Institution: Newcastle University

Unit of Assessment: UoA5

Title of case study: Commercialisation of a unique widely-applicable high-fidelity DNA polymerase engineered in Newcastle

1. Summary of the impact

Through their study of DNA polymerases from organisms of the domain archaea, researchers at Newcastle University and University College London identified the mechanism by which these organisms avoid potentially damaging mutations in their DNA. As a consequence of this work they invented a novel genetically-engineered DNA polymerase. This enzyme has been patented and is the world's only high-fidelity, proofreading DNA polymerase that efficiently reads through uracil in the polymerase chain reaction (PCR). PCR is a very widely used technique in biomedical research. An international bioscience company [Text removed for publication, EV d] signed a licensing agreement with Newcastle University in 2008 to market the enzyme, and total sales since 2008 exceed [Text removed for publication, EV d]. Further commercial exploitation has begun through licensing agreements with other major companies.

2. Underpinning research

Key Newcastle University researcher

Professor Bernard Connolly conceived and led the research in Newcastle and contributed significantly to collaborative work, detailed where appropriate.

Background

Four chemical bases make up DNA: adenine (A), thymine (T), cytosine (C) and guanine (G). Cytosine is prone to a deamination reaction (the loss of an amine moiety), the product of which is uracil. Unless it is repaired to cytosine uracil remains in the DNA strand and, when this is replicated using a normal DNA polymerase, the uracil forms a base pair with adenine. When the DNA strand including this adenine is replicated in turn, it will form a pair with thymine. Thus, the presence of uracil eventually results in the replacement of the original C-G base pair with T-A. This mutation (error) in the DNA is irreversible and scrambles the genetic code (Hofreiter et al. 2001 PMID: 11726688).

The deamination of cytosine is the most common reason for mutation in DNA. The reaction proceeds at a low rate at room temperature, but rises significantly at higher temperatures, such as those that are used during the polymerase chain reaction (PCR).

Archaea are single-celled organisms that are classified in their own domain, having been shown to have life-processes that are neither exclusively like bacteria nor eukaryotes (the other domains). Many archaea are found in extreme environments such as the high temperature water around deep-sea thermal vents. When they were discovered, it was suggested that such hyperthermophilic archaea would be particularly vulnerable to mutations produced through cytosine deamination caused by the high temperatures to which they are exposed in their natural habitat. Like all other organisms whose genetics is based on DNA, archaea have an absolute requirement for some type of uracil detection and repair system. However, no homologues of the two known families of uracil base excision-repair enzymes ubiquitous in the other domains have so far been identified in archaeal genomes, suggesting that a novel mechanism of error checking might be discovered in the archaea.

Underpinning research

Connolly's research at Newcastle University concerns the nature of protein-DNA interactions. In the late 1990s his group began to study organisms in the domain archaea, aiming to identify the molecular mechanism by which they recognise uracil in their DNA. This involved the study of enzymes, termed DNA polymerases, which are involved in the replication of DNA.

In 1999, Connolly and co-investigator Professor Laurence Pearl (University College London) published results which showed that DNA polymerases from several archaea stop DNA replication (stall) at uracil in the strand being copied (the template strand; R1). This stalling pointed to a possible mechanism that had evolved to protect archaea from harmful rates of mutation caused by cytosine deamination in the high temperature environments where they live. Stalling was achieved

by the DNA polymerase recognising uracil in the strand ahead of the point at which it was currently copying. Importantly, this was the first publication to show the existence of a read-ahead template-checking function in any DNA polymerase, from any type of organism.

Further studies of the structure of these DNA polymerases by Connolly and colleagues revealed the molecular basis of the template-checking function. The researchers demonstrated the presence of a 'pocket' in the region of the DNA polymerase away from the active site that interacts with the template DNA strand. By introducing mutations into the gene sequence coding for the DNA polymerase they demonstrated that chemical groups in the 'pocket' discriminate uracil from the other bases in DNA and, moreover, that it is this specificity that causes the DNA polymerase to stall at uracil but still progress unimpeded past the four normal bases in DNA. Significantly, these studies led Connolly and colleagues to genetically engineer the uracil binding pocket in the DNA polymerase to create a new type of DNA polymerase that could read through template strand uracil (R2).

An advantage of archaeal DNA polymerases is that, unlike bacterial DNA polymerases, they have a 3'-5' exonuclease III (proofreading) activity (Cline et al., 1996 PMID: 883618). This activity together with the Newcastle research into uracil recognition resulted in the production of a high-fidelity proofreading DNA polymerase, stable at high temperatures, which could read through uracil. These properties of the unique DNA polymerase make it a valuable laboratory tool.

3. References to the research

(Newcastle researchers in bold. Citations from Scopus as at July 2013.)

