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Review article

An overview on the strategies to exploit rice endosperm as production platform for biopharmaceuticals

Fumio Takaiwa*, Yuhya Wakasa, Shimpei Hayashi, Taiji Kawakatsu

Plant Molecular Farming Unit, Division of Biotechnology, Institute of Agrobiological Sciences, National Agriculture and Food Research Organization (NARO), Kannondai 2-1-2, Tsukuba, Ibaraki 305-8602, Japan

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ABSTRACT

Cereal seed has been utilized as production platform for high-value biopharmaceutical proteins. Especially, protein bodies (PBs) in seeds are not only natural specialized storage organs of seed storage proteins (SSPs), but also suitable intracellular deposition compartment for recombinant proteins. When various recombinant proteins were produced as secretory proteins by attaching N terminal ER signal peptide and C terminal KDEL endoplasmic reticulum (ER) retention signal or as fusion proteins with SSPs, high amounts of recombinant proteins can be predominantly accumulated in the PBs. Recombinant proteins bioencapsulated in PBs exhibit high resistance to digestive enzymes in gastrointestinal tract than other intracellular compartments and are highly stable at ambient temperature, thus allowing oral administration of PBs containing recombinant proteins as oral drugs or functional nutrients in cost-effective minimum processed formulation.

In this review, we would like to address key factors determining accumulation levels of recombinant proteins in PBs. Understanding of bottle neck parts and improvement of specific deposition to PBs result in much higher levels of production of high quality recombinant proteins.

1. Introduction

When cereal seeds are utilized as production platform of high-value biopharmaceutical proteins such as vaccines, antibodies, cytokines and bioactive peptides etc., they offer many advantages over the conventional fermenting system [1–3]. Especially, recombinant proteins produced specifically in seeds accumulate at high levels and are very stable at ambient temperature for several years without loss of bioactivity, resulting in no need for cold-chain for stock and transportation. When recombinant proteins are produced for molecular farming, enhancement of accumulation levels are inevitably desired. The accumulation levels of recombinant proteins are influenced by multiple steps, i.e. transcription, translation, post-translation modification including glycosylation, folding, assembly and proteolysis processing, transport, intracellular localization and genetic background (host genome) [4–8] (Fig. 1). Copy number of transgene and its integration site in host genome (position effect) also have influence on production yield [5,9]. Chromosome position effect can be blocked by flanking the transgene with the matrix attachment regions (MARs). On the other hand, transgene silencing sometimes occurs owing to introduced transgene copy number, repeated sequence and unusual transgene sequence itself etc [5,6,10]. In order to enhance the yield of recombinant protein, general approaches are primarily to increase transcription of the target gene by choosing strong tissue-specific promoters. Translation can be further enhanced by codon-optimization of the targeted transgene gene by especially avoiding rare codons, mRNA destabilizing sequence and cryptic intron splice sites. Specific 5′ and 3′ UTRs that enhance translation initiation and mRNA stability can also be employed. Improvement of key factors implicated in the transcriptional and translational regulation is relatively simple for expression in cereal grains. Various seed specific promoters that confer endosperm-specific expression in the desired spatial and temporal specific manner in the transgenic rice seed are now available [11,12]. Rice seed specific promoters are mainly grouped into several types based on their expression patterns; endosperm specific promoters directing mainly expression in subaleurone layer, inner starchy endosperm region and whole endosperm, embryo- and aleurone layer-specific promoter, transfer cell specific promoter and whole seed expression promoter.

In addition to the site of tissue accumulation, the extent of protein accumulation also depends on the intracellular site [13]. The available evidences indicate that optical accumulation of many biopharmaceutical proteins occurs when they are targeted to the secretory system. However, it is difficult to predict how much levels of the recombinant proteins can be finally accumulated in the endomembrane system, since

* Corresponding author.
E-mail address: takaiwa@affrc.go.jp (F. Takaiwa).

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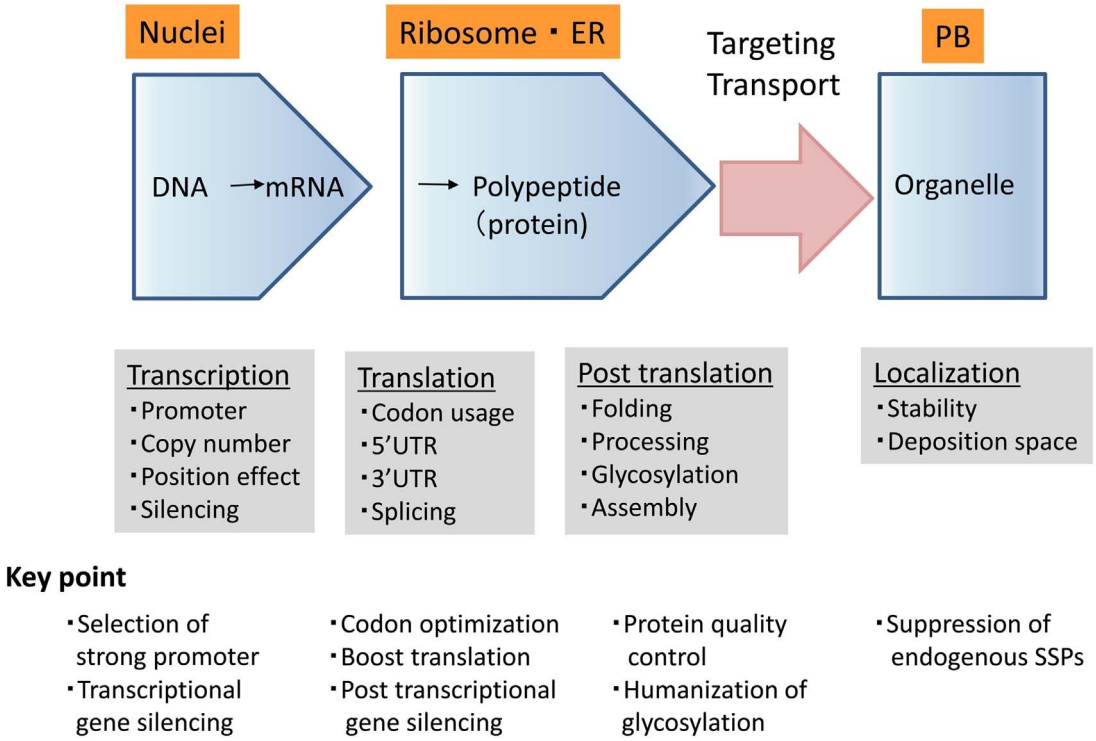

