Methods 60 (2013) 55–69

Contents lists available at SciVerse ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

In vitro selection of proteins with desired characteristics using mRNA-display

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ARTICLE INFO

Article history:
Available online 28 November 2012

Communicated by Kenneth Adolph

Keywords:
mRNA-display
Selection
In vitro
Library
Proteome
Networks

ABSTRACT

mRNA-display is an amplification-based, iterative rounds of *in vitro* protein selection technique that circumvents a number of difficulties associated with yeast two-hybrid and phage display. Because of the covalent linkage between the genotype and the phenotype, mRNA-display provides a powerful means for reading and amplifying a peptide or protein sequence after it has been selected from a library with very high diversity. The purpose of this article is to provide a summary of the field and practical framework of mRNA-display-based selections. We summarize the advantages and limitations of selections using mRNA-display as well as the recent applications, namely, the identification of novel affinity reagents, target-binding partners, and enzyme substrates from synthetic peptide or natural proteome libraries. Practically, we provide a detailed procedure for performing mRNA-display-based selections with the aim of identifying protease substrates and binding partners of a target protein. Furthermore, we describe how to confirm the function of the selected protein sequences by biochemical assays and bioinformatic tools.

1. Introduction

1.1. Principle of mRNA-display selection

mRNA-display permits the efficient identification of peptide sequences, via *in vitro* selection, with desired characteristics from a natural protein or a combinatorial peptide library [1–3]. The key feature of the technique is the peptide covalent linkage to its own mRNA (at 3′ end). This is accomplished by synthesis and *in vitro* translation of an mRNA template with puromycin attached to its 3′ end via a short DNA linker. During *in vitro* translation, when the ribosome reaches the RNA-DNA junction and translation pauses, puromycin, an antibiotic that mimics the aminoacyl moiety of tRNA, enters the ribosome "A" site and accepts the nascent polypeptide by forming a peptide bond. This results in tethering the nascent peptide to its own mRNA (Fig. 1). Since the mRNA (genotype) and peptide (phenotype) sequences are covalently linked in a single molecule, mRNA-display permits effective amplification of a peptide or protein sequence following selection based on its function. Iterative rounds of selection and amplification enable enrichment of rare sequences with desired characteristics. In theory, any peptide or protein sequence with desired characteristics may be selected by mRNA-display. Thus far, the applications of mRNA-display notably include, but are not limited to, mapping of the protein–protein and nucleic acid–protein interaction networks, identification of drug-binding targets, elucidation of the enzyme-substrate interactions, the development of novel and improvement of existing affinity reagents [4–11]. Fig. 1 shows the general procedure for the selection of the downstream substrates of a protease of interest (option A) and for the identification of the binding partners of a target of interest (option B).

Here, we briefly summarize the major developments, advantages, limitations and applications of mRNA-display, provide a practical step-by-step procedure of the selection process from mRNA-displayed protein libraries, focusing on the selection for binding partners and protease substrates, and conclude by pointing out the future direction of the field.

1.2. Advantage of mRNA-display selection

Compared to related protein selection methods, mRNA-display has notable advantages. First, the covalent linkage of the genotype to the phenotype allows rapid identification of isolated proteins with wide variety of desired characteristics following a functional selection. Second, the library complexity, with as many as 10^{12}–10^{14} unique sequences, close to that of the RNA or DNA pools,

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1046-2023/$ - see front matter © 2012 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.ymeth.2012.11.004

