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Research review paper

Production of heterologous proteins in plants: Strategies for optimal expression

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ABSTRACT

Plants are a promising expression system for the production of heterologous proteins, especially therapeutic proteins. Currently the majority of therapeutic proteins are produced in mammalian cell lines or bacteria. In a few cases insects, yeast and fungi have been developed for production of human proteins. However, these expression systems have limitations in terms of suitability, cost, scalability, purification and post-translational modifications. Therefore, alternative expression systems are being developed in transgenic animals and transgenic plants. Transgenic plants could provide an attractive alternative in terms of low production cost and lower capital investment in infrastructure, and with appropriate post-translational modifications. The potential of plants as an expression host has not been capitalized, primarily due to lower level of expression of transgenes in plants. The present review will evaluate the rate limiting steps of plant expression systems and suggest strategies to optimize protein expression at each of the steps: gene integration, transcription, translation and protein accumulation.

Contents

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1. Introduction

1.1. Heterologous proteins

In vivo production of protein is a very complex process, which also involves post-translational modifications of protein, required for its stability and biological activity: like glycosylation, phosphorylation and proper folding. Protein synthesis is a tightly regulated process involving many enzymes and co-factors at various steps. Production of a protein outside of its natural host system is called heterologous protein production and the protein is termed as heterologous protein (Mahmoud, 2007; Rai & Padh, 2001). Heterologous proteins are divided into three major groups: therapeutic proteins or those used for clinical diagnosis, proteins used as reagents for research and study purposes and those proteins with various industrial applications. Among these proteins, proteins used for therapeutic purposes constitute a special class with the stringent quality standards and therefore demand high value.

1.2. Expression systems

Molecular biology tools have helped us cross the species barrier and produce proteins of our interest in species of our choice. This single capability has provided us with more than hundred protein therapeutics and numerous other proteins/enzymes for various industrial applications and as diagnostics. The essential tools for production of heterologous proteins are a gene or cDNA encoding desired protein, a suitable vector and a biological system (expression system) which can transcribe and translate the transgene into a desired protein. A valuable expression system should (i) be capable of producing the required protein with right conformation, (ii) have good productivity, (iii) be easy to handle and maintain, (iv) be safe and economic, and (v) afford easy downstream processing. Needless to say that, no single biological system conforms to all these criteria (Rai & Padh, 2001). Hence, over a period of time a repertoire of need based expression systems have been developed as illustrated in Fig. 1 *E. coli* was the pioneer expression system in which first therapeutic protein, somatostatin, was successfully expressed (Itakure et al., 1977). Insulin was the first FDA approved therapeutic protein produced from *E. coli* expression system (Pillai and Panchagnula, 2001). Gradually various changes were introduced in the *E. coli* expression system to produce more complex therapeutic proteins (Sorensen and Mortensen, 2005). Likewise other expression systems like mammalian cells (Moreira, 2007; Wurm, 2004), yeast (Hitzeman et al., 1981; Walsh, 2003; Wildt and Gernfross, 2005), insects (Smith et al., 1983; Donald and Jarvis, 2003), transgenic plants (Barta et al., 1986; Strasser et al., 2004) and transgenic animals (Simons et al., 1988) were gradually involved. However, *E. coli* and mammalian cell expression systems are main bioreactors for the production of therapeutic proteins. Proteins expressed in transgenic plants and animals have reached to various clinical and preclinical stages (Ma et al., 2005; Echelard et al., 2006). ATryn was first approved as a therapeutic protein produced from transgenic animal (http://www.

