INTRODUCTION OF DNA INTO HOST CELLS

The next step in a gene cloning experiment is to introduce the recombinant molecule into living cells (usually bacteria), which will then grow and divide to produce clones (Brown, 1990).

Transformation: The uptake of DNA by bacterial cells

Transformation is the process of uptake of foreign DNA (normally plasmids) by bacteria. In nature, transformation is probably not a major process by which bacteria obtain genetic material. This is reflected by the fact that only a few species (notably members of the genera *Bacillus* and *Streptococcus*) can be transformed with ease in the laboratory, and studies have revealed that these species possess sophisticated mechanisms for DNA binding and uptake. Under normal circumstances, most bacterial species (including *E. coli*) will only take up limited amounts of DNA. In order to transform these species efficiently, the bacteria have to undergo some form of physical and/or chemical treatment that will enhance their ability to take up DNA. Cells that have undergone treatment are referred to as competent cells (Brown, 1990; Turner *et al.*, 1997).

It was discovered in the early 1970s (cited by Old & Primrose, 1994) that *E. coli* cells treated with solutions containing Ca$^{2+}$ ions were rendered susceptible to take up exogenous DNA. The precise mechanism of this is not understood. Ca$^{2+}$ ions possibly cause the DNA to precipitate on the outside of the cells, or perhaps they are responsible for some kind of change in the cell wall that improves DNA binding. Thus, treatment with Ca$^{2+}$ ions affects only DNA binding, and not the actual uptake of DNA (Brown, 1990). Furthermore, when DNA is added to treated cells, it remains attached to the exterior, and is not at this stage transported into the cytoplasm. The actual movement of the DNA into competent cells is stimulated by briefly rising the temperature to 42°C (referred to as heat shock) (Figure 6). This process induces enzymes involved in the repair of DNA and other cellular components, which allow the cell to recover from the unusual conditions of the transformation process, and increases the efficiency. After this, cells are incubated in a growth medium and finally spread out on an agar plate and incubated until single colonies of bacteria grow (Brown, 1990; Turner *et al.*, 1997).
The quality of a given preparation of competent cells may be measured by determining the transformation efficiency. This can be defined as the number of colonies formed (on a selective plate) per microgram of input DNA, where that DNA is a pure plasmid, most commonly the vector to be used in a cloning experiment. Transformation efficiencies can range from $10^3$ per μg for crude transformation protocols, to more than $10^8$ per μg for very carefully prepared competent cells to be used in the construction of libraries. A transformation efficiency of $10^5$ per μg would be adequate for a simple cloning experiment (Turner et al., 1997).

**Introduction of phage DNA into bacterial cells:**

A recombinant DNA molecule, constructed with a phage vector, can be introduced into a bacterial cell by transfection or *in vitro* packaging.

**Transfection**

The process of transfection is equivalent to transformation, the only difference being that phage DNA rather than a plasmid is involved. As with transformation, the purified recombinant phage molecule is added to competent *E. coli* cells and DNA uptake is induced by heat shock (Brown, 1990).
In vitro packaging

Another method of introducing a recombinant DNA molecule, constructed with a phage vector, is in vitro packaging. To introduce such recombinants into the host cell, they are first “packaged” into phage particles in vitro, and then the bacteria are infected (transfected) with the packaged recombinant (Adams et al., 1992; Brown, 1990).

Transformation of non-bacterial cells:

Ways of introducing DNA into yeast, fungi, animals and plants are also needed if these organisms are to be used as hosts for gene cloning (Brown, 1990). The uptake of DNA into eukaryotic cells is more challenging than bacterial transformation, and the efficiency of the process is much lower. For example, in yeast and plant cells the cell wall must be digested with degradative enzymes to yield fragile protoplasts, which may then take up DNA quite readily. The cell walls are re-synthesized once the degrading enzymes are removed. In contrast, animal cells in culture, which have no cell wall, will take up DNA at low efficiency if it is precipitated on their surface with calcium phosphate. Treating the cells with a high voltage, which is believed to open transient pores in the cell membrane, may increase the efficiency of the process. This process is referred to as electroporation and can also be used to introduce cloned genes into bacterial cells. In addition, direct physical methods have been used. DNA may be microinjected directly into the cytoplasm or even the nucleus of individual animal or plant cells in culture. Alternatively, DNA may be introduced by the bombardment of the target cells with metallic microprojectiles coated with DNA. This technique is referred to as biolistics (Brown, 1990; Turner et al., 1997).