EXPRESSION OF RECOMBINANT PROTEINS IN THE MILK OF TRANSGENIC MICE AND RABBITS. IMPLICATIONS FOR THE USE OF TRANSGENIC RABBITS AS BIOREACTORS

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Recent advances in mammalian gene transfer, had made it possible to produce transgenic animals with new functions. Among these is specially remarkable the targeting of transgene expression to the mammary gland. Transgenic proteins are thus secreted to the milk during lactation. The optimal end point of the above mentioned process will be the expression of the foreign gene at levels similar to those of the endogenous milk genes. At present, transgenic proteins have been expressed in the milk of transgenic mice, rats, rabbits, pigs, goats, sheep and cattle. While transgenesis in rodents is easy to acchieve, and relatively cheap, these animals can be used mainly for testing gene constructs and research purposes. Transgenesis in farm animals is handicaped by several factors ranging from difficulties in embryo availability, long reproductive intervals and lower efficiciency of transgenesis. Furthermore the cost of transgenesis in farm animals is rather high. Rabbits are indistinctively used as laboratory and farm animals. However, from the point of view of transgenesis, rabbits offer several advantages over both laboratory and farm animals; small size and relative low cost of manteinance, short reproductive cycles; high embryos yield, average milk yield of around 20 litters per doe per year. As a major drawback, not every transgene can be expressed in rabbit milk, but only those that are needed in small amounts.

We generated several lines of transgenic mice and rabbits for mammary gland specific expression. As a general rule we test gene construct in mice before making transgenic rabbits. Our basic expression cassette consists of a 6.3 kb rabbit WAP promoter and 3' sequences from the rabbit WAP gene. Chromosomal, genes, or cDNAs are then inserted. The expression levels depend on the gene construct and the species we used for transgenesis. Very low levels of biologically active human EPO were obtained in mouse (0,01 mgl^{-1}) and thereafter in rabbit milk (0,00003 mgl^{-1} {1}). The cause of such low expression is not clear, since the same gene have been expressed at much higher levels in CHO cells *in vitro*. The role of an hypothetic early fetal ectopic expression of hEPO during pregnancy, and its teratogenic activity is under investigation at present. In the case of EPO transgenic mice served nicely as predictive system.

The next step in our transgenic program is the expression of monoclonal antibodies in transgenic rabbit's milk. Transgenic mice expressing high levels (300 mgl^{-1}) of active chimaeric humanized antibodies were produced (2), and Limonta *et al.*, this book). On the other hand, we acchieved high expression of human growth hormone in the milk of transgenic rabbits {3} using a 2,6 kb mouse WAP promoter. Therefore we believe that transgenic rabbits could be capable of secreting high levels of recombinant Mabs in their milk, when appropriate gene construcs will be used.

It is well known that mosaisicism is very high (up to 25 % in microinjected rabbit embryos, {4}). Thus the number of founder animals is crutial in the search for high expressing transgenic rabbits. In our hands, in the case of two different hEPO gene constructs introduced into rabbits, only three (one male and two females) and five (three males and two females) founder animals were obtained, and none of them expressed high levels of the protein, while 11 founders (five females) were produced for the hGH transgenic rabbit line, and 2 of them expressed levels of hundreds of miligrams per liter of milk.

In conclusion, testing gene constructs in transgenic mice, and maximizing the number of non-mosaic transgenic founder rabbits, are important steps toward the use of transgenic rabbits as bioreactors.

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