2A - The “go-to” Technology for Transgene Co-expression

Ekaterina Minskaia* and Garry A. Luke2

1Institute of Molecular Medicine, Av. Prof Egas Moniz, 1649-028, Lisbon, Portugal
2Biomedical Sciences Research Complex, North Haugh, University of St. Andrews, St. Andrews, Fife, Scotland

Abstract

In order to co-express multiple genes for biotechnological and biomedical applications, several approaches have been used with varying degrees of success. Currently, internal ribosome entry site (IRES) elements and “self-cleaving” 2A peptides are the most widely used. The length of the IRES can be prohibitive and IRES-dependent translation of the second open reading frame is often significantly reduced. 2A peptides have gained in popularity due to their small size and ability to consistently produce discrete proteins at an equal level. Here, we promote the use of these sequences as the “go-to” technology for co-expression of multiple proteins.

Keywords: Protein co-expression; 2A; Biotechnology; Biomedicine

Commentary

Many biotechnological and biomedical applications rely on the effective co-expression of multiple proteins. So far, multiple genes have been expressed via: (i) monocistronic vectors e.g. viral co-infection or co-transfection of plasmids expressing one protein each; fusion proteins; fusion proteins incorporating proteinase cleavage sites and (ii) polycistronic vectors where multiple genes are assembled either under the control of multiple promoters or a single promoter. IRES sequences have been used as a method to separate two coding sequences under the control of a single promoter. Despite their widespread use, they are relatively large (~600 base pairs) and expression of the downstream gene is significantly less efficient than the upstream gene [1]. A different approach using the 2A oligopeptide sequence allows multiple discrete proteins to be synthesized from a single strand of RNA, which also functions as a messenger RNA (mRNA). Not only is the 2A sequence smaller (54-174bp) than IRES elements, co-expression of proteins linked via 2A is independent of the cell type (cleavage activity is only dependent on eukaryotic ribosomes) and proteins are produced with equimolar stoichiometry [2-4]. The potential of this system is vividly demonstrated in the yeast Pichia pastoris equimolar stoichiometry [2-4,36,38-43]. Thosea asigna virus (¨T2A¨) (Table 1) [2-4,36,38-43]. Of the many 2A peptides identified to date [10,27,37], four have been widely used in biotechnology and biomedicine: F2A, equine rhinitis A virus (¨E2A¨), porcine teschovirus-1 (¨P2A¨) and Thosea asigna virus (¨T2A¨) (Table 1) [2-4,36,38-43].

The Foot-and-mouth disease virus (FMDV) 2A sequence (hereafter “F2A”) mediates “self-processing” by a novel translational effect “self-cleavage” 2A peptide. Gene sequences 1 (stop codon removed) and 2 are linked together into a single (trans) gene via a 2A sequence. The translation products are synthesized in an equimolar ratio, although, protein 1 upstream of 2A bears a C-terminal extension of 2A, and protein 2 bears an N-terminal proline residue. Finally, it should be noted that (i) 2A remains as a C-terminal extension of the upstream gene, and (ii) proline forms the N terminus of the downstream gene. The presence of N-terminal proline does not seem to affect proteins which are metabolically stable [44], but when the authentic C-terminus is required for activity or subcellular targeting of certain proteins, they should either be encoded at the C-terminus of the polyprotein or followed by cleavage sequences of the mammalian Kex2p homologue, furin (+RRRR-), -RRKR-, -RRKR-), which removes the 2A “tag” [21,38]. The presence of 2A, however, can be used for detection of protein expression and localization using anti-2A antibodies [2,3].

*Corresponding author: Ekaterina Minskaia, Institute of Molecular Medicine, Av. Prof Egas Moniz, 1649-028, Lisbon, Portugal, Tel: +351 217999566; E-mail: minskaiaek@hotmail.com

Received August 21, 2015; Accepted August 24, 2015; Published August 26, 2015


Copyright: © 2015 Minskaia E, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Biotechnological and Biomedical Applications

F2A is the most widely used 2A sequence in plant biotechnology and has been used to target multiple proteins to various subcellular compartments [13,34,45,46], to improve disease resistance [47,48], drought-resistance [49] and nutritional value through metabolome engineering [10,50]. Vitamin A deficiency (VAD) is a major global health issue which affects hundreds of millions of people. This problem arises because rice, the staple food source in countries where VAD is prevalent, does not produce vitamin A or its precursor β-carotene, which have a number of vital functions in the body including growth.

Since 2000, researchers have been engineering a transgenic variety of rice referred to as “golden rice” (Orzya sativa, GR) that includes the biosynthetic pathway for production of β-carotene [51,52]. Engineering GR, which has a number of vital functions in the body including growth, does not produce vitamin A or its precursor β-carotene, which have a number of vital functions in the body including growth. Biotechnological and Biomedical Applications

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Source</th>
<th>2A/2A-like sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>F2A</td>
<td>Foot-and-mouth disease virus (FMVD)</td>
<td>-PKYKLLNFDLDKLLAGDVEISPNGP-</td>
</tr>
<tr>
<td>E2A</td>
<td>Equine rhinitis A virus</td>
<td>-QCTYALLLKLAGDVEISPNGP-</td>
</tr>
<tr>
<td>P2A</td>
<td>Porcine teichovirus-1</td>
<td>-ATFSLILAGDVEISPNGP-</td>
</tr>
<tr>
<td>T2A</td>
<td>Thosea asigna virus</td>
<td>-EGRGSLITCGDVEISPNGP-</td>
</tr>
</tbody>
</table>

Table 1: 2A and ‘2A-like’ sequences used for protein co-expression. The –DxExnPgp- motif conserved among 2A/2A-like sequences is shown in red.

localization throughout the cell and mutated Sp2A acting as a signal lead to cheryFP localization in the exocytic pathway [37,61].

In conclusion, with the number of studies that have successfully used F2A and ‘2A-like’ sequences approaching 1000, these small peptides are proving to be the ‘go-to’ technology for co-expression of multiple proteins.

References


