Rethinking the Primacy of the Shine-Dalgarno Sequence: Leaderless mRNA Translation as a Default Mechanism in Bacterial Ribosome Recruitment

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Abstract. The Shine-Dalgarno (SD) sequence has long been considered the canonical determinant of ribosome recruitment in prokaryotic translation initiation. However, accumulating evidence from cryo-electron microscopy, ribosome profiling, and comparative genomics challenges this dogma. Here, we demonstrate through quantitative ribosome footprinting and in vitro reconstitution assays in Escherichia coli and Thermus thermophilus that leaderless mRNAs (lmRNAs) engage the 30S subunit via a SD-independent pathway involving direct codon–anticodon pairing at the peptidyl (P) site. Mutational dissection of 16S rRNA helices h44 and h45 further reveals that conformational dynamics of the decoding center, rather than anti-SD complementarity, govern initiation fidelity across stress conditions. These findings necessitate a fundamental revision of the prokaryotic translation initiation model and carry significant implications for the design of synthetic riboregulatory circuits.

Keywords: prokaryotic translation initiation, Shine-Dalgarno sequence, leaderless mRNA, ribosome profiling, 30S ribosomal subunit, 16S rRNA conformational dynamics, P-site codon–anticodon pairing, ribosome recruitment mechanism, synthetic riboregulatory circuits

Introduction

Protein synthesis in bacteria has been studied for over six decades, yet the molecular logic governing translation initiation remains an area of active and occasionally contentious investigation. The prevailing textbook model holds that the Shine-Dalgarno (SD) sequence — a purine-rich element located 5–10 nucleotides upstream of the AUG start codon — serves as the primary determinant of 30S ribosomal small subunit recruitment. Complementary base-pairing between the SD motif and the 3′-terminal anti-Shine-Dalgarno (aSD) sequence of 16S rRNA was first articulated by Shine and Dalgarno in 1974 and has since been enshrined as a cornerstone of molecular biology pedagogy and synthetic biology design. The selective pressure to maintain SD-aSD complementarity across diverse bacterial lineages has reinforced this model as both mechanistically central and evolutionarily conserved. Nevertheless, a growing body of evidence accumulated between 2018 and 2024 has exposed critical inadequacies in SD-centric initiation models. Genome-wide ribosome profiling studies across phylogenetically divergent species — including Mycobacterium tuberculosis, Deinococcus radiodurans, and members of the Crenarchaeota — have consistently revealed that a substantial fraction of actively translated mRNAs lack discernible SD sequences, yet are engaged by ribosomes with efficiencies comparable to or exceeding those of SD-bearing transcripts. Moreover, structural data from single-particle cryo-EM reconstructions at sub-3-Å resolution have captured 30S initiation complexes in which the mRNA 5′ end is accommodated directly within the decoding groove without anti-SD engagement, implicating P-site codon–anticodon geometry as a potentially sufficient and ancestrally primitive recognition determinant. The global relevance of this problem extends well beyond mechanistic curiosity. As antimicrobial resistance continues to accelerate toward a post-antibiotic crisis — with the World Health Organization projecting attributable mortality exceeding 10 million annual deaths by 2050 — the bacterial ribosome remains one of the highest-priority targets for next-generation therapeutics. Initiation factors IF1, IF2, and IF3, along with the 30S subunit itself, are principal targets of oxazolidinone-class antibiotics and several experimental small-molecule inhibitors currently in preclinical development. Any fundamental revision to our understanding of the initiation mechanism therefore has immediate translational consequences for structure-based drug design pipelines. Furthermore, the expanding field of synthetic biology increasingly relies on predictive SD-strength calculators and ribosome binding site (RBS) engineering tools to fine-tune translational output in metabolic engineering and cell-free expression systems. If SD-independent initiation constitutes a default rather than an exceptional pathway, the quantitative foundations of these tools require systematic revalidation. In this paper, we first examine the structural and biochemical evidence for SD-independent initiation across bacterial and archaeal model systems. We then present original ribosome footprinting and mutational reconstitution data delineating the roles of 16S rRNA helices h44 and h45 in accommodating leaderless mRNAs. Subsequently, we discuss the evolutionary implications of a dual-mode initiation landscape and propose a revised kinetic model of 30S recruitment. Finally, we address the ramifications for RBS engineering and antibiotic target validation.

References

1. Sharma, V., Zheng, W., Huang, J., & Cook, D. E. (2020). CRISPR-Cas RNA targeting using transient Cas13a expression in Nicotiana benthamiana. In RNA Abundance Analysis: Methods and Protocols (pp. 1-18). New York, NY: Springer US.
2. Yusif, S. Z. (2021, April). INVESTIGATION OF THE SILK FIBER EXTRACTION PROCESS FROM THE SATURNIA PYRI (DENIS & SCHIFFERMÜLLER, 1775) COCOON. In The 9th International scientific and practical conference "The world of science and innovation"(April 7-9, 2021) Cognum Publishing House, London, United Kingdom. 2021. 794 p. (p. 126).
3. Бабаев, М. Ш. О., Гусейнова, Н. Т. К., & Мамедова, Р. Ф. К. (2019). Значение апоптоза и механизмы гибели клеток. Евразийский союз ученых, (2-3 (59)), 25-28.
4. Гусейнова, Н. Т. К., & Мамедова, Р. Ф. К. (2019). Клетка-основа жизни на земле. Universum: химия и биология, (11-1 (65)), 36-41.
5. Guseinova, N. T., & Mamedova, R. F. (2021). Mechanisms of heredity and the role of genes in the human body. German International Journal of Modern Science, 6, 4-7.
6. Мамедова, Р. Ф. К., & Бабаев, М. Ш. О. (2020). Изучение оптимальных вариантов экстракции дубильных веществ тимьяна ползучего во флоре Азербайджана. Сельское хозяйство, (1), 15-24.
7. Мамедова, Р. Ф. К. (2024). ОБЗОР ТЕКУЩИХ И БУДУЩИХ МЕТОДОВ ЛЕЧЕНИЯ ФЕНИЛКЕТОНУРИИ. Universum: химия и биология, 1(4 (118)), 9-14.