PATENTS AND LITERATURE

Protein Engineering and Site-Directed Mutagenesis

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ABSTRACT

Protein engineering and site-directed mutagenesis is becoming immensely important in both fundamental studies and commercial applications involving proteins and enzymes in biocatalysis. Protein engineering has become a powerful tool to help biochemists and molecular enzymologists elucidate structure-function relationships in enzymic active sites, to understand the intricacies of protein folding and denaturation, and to alter the selectivity of enzymatic catalysis. Commercial applications of engineered enzymes are being developed to increase protein stability, widen or narrow substrate specificity, and to develop novel approaches for use of enzymes in organic synthesis, drug design, and clinical applications. In addition to protein engineering, novel expression systems have been designed to prepare large quantities of genetically engineered proteins. Recent US patents and scientific literature on protein engineering, site-directed mutagenesis, and protein expression systems related to protein engineering are surveyed. Patent abstracts are summarized individually and a list of literature references are given.

INTRODUCTION

The objective of the Patents and Literature Section is to summarize and cite recent developments in industrial and academic research as portrayed within the scope of current patents and literature and to highlight emerging biotechnological research areas. The subject of the first Patent and Literature Section of 1990 is Protein Engineering and Site-Dirtected Mutagenesis.

PATENTS

This section covers US patents concerning protein engineering and site-directed mutagenesis from the period January 1985 to December 1989. The search headings were protein engineering, site-directed mutagenesis, and nucleotide modification or alteration. Both patent titles and abstracts were searched. Within this time period, over 100 patents were recovered, however, only relevant US patents are detailed herein. Patent applications are not included. Some abstracts have been edited for clarity. Copies of US patents can be obtained from the Commissioner of Patents and Trademarks, Washington, DC 20231.

Backman, K. C., Balakrishnan, R.
ENZYME DEREGULATION
US 4,753,883, Jun. 28, 1988
Assignee: Biotechnica International, Inc.

Proteins having chorismate mutase-prephenate dehydratase (CMPD) activity, but lacking phenylalanine sensitivity are produced by genetic engineering. The proteins contain a sequence

substantially corresponding to the N-terminal 337 amino acids of *Escherichia coli* enzyme Expression vectors including genes coding for those proteins and regulatory DNA enabling their expression are used to transform host microorganisms, which are cultured to produce phenylalanine.

Brent, R. and Ptashne, M. S.
REGULATION OF EUCARYOTIC GENE EXPRESSION; PEPTIDES, PROTEINS, GENETIC ENGINEERING
US 4,833,080, May 23, 1989
Assignee: President and Fellows of Harvard College

Regulation of eucaryotic gene expression is controlled by procaryotic peptides. The peptides recognize specific DNA sequences present in the gene, which may be derived from procaryotic genes, and either activate or repress gene transcription. Hybrid procaryotic peptides may by used containing both repressor and activator peptides.

Casson, L. P., Goff, S.A., and Goldberg, A.L.
HOSTS AND METHODS FOR PRODUCING RECOMBINANT PRODUCTS IN HIGH YIELDS
US 4,758,512, Jul. 19, 1988
Assignee: President and Fellows of Harvard College

This invention relates to improved host organisms and methods for producing recombinant products in high yields. More particularly, the present invention relates to cell strains carrying specific mutations within their DNA sequences which cause the organism to exhibit a reduced capacity for degrading foreign products and the use of these strains to produce increased yields of genetically engineered foreign proteins, polypeptides and other products. The methods disclosed in this invention advantageously permit the production, in high yields, of foreign recombinant proteins, polypeptides or other products in hosts which do not usually produce such products.

Cornelius, P. A., Hochstrasser, R. M., Kallenbach, N. R., Rubin, H., and Todaro, G. J.
PULSED LIGHT SELECTIVE PHOTOLYSIS PROCESS FOR TREATMENT OF BIOLOGICAL MEDIA AND PRODUCTS MADE THEREBY
US 4,880,512, Nov. 14, 1989
Assignee: Kollmorgan Corp

This invention describes a novel irradiation process and products made thereby. The process treats biological media such as blood fractions. In general, the process comprises irradiating the medium with pulsed light of wavelength and flux selected so that (1) the nucleic acids in their ground state absorb radiation and thereby rise to an excited state or states, (2) the nucleic acids in their excited states absorb radiation and thereby rise to higher energy states and undergo photolysis, and (3) the proteins in their ground or their excited states do not absorb sufficient radiation to undergo substantial photolysis. It is surprising and unexpected that with pulsed light of wavelength and flux nucleic acids in their excited states undergo efficient photolysis whereas proteins under the same conditions in the same meduim are kept substantially intact.

De Boer, H. A.
DEDICATED RIBOSOMES AND THEIR USE; GENETIC ENGINEERING, PROTEIN SYNTHESIS
US 4,772,555, Sep. 20, 1988
Assignee: Genentech, Inc.

A method is described that dedicates specialized ribosomes to the synthesis of desired proteins. DNA which encodes rRNA having a mutant anti-Shine-Dalgarno sequence is used to transform a host cell in combination with DNA encoding messenger RNA for the desired protein having a complementary mutant Shine-Dalgarno sequence. Since the mutant sequences are selected so as to be substantially unrecognized by either endogenous messenger RNA or ribosomes, respectively, the synthesis of protein proceeds in a dedicated system without interference from other host cell protein synthetic machinery.

