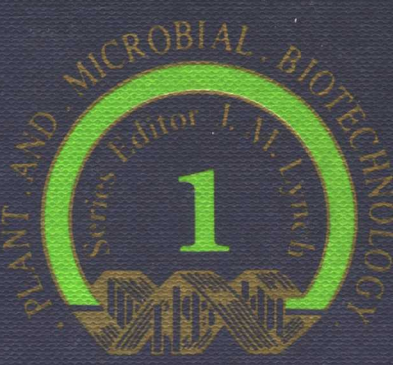

PLANT PROTEIN ENGINEERING

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Plant Protein Engineering

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Series Preface

Plant and Microbial Biotechnology

The primary concept of this Series of books is to produce volumes covering the integration of plant and microbial biology in modern biotechnological science. Illustrations abound, for example the development of plant molecular biology has been heavily dependent on the use of microbial vectors and the growth of plant cells in culture has largely drawn on microbial fermentation technology. In both of these cases the understanding of microbial processes is now benefiting from the enormous investments made in plant biotechnology. It is interesting to note that many educational institutions are also beginning to see things in this way and integrating departments previously separated by artificial boundaries.

Having set the scope of the Series, the next objective was to produce books on subjects which had not been covered in the existing literature and, it was hoped, set some new trends. At an early stage in the planning of the Series, I had the opportunity to discuss with Peter Shewry the potential of plant protein engineering and suggested to him that a book on the subject would be an ideal flagship of the new Series; when he suggested Steve Gutteridge, with his commercial as well as academic interest in the subject, as co-editor we became very excited.

There is no need for me to re-iterate the summary given in the Editors' Preface but for my part I am delighted that Peter and Steve were able to bring together a team of international contributors to make an outstanding foundation volume for the Series. Plant protein engineering is with us, its potential is enormous and I hope that this volume will help students and researchers to realize its potential.

Jim Lynch

Preface

Why engineer plant proteins?

Steven Gutteridge and Peter R. Shewry

A book that deals with protein engineering relevant to plant systems might be considered premature. Very few plant proteins have been characterized at the molecular level and even less have been subjected to detailed protein engineering studies. In addition, plant cell and molecular biologists are still struggling to develop methods for the routine transformation of agronomically important crop plants. Nevertheless the ability to alter protein structure and function by rational design using *in vitro* techniques is now well established. The natural extension of this is to modify the phenotype of plants through selective alterations to the genetic constitution, and this is the goal of research programmes in many publicly and commercially funded laboratories.

It is possible to identify many desirable traits that would increase the potential of crop plants. Plants have not evolved to satisfy the requirements of the human race and only modern farming practices, with their inherent disadvantages, allow these needs to be satisfied, at least in the developed world. Engineering plants to achieve this potential without high inputs would, therefore, be economically and environmentally advantageous.

A major aim of any plant breeding programme is to increase yield, and achieve yield stability across a range of environmental conditions. This includes resistance to stress such as drought, high or low temperatures, and salinity. Hardly less important is resistance to pests and pathogens, which range from insects and nematodes to fungi, bacteria and viruses. Finally, crop quality is an important consideration, especially when the harvested organ is destined for a complex industrial process such as breadmaking or malting and brewing. In some cases (e.g. resistance to fungal pathogens) it is possible to identify single genes which determine these traits, in other cases (e.g. yield) they are quantitative and presumably determined by the interactions of several genes distributed over the genome.

The classical approach to crop improvement is genetical, via plant breeding. Favourable combinations of genes are selected from the progeny of crosses, and specific genes may be introduced from exotic lines or wild relatives by wide crossing. This procedure is time consuming, and depends greatly on the skill and intuition of the plant breeder and his ability to recognize and select for the phenotype of interest. The latter may be limited by complex interactions between the genes of interest, the environment and the genetic background. The application of DNA restriction fragment length polymorphism (RFLP) analysis offers an opportunity to streamline plant breeding, especially for quantitative traits, but this is essentially an adjunct to the classical methods.

Genetic engineering should enable us to make specific alterations to the crop genotype and phenotype. Introducing mutant or novel genes, or down-regulating endogenous genes by anti-sense (for a review see Krol *et al.*, 1988) or 'gene shear' (Hazelhoff and Gerlach, 1988) approaches, will allow us to produce the same range of genotypes as by classical plant breeding, but more quickly and economically. The ability to introduce genes from other species including animals and micro-organisms will extend the range of variation far beyond that currently available to the plant breeder.

