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Review

Strategies for multigene expression in eukaryotic cells

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ABSTRACT

Multigene delivery systems for heterologous multiprotein expression in mammalian cells are a key technology in contemporary biological research. Multiprotein expression is essential for a variety of applications, including multiparameter analysis of living cells *in vitro*, changing the fate of stem cells, or production of multiprotein complexes for structural biology. Depending on the application, these expression systems have to fulfill different requirements. For some applications, homogenous expression in all cells with defined stoichiometry is necessary, whereas other applications need long term expression or require that the proteins are not modified at the N- and C-terminus. Here we summarize available multiprotein expression systems and discuss their advantages and disadvantages.

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1. Introduction

Multigene expression systems are central tools for many applications in biology. Therefore many new strategies for multigene expression have been established in recent years (Fig. 1). Multigene expression systems were initially developed for production of protein complexes needed for structural and biochemical analysis. Bacterial and insect cell systems were mainly used for this purpose because they are fast, cost-efficient and easy to handle (Vijayachandran et al., 2011). Mammalian systems are of course also suitable for protein production, but they are mainly used to study or to manipulate physiological processes within cells (Geisse and Kocher, 1999; Perrakis and Romier, 2008). Therefore, additional requirements have to be fulfilled by these systems.

The expression of multiple fluorescently-tagged sensors is a useful strategy to monitor several parameters simultaneously in living cells. For example, we use our MultiLabel

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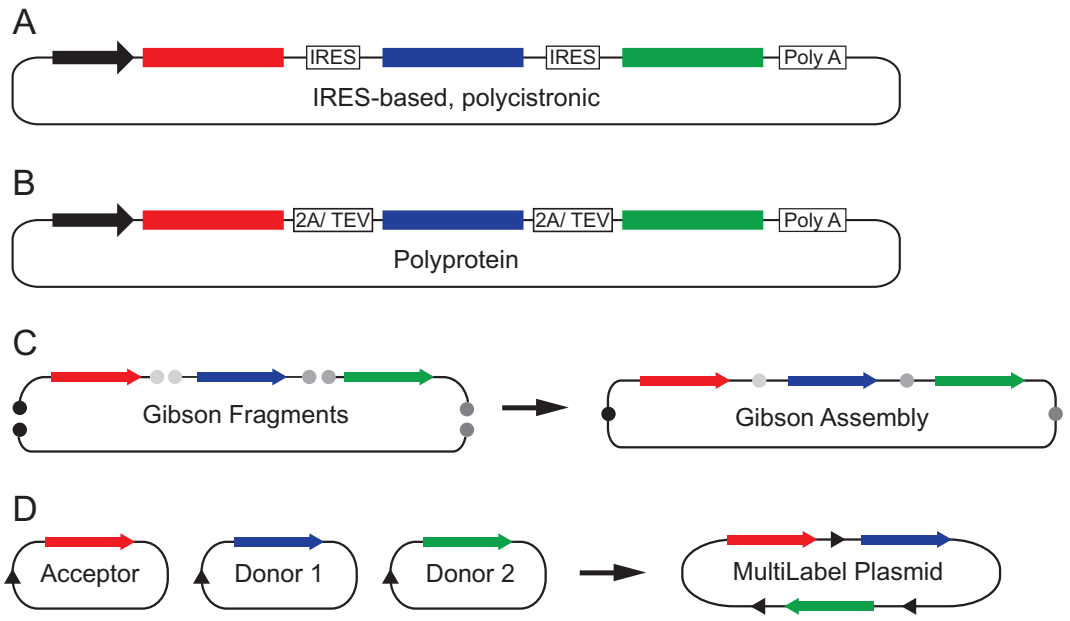

Fig. 1. Strategies for multigene expression. (A) IRES-based: a single transcript can lead to multiple proteins if the coding regions (colored boxes) are separated by an IRES element. (B) Polyprotein: a single transcript leads to a long polyprotein. The individual proteins are then released either by "self-cleavage" (see text) or by a coexpressed protease such as TEV. (C) Gibson assembly allows the assembly of independent expression cassettes (colored arrows). Assembly occurs at homologous ends of the fragments (indicated by grey-shaded circles). (D) cre/LoxP-based: Independent expression cassettes on Acceptor and Donor plasmids are assembled by a cre/LoxP reaction to yield a single plasmid. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

expression system to express Rab GTPases and phosphoinositide sensors to follow trafficking of activated receptors in living cells (Ballmer-Hofer et al., 2011; Kriz et al., 2010). Sensors for monitoring protein–protein binding are often composed of two subunits that transiently interact (Massoud et al., 2007). Fluorescence resonance energy transfer (FRET) and bioluminescence resonance energy transfer (BRET) sensors require coexpression of two fluorescent proteins or of luciferase together with a fluorescent protein (Sun et al., 2011). For bimolecular fluorescence complementation (BiFC) assays, two parts of a fluorescent protein reconstitute upon binding (Kerppola, 2013). Multiprotein expression systems allow in these assays the expression of the two proteins in a defined stoichiometric ratio.

The manipulation of cell fates is another interesting application area for multiprotein expression systems. It was shown that the expression of the transcription factors Oct3/4, Sox2, c-Myc, and Klf4 allows the reprogramming of somatic cells into pluripotent stem cells (Takahashi and Yamanaka, 2006). In the meantime several other combinations of transcription factors have been used for the reprogramming of cells. For example, it was shown that the coexpression of Asc1, Brn2, and Myt1l allows direct conversion of fibroblasts into functional neurons (Vierbuchen et al., 2010). So far these studies mainly involved coinfecting several Lentiviruses, but the coexpression from a single plasmid might be an interesting strategy to overcome the low efficiency of the process.

Drug development is another important application field for multiprotein expression systems. First, multiprotein complexes themselves might act directly as potential drugs. Virus-like particles (VLPs) consisting of components of a virus without its genetic material can be used to produce safe vaccines (Roy and Noad, 2008). VLPs were shown to stimulate the immune response and could replace attenuated viruses that are currently used for vaccination (French et al., 1990; Gheysen et al., 1989; Noad and Roy, 2003). Second, multiprotein expression systems can be used to develop screening platforms for drug discovery. Libraries of small molecules are usually used to identify new agonists or antagonists of GPCRs and receptor tyrosine kinases. Very often, receptors form heterodimeric complexes and a suitable primary cell type is not available. For example, the serotonin receptor 2A (5-HT2A) forms either a complex with the dopamine receptor D2 (D2DR) or the metabotropic glutamate receptor-2 (mGluR2) (Borroto-Escuela et al., 2010; Gonzalez-Maeso et al., 2008). In this situation, a heterologous cell line is a suitable tool. In such a cell line it is important that all cells express both receptors, since otherwise a mixed response is measured in the assay.

As described above, mammalian expression systems have to fulfill special requirements depending on the application. Many strategies have been developed in the last years to fulfill these requirements, and all systems have their advantages and disadvantages. Viral systems usually lead to high infection rates (up to 100%), but their capacity as carriers of foreign DNA is limited and the expression is usually transient. The translation of a polyprotein followed by proteolytic cleavage allows a short mRNA, but the N- and C-terminus of the mature protein are then modified which may lead to problems with proteins that need a native N- and C-terminus (e.g. Rab GTPases). Here we