

SRP19 Is a Dispensable Component of the Signal Recognition Particle in *Archaea*[▽]

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In vitro, archaeal SRP54 binds SRP RNA in the absence of SRP19, suggesting the latter to be expendable in *Archaea*. Accordingly, the *Haloflex volcanii* SRP19 gene was deleted. Although normally transcribed at a level comparable to that of the essential SRP54 gene, SRP19 deletion had no effect on cell growth, membrane protein insertion, protein secretion, or ribosome levels. The absence of SRP19 did, however, increase membrane bacterioruberin levels.

Despite their structural similarities (9, 28), eukaryal and archaeal signal recognition particles (SRP) seemingly follow very different pathways of assembly. In *Eukarya*, SRP19 binding both to the capping tetraloop of SRP RNA helix 6 and to a lower-affinity binding site near the tip of helix 8 brings these helices into close proximity, leading to a conformational change in helix 8 and exposure of the normally cryptic SRP54-binding site (16, 21). By contrast, structural and biochemical investigations of archaeal SRP raise questions regarding the role of SRP19 in the assembly of the particle in *Archaea*. While crystallographic studies have shown the SRP54 binding site to be fully presented in the SRP19-SRP RNA complex (11), structural analysis of free archaeal SRP RNA has shown that helices 6 and 8 normally lie parallel to each other and interact (12, 27) and that there is partial exposure of the SRP RNA helix 8 SRP54-binding site (12). Accordingly, in vitro reconstitutions with purified components from *Archaeoglobus fulgidus* (4, 7), *Methanococcus jannaschii* (12), *Pyrococcus furiosus* (18), or *Haloflex volcanii* (26) have shown binding of archaeal SRP54 to SRP RNA in the absence of SRP19. Although these studies showed that SRP19 was required for high-affinity binding, SRP54 clearly presented inherent affinity for SRP RNA (7, 12). Thus, although polyhistidine-tagged SRP19, SRP54, and SRP RNA could be cocaptured from transformed *H. volcanii* cells (22), the need for SRP19 in vivo merits further investigation.

To discern whether SRP19 is essential in *H. volcanii*, the encoding gene was exchanged for the tryptophan synthase-encoding *trpA* gene by a gene knockout technique developed for *H. volcanii* (2, 5). Briefly, a *pyrE*-containing vector plasmid encoding 400 nucleotides flanking each end of the *SRP19* gene, separated by *trpA*, was integrated into the genome of *H. volcanii* strain WR536, a uracil and tryptophan auxotroph. To replace *SRP19*, the transformed cells were grown in the presence of uracil and 5-fluoroorotic acid, without tryptophan. PCR using primers directed against the *SRP19* flanking regions, designed to follow the exchange of *trpA* for *SRP19*,

revealed plasmid integration, with bands corresponding to genome- and plasmid-derived sequences being detected in the transformed cells (Fig. 1A, lane 2). Upon expulsion of *SRP19* and the integrated plasmid, only *trpA* with its *SRP19* flanking regions were detected (lane 3). Using genomic DNA from untreated and *SRP19*-lacking cells as a template and primers directed against the *SRP19* coding region, PCR revealed a reaction product in the background but not in the deletion strain (Fig. 1B). To ensure the ability of the deletion technique to report essential *H. volcanii* genes, *SRP54* was replaced by *trpA*. In agreement with earlier studies (22), no growth was detected in the absence of *SRP54* (Fig. 1C, lane 3). The absence of *H. volcanii* *SRP19* was next confirmed at the RNA level by reverse transcription (RT)-PCR (1), employing primers against *SRP19*. A PCR product of the expected size was obtained with cDNA from the background but not from the *SRP19*-lacking cells as a template (Fig. 1D; compare lanes 4, upper panels). By contrast, when primers directed against *SRP54* were employed, the expected PCR product was obtained with cDNA from either background or *SRP19*-deleted cells as a template (lanes 4, lower right and left panels, respectively).

