'No-charge' journals: A choice for low-quality science?

From Narayan's letter[1], a clear message emerges that only journals demanding publication charges are worth publishing in. In his opinion, reputable scientists choose journals that charge for publication, and those who do otherwise represent a group of poor-quality scientists. One particular instance where the author mentions that 'not … all journals which do not charge for publication are of poor quality' does not weaken this message.

I do not agree with this opinion whatsoever. Indeed, there are journals that charge and those that do not charge for publication. Narayan's viewpoint is unfair to both the journals and their authors. His suggestion to spend about 20–25% of project funds on publication charges really cannot be disregarded so easily – in fact this much share of the project funds may help a researcher conduct a significantly more thorough and complex experiment. I do think that if a researcher has a limited amount of money, a common situation nowadays, he/she should spend it on research instead of on publication charges, provided that there are high-quality journals that do not demand publication charges and are of interest to the researcher. There are such journals.

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NEWS

Gene targeting in mice using embryonic stem cells: The 2007 Nobel Prize in Medicine or Physiology

Genetic variation, which is the basis of all genetic studies and breeding programmes, results either due to recombination causing reshuffling of genes/alleles, or due to mutations generating *de novo* genetic variation. Therefore, mutations are obviously more important as a source of genetic variation, but these events are often random and undesirable. Efforts in the past, therefore, have been made to induce useful and directed mutations in the desired direction, as was done by Michael Smith, who shared with Karry Mullis the 1993 Nobel Prize in Chemistry. Efforts have also been made in the past to insert foreign DNA in a living cell and then facilitate homologous recombination between this foreign DNA and the corresponding endogenous gene in order to repair or disable this gene in a desired manner; this phenomenon is described as gene targeting.

Although in the past, the major objective of producing mutations has been improvement of crops and livestock, a renewed interest in mutation research has been witnessed in recent years due to their utility in genomics research. As we know, during the last more than a decade, a large number of species of microbes, animals and plants have been subjected to whole genome sequencing, which is generally followed by functional genomics approach involving annotation of all genes, thus assigning function to each individual gene. A number of approaches for the study of functions of genes with known sequences and unknown functions are now available, the most important being the inactivation of genes through production of knockout mice. It is this research area that has been selected for the award of the 2007 Nobel Prize in Medicine or Physiology to three scientists, including two American scientists, namely Mario Capecchi (71 years old), University of Utah at Salt Lake City, USA and Oliver Smithies (82 years old), University of North Carolina at Chapel Hill, USA and one British scientist, Sir Martin J. Evans (66 years old), Cardiff University, Wales, UK. As is often the case, these three scientists had earlier won the prestigious Lasker Award in 2001.

Production of knockout mice

Production of knockout mice, each with a specific gene disabled, became possible due to the ground-breaking discovery of homologous recombination in cultured somatic cells by Capecchi and Smithies and due to the development of the technique for culturing embryonic stem cells (ESCs) by Evans. Later, the two techniques were combined and in 1989 several laboratories reported the successful production of knockout mice, which carried each a disabled gene[1–3]. The technique allowed the study of function of individual genes with far-reaching implications in biomedicine. Knockout mice for as many as 11,000 genes (almost half of the total number of protein-coding genes in a mouse) are now available. Hopefully, knockout mice for each individual mouse gene will become available within the next few years.

Transgenic mice, homologous recombination and gene targeting

During early attempts for the production of transgenic mice, integration of foreign DNA within the genome was found to be random, and the number of copies of integrated gene varied. Therefore, this could not be used for the manipulation of endogenous genes in a desired manner. Instead, transgenic mice with specific disabled genes could be produced using homologous recombination, a characteristic of meiotic cells in higher organisms and of sexual conjugation in bacteria. The

phenomenon of homologous recombination in cultured cells was discovered with the hope that it may prove useful for either repairing defective genes or for disabling healthy genes in mice and other mammals, including humans. The first successful attempt in this direction was made as early as 1977 by Richard Axel (2004 Nobel Prize winner), who repaired cells that were defective in thymidine kinase (tk) activity by introducing a wild type herpes virus *tk* (*HSV-tk*) gene. The technique was improved by Mario Capecchi, who used a fine glass pipette to inject DNA directly into the nucleus, thus improving the efficiency of transgenesis many fold (Figure 1). Subsequent experiments conducted by Capecchi allowed detection of homologous recombination between the defective endogenous genes like *tk* and neo^R and the corresponding functional genes introduced exogenously. The event occurred at an approximate frequency of 1 in 1000 cells. Smithies similarly used homologous recombination to introduce a plasmid into the human erythroleukaemia cells. Thus, both Capecchi and Smithies demonstrated for the first time that homologous recombination does occur in cultured mammalian somatic cells at a reasonably high frequency to be useful and may therefore be utilized for repairing defective genes in mice and other mammals, including humans[4].