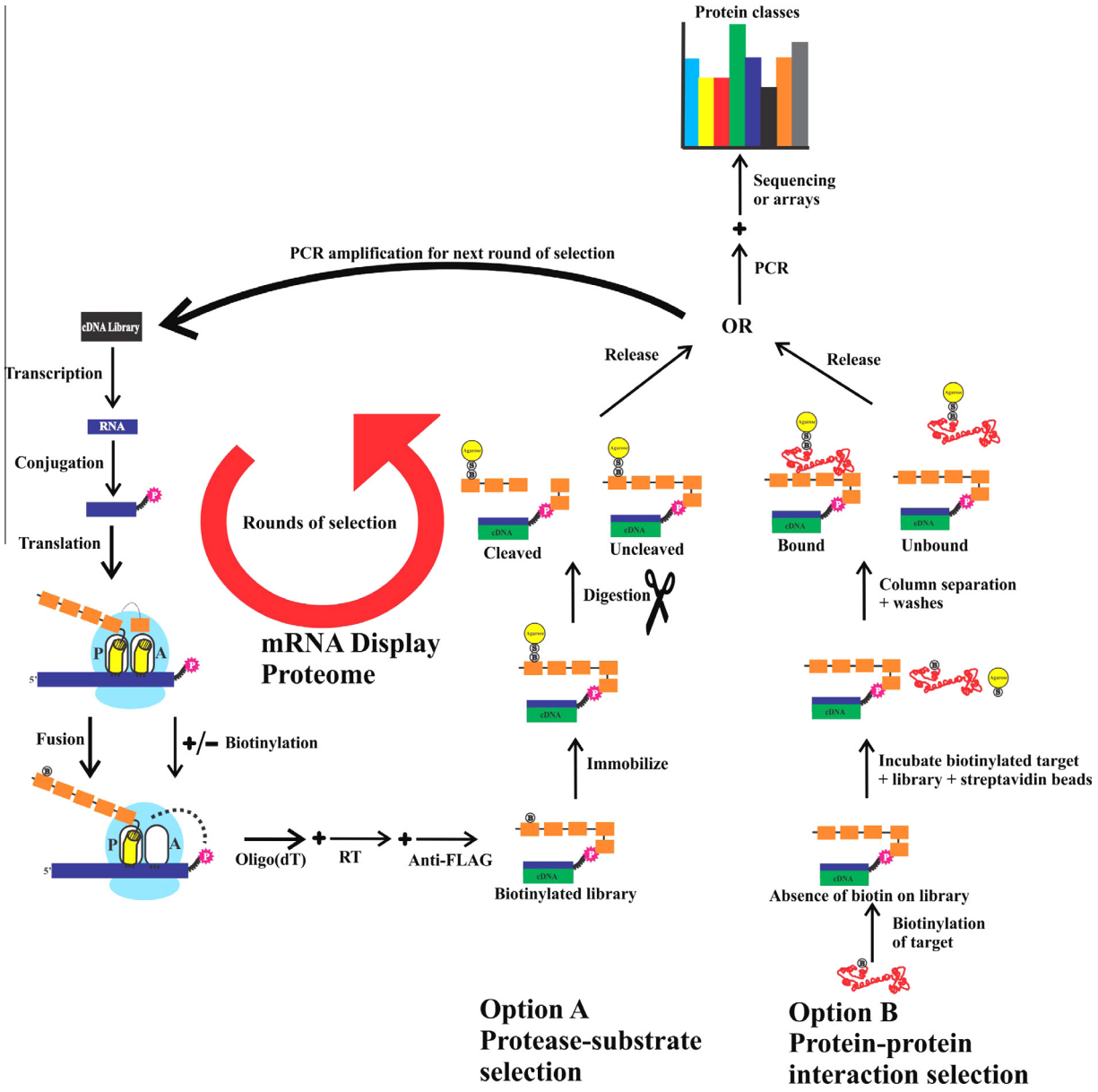

Fig. 1. Sample selection types. Schematic representation of the selection procedure for the natural substrates of a protease of interest from an mRNA-displayed proteome library (Option A). Schematic representation of the selection procedure for the binding partners of a target of interest from an mRNA-displayed proteome library (Option B). mRNA, blue; DNA, black; protein, orange; puromycin, pink circle labeled with a P; biotin, black circle labeled with a B; streptavidin, black circle labeled with an S; agarose beads, yellow circle labeled as agarose; protease, black scissors; target protein, red.

can be readily generated and selected, several orders of magnitude higher than phage display can reach. In this case, the main advantages are that the likelihood of selecting rare sequences and that the diversity of the selected sequences isolated is significantly increased. Third, the covalent linkage between the protein and its mRNA permits the utilization of any arbitrary condition in a functional selection with tunable stringency without compromising the recovery of the selected sequences. The success of a selection is highly dependent on the development of a selection scheme that allows for specific enrichment of sequences with desired characteristics, while minimizing nonspecific sequences. Many of the current technologies, including ribosome-display, are limited in terms of the binding conditions that may be used in a given screen. For example, ribosome-display with the fragile noncovalent conjugation between genotype and phenotype requires mild selection conditions. In contrast, the phenotype–genotype linkage in mRNA-display is covalent and highly stable, making it possible to perform many types of selections under very stringent conditions. mRNA-display is an entirely *in vitro* selection technique that exploits cell-free translation systems for the efficient generation of peptide sequences. Since the expression and selection steps are both performed *in vitro* and transformation is not necessary, the library complexity size is determined by the genetic material, which can be scaled up in the laboratory to increase library diversity. The amount of DNA oligonucleotides that can be synthesized, the volume of PCR that can be performed to generate the cDNA library and the volume of *in vitro* translation reaction to express the protein library can also be scaled up and tailored to increase the diversity [12]. As such, peptide or protein libraries with 10^{12}–10^{14} unique sequences can be obtained. In contrast, *in vivo* protein selection methods are limited to small, low complexity libraries due to inefficient transformations or transfections of the starting cDNA library into the organism of choice. The phage display is typically around 10^{9}, whereas the library size for cell-based selections,