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Press Release

20 March 2003

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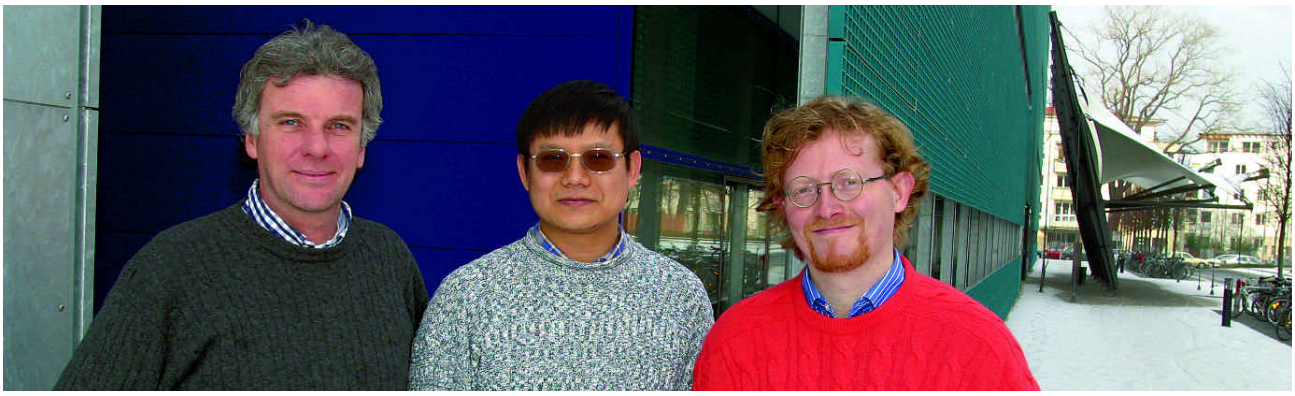

Francis Stewart, Youming Zhang and Giuseppe Testa
Photo by C. Panagiotidis, MPI-CBG photolab

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Source article:
Engineering the mouse genome with bacterial artificial chromosomes to create multipurpose alleles
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Published online: Nature Biotechnology, 10 March 2003, doi:10.1038/nbt804

A subtle tool to study mankind's diseases in the mouse

One of the most powerful tools in today's biological and medical science is the ability to artificially remove and add bits of DNA to an organism's genome. This has helped scientists to understand problems caused by defective genes, for example, which have now been linked to thousands of human diseases. So far the technology has been limited to small segments of DNA. But four years ago, Francis Stewart and his colleagues at the European Molecular Biology Laboratory (Heidelberg) developed a new technique to engineer greater stretches of DNA in bacteria. The researchers, now working at the Biotec-Technical University in Dresden, have just used this method to engineer a complex set of changes in a mouse gene, in hopes of shedding light on human leukemias. Their work appears in the current edition of the journal *Nature Biotechnology*.

Over two decades ago, researchers learned to use bacteria as "copy machines" for DNA taken from other organisms. This was a huge step for biotechnology, because most types of

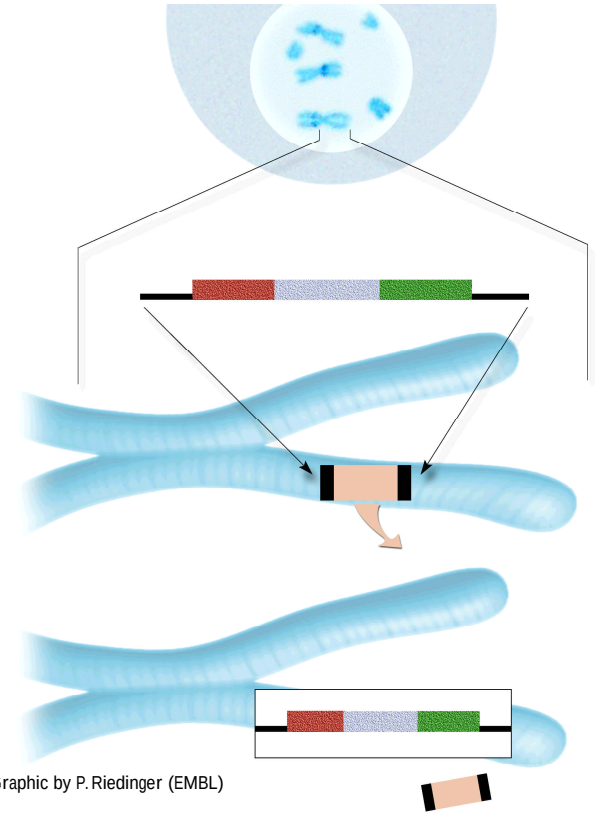
research require billions of copies of a molecule under investigation. However, there was a limitation: researchers need to change the DNA molecules in precise ways and for large molecules – such as whole genes – this was tremendously difficult.

