Transgenic chickens as bioreactors for protein-based drugs

Simon G. Lillico, Michael J. McGrew, Adrian Sherman and Helen M. Sang

The potential of using transgenic animals for the synthesis of therapeutic proteins was suggested over twenty years ago. Considerable progress has been made in developing methods for the production of transgenic animals and specifically in the expression of therapeutic proteins in the mammary glands of cows, sheep and goats. Development of transgenic hens for protein production in eggs has lagged behind these systems. The positive features associated with the use of the chicken in terms of cost, speed of development of a production flock and potentially appropriate glycosylation of target proteins have led to significant advances in transgenic chicken models in the past few years.

The possibility of using transgenic animals for the production of therapeutic proteins was raised shortly after the development of the first method for the genetic modification of mice. Significant progress has been made towards this goal based on targeting expression of pharmaceutical proteins to secretory organs of animals, particularly the mammary gland of livestock mammals [1–4]. The predicted advantages of such production systems compared with synthesis in microbial cells or mammalian tissue culture cells include the ability to produce large quantities of posttranslationally modified and complex proteins and the possibility of providing a cheaper alternative to the use of large-scale fermentors. The cost of production of glycosylated proteins from large-scale cell cultures is considerable, and it is predicted that transgenic animal production will be more cost effective [5]. A key requirement for the production of human proteins for therapeutic purposes is that the production method should result in a protein that incorporates the posttranslational modifications of the naturally expressed protein [6]. The posttranslational modification of many proteins is essential for protein function, and if a protein is not appropriately modified it could have a short half-life in the patient and poor therapeutic efficacy. Absence of specific glycosylations can result in a protein that is immunogenic or unmask peptide epitopes that might be antigenic. The requirement for appropriate glycosylation of each therapeutic protein for overall efficacy must be evaluated for each target protein.

The basic strategy for targeting expression of a foreign protein to, for example, the mammary gland, has been to identify the regulatory sequences of milk protein genes required for high-level, tissue-specific expression and subsequently link these to the coding sequence of a therapeutic protein. Next, various methods can be used to introduce the resultant transgene into the genome of the host species [2,3]. Over the past decade, various recombinant human proteins, for example, proteins that are typically isolated from human blood products and monoclonal antibodies [7], have been successfully produced in the milk of transgenic mammals such as cows, goats, sheep and rabbits [5]. With the promise of cheaper production cost and ease of purification, it was expected that biopharmaceutical products produced in transgenic animals would quickly pass from the barnyard to the market place. Although progress has been slower than predicted, significant

Simon G. Lillico
Michael J. McGrew
Adrian Sherman
Helen M. Sang*
Roslin Institute,
Roslin,
Midlothian,
UK, EH25 9PS
*e-mail:
helen.sang@bbsrc.ac.uk

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TABLE 1

Composition of an egg produced by a hen

Constituent	Proportion of egg by weight (%)	Components of individual constituents (%)	
Albumen	60	Water	88
		Protein	11
Yolk	30	Water	50
		Lipid	30
		Protein	20
Shell	10		–

Major albumen proteins in a 60 g egg[a]

Constituent	Weight (g)	Fraction of total protein (%)
Ovalbumin	2.20	54.0
Ovotransferrin	0.50	12.0
Ovomucoid	0.50	12.0
Lysozyme	0.15	3.4

[a]Figures expressed as percentages of the total albumen proteins.
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advances are now being made and several products have reached various stages of clinical trials. Recombinant C1 inhibitor (Pharming Group NV) purified from the milk of transgenic rabbits has recently entered Phase III clinical trials. If successful, market launch for this product is expected in 2005. In addition, recombinant human antithrombin III protein produced in the milk of transgenic goats (GTC Biotherapeutics) has completed Phase III clinical trials. This product is currently under review for market authorization in Europe, the successful completion of which promises to galvanize the field of animal biopharming.

More recently, the development of genetically modified plants for production of pharmaceuticals, a potentially competitive approach to that of using animal bioreactors, has made significant progress, with many companies currently in start-up phase [8]. These methods entail the production of recombinant human proteins in the leaves or seeds of transgenic plants. The advantages of this system include low production costs and absence of mammalian-derived viral sequences and pathogens. Several recombinant proteins produced in plants have entered clinical trials and lipase from transgenic maize has been granted orphan drug status (www.meristem-therapeutics.com). However, there are two key issues that need to be addressed before plant-derived biopharmaceuticals will reach the market place. First, the glycan groups that are added to proteins are not the same in animals and plants. This problem could be circumvented by additional manipulation of the production plant species to 'humanize' the glycosylation patterns of the proteins produced. Second, and more importantly, the real or perceived issue of environmental biosafety, which involves the risk of food crop contamination by the horizontal spread of the introduced transgene to the wild-type population, must be resolved.

Why develop chickens as bioreactors?

Although there are an increasing number of options for production systems for therapeutic proteins, it is recognized that the resources of commercial production create a bottleneck. Pharmaceutical proteins produced in eggs might have significant advantages for specific target drugs, including appropriate glycosylation, lower costs than either cell culture or transgenic mammalian systems and faster scale-up.

Production of proteins in eggs

Modern layer hens produce eggs in a 20–24 h cycle, with each ovulated yolk initially acquiring layers of egg white, followed by shell membranes and eventually a shell during its passage through the 50–70 cm length of the mature oviduct. At lay, a typical egg weighs around 55–60 g, with the yolk constituting ~30%, the white 60% and the shell 10% of the total weight (Table 1) [9]. Albumen (protein) is the main constituent of dried egg white, and is biochemically relatively simple. Nine different proteins account for 87% of the total protein mass, with ovalbumin, ovotransferrin and ovomucoid being the most abundant (54%, 12% and 12%, respectively). The relatively low complexity of egg white should facilitate purification of recombinant proteins from albumen. There is considerable commercial expertise available in processing of eggs and purification of some components, for example, lysozyme.

The genes encoding egg white proteins are translated in the secretory cells of the magnum of the oviduct of the laying hen (Figure 1a,c). Secretion is stimulated as the yolk passes down the oviduct (Figure 1b). Transgenic expression of therapeutic proteins in egg white is likely to be achieved by using the regulatory sequences of the genes encoding egg white proteins to drive expression of a sequence that encodes a therapeutic protein and that has been modified to promote secretion. The regulatory sequences of two egg white protein genes (ovalbumin and lysozyme) have been characterized in detail at the molecular level and are therefore the most obvious candidate genes to modify to target expression to the oviduct. Ovalbumin has been less well-characterized because the only system in which the regulatory elements can be studied is through transient transfection of cells isolated from the oviduct of female chicks that have been stimulated by hormone injections to develop prematurely. Significant regulatory elements have been identified, including steroid response elements and a negative regulatory region that inhibits expression of ovalbumin in tissues other than the oviduct [10,11]. There is also evidence that sequences within the transcribed region of the ovalbumin locus contribute to the stability of the ovalbumin mRNA, and therefore the total amount of ovalbumin protein synthesized. It could be important to include such sequences in an