Our success in choosing the most desirable traits will also depend on our understanding of the equilibria that exist among intermediates of the

primary and secondary metabolic pathways and how they are controlled. An important application of down-regulation will be to identify the enzymes that determine which pathways operate under different conditions. Determining the functional characteristics of the enzymes catalysing the reactions in the pathways will be an essential prerequisite for manipulating the rates of flux and concentrations of intermediates. Manipulation of enzyme structure by *in vitro* mutagenesis has provided a powerful method of describing the role of protein structure in determining function, not only at the level of the primary sequence but also, through sub-domain alterations, the contributions of higher level structures. The challenge is to return these proteins with altered characteristics back to the plant, and thus change the phenotype in a predictable fashion.

The ultimate aim is to produce an ideal crop which would be sown at any convenient time, grow with little requirement for agrochemicals (pesticides, fertilisers and growth regulators) and show high resistance to environmental stresses. The harvested organ would be easy to handle, have good storage properties (in the field and after harvest) and have good nutritional and/or technological quality. It should also possess an attractive flavour and aroma, features absent from some commercial fruits. The yield and harvest index of the crop should be high, and the remaining plant parts utilizable on the farm (e.g. as feed) or in industrial processes (e.g. as fibre), or readily degraded *in situ* to enrich the soil for subsequent crops.

In this book we have brought together a number of chapters written by prominent researchers, who are involved in protein engineering relevant to understanding plant protein structure or improving plant properties. The targets include proteins of plant origin, and foreign proteins tailored to enhance particular characteristics. We have not included details of plant transformation methods as these are covered by a number of recent reviews (see, for example *Plant Molecular Biology Reporter*, Volume 13 (1989), issue no. 3).

A number of obstacles have delayed the development of plant protein engineering, including the absence of plant-based expression systems equivalent to those available in bacteria, yeast and cultured animal cells (mammalian and insect). It is, therefore, necessary to express plant proteins in heterologous systems, in which signals for post-translational processing and assembly may not be recognized. The sections on plant proteins are, therefore, preceded by introductory chapters, by Sarah Eccles and by Graham Belfield and Mick Tuite, which discuss the development and characteristics of expression systems based on *E. coli*, yeast and cultured mammalian cells. In addition Gunter Schneider and Ylva Lindqvist provide a broad introduction to the forces that stabilize protein structure, and how these can be explored by protein engineering.

Although plant genes may be difficult to express in heterologous organisms, equivalent genes may be present in photosynthetic bacteria, allowing studies of protein structure and function which can be extrapolated to the higher plant homologues. In some cases photosynthetic bacteria may themselves serve as substitute hosts for higher plant genes, where a selectable regime can be devised to assess *in vitro* generated mutations. This has been revealing with site specific mutations in proteins that compose the photosystem II complex of the cyanobacterium *Synechocystis* 6803, described by Bruce Diner and colleagues, where the function of the protein is very much dependent on the nature of the complex in which it is associated. No such selectable systems exist for ribulose bisphosphate carboxylase/oxygenase (Rubisco), the primary catalyst of carbon dioxide assimilation. Attempts to engineer cyanobacteria to act as a reporter for mutations in Rubisco structure served mainly to highlight the essential nature of the protein in other metabolic processes of the bacterium (Pierce *et al.*, 1989). Fred Hartman describes how mutagenesis of this enzyme has proceeded, by rational design relying on the identification of critical amino acids with novel active site affinity probes and aided most recently by a high resolution crystal structure for the enzyme. Nevertheless it is frustrating to have identified a region of the protein that is involved in the reaction mechanism yet have no system for rapid selection of more efficient mutants.

