Biotechnology and Healthcare

Accumulation of metabolic products in bacteria

Professor Martin Warren is among the principal inventors of this technology.
Reference: 038-PM (co-owned with University College Cork - National University of Ireland)
Protected Intellectual Property: follow link here.

Summary: Bacterial microcompartments are closed polyhedral shells made of thin protein sheets, enclosing enzymes and cofactors for various forms of fermentative metabolism. The present technology concerns bacteria that are genetically modified or transformed to express a heterologous bacterial microcompartment containing an enzyme that is capable of converting a low molecular weight substrate into a high molecular weight or polymeric metabolic product.

Background: Bacterial microcompartments (metabolosomes) are closed polyhedral shells 100-150 nm diameter made of thin protein sheets (with pores less than 1 nm in diameter which can be positively or negatively charged), enclosing enzymes and cofactors for carbon fixation (carboxysomes) or various forms of fermentative metabolism. 20% of bacterial genome sequences contain microcompartment structural genes, in many cases associated with enzymes of unknown function. It is believed that these structures help bacterial metabolic efficiency by selective limitation of the shell pores on the passage of reactants, by metabolic channelling, or other mechanisms achieving temporary retention of small reaction intermediates within the structure, but these advantages have not been fully quantified.

Technology: The present technology provides a non-therapeutic method to accumulate polymeric metabolic products or metabolic products of high molecular weight within bacterial microcompartments in bacterial cytoplasm. The technology employs bacteria that are genetically modified or transformed to express a heterologous bacterial microcompartment containing an enzyme that is capable of converting a low molecular weight substrate into a high molecular weight or polymeric product. Two specific applications of this technology are given as an illustrative example of the utility of this biological system. The first application is a method of removing or reducing the levels of toxic inorganic phosphate (Pi) from a system or environment by employing recombinant bacteria which are capable of non-reversibly accumulating phosphate in the form of the polymer polyphosphate. The second application is a method for accumulating an amino acid polymer (cyanophycin) with an increase in chain length.

Biopharmaceutical Production Method

Reference: 048
Protected Intellectual Property: follow link here.

Summary: The invention resides in a method for the manufacture of a disulphide- requiring biopharmaceutical having an element of at least tertiary structure using wild type E. coli.

Background: Escherichia coli is a popular host for the production of recombinant proteins, underpinning the production of over a third of currently-licensed therapeutic proteins (Walsh (2010) Drug Discov. Today 15:773-780). There are several strategies for production of these biopharmaceuticals in E. coli, including expression of soluble proteins in the cytoplasm, expression as insoluble inclusion bodies or export to the periplasm followed by selective rupturing of the outer membrane to release the protein (Pierce et al (1997) J. Biotechnol. 58:1 -1 1). The latter is a favoured approach for many protein products because it offers major advantages in downstream processing, including a reduction in the release of contaminant proteins and proteases, a lack of DNA release and less debris micronisation, hence better clarification performance (reviewed in Balasundaram ef a/ (2009) Trends Biotechnol. 27:477-485; Harrison and Keshavarz-Moore (1996) Ann. NY Acad. Sci. 782:143-158). In addition, the periplasm is an oxidising environment which is essential for the production of disulphide bond-containing proteins. In wild type E. coli host strains, these cannot form in the cytoplasm.

Technology: The invention encompasses a method for the manufacture of a disulphide-requiring biopharmaceutical having an element of at least tertiary structure, preferably a substantially active and/or natural conformation, using wild type E. coli. The method is particularly applicable to proteins and fragments thereof, such as antibody fragments.
In particular, the method comprises use of Tat-dependent export from the cytoplasm to the periplasm and subsequent extraction and purification. It is believed the exported proteins and fragments acquire disulphide bonds in the periplasm, indicating that the normal disulphide oxidation machinery in the bacterium is able to recognise and act on the proteins and fragments thereof.

Cancer Diagnostic

Dr Vadim Sumbayev is the inventor of this technology.
Reference: 054
Protected Intellectual Property: follow link here.

Summary: The invention relates to the diagnosis of haematopoietic cell cancer using expression of latrophilin as a biomarker and methods and kits for the detection and stimulation of latrophilin.