Given that *SRP19* can be deleted from *H. volcanii*, the possibility that cells express less SRP19 than SRP54 was investigated by real-time RT-PCR. Using SYBR green PCR 2× Master Mix (Applied Biosystems) and primers designed with Primer Express 2.0 software (Perkin-Elmer Life Sciences), real-time RT-PCR was performed in an ABI Prism 7300 light cycler (Applied Biosystems). Relative quantification of mRNA levels was calculated by the standard formula $2^{-\Delta\Delta C_T}$, where C_T is the cycle threshold. Based on five experiments, each involving triplicate samples, *H. volcanii* grown to exponential phase was shown to contain twofold (2.06 ± 0.3)-more SRP19- than SRP54-encoding mRNA. The effect of *SRP19* deletion on SRP54 mRNA and SRP RNA levels was next considered in experiments in which 16S rRNA or S-layer glycoprotein mRNA served as a housekeeping marker. When SRP RNA expression was measured relative to either marker (four experiments involving triplicate samples with each), it was shown that the deletion strain expresses only 1.7- to 2.1-fold-more SRP RNA than do background cells. Similarly, five experiments each involving triplicate samples for each house-

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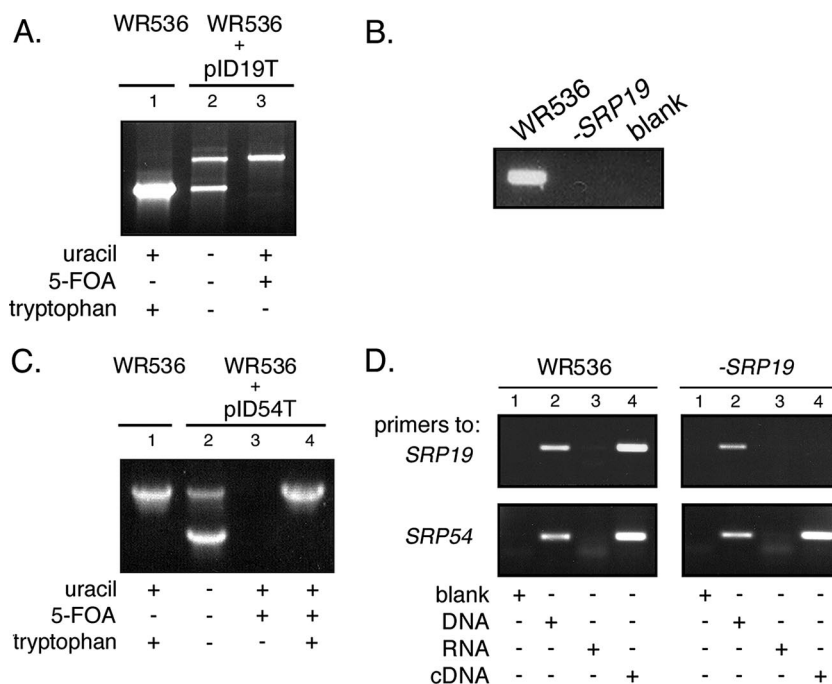


FIG. 1. *SRP19* is not an essential gene in *H. volcanii*. (A) PCR amplification of *H. volcanii* cells transformed with plasmid pID19T, designed to delete *SRP19*. Shown are PCR products from genomic DNA (lane 1), genomic DNA including the integrated pID19T plasmid (lane 2), and the same DNA taken from cells grown in tryptophan-free, 5-fluoroorotic acid (5-FOA)- and uracil-containing medium, i.e., following expulsion of the plasmid and native *SRP19*-encoding gene (lane 3), as a template together with primers against sequences within the regions flanking *SRP19*. (B) PCR amplification was performed with primers directed against the sequences within the *SRP19* coding region and DNA from the background strain (WR536) or the *SRP19* deletion strain as a template. In the lane marked "blank," no template was added to the reaction. (C) PCR amplification of *H. volcanii* cells transformed with plasmid pID54T, designed to delete *SRP54*. The lanes are as described for panel B, except that lane 4 shows the PCR product of the cells considered in lane 3 but returned to tryptophan-containing medium such that integrated plasmid is expelled in its entirety. (D) RNA was extracted from the background strain (WR536) or from *H. volcanii* cells from which the *SRP19*-encoding gene had been deleted and used to direct the synthesis of cDNA. PCR was then performed in the absence of nucleic acids (lane 1) or in the presence of genomic DNA taken from the background strain (lane 2) or of total RNA (lane 3) or cDNA (lane 4) from the background or deletion strain, with primers against either *SRP19* (upper panels) or *SRP54* (lower panels).

keeping marker showed *SRP54* mRNA expression to be just 1.8-fold higher in the deletion strain than in the background strain.