Many proteins have evolved in plants that might be exploited for therapeutic use. These include inhibitors of mammalian proteases and α-amylases and Alison Campbell describes the structure of the chymotrypsin inhibitor CI-2 from barley seeds, and how site directed mutations of its reactive (inhibitory) site can be used to alter its activity and specificity for different serine proteases. Another such family of proteins is the protein synthesis inhibitors, the most well-characterised being ricin from castor bean. The lethal function of this protein is being tamed by Mike Lord and colleagues in ways that should prove valuable for the treatment

of carcinomas. Enno Krebbers and colleagues have adopted a different approach to producing therapeutic proteins in plants. They have isolated the genes for a group of albumin storage proteins and engineered them to replace a variable region of the encoded protein with peptides of therapeutic value. The engineered storage protein is robust to such alterations, and is synthesized to high concentrations in the seeds of transgenic plants. It is also possible to produce other proteins of animal origin in transgenic plants, including antibodies which are correctly assembled and exhibit biological activity (Hiatt *et al.*, 1989).

Storage proteins have proved to be attractive targets for genetic and molecular analyses, because of their role in determining grain quality. Attempts to improve their quality by protein engineering have been less successful. Craig Lending and colleagues describe elegant experiments of mutagenesis, to improve the lysine content, on the properties and packaging of the zein storage proteins of maize, but the application of these studies to the improvement of seed quality may well be limited by the complexity of the zein genetic system as well as the difficulty in routinely transforming maize. Even more problems are encountered in trying to understand and manipulate the structures of legume and gluten proteins that determine technological quality. The 7S and 11S globulins of legumes undergo extensive post-translational processing (disulphide bond formation, glycosylation and proteolytic nicking), while the functional properties of gluten proteins depend on complex interactions between over 50 individual components. The chapters by Pippa Madgwick and colleagues and by Nigel Lambert and Jenny Yarwood are essentially state-of-the-art accounts of attempts to use protein engineering to get to grips with these daunting systems. Neil Bulleid and colleagues describe an alternative approach to study wheat gluten protein assembly, *in vitro* transcription and translation. Finally, Theo Verrips provides a fascinating account of sweet-tasting proteins, which are yet to find a significant role in the food industry.

An important determinant of yield is the ability of the plant to survive infestations with pests and diseases, and to compete with non-crop species (i.e. weeds). At present the major control of pests, pathogens and weeds is by the application of agrochemicals. Two developments have had an important impact on the use of agrochemicals. The first is the identification of the sites of action of many herbicides, showing that some specifically inhibit one enzyme or protein unique to plants. Dan O'Keefe and colleagues briefly discuss the exploitation of these enzymes that catalyse essential biochemical processes in plants. They then describe in detail the use of cytochrome P450 to detoxify specific herbicides, and the potential for engineering this protein. For this approach to be used for weed control it is necessary to engineer resistance into the crop plant, by the introduction of mutated resistant enzyme or enzymes that detoxify the chemical, or by blocking the enzymes that convert an inactive precursor into the active compound. In this context, the details of enzyme mechanisms elucidated by *in vitro* mutagenesis could lead to *de novo* design of potent and specific inhibitors. Our expanding knowledge of plant gene regulation will allow the expression of the inserted genes to be restricted to specific tissues or stages of development, giving a wide choice of weed control strategies.

A second approach is to provide plants with a form of biological protection against pests. This has been successfully used to provide resistance to viruses, using coat protein (Beachy, 1988; Baulcombe, 1989) or satellite DNA (Harrison *et al.*, 1987; Gerlach *et al.*, 1987), and to insects using a serine protease inhibitor derived from cowpea (Hilder *et al.*, 1987), and other possible strategies involve the use of other protease inhibitors (e.g. CI-2), amylase inhibitors, endochitinases and lysozyme. A particularly successful application, which has included detailed protein engineering studies, is the use of insecticidal toxin derived from *Bacillus thuringiensis*, reviewed by Don Dean.

In many cases, it might be desirable that the proteins and enzymes of interest be transported and localized in specific sub-cellular organelles, e.g. storage proteins, enzymes of photosynthesis or amino acid metabolism. Newell Bascomb and Tom Lubben describe the contribution that protein engineering has made to unravelling the targeting information buried in those proteins destined for specific intraorganellar locations.

We hope that this discussion and the chapters that follow will convince the reader that a book on plant protein engineering is not premature, but timely in that it summarizes the current situation and points the way to a range of exciting applications. There are many examples in the literature of proteins with improved characteristics produced by *in vitro* engineering, but relatively little work of this type on plant proteins. It is therefore possible to cover the whole range of work in the area in one

book. We anticipate that the rate of growth will be rapid as many of the obstacles associated with the manipulation of plant systems are surmounted, and a high degree of selectivity will be required should we consider the preparation of future editions.