Fig. 1. Approach to enhance accumulation levels of foreign recombinant proteins in transgenic rice seeds.

they are initially checked by the ER protein quality control system after translation, are exposed to potential post-translational modification (e.g. glycosylation) or processing, and then transported to their designated intracellular compartment. Therefore, understanding the molecular mechanisms regarding ER protein quality control, targeting to the suitable intracellular compartment and its deposition is required to optimize the final product yield of recombinant proteins. These areas are the next targets to obtain high amounts of good quality recombinant proteins that can be used as biopharmaceutical proteins. In this review, we would like to address the present state of this field and to give a new prospective on production of PB-based pharmaceutical proteins.

2. Rice endosperm as production platform

Rice is one of the most important staple foods for more than half of world population. Rice seed has been utilized as an efficient bioreactor for the production of recombinant proteins due to its high biomass yield, low risk of gene flow due to self-pollination, ease of transformation and convenience of scale-up production by established cultivation system [1,14,15]. Cereal seeds including rice, wheat, barley and maize accumulate nitrogen, carbohydrate and lipids in protein bodies (PBs), amyloplasts and oil bodies, respectively. These storage products are used as nutrients for seed germination and development of the young seedling. These storage products are not uniformly accumulated in developing seeds. Amyloplasts containing the starch granules and PBs containing storage proteins accumulate predominantly in the endosperm, whereas oil bodies are mainly found in aleurone layer and embryo. PBs containing seed storage proteins (SSPs) are distributed as a gradient within the endosperm with the heaviest concentration of these storage organelles on the outer layers of endosperm cells. Inner starchy endosperm cells are filled with amyloplasts containing large starch granules, while the peripheral layers containing smaller amyloplasts.

Endosperm at the mature stage consist of four tissues; starchy endosperm, aleurone layer, basal endosperm transfer cells and embryo surrounding region cells (ESRC). Rice endosperm comprises about 90% of the total seed weight, in which starch is stored as the main reserve (85–90%). Seed proteins account for 7–15% of total seed weight which are predominantly localized in starchy endosperm cells. The SSPs are divided into four groups; albumin, globulin, prolamin and glutelin, based on difference in physico-chemical properties. The major rice seed protein is glutelins sharing homology to 11–12S globulin, and account for 60–80% of total seed protein. Glutelins are classified into four groups (GluA, GluB, GluC and GluD) [16]. Prolamins and 26 kDa α-globulin comprise 10–20% and 5% of total seed proteins, respectively. Rice prolamins are composed of four types; 16 kDa, 14 kDa and 10 kDa cysteine (Cys)-rich prolamins and 13 kDa cysteine-poor prolamins. Fourth minor class of seed proteins is water-soluble albumin such as α-amylase/trypsin inhibitors. These SSPs are synthesized in the starchy endosperm and are deposited in two distinct protein bodies called PB-I and PB-II [17]. Prolamins are packaged in PB-I which consists of ER-bounded intracisternal granules. Glutelins and globulins accumulate in PB-II which are protein storage vacuoles (PSVs) [18]. PB-I and PB-II are readily distinguishable morphologically. PB-I are spherical with a size of 1–2 μm diameter. The four types of prolamins are differentially distributed within the intracisternal granule. The 10 kDa prolamins occupy the central core while the rest forms concentric layers and rings of varying electron density [19,20]. PB-IIs are irregular-shaped high-density structure with a size of 2–4 μm. Glutelins and 26 kDa α-globulin are localized as the cystalloid region and peripheral matrix region of PSV.

Glutelin is initially synthesized as a proglutelin on the rough ER and then transported to the PSVs through the Golgi apparatus and the dense-vesicle-mediated post-Golgi trafficking pathway [21]. At PB-II, the proglutelin is proteolytically cleaved to generate mature acidic and basic subunits.

3. Targeting of high value products to PBs as deposition site in endosperm cell

Up to date, various high value recombinant proteins and peptides have been produced in rice endosperm as a production platform [22–31]. It has been known that production yields of recombinant