Chapter 1

Background and Perspective

Protein synthesis is one of the fundamental events in every living cell. The central dogma of biology (Figure 1.1) shows that our genetic material, encoding ~30,000 genes (1, 2), can be transformed into protein through RNA. A specialized type of RNA called mRNA is deciphered with the aid of several translation factors on ribosomes, the molecular decoding machinery of the cell. Ribosomes are RNA and protein complexes that make up more than 25% of the total mass of *Escherichia coli* cells and carry a M_r of 3 million daltons. In an *E. coli* cell, 3 x 10^4 ribosomes select the correct amino acid from over 41 aminoacyl-tRNAs containing a unique anticodon sequence and assemble amino acids into protein at a rate of $10 - 20$ sec^{-1} (3). Yet the macromolecular concentration of an *E. coli* cell is 340 mg/mL, which in terms of

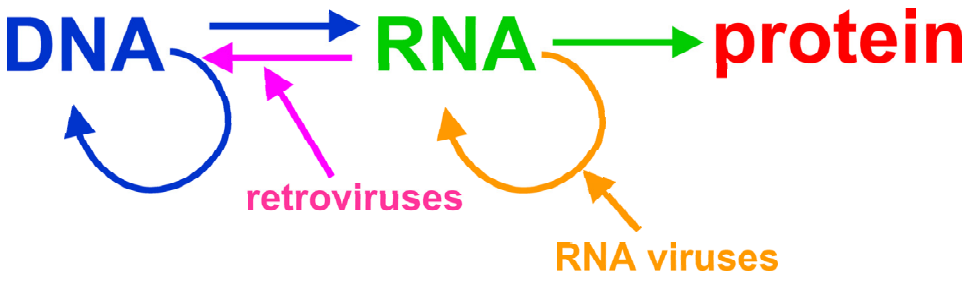

Figure 1.1: Central dogma of biology.

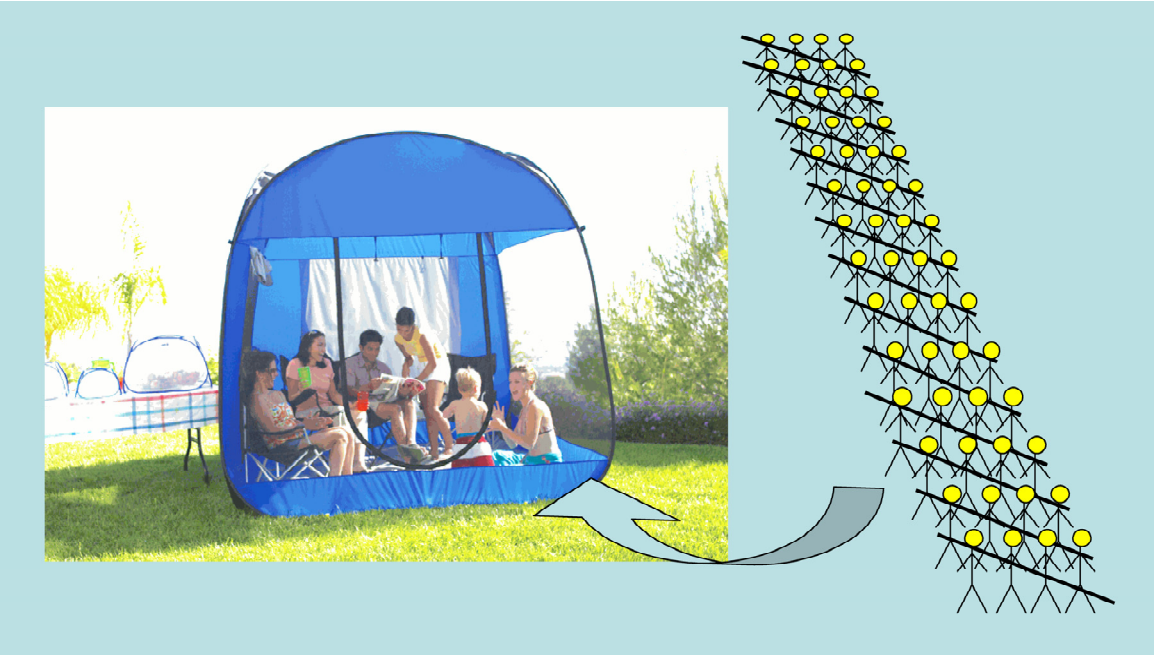

Figure 1.2: The macromolecular concentration of the *E.coli* cell is equivalent to packing 70 humans into this small tent.

volume is equivalent to packing more than 70 humans into a space of a small tent with volume 500 ft^3 (Figure 1.2) (4)! So how can protein synthesis proceed at a rate necessary to sustain life with an error of only 1 in 10 thousand amino acids? This question is one of many that remain unanswered in the area of protein synthesis.