Embryonic stem cells

In parallel with the discovery of homologous recombination for gene targeting, the technique of culturing ESCs was also being developed by Evans in the UK. Initially, during the early 1970s, Evans developed the techniques for culturing embryonal carcinoma (EC) cells from murine testicular teratocarcinoma. These EC cells were also shown to differentiate into all kinds of cells, including skin, nerve and cardiac cells. However, one major limitation in using these EC cells for genetic modification of mice was the frequent occurrence of chromosomal abnormalities in these cells, which led to the search for cells that were karyotypically more stable; these stable cells were ESCs[5]. The technique for injecting genetically modified ESCs into blastocysts for successful production of transgenic mice using these ESCs was also developed by Evans.

Early efforts of gene modification using ESCs

Once the technique for culturing ESCs was available, the next step was to find out if these cells can be genetically manipulated through injecting foreign DNA of known specificity. Initially, during the mid-1970s, DNA from retrovirus and SV40 virus was tried and successful transfer and integration of foreign DNA in the mouse genome was demonstrated. Later, mutant genes for neomycin resistance (neo^R), hypoxanthine phosphoribosyltransferase (*HPRT*) and other genes were also used. In each case, genetically modified ESCs were used for injection into eggs or blastocysts, which were implanted into pseudopregnant foster mothers for development to term leading to the production of chimaeric transgenic mice; the chimaeric mice were then mated with normal mice to obtain the desired progeny with modified gene (Figure 1).

Combining ESC cultures with homologous recombination

Once Capecchi and Smithies were ready with the discovery of homologous recombination in cultured cells, they were looking for a technique to repair/disable

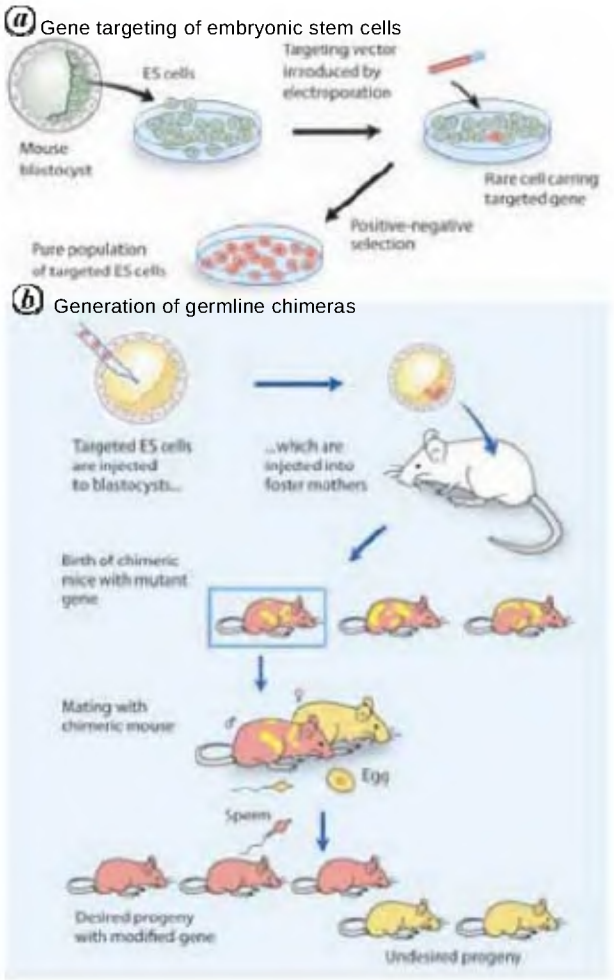

Figure 1. General strategy for gene targeting in mice. ***a***, Steps involved in gene targeting of embryonic stem cells (ESCs) involving isolation, injection of foreign DNA, enrichment for ESCs having modified genes and cloning of modified ESC (normal ESCs derived from blastocyst are shown in green and genetically modified ESCs are shown in red). ***b***, Steps involved in using genetically modified ESCs for the production of knockout mice (genetically modified ESCs and their progeny are shown in red and blastocysts shown in yellow; Source: http://nobelprize.org/nobel_prizes/medicine/laureates/2007/adv.html).