Background: Haematopoietic cells, haematopoietic stem cells (HSCs) or haemocytoblasts are the cells that give rise to all the other blood cells through the process of haematopoiesis. They are derived from mesoderm and located in the red bone marrow, which is contained in the core of most bones. The cells give rise to both the myeloid and lymphoid lineages of blood cells. Myeloid cells include monocytes, macrophages, neutrophils, eosinophils, erythrocytes, dendritic cells, and megakaryocytes or platelets. Lymphoid cells include T cells, B cells, and natural killer cells. Leukaemia is a group of cancers that usually starts in blood-forming tissue, including the bone marrow. It leads to the over-production of abnormal white blood cells, the part of the immune system that defends the body against infection.

Technology: The present invention resides in the use of expression of one or more latrophilin isoforms as a biomarker for the diagnosis of haematopoietic cell cancer in a subject. Latrophilins form a group of structurally similar adhesion G-protein-coupled receptors (GPCRs) that function as exocytosis promoters acting through calcium mobilisation/signalling machinery (Capogna M. ef a/ (2003) J. Neurosci. 23 4044- 4053; Volynski K.E. er a/ (2004) EMBO J. 23 4423-4433; Ushkaryov Y.A. er a/ (2008) Pharmacology of Neurotransmitter Release 184 171 -206; Silva J. -P. et al (2009) J. Neurochem. ± 275-290; Silva J. -P. and Ushkaryov Y. (2010) Adhesion- GPCRs: Structure to Function. 59-75). This group contains three proteins with 48- 63% of structural homology, known as latrophilin 1 (LPHN1), latrophilin 2 (LPHN2) and latrophilin 3 (LPHN3). Initially, LPHN1 and LPHN3 were detected only in neurons (Davletov B.A. et al (1996) J. Biol. Chem. 271 23239-23245; Lelianova V.G. et al (1997) J. Biol. Chem. 272 21504-21508). However, LPHN2 is more ubiquitously expressed and its role in normal and pathological processes has been discussed (Sugita S. er a/ (1998) J. Biol. Chem. 273 32715-32724; Matsushita H. er a/ (1999) FEBS Lett. 443 348-352; White G.R.M. et al (1998) Oncogene 17 3513-3519; Bushel P.R. er a/ (2012) PLoS ONE 7 e34286; Zhang S. et al (2014) PLoS OΛ/E 9 e91466). In addition, LPHN3 has also recently been linked to some cancers (Kan Z. et al (2010) Nature 466 869-875; Kotepui M. et al (2012) Asian Pacific J. Cancer Prev. 13 5879-5882), while LPHN1 has been found in non-small cell lung cancer (Hsu Y.-C. er a/ (2009) Clin. Cancer Res. 15 7309-7315). However, the expression of latrophilin in malignant myeloid leucocytes has not been investigated or even profoundly discussed.
The inventors have found that latrophilin is not expressed in healthy human leucocytes, even at the mRNA level, and expression is not induced by proinflammatory agents and growth factors in healthy white blood cells. Functionally, activation of latrophilin induces intracellular calcium mobilisation, thereby significantly promoting exocytosis. Exocytosis is crucial not only for neuronal function but also for the formation of myeloid cells, a process

termed "haematopoiesis" that includes the proliferation and differentiation of stem cells into blood cells.

Genetically Modified Microorganisms

Professor Martin Warren is the inventor of this technology.
Reference: 056
Protected Intellectual Property: follow link here.

Summary: The invention relates to genetically modified microorganisms comprising one or more heterologous nucleic acid molecules together encoding at least three different proteins, each protein comprising an enzymatic domain and a bacterial microcompartment-targeting signal polypeptide, wherein said enzymatic domains each catalyse a different substrate to product conversion in the same metabolic pathway, and wherein said microorganisms are essentially free of bacterial microcompartments (BMCs); and to cell free systems comprising aggregates comprising at least three different proteins, each protein comprising an enzymatic domain and a bacterial microcompartment-targeting signal polypeptide, wherein said enzymatic domains each catalyse a different substrate to product conversion in the same metabolic pathway, and wherein said system does not comprise bacterial microcompartments; and to methods for the production of said microorganisms and cell free systems and their use in methods of producing a product of interest.

Background: Bacterial microcompartments (BMCs) are metabolosomes, i.e. discrete protein-based organelles in which steps of a particular metabolic pathway occur. BMCs are typically 40 to 200 nm in diameter and consist of a semipermeable proteinaceous outer layer that encases the enzymes that catalyse steps of a particular metabolic process. Thus, within a BMC, enzymatic activity of a particular metabolic pathway or part thereof is concentrated. The encapsulated environment is ideal for the channelling of toxic/volatile intermediates.

