Preface

Protein targeting — how proteins find the correct, functional location in the cell — is one of the fundamental processes of life. Within the pages of this book the molecular mechanisms of several protein targeting events are described. Because the majority of proteins are manufactured in the cytosol, most of the targeting processes outlined look at the process by which such proteins find their way to the correct intracellular destination from the cytosol. All of the chapters concentrate on protein targeting within eukaryotes; in order to keep the book to a manageable size, protein targeting within bacteria and virus assembly have been neglected. Also I must apologize to those of you whose first love is plant biology. While much has been learnt recently about protein targeting to the chloroplast, the general principles that are emerging are very similar to what has been learnt by looking at mitochondrial import (Chapter 1). Similarly, studies on targeting to other organelles within plant cells are yielding results similar to those found in other eukaryotic cells.

Throughout the pages of the book similar principles will emerge for protein targeting to distinct organelles. Newly synthesized proteins contain protein targeting signals which act as molecular address tags to allow the protein to engage with the protein targeting machinery on any particular organelle. Sometimes targeting actually occurs co-translationally (Chapter 4). Targeting signals may consist of linear sequences of amino acids, as found in signal sequences for targeting to the endoplasmic reticulum (Chapter 4) or to the mitochondria (Chapter 1). Alternatively, they may consist of more complicated motifs such as the tyrosine-containing β-turns that cause plasma membrane proteins to be targeted to coated vesicles during endocytosis (Chapter 7). Once in the correct organelle, proteins may undergo further trafficking that will require the sequential reading of multiple targeting signals, which may consist of sugars, the oligomeric state of a protein, or other signals, some of which have yet to be identified (Chapters 5–7). The various molecular machineries that recognize targeting signals also have similar basic characteristics. For proteins which must be translocated across one or more membranes to achieve their final, functional location, cytosolic proteins recognize and bind to the newly synthesized targeting signals and often act as molecular chaperones to maintain the protein in a transport-competent state. At the target organelle a receptor molecule recognizes and binds to the protein–chaperone complex and translocation will be initiated. The mechanism of translocation itself is generally thought to involve some sort of 'pore' in the target membrane to allow transport. Energy, in the form of ATP, will generally be required to drive translocation and to ensure that the transport reaction remains vectorial. Inside the target organelle the translocating molecules will often be met by further chaperone molecules which may play a role

both in the folding and assembly of the newly translocated proteins and also in providing the energy-source for translocation itself. At the final destination targeting signals will often be destroyed by proteolysis or other post-translational modifications which may be involved both in making transport irreversible, and in producing a mature, functional protein.

The initial targeting of a newly synthesized protein will often not be the end of a protein's requirement for targeting. Nearly all of the proteins found in the Golgi apparatus, secretory vesicles and granules, plasma membrane, endosomes, and lysosomes start out by being co-translationally translocated into the endoplasmic reticulum (Chapter 4). They will then need further sorting signals to allow them to be transported by vesicle-mediated intracellular transport to the correct destination. Both signal-mediated transport and retention mechanisms are required to maintain the distinctive protein compositions of the various organelles, which continually exchange components by vesicular traffic (Chapters 5–7).

Import into the nucleus provides a variation on the general theme. The nucleus is surrounded by the nuclear envelope which is perforated by pores which could allow the free diffusion of molecules from the cytosol into the nucleus and vice versa. However, most nuclear proteins, are in fact, specifically targeted to the nucleus by a mechanism that involves the recognition of targeting signals in the protein by cytoplasmic receptor molecules, transport to the nucleus, and binding to and active transport across the nuclear pore (Chapter 2). Because the nucleus actually disassembles during cell division, releasing its contents to the cytosol, the signals on nuclear proteins are not destroyed and remain active for multiple rounds of nuclear import and release over the lifetime of the protein.

A general failure of protein targeting would be incompatible with life. However, defects in the targeting of specific proteins to specific destinations are known, and will generally lead to relatively severe disease. For example, lysosomal storage diseases are often associated with defects in the recognition of the mannose-6-phosphate signal found on lysosomal enzymes. Similarly the defect in some other disorders has been shown to involve protein import into peroxisomes (Chapter 3). The characterization of certain disorders has been one of the major keys in the elucidation of the mechanisms involved in protein targeting---for example, the characterization of the defect in the low-density lipoprotein receptor which can lead to familial hypercholesterolaemia was one of the key findings in the elucidation of the mechanism of protein targeting to clathrin-coated endocytic vesicles.

Yeast strains with general defects in protein targeting have also been isolated. As would be expected the defects are generally conditional lethal mutations, but characterization of such mutants has been invaluable in advancing our knowledge of the molecular mechanisms of protein targeting.

In addition to knowledge gained from studying cells with defects in protein targeting, much molecular insight into the steps involved in protein targeting has been gained from the reconstitution of protein targeting in cell-free systems. Many protein targeting steps have been reconstituted *in vivo* including protein translocation into the ER (Chapter 4), mitochondria (Chapter 1), chloroplasts and

peroxisomes (Chapter 3); protein import into isolated nuclei (Chapter 2); and also multiple stages of intracellular transport (Chapters 5–7).

I hope that through reading this book you will be infected with the enthusiasm of all of the authors for getting to grips with the molecular details of protein targeting. While much has been learnt, the area is still a very active one for numerous molecular and cell biologists world-wide.

S. M. H.

Cambridge
June 1995