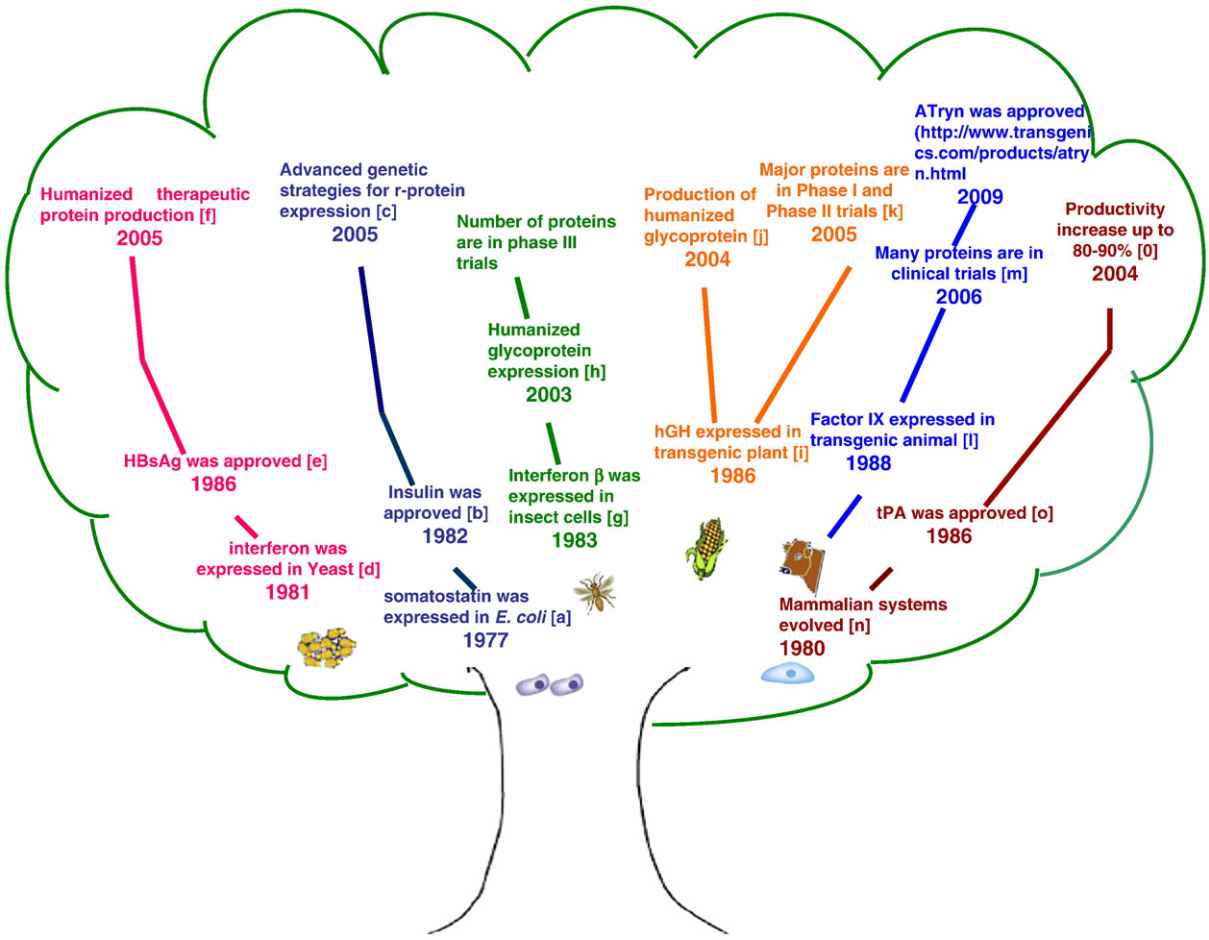

Fig. 1. Demonstrates advances in various expression systems with reported milestones. HBsAg (Hepatitis B surface antigen), r-Protein (recombinant protein), hGH (Human growth hormone), ATryn (recombinant human anti thrombin produced in goat by GTC Biotherapeutics), tPA (human tissue plasminogen activator). References: (a) Itakure et al., 1977; (b) Pillai & Panchagnula, 2001; (c) Sorensen & Mortensenl, 2005; (d) Hitzeman et al., 1981; (e) Walsh, 2003; (f) Wildt & Gernfross, 2005; (g) Smith et al., 1983; (h) Donald & Jarvis, 2003; (i) Barta et al., 1986; (j) Strasser et al., 2004; (k) Ma et al., 2005; (l) Simons et al., 1988; (m) Echelard et al., 2006; (n) Moreira, 2007; (o) Wurm, 2004.