Stewart and his colleagues thought that bacteria could be taught to do better, so they "borrowed" a strategy that organisms such as mice and yeast use to repair breaks in DNA. Proteins called *recombinases* circulate through their cells, looking for loose DNA fragments that have familiar sequences.

"Recombinases assume that the fragments have been cut out of the DNA by mistake, so they try to glue them back into the genome in the right place," says Giuseppe Testa, who headed the current study. "Sometimes they're a bit over-industrious; they put in pieces that look right, such as variations of a gene that have been put into the cell by a researcher."

Called *homologous recombination*, this process works a bit like a "find-and-replace" command in your word processor. Imagine you have typed "Stephen Q.Gould" everywhere, and suddenly discover that the middle initial should be "J".The computer can be told to look for "Stephen" and "Gould" and replace what comes between them. In the same way, recombinases find recognizable sequences of DNA to the left and right of a target and replace what comes in between with the new sequence.

Homologous recombination was known to occur in bacteria, but it hadn't been possible to use it to engineer DNA, as was the case in yeast and mouse stem cells.Stewart's team decided to try to find a strain that could do it. "We ordered as many types of *E. coli* as we could, looking for defects in the way they repair their DNA," he says. "After five months of work, Youming Zhang, a postdoc in the lab, found the strain."



Graphic by P. Riedinger (EMBL)

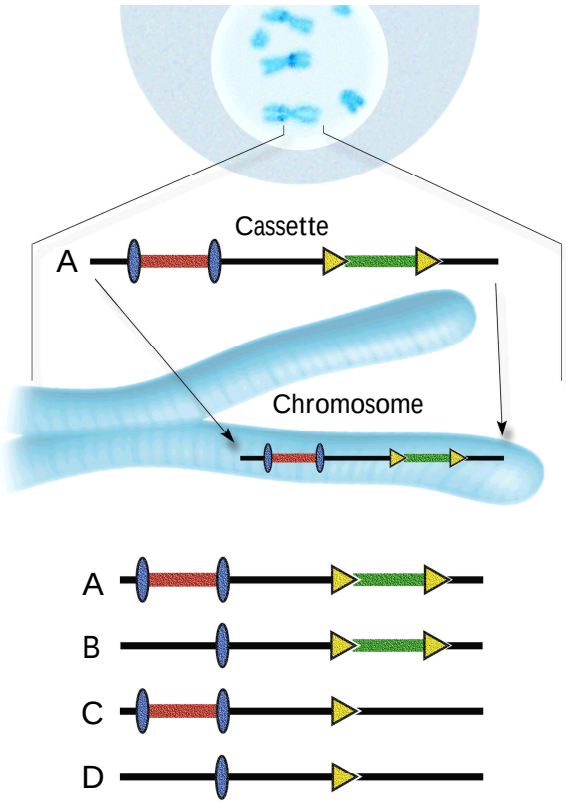
Red/ET combination relies on proteins that recognize specific DNA sequences (black) and replaces the DNA between them with genetic material engineered by scientists.

The group quickly identified the bacterial factors involved and turned them into a new tool called *Red/ET recombination* that is now being adopted by biologists all over the world.Red/ET recombination is proprietary technology of the EMBL and is one of the mainstays of Gene Bridges GmbH, an EMBL spin out company that Stewart and his colleagues founded in 2000 to develop the commercial implications of the breakthrough. The technology is licensed exclusively to Gene Bridges by EMBL Enterprise Management GmbH (EMBLEM), the subsidiary and commercial arm of the EMBL.

