Seminars in Cell & Developmental Biology 23 (2012) 863–871

Contents lists available at SciVerse ScienceDirect

Seminars in Cell & Developmental Biology

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

Review

Quantitative phosphoproteomics to characterize signaling networks

Kristoffer T.G. Rigbolt**, Blagoy Blagoev*

Center for Experimental BioInformatics (CEBI), Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark

ARTICLE INFO

Article history:
Available online 5 June 2012

Keywords:
Phosphoproteomics
Signal transduction
Mass spectrometry

ABSTRACT

Reversible protein phosphorylation is involved in the regulation of most, if not all, major cellular processes via dynamic signal transduction pathways. During the last decade quantitative phosphoproteomics have evolved from a highly specialized area to a powerful and versatile platform for analyzing protein phosphorylation at a system-wide scale and has become the intuitive strategy for comprehensive characterization of signaling networks. Contemporary phosphoproteomics use highly optimized procedures for sample preparation, mass spectrometry and data analysis algorithms to identify and quantify thousands of phosphorylations, thus providing extensive overviews of the cellular signaling networks. As a result of these developments quantitative phosphoproteomics have been applied to study processes as diverse as immunology, stem cell biology and DNA damage. Here we review the developments in phosphoproteomics technology that have facilitated the application of phosphoproteomics to signaling networks and introduce examples of recent system-wide applications of quantitative phosphoproteomics. Despite the great advances in phosphoproteomics technology there are still several outstanding issues and we provide here our outlook on the current limitations and challenges in the field.

© 2012 Elsevier Ltd. All rights reserved.

Contents

1. Introduction 863
2. Experimental strategies in mass spectrometry-based phosphorylation analysis of signaling networks 864
3. Applications of phosphoproteomics in eukaryotic cell signaling studies 865
   3.1. G-protein coupled receptor signaling 866
   3.2. Immunology and infection 866
   3.3. Cell cycle regulation 866
   3.4. DNA damage response 867
   3.5. Stem cell differentiation and reprogramming 867
4. Concluding remarks 868
   Acknowledgements 869
   References 869

1. Introduction

Most, if not all, signal transduction pathways depend on protein phosphorylation to relay information through signaling cascades or regulate effector proteins, such as kinases, transcription factors or ubiquitin ligases, to elicit the end result of pathway activation [1]. During the last decade it became apparent that analysis of signaling networks at a system-wide level is required for understanding the dynamic and complex mechanisms of cellular signaling. This aspiration to study signaling pathways on a global scale has been among the principal motivations for developing and improving strategies in mass spectrometry (MS)-based phosphoproteomics

Abbreviations: AT1R, angiotensin II type 1A receptor; cAMP, cyclic AMP; CXCR, C-X-C chemokine receptor; DDR, DNA damage response; EGFR, epidermal growth factor receptor; ESC, embryonic stem cell; GPCR, G-protein coupled receptors; IMAC, immobilized metal affinity chromatography; iPSC, induced pluripotent stem cell; LPA, lysophosphatidic acid; MS, mass spectrometry; PTM, post translational modification; pTyr, phospho-tyrosine; RTK, receptor tyrosine kinase; TCR, T cell receptor.

* Corresponding author. Tel.: +45 6550 2366; fax: +45 6593 3018.
** Corresponding author. Current address: Freiburg Institute for Advanced Studies, School of Life Sciences – LifeNet, Albertstr. 19, 79104 Freiburg, Germany. Tel.: +49 761 203 97152; fax: +49 761 203 97451.
E-mail addresses: kristoffer.rigbolt@frias.uni-freiburg.de (K.T.G. Rigbolt), bab@bmb.sdu.dk (B. Blagoev).

1084-9521/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.semcdb.2012.05.006

[2]. Thus, the aim of phosphoproteomics and cell signaling studies converge on the need for an efficient, reliable and reproducible platform for quantitative phosphorylation analysis [3]. Numerous developments in enrichment procedures, instrumentation, quantitation strategies and software tools have been essential to enable routine application of phosphoproteomics [4] and as a consequence phosphoproteomics has matured from an exotic approach applied by only few labs to the method of choice for studying global phosphorylation in signal transduction. In this review we will focus on the improvements in experimental strategies for studying signal transduction pathways and provide an overview of the application of this technology and finally address some outstanding issues in this field.

2. Experimental strategies in mass spectrometry-based phosphorylation analysis of signaling networks

A fundamental challenge in analyzing protein phosphorylation by mass spectrometry is the low stoichiometry of phosphorylated proteins arising from the fact that usually only a small fraction of the complete complement of a given protein will exist in a particular phosphorylated form [5,6]. This constitutes a large obstacle for detection of phosphorylation sites by MS because this technology is biased toward high abundant sample components [7]. In the context of signal transduction this challenge is exacerbated by the generally low copy number of many proteins with pivotal roles in signaling cascades [8].

