

All That Glisters is Not Gold - Genes, Transgenesis and Behavior

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Despite the power and promise of transgenic experiments, in many cases it has not been possible to draw firm conclusions from them. A series of problems will be discussed, together with possible solutions.

1. Genetic background. ES cells are most commonly made from mouse strains (such as 129) that harbour a mutation or mutations, so far unknown, that permit the outgrowth and proliferation of cells from the early embryo. ES cells are reported to have been prepared from other strains, including BL/6, but these have not been widely used. There are also potential problems of strain typing that could confound the issue (Simpson et al., 1997). Because 129 mice perform poorly on a variety of tests, transgenic mice are routinely backcrossed to a more robust strain (e.g. BL/6). This presents a problem because the control mice will tend to have the BL/6 allele (and flanking BL/6 genes) where modified or knockout mice will contain the 129 allele. This may explain, in part, irreproducibility of some transgenic experiments (Routtenberg, 1995; Gerlai, 1996; Lathe, 1997).

2. Non-specific effects. In a study addressing the relationship between gene X and behavior Y a clear problem occurs if gene X affects another aspect of body physiology (blood pressure, liver function, ...) that themselves can affect behavior Y (see Lathe, 1997)

3. Sex and other differences. Males and females perform differently (see Lathe, 1997 and references therein) – in other tests behavior evolves as a function of the age of the animal. Animals may behave differently according to the location of the apparatus (Crabbe et al., 1999). For these reasons experimental group sizes must be increased. In addition, small differences in rearing conditions can have relatively large effects on certain aspects of brain development.

4. Variegation. When genes are added to the mouse genome by the technique of pronuclear microinjection, the most common outcome is that multiple copies integrate at a single site. This has the unfortunate consequence that the expression of the transgene becomes unreliable, and mosaic 'patching' of expression takes place in most if not all lines (Dobie et al., 1996, 1997; Festenstein et al., 1996). Thus the expression status of a given transgene, during a behavioral experiment, cannot easily be assessed, and inter-animal variations are maximized. 'Knock-in' insertion of single copies at a pre-chosen site is recommended.

5. Inducibility. Ideally, one would wish to be able to turn on or off a target gene, and only at a predetermined site, by exposing the animal to a chemical inducer (Lathe and Morris, 1994). A number of techniques have emerged that should, in principle, permit the induction of transgene expression. These include the use of the derivatives of the *E. coli* tetracycline repressor protein (Gossen et al., 1995; Shockett et al., 1995; Baron et al., 1997; Kistner et al., 1996; Mansuy et al., 1998) and the receptor for the insect steroid hormone

ecdysone (Christopherson et al., 1992; No et al., 1996; Suhr et al., 1998). Transgene expression is then regulated by administration of derivatives of tetracycline or ecdysone to animals. Unfortunately, brain effects of have been reported for derivatives of both tetracyclines (Clark et al., 1997; Yrjanheikki et al., 1998) and steroids related to ecdysteroids (e.g. Catalan et al., 1984), and indirect effects of drug administration are expected. Tetracycline derivatives are also toxic (see presentation by Dr. [Isabelle Mansuy](#)).

6. Region specificity. Despite some claims, no truly region-specific gene yet exists (see Pickard et al., 1999), and expression of a given transgene cannot yet be restricted to a brain region under study.

7. Inefficiency of recombination. The efficiency of CRE-lox recombination is sometimes dependent on the tissue, with low rates of deletion reported in brain (Kuhn et al., 1995), and in some circumstances CRE expression can lead to chromosome loss (Lewandoski and Martin, 1997). It is hard to interpret the outcome of experiments in which deletion or inactivation of a target gene is driven by recombination.

8. Hybrid transcriptional / recombinational elements. These depend on receptor-recombinase fusions to produce hormonal activation of deletion (Feil et al., 1997; Brocard et al., 1998; Schwenk et al., 1998; Kellendonk et al., 1999). However, the steroids used to produce activation are brain active and can have persistent effects on behavior. With these caveats, and some others not discussed here, the outcome of transgenic experiments must be interpreted most critically.

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