References

Baulcombe, D. (1989). Strategies for virus resistance in plants. *Trends in Genetics*. **5**: 56–60.

Beachy, R.N. (1988). Virus cross-protection in transgenic plants. In: *Plant Gene Research: Temporal and spatial regulation of plant genes*, (ed. D.P.S. Verma and R.B. Goldberg), pp. 313–27. Springer Verlag, New York.

Gerlach, W.L., Llewellyn, D. and Haselhoff, J. (1987). Construction of a plant disease resistance gene from the satellite RNA of tobacco ringspot virus. *Nature*. **328**: 802–5.

Harrison, B.D., Mayo, M.A. and Baulcombe, D.G. (1987). Virus resistance in transgenic plants that express cucumber mosaic virus satellite RNA. *Nature*. **328**: 799–802.

Hazelhoff, J. and Gerlach, W.L. (1988). Simple RNA enzymes with new and higher specific endoribonuclease activities. *Nature*. **334**: 585–91.

Hiatt, A., Cafferkey, R. and Bowdish, K. (1989). Production of antibodies in transgenic plants. *Nature*. **342**: 76–8.

Hilder, V.A., Gatehouse, A.M.R., Sheerman, S.E., Barker, R.F. and Bovett, D. (1987). A novel mechanism of insect resistance engineered into tobacco. *Nature*. **300**: 160–3.

Krol, A.R. van der, Mol, J.N.M. and Stuitje, A.R. (1988). Modulation of eukaryotic gene expression by complementary RNA or DNA sequences. *Biotechniques*. **6**: 958–76.

Pierce, J., Carlson, T.C. and Williams, J.G.K. (1989). A cyanobacterial mutant requiring the expression of ribulose bisphosphate carboxylase from a photosynthetic anaerobe. *Proceedings National Academy of Science U.S.A.* **86**: 5753–7.

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Contents

Part I INTRODUCTION TO PROTEIN EXPRESSION AND STRUCTURE

1

Development of Expression Systems for Eukaryotic Proteins in *E. coli* and Mammalian Cells

Sarah Eccles

Introduction

During the past two decades technical advances in DNA manipulation *in vitro*, together with the development of efficient transformation systems for a wide variety of prokaryotic and eukaryotic cells, have allowed an extensive study of the expression of genes in both homologous and heterologous systems. Such studies have contributed widely to our understanding of the regulation of gene expression via transcriptional, translational and post-translational mechanisms and have allowed the exploitation of recombinant gene expression for protein production and purification. The increase in protein yield obtained in many recombinant expression systems has facilitated studies of protein structure and biological activity that were formerly limited by the ability to purify sufficient quantities of many biologically active proteins from their native systems. Commercial application of recombinant gene expression technology has grown rapidly and many proteins are now produced commercially using recombinant systems. These range from bacterial proteins such as restriction enzymes, cloned from a variety of bacterial species and expressed in *Escherichia coli*, to human proteins such as insulin and tissue plasminogen activator cloned from human tissue and expressed in *E. coli* or mammalian cells.

Much of the early effort on producing mammalian proteins was invested in the development of expression systems in *E. coli*. *E. coli* has obvious advantages as a host for gene manipulation and protein production, related to our extensive knowledge of its genetics and the ease with which it can be grown to very high cell densities in simple growth media. Indeed, for the production of bacterial proteins it has been very successful. However, attempts to express eukaryotic proteins in *E. coli* have met with a number of problems, the most important of which is the lack of post-translational modification of proteins in bacterial cells. Processes such as glycosylation and phosphorylation are necessary for the biological activity, stability and antigenicity of many mammalian proteins and their production in *E. coli* may not result in a product with all the required properties.