My Ph.D. tenure has overlapped with several spectacular achievements in the area of protein translation. On August 11, 2000, Tom Steitz and co-workers published an atomic resolution crystal structure of the large ribosomal subunit (5). This work unambiguously assigned the location of peptide bond formation to be completely within a region of RNA. This confirmed a theory backed by biochemical evidence (6) that the ribosome is a ribozyme (4). Soon thereafter, a 5.5 $\AA$ map of the entire ribosome assembled with mRNA and aa-tRNAs was published by Harry Noller and colleagues. This account yielded a snapshot of protein synthesis that continues to stand at the structural forefront of biology. In addition to structural insights, kinetic

and equilibrium measurements of ribosomes at discrete steps during translation were published (7). These structural and biochemical data presented in just the last few years have advanced our understanding of translation beyond what could even be imagined just 10 years ago. What lies ahead in this field is the development of tools and reagents to elucidate the proteome at the molecular level within the cell.

In the pages that follow, I describe the design and characterization of compounds to directly study protein synthesis both *in vitro* and in live cells. Much of what we know about translation has been derived from the study of prokaryotic organisms, such as *E. coli*. The lack of comparable insight about translation in higher organisms was the impetus behind the work presented in this thesis.

Chapter 2 reviews the historical use of reagents to probe protein synthesis and examines their drawbacks for understanding protein translation in the context of cells. A re-evaluation of puromycin action using a reticulocyte lysate cell-free translation system is presented, which takes into account the potency, product distribution, and mechanism of various puromycin-oligonucleotide conjugates. Insights derived from these studies lay the framework for the design of reagents to study translation in live cells. Chapter 3 describes the implementation of these reagents to study protein synthesis *in vivo*. Fluorescent puromycin derivatives (of the form X-dC-puromycin) enable direct monitoring of protein expression and provide the potential for both spatial and temporal resolution in living cells and tissues. Chapter 4 revisits the issues surrounding the stereo- and regiochemistry of protein translation and presents a set of data that show the specificity of the ribosome (compared to typical enzymes) to be more relaxed than previously recognized. These new observations support the notion that the ribosome could synthesize peptides and proteins using D- and $\beta-$amino acids. Chapter 5 continues to examine the specificity of the ribosome and highlights the sensitivity of translation to substitution of the reactive amine. Chapter 6 is an *in vivo* analysis of ribosome specificity using the number of live cells as a measurement of ribosome specificity. Further, the effect of sidechain identity on ribosome stereos-

electivity is analyzed in live cells. These results extend the theory that the protein synthesis machinery should be capable of generating polymers that differ substantially from natural peptides and proteins. Finally, in Chapter 7, data is presented that provides prebiotic evidence for the evolution of amino acid homochirality. In summation, these data offer new insights into the chemical nature of eukaryotic protein synthesis and advance our understanding of the proteome.

References

[1] The International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. *Nature*, 409:860–921, 2001.

[2] J.C. Venter, M.D. Adams, E.W. Myers, P.W. Li, R.J. Mural, G.G. Sutton, H.O. Smith, M. Yandell, C.A. Evans, and R.A. et al. Holt. The sequence of the human genome. *Science*, 291:1304–1351, 2001.

[3] K.H. Nierhaus. *Gene Expression: Decoding and Accuracy. In: Nature Encyclopedia of Life Sciences.* Nature Publishing Group, London, 1999.

[4] N. Ban, P. Nissen, J. Hansen, P.B. Moore, and T.A. Steitz. The complete atomic structure of the large ribosomal subunit at 2.4 angstrom resolution. *Science*, 289:905–920, 2000.

[5] P. Nissen, J. Hansen, N. Ban, PB. Moore, and Steitz TA. The structural basis of ribosome activity in peptide bond synthesis. *Science*, 289:920–930, 2000.

[6] HF. Noller, V. Hoffarth, and Zimniak L. Unusual resistance of peptidyl transferase to protein extraction procedures. *Science*, 256:1416–1419, 1992.

[7] M.V. Rodnina and W. Wintermeyer. Fidelity of aminoacyl-tRNA selection on the ribosome: Kinetic and structural mechanisms. *Ann. Rev. Biochem.*, 70:415–435, 2001.