R1. Greagg MA, **Fogg MJ**, Panayotou G, **Evans SJ, Connolly BA** and Pearl LH (1999) A read-ahead function in archaeal DNA polymerases detects promutagenic template-strand uracil. *Proceedings of the National Academy of Sciences USA* 96(16):9045-50. DOI: 10.1073/pnas.96.16.9045. **75 citations.**

R2. **Fogg MJ**, Pearl LH and **Connolly BA** (2002) Structural basis for uracil recognition by archaeal family B DNA polymerases. *Nature Structural & Molecular Biology* 9(12):922-7. DOI: 10.1038/nsb867. **83 citations.**

Key research grants

MRC. 1993–6. £101,000. Joint award with Laurence Pearl, UCL. *Mapping of the DNA binding site.*

BBSRC. 2000-3. £193,000. *Recognition of DNA containing DU bases by archaeal DNA polymerases.*

BBSRC. 2004-6. £222,000. *Improving archaeal DNA polymerases for molecular biology applications.*

4. Details of the impact

The DNA polymerases of hyperthermophilic archaea were of interest to molecular biologists because of their inherent ability to withstand high temperatures. Such a property was valuable for the amplification of DNA in the laboratory, using the polymerase chain reaction (PCR). PCR makes use of DNA polymerases to replicate DNA and involves repeated heating and cooling cycles, amplifying a small quantity of DNA into one large enough for meaningful analysis and for many experimental approaches. However, the high temperature phases of the PCR increase the rate of the deamination of cytosine and lead to a build-up of DNA strands incorporating uracil during the amplification process. The commonly used heat-stable DNA polymerase, Taq (from ***T**hermus **aq**uaticus*) cannot proofread, so uracil generated during PCR using Taq polymerase leads to the accumulation of T-A for G-C substitutions and loss of fidelity. Archaeal polymerases have a proof-reading activity, but the fact that archaeal polymerases stall at uracil make them less suited to PCR in their native form.

In the course of their work, not only had Newcastle researchers and their colleagues identified a unique property of certain hyperthermophilic archaeal DNA polymerases but, through their discovery that the uracil recognition function was not present at the active site, they were able to engineer a change in the structure of the uracil binding pocket to create a novel DNA polymerase that was capable of reading through DNA strands that included uracil. This combination of

properties - both the high fidelity and stability at high temperatures - was recognised as having potential for commercial exploitation.

Patenting a new enzyme

The new DNA polymerase, hereafter referred to as V93Q Pol, was co-invented by Connolly and Mark Fogg (Connolly's PhD student) at Newcastle University, and Professor Laurence Pearl at UCL, in 2003. The invention was underpinned by insights gained through the researchers' studies into how archaeal DNA polymerases recognise the base uracil. A patent to protect the invention, *Mutation of DNA polymerases from archaebacteria*, was filed in April 2003, and it has now been granted in many countries worldwide (Australia, Canada, Japan, USA, UK and other European countries; Ev a). The enzyme is unique.

Some DNA polymerases used in PCR have been derived from hyperthermophilic archaea but they become less efficient in the presence of even small amounts of uracil in the reaction mixture, because their structure causes them to stall at uracil. V93Q Pol was engineered to be uracil-insensitive and is therefore not affected in the same way. Because of its broad tolerance to the presence of uracil, V93Q Pol is often used for reactions in which high-fidelity copying is required but reaction conditions have not been optimised, or where the template is relatively long and contains many cytosine residues (which can be deaminated to uracil, as described above). Under equivalent reaction conditions, V93Q Pol typically yields more DNA product than standard enzymes used in PCR.

The V93Q Pol enzyme is particularly useful in DNA methylation studies, which are important in understanding why genes are switched off in cells (Ev b). Methods that target DNA methylation sites (bisulphite sequencing) lead to degradation of much of the DNA, leaving very few intact copies of the DNA sequence. Amplifying these few copies using PCR requires many cycles of heating and cooling before sufficient DNA is available for analysis and generates large amounts of uracil that other polymerases cannot overcome.

Commercial impact

The V93Q Pol enzyme has been on the market since 2003, but the substantial commercial impact has only occurred since an exclusive licensing agreement was signed between Newcastle University and [Text removed for publication, EV d] on 1 November 2008. [Company name removed for publication] sell V93Q Pol using the brand name [Text removed for publication] (Ev b and c).

Income to Newcastle University from [Company name removed for publication, EV d] in 2008 and 2009 was in the form of an agreed fee. From 2010 onwards income has been in the form of royalties on sales. Royalties are shared 75% to Newcastle University and 25% to UCL, reflecting the fact that Professor Pearl at UCL was an important collaborator in the early stages. Most of the research that underpinned the development of the enzyme was carried out at Newcastle University.

[Sales figures removed for publication, EV d]

Expanding commercial impact

At the end of 2011, the licensing agreement with [Company name removed for publication] became non-exclusive. Two further licensing arrangements have now been signed, one in November 2012 and one in February 2013, expanding the commercial impact of the invention [Text removed for publication, EV d].

5. Sources to corroborate the impact

Ev a. Patent: *Mutation of DNA polymerases from archaeobacteria*.

US: http://www.google.com/patents/US20060057682

Worldwide: http://patentscope.wipo.int/search/en/detail.jsf?docId=WO2003089637

Ev b. [Text removed for publication]

Ev c. [Text removed for publication]

Ev d. The contact details for the Newcastle University lawyer responsible for managing the

agreements can be made available on request, as can confidential information confirming the commercial agreements.