Technology: The present inventors have now found that increased levels of a product of interest can be obtained using microorganisms that comprise polypeptides having enzymatic domains and BMC-targeting signal sequences, wherein the enzymatic domains catalyse steps of the same metabolic pathway for the production of said product of interest, but wherein the cell lacks the ability to produce BMCs.

Increased production of disulphide-bonded recombinant proteins in the yeast *Saccharomyces cerevisiae*

Professor Mick Tuite is among the principal inventors of this technology
Reference: 021-MFT (co-owned with Merck)
Protected Intellectual Property: follow link here.

Summary: Most mammalian secretory proteins (e.g. pituitary hormones, interleukins, immunoglobulins) contain multiple intramolecular and/or intermolecular disulphide bonds. Such proteins are among the prime targets for commercial genetic engineering and disulphide bond formation, which, *in vivo*, is catalyzed by the enzyme protein disulphide-isomerase (PDI), is required for their proper protein folding. The present technology concerns the process for increasing the yield of disulphide-bonded recombinant proteins produced by yeast, especially recombinant secreted proteins. Recombinant strains of the yeast *Saccharomyces cerevisiae* have been constructed, which overproduce either human PDI or yeast PDI in a regulated fashion.

Background: The vast majority of mammalian secretory proteins (e.g. pituitary hormones, interleukins, immunoglobulins, proteases and other serum proteins) contain multiple intramolecular and/or intermolecular disulphide bonds. Such proteins are among the prime targets for commercial genetic engineering and disulphide bond formation is required for their proper protein folding, which is necessary for their function. The process of disulphide bond formation *in vivo* occurs co-translationally or as a very early post-translational event and is catalyzed by the enzyme protein disulphide isomerase (PDI). PDI is an abundant protein in secretory cells and is located at the luminal face of the endoplasmic reticulum.

Technology: The present technology concerns the process for increasing the yield of disulphide-bonded recombinant proteins produced by yeast, especially recombinant secreted proteins. Recombinant strains of *Saccharomyces cerevisiae* have been constructed which overproduce either human PDI or yeast PDI in a regulated fashion. These strains show greatly increased secretion of disulphide-bonded proteins of potential therapeutic significance.

Soluble adenyl cyclase inhibitors

Professor Fritz Muehlschlegel is among the principal inventors of this technology.
Reference: 007-PM (co-owned with Cornell University, USA)
Protected Intellectual Property: follow link here.

Summary: cAMP is a nearly ubiquitous second messenger molecule that affects a multitude of cellular functions. The present technology relates to the soluble adenylyl cyclase (sAC)-mediated generation of cAMP that exhibits nuclear signalling and is sufficient for eliciting insulin release. This technology provides a chemical inhibitor that specifically modulates the sAC, and thus can potentially be used as medication for learning or memory disorders, malaria, fungal infection, spinal cord injury, diabetes, Alzheimer's disease, amyotrophic lateral sclerosis, and peripheral neuropathy.

Background: cAMP is a nearly ubiquitous second messenger molecule that affects a multitude of cellular functions. In mammalian cells, cAMP is generated by transmembrane adenylyl cyclases (tmACs), which are tethered to the plasma membrane and regulated by heterotrimeric G proteins in response to hormonal stimuli, and by so-called "soluble" adenylyl cyclases (sACs), which reside in discrete compartments throughout the cell and are regulated by the intracellular signaling molecules bicarbonate and calcium. The cAMP generated by tmACs appears to act locally, however cAMP targets are also located to subcellular compartments that are distant from the plasma membrane (e.g. exchange proteins activated by cAMP are localized to the nuclear membrane and mitochondria). Thus far, the source of cAMP in certain cellular processes and its connection to those processes has remained undefined.

Technology: The present technology demonstrates the existence of a nuclear cAMP signaling microdomain that mediates bicarbonate/sAC-dependent activation of the transcription factor CREB which constitutes a classical cAMP-downstream target. Bicarbonate activation of CREB represents an example of a mammalian cAMP-dependent pathway solely modulated by intrinsic cellular signals. Furthermore, the present technology shows that, unlike cAMP synthesized by tmACs, sAC-generated cAMP is sufficient to elicit insulin release. In this regard, this technology concerns a method of treating a disorder mediated by sAC in a subject. The method involves administering to a subject an effective amount of a sAC specific chemical compound that modulates sAC, under conditions effective to treat the disorder mediated by sAC. Diseases mediated by sACs and in which this specific compound may be used as medication include learning or memory disorders, malaria, fungal infection, spinal cord injury, diabetes, Alzheimer's disease, amyotrophic lateral sclerosis, and peripheral neuropathy.