Research efforts have therefore diversified into developing eukaryotic expression systems. Yeast has several advantages over *E. coli* as an expression system. In particular, yeast secretes proteins into the medium which aids protein purification and also allows disulphide bond formation and glycosylation of mammalian proteins to occur. The glycosylation pathways in yeast are not identical to those used in mammalian cells, but human albumin is an example of a protein which has been produced successfully using yeast (Collins 1990). Insect cells have also been used to produce mammalian proteins. High yields of foreign protein can be obtained from *Spodoptera frugiperda* cells when they are infected with recombinant baculoviruses, in which the foreign gene is expressed from the strong viral polyhedrin promoter. Recombinant proteins produced in this system are generally functional (Lucknow and Summers 1988) but their glycosylation patterns are not identical to the authentic proteins. Certain mammalian proteins

cannot be produced in heterologous systems because of the requirement for very specific modifications, for example, factor IX, which is subject to γ-carboxylation. Hence, the past decade has witnessed the development of efficient mammalian cell expression systems for mammalian proteins in a variety of cell types. Considering the various problems encountered in expressing mammalian genes in heterologous systems, it seems likely that in many cases these will be the systems of choice for producing fully active therapeutic mammalian proteins in the future. A further extension of the use of mammalian cell expression systems is to express cloned genes in whole animals, and the possibility of using transgenic domestic animals to produce therapeutic human proteins is being explored actively.

Expression of exogenous genes in plants has been successfully achieved using both *Agrobacterium tumefaciens* T-DNA mediated gene transfer and transformation of protoplasts with free DNA. More recent procedures for introducing DNA into intact plant cells include microinjection and the use of particle guns. Together these techniques have allowed the production of transgenic plants from a wide variety of species (Gasser and Fraley 1989). Genes introduced into plants include those conferring herbicide, virus and insect resistance as well as a number of mammalian genes.

The ability to modify genes using a combination of *in vitro* recombination and mutagenesis has led to the growth of the field of protein engineering and the possibility of producing protein products with novel activities. The exploitation of this technology requires that appropriate expression systems are chosen. These should allow efficient transcription and translation of engineered genes and appropriate post-translational modifications (when these are required for the desired characteristics of the engineered protein). In this chapter the development of expression systems for eukaryotic genes in bacteria (*E. coli*) and mammalian cells will be reviewed. The intention is to illustrate the types of problems encountered in achieving efficient expression of cloned genes in both homologous and heterologous systems.

Expression of eukaryotic genes in *E. coli*

Two fundamental requirements for an efficient expression system are that the chosen gene is efficiently transcribed and translated. Since prokaryotic genes do not generally contain introns, the eukaryotic gene either must be in the form of a cDNA or, alternatively, must be synthesized chemically using the known protein or genomic sequence. Transcription is achieved by cloning the eukaryotic sequence downstream from a strong prokaryotic promoter, most commonly the *E. coli* *lac* or *trp* promoter or the leftward promoter of phage lambda (P_L). These promoters contain conserved regions 35 bp and 10 bp upstream of the transcription initiation site (Rosenberg and Court 1979) that are involved in the binding of *E. coli* RNA polymerase, and may also contain regulatory elements such as the *lac* operator which allows inducible expression through the action of the *lac* repressor.

Efficient translation in *E. coli* requires the presence of a ribosome binding site. This consists of the initiation codon, AUG, together with a sequence (lying 3–12 bases upstream) known as the Shine–Dalgarno (SD) sequence. The SD sequence (3–9 bases) is complementary to the 3′ end of 16S rRNA and it is believed that hybridization of the 5′ end of the mRNA to the 3′ end of 16S rRNA locates the message on the 30S ribosomal subunit (Steitz 1979). Translation also depends on the codon composition of the mRNA. *E. coli* shows a codon preference reflected in the abundance of particular tRNA species (Ikemura 1981). The occurrence in eukaryotic mRNA of codons corresponding to rare *E. coli* tRNA species results in poor translational efficiency. For this reason it is generally advantageous to synthesize genes chemically, incorporating the appropriate *E. coli* codons, rather than to express cDNAs. However, this is not entirely without problems because such chemically synthesized genes may give rise to unpredicted secondary structures in the mRNA, which themselves lead to poor translational efficiency (Bialy 1987). A further potential problem raised by codon bias is the importance of translational pauses in protein folding. It is believed that certain proteins utilize clusters of rare codons to slow down the rate of chain elongation, and hence influence the way in which the protein folds (Purvis *et al.* 1987). This mechanism may contribute to the high frequency of heterologous proteins that are insoluble in *E. coli* (Harris 1983).

Many eukaryotic proteins have been produced in *E. coli* as fusion proteins with *E. coli* polypeptides such as β-galactosidase. In this case the promoter, ribosome binding site and initiation codon all come

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