European Journal of Cell Biology 91 (2012) 349–356

Contents lists available at ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.de/ejcb

Review

Mapping receptor–ligand interactions with synthetic peptide arrays: Exploring the structure and function of membrane receptors

Rudolf Volkmer*, Ines Kretzschmar, Victor Tapia

Charité-Universitätsmedizin Berlin, Institut für Medizinische Immunologie Berlin, Molecular Libraries and Recognition Group, Hessische Str. 3-4, 10115 Berlin, Germany

ARTICLE INFO

Article history:
Received 27 December 2010
Received in revised form 15 March 2011
Accepted 17 March 2011

Keywords:
SPOT technology
Peptide arrays
Peroxisomal membrane proteins
Maltose importer
Protein interaction domains
Protein–protein interaction

ABSTRACT

Development of synthetic peptide array technology started in the early 1990s. The technique originally developed by Ronald Frank has become a powerful tool for high throughput approaches in biology and chemistry mapping protein interaction sites. In this review we focus on peptide arrays applied to investigate receptor–ligand interactions, such as peroxisomal membrane receptor proteins, the maltose importer machinery and receptor proteins recognizing short linear motifs of their partners. We present several systematic sets of peptide arrays useful for mapping protein–protein- or receptor–ligand binding sites. Besides a more technical description of the peptide array preparation we discuss in detail the reliability and improvement of mapping protein–protein interactions by synthetic peptide arrays. At least proteomic approaches for mapping protein–protein interactions by peptide arrays are shown especially for the case of protein interaction domains.

Introduction

The organization of living systems depends on complex networks of molecular interactions. Proteins are a central component of such networks, since they can bind not only to other proteins, but also to phospholipids, nucleic acids and small molecules to link the diverse physiological functions of the cell. Based on these observations, it is tempting to suggest the existence of a molecular recognition code for cellular organization (Sudol, 1998). In fact, structural modules and motifs may have isolated functional "meaning" like words in human language (Smith, 1970; Jacob, 1994). To pursue this analogy, we can think of cellular wiring as a masterpiece of evolutionary tinkering, with structural elements used many times in different protein contexts, where by trial and error some rules of interconnectivity have achieved a favorable feature or message (Lichtarge et al., 1996). Therefore, it is not surprising that the idea of independent protein "linguistics" arose in the protein–protein interaction community (Gimona, 2006). Moreover, as in human language, one can interpret an analogous hierarchical organization: linear sequence of polypeptide chains, fold elements, compound structural motifs, protein complexes, and protein "machines" (Searls, 1997, 2002; Phizicky et al., 2003). The idea of protein linguistics is supported by the fact that protein architecture is modular. A protein is composed of single domains (modules) separated on discrete sequence patterns, which in turn comprise folding motifs. In general, isolated protein modules have the same globular folding as in the context of the whole protein, and therefore a reductionist approach could be applied in practice (Holm and Sander, 1998; Das and Smith, 2000).

Structural analysis of functional protein complexes suggests at least two classes of protein–protein interactions (Jones and Thorton, 1996; Ma et al., 2003; Reineke, 2009). In the first class which reflects the majority of protein–protein interactions, the complementary surfaces of the interacting partners are both extensive. This means that the residues involved in each interacting surface only come together upon protein folding (discontinuous binding sites). The second class comprises asymmetric interactions, where a protein interaction domain (PID) may dock a short linear sequence motif on the partner protein. It is a great challenge to map discontinuous binding sites (Reineke et al., 1998, 1999); however, the concept of hot spots (Bogan and Thorn, 1998; Clackson and Wells, 1995) shows the principal feasibility to interfere with interactions given by extensive surfaces. In contrast, the binding determinants of a PID may be mapped to short linear motifs matching the sequence of the ligand peptide. The importance of small recognition domains in the formation of protein complexes involving binding to short linear peptides was demonstrated in the late 1980s and early 1990s.

Since the early 1990s biological library techniques such as phage display (Smith, 1985; Scott and Smith, 1990), yeast two-hybrid (Fields and Song, 1989) and pull-down assays (affinity chromatography) in combination with mass spectrometry (Gavin et al.,

* Corresponding author. Tel.: +49 030 450 524267; fax: +49 030 450 524942.
E-mail address: rve@charite.de (R. Volkmer).
URL: http://immunologie.charite.de (R. Volkmer).

