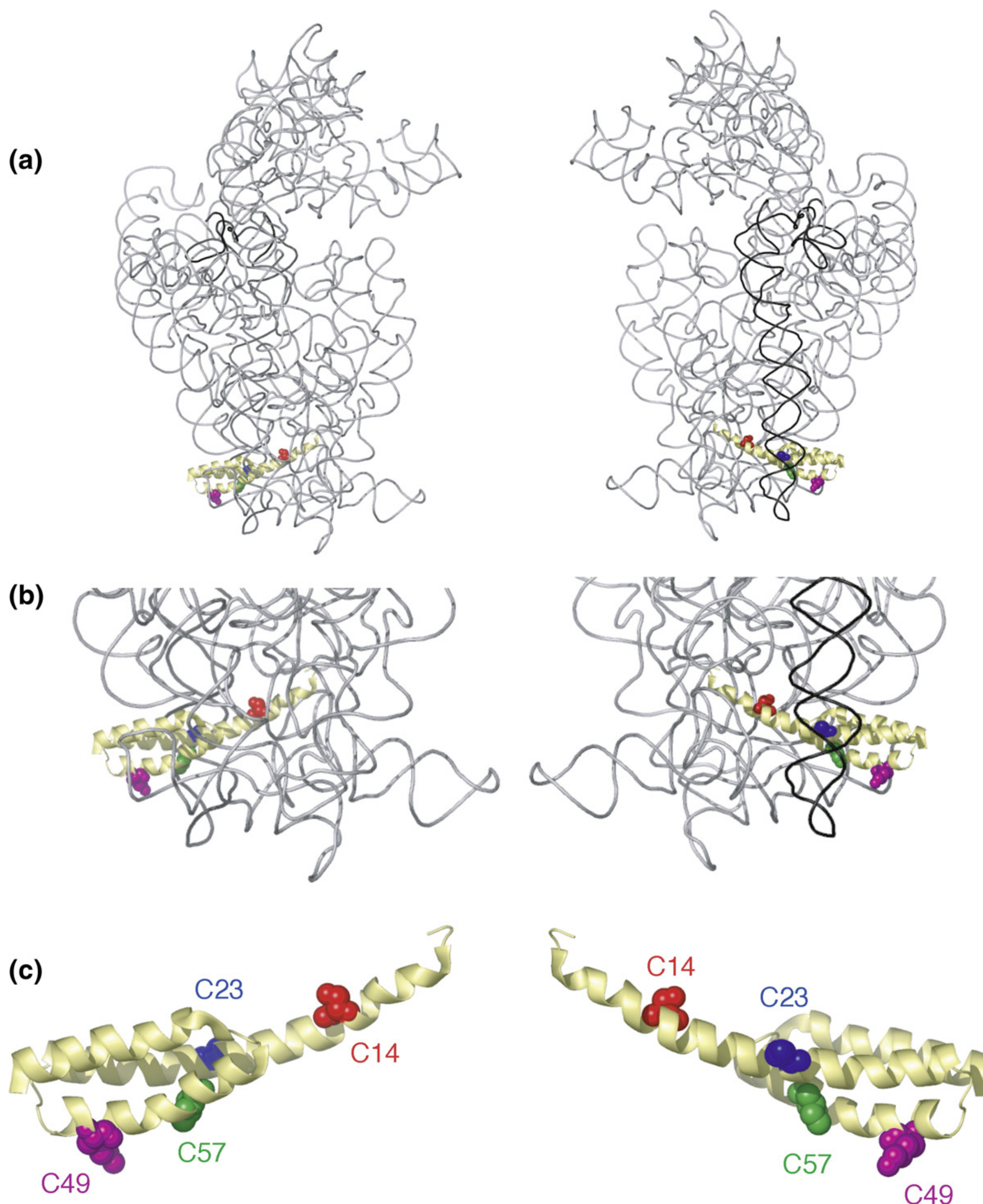

Fig. 1. Three-dimensional structure of selected components of the *E. coli* 30S ribosomal subunit. (a) The entire 16S rRNA is shown in light gray, with the 3′ minor domain shown in black; S20 is shown in yellow, with the cysteine-substituted sites colored; position 14 is shown in orange, position 23 is shown in blue, position 49 is shown in magenta, and position 57 is shown in green. Other small subunit r-proteins were omitted for clarity. Two views are presented for clarity. (b) Close-up view of the position of S20 taken from (a). (c) Structure of S20 taken from (a) and (b). All the figures containing three-dimensional structures were prepared using PyMOL[5] and the Protein Data Bank file 2AW7.[4]

result of r-protein binding.[11,12] Thus, roles of different r-proteins in the assembly process were elucidated. This is a major advantage of the directed hydroxyl radical probing strategy compared with traditional footprinting studies; much can be learned about the roles of many different r-proteins in one set of experiments, and not just regarding the protein that is being footprinted or used as a probe. Another advantage of directed hydroxyl radical probing is the fact that the region of RNA that interacts with the area of the protein where the derivatized residue is present can be determined, thus affording information about the orientation and directionality of the interactions. This approach made possible a better

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