To assess whether the absence of *SRP19* had any effect on protein biogenesis, the nascent cytosolic, membrane, and secreted protein populations of metabolically ^{35}S -radiolabeled background and *SRP19*-lacking cells were examined. Coomassie staining confirmed that comparable amounts of the cytoplasmic and membrane fractions were obtained from both strains (Fig. 2, upper panels). No secreted proteins were detected upon staining of the growth media. When these newly synthesized protein populations were examined by fluorography, no obvious differences were apparent in the profiles of the nascent cytosolic, membrane, or secreted protein pools from either strain (Fig. 2, lower panels). Similarly, when the ribosome content of the background and *SRP19*-lacking cells was compared via densitometric quantitation of agarose gel-separated rRNA, the deletion strain was shown to contain 23S and 16S rRNA levels similar to those of the background ($106\% \pm 13\%$ [$n = 3$] and $109\% \pm 15\%$ [$n = 3$], respectively). Moreover, the absence of *SRP19* had no effect on growth rates at 40°C (not shown).

It was observed that exponentially growing *SRP19*-lacking cells were more brightly red colored than was the background

strain. Haloarchaea such as *H. volcanii* are naturally red/pink, due to a neutral lipid pool containing large amounts of isoprene-based carotenoid pigments, with bacterioruberin predominating (14, 19). The absorbance profile of nonpolar lipids showed that, relative to the background strain, *SRP19*-lacking cells presented major absorption peaks at 490 nm and 530 nm (Fig. 3A), likely corresponding to bacterioruberin (17). Elevated bacterioruberin levels are noted in haloarchaea facing various stress conditions (3, 8, 10, 15, 20, 24). The observation that the acetone-extracted lipid profile of *H. volcanii* lacking Stt3 (1), the sole known component of the archaeal oligosaccharide transferase, was almost indistinguishable from that of the background strain confirmed that the enhanced absorbance of the neutral lipid pool extracted from the *SRP19* deletion strain was not an artifact of the gene deletion protocol (Fig. 3A). In addition, the nonpolar lipid contents of *H. volcanii* WR536 and *SRP19*-deleted cells were identical (Fig. 3B).

Prior to these studies, the essentiality of *SRP19* had been considered only for *Eukarya*, where species-specific differences were observed. In *Saccharomyces cerevisiae*, the absence of the *SRP19* homologue *Sec65* did not prevent cell survival but led to slower growth and defective secretory preprotein processing (6, 13, 25). In contrast, deletion of *sec65* was lethal to *Yarrowia lipolytica* (23). In *H. volcanii*, the absence of *SRP19* had no

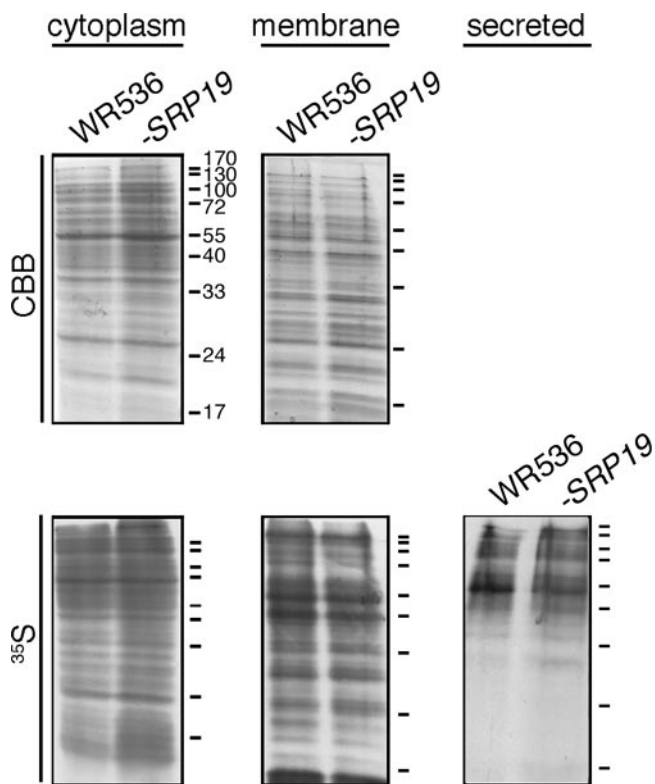


FIG. 2. The absence of *H. volcanii* SRP19 does not affect global protein biogenesis. *H. volcanii* cells deleted of *SRP19* and the WR536 background strain were grown with metabolically ^{35}S -radiolabeled cysteine/methionine at 40°C. The cytoplasm, membrane, and secreted protein pools were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and examined by Coomassie staining (CBB) or by fluorography (^{35}S). The positions of molecular weight markers (identified in the upper left-hand panel) are shown to the right of each panel.