Delgoffe, J.C., Lobmann, M., and ZyGraich, N.
TEMPERATURE SENSITIVE STRAINS OF BOVINE VIRAL DIARRHEA VIRUS, PREPARATION THEREOF AND VACCINES CONTAINING THEM; MUTATION WITH NITROUS ACID
US 4,618,493, Oct. 21, 1986
Assignee: Smithkline-RIT BE

A live bovine viral diarrhea (bvd) virus vaccine capable of producing immunity in bovines without causing significant side effects and comprising of a temperature sensitive (TS) mutant of bvd virus is obtained by mutating a strain of bvd virus with nitrous acid. The temperature sensitive mutants show a replication ability that is considerably limited at the animal body temperature of 39.5°C. The vaccine may be combined with other live bovine vaccines such as respiratory virus vaccines and then administered to bovines.

Gehrke, L.
PROCESS FOR ENHANCING TRANSLATIONAL EFFICIENCY OF EUKARYOTIC MRNA; INCREASED PROTEIN PRODUCTION-GENETIC ENGINEERING
US 4,820,639, April 11, 1989
Assignee: Massachusetts Institute of Technology

A process and means for increasing the production of protein translated from eukaryotic messenger ribonucleic acid (mRNA) is comprised by transferring a regulatory nucleotide sequence from a viral coat protein mRNA to the 5' terminus of a gene or complementary deoxyribonucleic acid (cDNA) encoding the protein to be produced to form a chimeric DNA sequence. The regulatory DNA sequences are generated *de novo* using genetic engineering procedures to produce synthetic double-stranded oligonucleotides representing the regulatory viral sequence. Nucleotide sequences which encode a regulatory sequence or structure conferring enhanced competitive activity and increased rate of translation upon the chimeric DNA sequences include the nucleotide sequence preceding the initiator AUG codon at the 5' terminus of coat protein messenger RNA from alfalfa mosaic virus, brome mosiac virus, black beetle virus, turnip yellow mosaic virus, and satellite tobacco necrosis virus.

Kunkel,T.
PROCESS FOR SITE SPECIFIC MUTAGENESIS WITHOUT PHENOTYPIC SELECTION
US 4,873,192, Oct. 10, 1989
Assignee: U.S.Health & Human Services

The present invention discloses several DNA mutagensis processes using a DNA template containing several uracil residues in place of thymine. These replacements can be applied without selection techniques to produce altered DNA sequences with approximately 10-fold greater efficiency than current methods of site-specific mutagenesis. This template has relatively normal coding potential in the *in vitro* reatctions typical of standard site-directed mutagenesis protocols but is not biologically active upon transfection into a wild type *E. coli* host cell. Expression of a desired change, present in the newly synthesized non-uracil-containing covalently closed circular complementary strand, is thus favored. The procedure has been applied to mutations introduced via both oligonucleotides and error-prone polymerization. The inclusion of two additional simple treatment steps before transfection results in a site-specific mutation frequency approaching 100%.

Paau A., Platt S. G., and Sequeira, L.
ASSAY METHOD AND PROBE FOR POLYNUCLEOTIDE SEQUENCES; EXPOSING TEST SAMPLE TO MODIFIED PROBE AND BINDING PROTEIN
US 4,556,643, Dec. 3, 1985
Assignee: Agracetus

An assay method for the detection of a specific nucleotide target sequence in a polynucleotide test extract is disclosed which utilizes a polynucleotide modified probe including both a cDNA sequence substantially complementary to the specific target sequence and a protein binding sequence. The assay is conducted by exposing the modified probe to the polynucleotide test extract for hybridization and then exposing the complex to the protein which binds to the protein binding

sequence. An assay, such as an immunoassay, can then be conducted on the test sample to indicate the presence of the specific target sequence by detecting the presence of the binding protein.

Palladino, M. A.
CHEMOTACTIC ASSAY FOR IMMUNOGENICITY; PREDICTION OF IMMUNE RESPONSE TO GENETICALLY ENGINEERED PROTEIN
US 4,714,674, Dec. 22, 1987
Assignee: Genentech, Inc.

The *in vitro* chemotactic activity of recombinant protein compositions is a predictive test for vivo immunogenicity. Recombinant synthesis methods and subsequent purification or processing techniques are modified in the light of the chemotactic assay results in order to reduce or enhance the *in vivo* immunogenicity of the compositions. The invention ameliorates a major cost and source of uncertainty in the development of recombinant protein compositions. Immunogenicity of substances is modulated by the binding of chemotactic polypeptides.

Roman, H. G., Hultmark, D., Rasmusan, TT., and Steiner, H.
BACTERIOLYTIC PROTEINS; LOW MOLECULAR WEIGHT, HEAT-RESISTANT PROTEIN AS BACTERICIDE FOR GRAMNEGATIVE BACTERIA
US 4,520,016, May 28, 1985
Assignee: Kabigen AB SE

A non-lysozyme highly active bacteriolytic protein which is heat stable and has a relatively low molecular weight is described. The protein may be produced by immunizing an insect against *E. coli* and recovering the protein from the insect. The proten is useful for extracting proteins from genetically engineered bacteria and as a pharmaceutical for inhibiting certain bacteria.

LITERATURE

This section surveys the literature in the area of protein engineering and site-directed mutagenesis. Space considerations limited the search to 1989. The search headings are the same as listed in the patent search. This section is not intended to be all encompassing and lists both review articles and research publications that highlight the varied nature of research in this field during the past year.

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