0171-9335/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.ejcb.2011.03.004

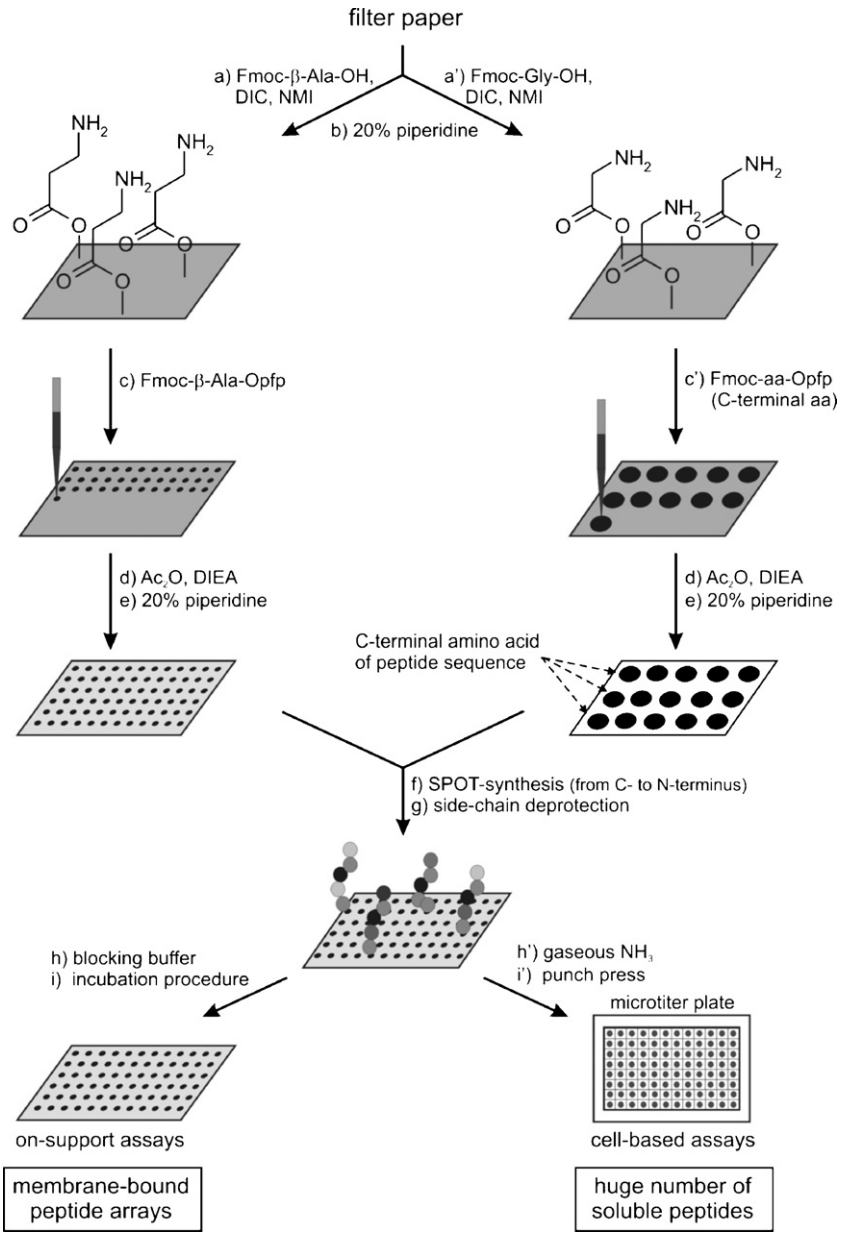

Fig. 1. The principals of SPOT synthesis. On the one hand thousands of immobilized cellulose membrane-bound peptides can be synthesized especially for on-support binding studies (left). On the other hand high numbers of soluble peptides can be generated in sufficient quality and yield suitable for several kinds of solution- and cell-based assays (right).

2002; Ho et al., 2002) have been predominately used to reveal cellular protein–protein interactions. In particular, phage display has become one of the major techniques for applying highly diverse combinatorial peptide libraries, e.g. to discover PID interaction networks. Additionally, bioinformatics and computational tools were developed to find modular domains and their cognate ligands (Linding et al., 2005). Nowadays, several databases are freely available, such as MINT (http://mint.bio.uniroma2.it/mint), a public repository for molecular interactions reported in peer-reviewed journals, and the SMART database (http://smart.embl-heidelberg.de). Array technologies, especially protein arrays, arrived late in the field of protein–protein interactions (MacBeath and Schreiber, 2000; Zhu et al., 2001) due to critical factors such as native folding stability or functionality. Peptides, in contrast, are easier to handle and retain partial features of protein function. Thus, peptide arrays are suitable to support proteomic research, particularly in the case of PID recognition, since PIDs recognize short linear amino acid strands that can be synthesized by high throughput synthetic approaches.

Here we focus strictly on protein–protein interaction mapping studies that were done within the scope of a researcher network on structure and function of membrane receptors. This includes investigation of the binding sites of membrane receptors but also mapping receptor–ligand interactions that are not membrane receptors in a classical sense.

Results and discussion

In situ peptide synthesis on cellulose membranes

Whatever the intended application of SPOT technology (Frank, 1992; Volkmer, 2009), the general strategy for parallel peptide assembly on a cellulose membrane is the same (Fig. 1). In a first step, hydroxyl groups of the cellulose membrane are transformed into