readily discernible impact on cell growth, on the biogenesis of metabolically radiolabeled nascent cytoplasmic, membrane, or secreted protein pools, or on ribosome levels under standard growth conditions. Thus, it appears that an SRP RNA-SRP54 complex, first observed in earlier in vitro SRP reconstitution studies (4, 7, 12, 18, 26), is sufficient to fulfill the principal roles assumed by the ternary SRP complex in archaeal cells. Furthermore, the deletion of *H. volcanii* *SRP19* had little effect on the transcription of SRP RNA or SRP54 mRNA, as judged by real-time RT-PCR. In *S. cerevisiae*, *sec65* deletion also had little effect on SRP RNA levels but led to a significant drop in the amount of SRP54 in the cell (6), in accordance with the SRP19 dependence of SRP54-SRP RNA binding in *Eukarya* (16, 21). Thus, it appears that although *H. volcanii* SRP19 can participate in a complex comprising SRP RNA, SRP54, and SRP19 both in vitro (26) and in vivo (22), SRP19 does not play a pronounced role in *H. volcanii* cells. Still, the possibility that archaea contain a presently unknown protein able to functionally replace SRP19 cannot be discounted, even though no other *H. volcanii* genes encoding SRP19-like proteins were detected in bioinformatics-based searches. Similarly, it remains possible that an alternate targeting pathway is enlisted by *H. volcanii* to overcome any putative damage to the SRP pathway

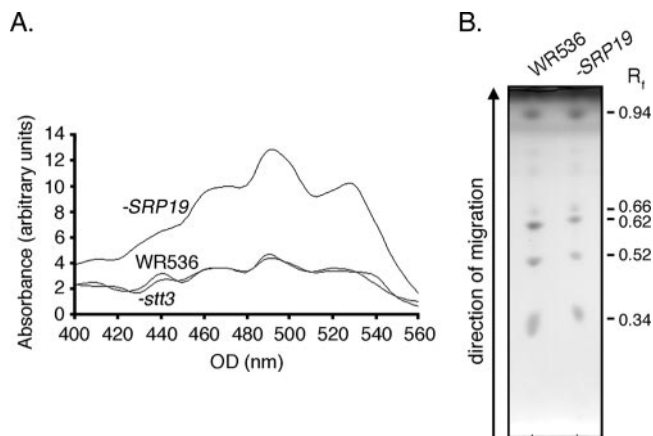


FIG. 3. Bacterioruberin levels are elevated in the absence of *H. volcanii* SRP19. (A) The acetone-soluble *H. volcanii* lipid fraction from WR536 and SRP19- and Stt3-lacking cells was spectrophotometrically examined between 400 and 590 nm. (B) The polar lipid content of *H. volcanii* WR536 and SRP19-deleted cells was examined by thin-layer chromatography in a chloroform-methanol-water (65:25:4 volume ratio) solvent system. The R_f values for the major species detected are shown.

arising from deletion of the SRP19-encoding gene. It is also plausible that the effect of SRP19 deletion on protein biogenesis or cell physiology would be magnified in *H. volcanii* cells grown under conditions other than those tested here. Despite the apparently dispensable nature of *H. volcanii* SRP19, it was observed that SRP19-encoding mRNA was nonetheless present in exponentially growing cells of the background strain at a level comparable to that of SRP54 mRNA, suggesting a function for SRP19. This likelihood is strengthened by the fact that membranes of SRP19-lacking cells are enriched in bacterioruberin, possibly reflecting an effect on a membrane protein involved in the metabolism of this compound.

Despite its omnipresence, the composition of SRP differs among the three domains of life. Whereas SRP RNA and SRP54 are found in all life forms, SRP19 is restricted to *Eukarya* and *Archaea* (9, 28). However, as demonstrated in the present report, SRP19 is not essential in *Archaea*.

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