A major breakthrough in the detection phosphorylated tyrosine residues came with the development of phospho-tyrosine (pTyr) specific antibodies which proved very suitable for immunoprecipitation of both pTyr containing intact proteins [9–11] and also pTyr peptides obtained from endopeptidic digestion of proteins (see Fig. 1A) [12]. Furthermore, if the enrichment is performed under native buffer conditions it is possible to enrich not only pTyr containing proteins but also additional secondary interactors (see Fig. 1B) [13–15]. Although powerful, the antibody-based strategies are inherently directed toward pTyr, while generic antibodies targeting phosphorylated serine and threonine residues in sequence independent manner prove unsatisfactory over the years. Therefore complementary techniques have been developed that allow also enrichment of phosphorylated serine and threonine containing proteins and peptides. For this task the use of immobilized metal affinity chromatography (IMAC) [16–18] or metal oxides [19–21], performed either off-line or in an automated setup [22–24], has proven very successful in providing near complete enrichment of phosphorylated peptides (see Fig. 1C). These enrichment techniques are often being used in combination with additional chromatographic approaches for sample fractionation in order to reduce sample complexity and thereby to further increase the coverage of the phosphoproteome [25–29].

In addition, to the developments in phosphopeptide enrichment and fractionation, also numerous improvements in mass spectrometry have greatly facilitated identification of phosphopeptides. In particular the emergence of instrumentation utilizing the Orbitrap analyzer [30] combined with a linear ion-trap [31–33] or by itself [34,35] have been very beneficial due to their high sensitivity, sequencing speed and excellent accuracy. Furthermore, several new innovations in peptide fragmentation technology [36–40] have greatly aided identification of phosphopeptides by overcoming the poor peptide backbone cleavage of phosphopeptides that impede peptide sequencing by conventional techniques [41].

Another challenge in phosphoproteomics relates to the highly dynamic nature of protein phosphorylation involved in signal transduction [42,43]. Although establishing whether a given protein or specific amino acid residue is phosphorylated is highly

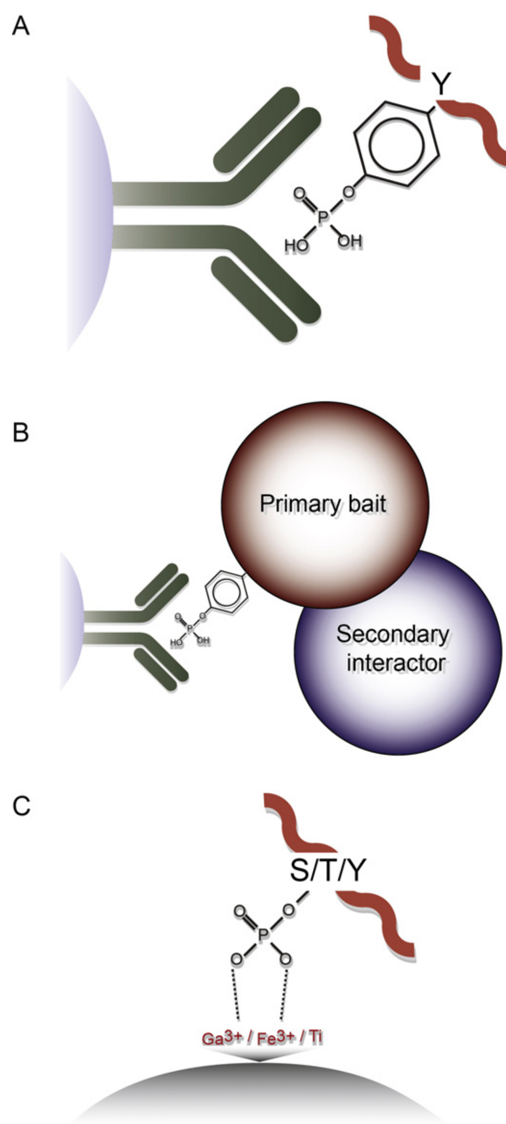

Fig. 1. Enrichment of phosphorylated proteins or peptides. To compensate for the low levels of most phosphorylated proteins in complex biological samples two general principles based on antibodies or metal affinity have been applied for enrichment of phosphorylated proteins or peptides. (A) Antibodies to phosphotyrosine residues can be used to enrich for either intact proteins or proteolytic peptides containing phosphorylated tyrosine. (B) By using phosphotyrosine specific antibodies for enrichment of intact proteins not only phosphotyrosine containing proteins (red) but also additional proteins physically interacting with the primary bait protein can be enriched (blue) providing information about protein–protein interactions. (C) To enrich for peptides phosphorylated on serine, threonine or tyrosine a number of procedures based on the affinity of different metals for phosphate groups has been developed.

informative by itself, the main goal when applying phosphoproteomics to study signal transduction pathways is usually also to quantitate the changes in phosphorylation associated with a given stimulus or cellular process. To this extent the development of a range of different strategies utilizing stable isotopes has been highly influential as those enable proteomics experiments to be performed in a quantitative manner. The basic concept of stable isotope labeling for MS-based quantitation is that isotopically different peptides behave virtually identically during mass spectrometry analysis, but are distinguishable due to the mass shift conveyed by the different isotopic composition and hence the ratio of observed intensities are directly proportional to the relative quantities of the peptides in the sample [44,45] (see Fig. 2A). Two different approaches have been the ones predominantly applied

Download English Version:

https://daneshyari.com/en/article/2202800

Download Persian Version:

https://daneshyari.com/article/2202800

Daneshyari.com