"We have been pushing it to work with larger and larger bits of DNA," Testa says, "and our latest project has been to engineer an entire artificial chromosome in bacteria. We've constructed a large, complex 'cassette' that we've now inserted into a mouse in place of its normal gene."

The gene that they chose is called *mixed-lineage leukemia (Mll)*, and is known to become defective in childhood leukemias in humans. By inserting the artificial version into the mouse, researchers hope to understand how the defects lead to disease.

"There are many things that can go wrong in this gene," Testa says,"and we wanted to construct a version of it that would allow us to test as many aspects of the problem as possible."



Graphic by P. Riedinger (EMBL)

The engineered form of Mll (A) comes in a "cassette" that has two sections (red and green) under the control of switches (ovals and triangles).This permits scientists to activate the genes simultaneously (A), independently (B and C), or to leave them switched off (D).

The artificial Mll that they have put into the mouse will permit a variety of experiments. It contains two defects in the genetic sequence that have been linked to leukemias.The cassette also contains control switches that allow each defect to be "switched on" whenever the researchers choose; they can also be left off. "We can study each mutation independently, or watch how they act together, or control the time at which each one acts," Testa says. "This will give us a new look at subtle relationships between multiple defects."

Many diseases are linked to single mutations; however, disease susceptibility also often relies on other sequence variations, known as *polymorphisms*, in the human population. "The Mll cassette shows, in principle, a simple way to study both a mutation and a related polymorphism in the gene of interest," Testa says. "This aspect of making mouse models will become increasingly more important for authentic modeling of human disease susceptibility and the way organisms respond to drugs and we think that our work shows the way to set up these models".

The new work also heralds a new era for genomic engineering in many living systems. "The Mll cassette is a first demonstration of what can be done with large DNA molecules," Stewart says. "Red/ET recombination increases the size of DNA that can be comfortably engineered by more than ten times and opens up new possibilities for genomic engineering that will filter into standard practice in the next few years."

- Russ Hodge

16 EMBL member States:

About EMBL

The European Molecular Biology Laboratory is a basic research institute funded by public research monies from 16 member states,including most of the EU, Switzerland and Israel. Research at EMBL is conducted by approximately 80 independent groups covering the spectrum of molecular biology. The Laboratory has five units: the main Laboratory in Heidelberg, and Outstations in Hinxton (the European Bioinformatics Institute), Grenoble, Hamburg, and Monterotondo near Rome. The cornerstones of EMBL's mission are:to perform basic research in molecular biology, to train scientists, students and visitors at all levels, to offer vital services to scientists in the member states, and to develop new instruments and methods in the life sciences.The Laboratory also sponsors an active Science and Society programme. Visitors from the press and public are welcome.

About EMBLEM

EMBL Enterprise Management Technology Transfer GmbH (EMBLEM) is a subsidiary and the commercial arm of the EMBL.EMBLEM,established in 1999 identifies,protects and commercialises the intellectual property developed in the EMBL-world, from EMBL-alumni and from non-EMBL third parties.The aim of EMBLEM is to facilitate and accelerate the transfer of innovative technologies from basic research to industry and hence contribute to the development of new diagnostics, drugs and therapies, to ultimately benefit the health of all.
www.embl-em.de

About the MPI for Cell Biolo gy and Genetics

The Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG) in Dresden is part of the Max Planck Society for the Advancement of the Sciences e.V.,an independent, non-profit organisation in Germany. The 80 research institutes of the Max Planck Society perform fundamental research in the interest of the general public into the natural sciences,life sciences,social sciences,and the humanities. In particular, the Max Planck Society takes up new and innovative research areas that German universities are not in a position to accommodate or deal with adequately.
Research at the MPI-CBG is conducted by 21 independent groups. Scientists from 32 nations have been working in the newly founded institute since February 2001.When the Institute has finished recruiting its scientists, about 350 people will be working in 25 research groups, concentrating on different topics of cell biology.