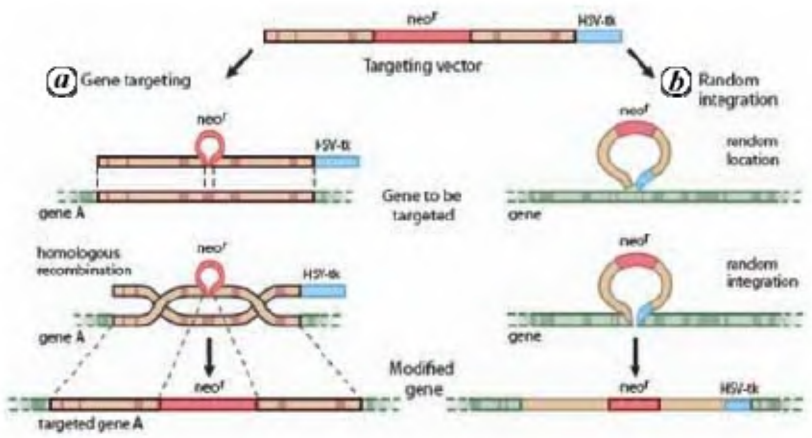

Figure 2. Basic principle involved in positive–negative selection strategy used for enrichment of ESCs containing a disrupted gene. ***a***, Steps involved in gene targeting through homologous recombination. ***b***, Steps involved in random integration of the insert (for details, see text; Source: http://nobelprize.org/nobel_prizes/medicine/laureates/2007/adv.html).

a gene in the germline. In this connection, the reported success of Evans with ESC cultures was timely and useful. Also they had available the technique used for hybridoma, where genetically modified cells could be selected on HAT (hypoxanthine aminopterin thymidine) medium. Therefore, Smithies and Capecchi first used the *HPRT* gene for repair of defective *HPRT* and later Capecchi used *neo*^{R} gene to demonstrate the repair of this gene by transformation involving homologous recombination.

In the above approach, HAT medium allows selection of repaired *HPRT*-positive cells, but this approach did not allow targeting of genes, whose function cannot be selected using a medium like HAT. Therefore, Capecchi devised a strategy described as positive–negative selection strategy for enriching ESCs, where the function of a specific gene is disrupted. In this approach, a replacement vector was used in which *neo*^{R} or any other gene intended to be introduced was present along with *HSV-tk* gene at a distance. While using this vector, one would expect that homologous recombination will allow the transfer of only the desired gene and not *HSV-tk*, but random integration without homologous recombination will transfer the entire segment carrying the desired gene and *HSV-tk*. Thus the presence and absence of thymidine kinase activity would allow detection of ESCs resulting from homologous recombination[6] (Figure 2). This technique thus allowed the production of gene-targeted mice for any gene of choice. Following this approach, several laboratories around the world reported the production of knockout mice in 1989, which is therefore considered to be the year of the birth of knockout mice[1–3].

Cre–lox system for gene targeting and conditional mutants

For the production of gene-targeted knockout mice, several other strategies were later suggested to be used in place of the positive–negative selection strategy of Capecchi. In one of these approaches, recognition sites (described as *lox*^{P} sites) for Cre recombinase enzyme were introduced first in the existing genes. When these mice with *lox*^{P} sites flanking the desired gene are mated with transgenic mice carrying the *Cre* gene, the targeted gene is modified through site-specific homologous recombination, which is facilitated due to the presence of *Cre* recombinase in the F_1 hybrids.

The *Cre–lox* system also allowed the production of conditional mutants, which made the knockout mice even more valuable to biologists, because it allowed the desired gene to be switched off (knocked-out) at a chosen time and/or in a chosen tissue, through the use of a promoter with *Cre* locus that will respond in a tissue-specific or stimulus-specific manner. This was necessary, first because 15% of genes are essential for embryo development thus causing lethality in knockout mice, and secondly because some genes become relevant for a particular disease only later in life.

Significance of gene targeting in medicine

The genomes of human and mouse, each contains as many as 22,499 genes. As mentioned earlier, ~11,000 of these genes have already been examined through gene targeting thus giving an unprecedented wealth of information that is relevant to medicine. In particular, the modelling of human diseases by gene targeting in mice has been informative and useful. Some of the diseases for which knockout mouse models and gene targeting were found to be useful include: (i) Lesch–Nyhan syndrome, (ii) cystic fibrosis, (iii) cardiomyopathy, (iv) hypertension, (v) atherosclerosis, and (vi) a variety of cancers. In recent years, Capecchi has also used the knockout approach to examine the role of specific genes in embryo development, and focused on the development of body organs and also on the way the body plan is designed.

Knockout mice could be produced for a large number of protein-coding genes through the techniques developed by Capecchi, Smithies and Evans. These knockout mice have already generated and will continue to generate a wealth of information that is relevant to medicine. Mouse models for a variety of diseases have already been produced to generate knowledge and more will certainly be produced in future.

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