Biochimica et Biophysica Acta 1783 (2008) 2080–2088

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Functional architecture of the cell nucleus: Towards comprehensive toponome reference maps of apoptosis

Walter Schubert [a,b,*], Manuela Friedenberger [a], Marcus Bode [a], Andreas Krusche [a], Reyk Hillert [a]

[a] Molecular Pattern Recognition Research Group, Institue of Medical Neurobiology, Otto-von-Guericke-University Magdeburg, Leiziger Street 44, 39120 Magdeburg, Germany
[b] CAS-MPG Partner Institute for Computational Biology, International Faculty, Shanghai, China

ARTICLE INFO

Article history:
Received 20 May 2008
Accepted 20 July 2008
Available online 31 July 2008

Keywords:
Apoptosis
Toponome
TIS
Cell nucleus
Fluorescence microscopy
Liver cell

ABSTRACT

We have recently described the MELC/TIS fluorescence robot technology that is capable of colocalizing at least a hundred different molecular cell components in one cell. The technology reveals new hierarchical properties of protein network organisation, referred to as the toponome, in which topologically confined protein clusters are interlocked within the structural framework of the cell. In this study we have applied MELC/TIS to construct a three-dimensional toponome map of the cell nucleus of a single human hepatocyte undergoing apoptosis. The map reveals six different spatially separated toponome domains in the nuclear interior of one apoptotic cell. In the drive to decipher the apoptosis-specific molecular network on the single cell level, the present toponome map is a first milestone towards the construction of much larger maps addressing hundreds of molecular cell components across the stages of apoptosis.

1. Introduction

The cell nucleus is the largest single organelle in the cell. It contains the overall functional plan for myriads of different functionalities, such as regulated transcription of single genes, coordination of large gene programmes during cell differentiation and DNA replication, maturation of ribosomes in the nucleolus, and transfer of factors across nuclear pores. Emerging views indicate that the genome and its functional domains are dynamically linked to the overall nuclear architecture [1]. For example, specific factors are organized in 20–40 speckled structures [2], and DNA is replicated only once in each cell cycle. Considering the concept that almost every function in the cell relies on the coordinated action of molecular machines [3] at highly defined subcellular locations [4,5], the precise detection and quantitative as well as functional analysis of these machines within the cell is one of the biggest challenges in the post-genome era [6]. For example, which clusters of transcription factors are formed at the promotor region of specific genes? Would we be able to decipher the overall functional plan of the nuclear DNA by studying transcription of hundreds of specific genes in one single cell nucleus across the whole genome? How is this machinery disturbed in individual cells undergoing apoptosis? Although apoptosis-specific molecular machines, such as the apoptosome, have been identified [7,8], significant gaps remain in our knowledge of this process. There must be an apoptosis-specific molecular network with clear cut topological constraints that finally leads to this particular form of cell death (in contrast to necrosis that induces inflammatory reactions of the surrounding tissue). How can this network be quantitatively described in individual apoptotic cells? These and many similar problems addressing the functional architecture of the cell nucleus place significant methodological constraints on molecular imaging technologies able to identify the topological properties of molecular networks *in situ*, by colocalizing a very large number of molecular cell components in one cell nucleus and cytoplasm [4].

We have recently described the microscopic fluorescence robot technology MELC/TIS that is capable of imaging at least 100 different molecular cell components (MCC) in one cell [4,5]. MELC/TIS overcomes the spectral limitation of traditional fluorescence microscopy by using large dye-conjugated tag libraries and automatically bleaching a dye after imaging and re-labelling the same or another MCC in the identical sample with the same dye coupled to a tag having the same or another specificity, and repeat similar cycles with other tags for multiple times revealing multidimensional colocation patterns [4,5,9–11]. By this approach we have addressed for the time as what we term the toponome (the functional protein networks, or, the biological code of the cell) [11–14]. After the first description of the technology in 1990 [9], the importance of this approach to cell function has been increasingly recognized [5,11,15–19]. The technology has proven to solve key problems in biology and therapy research: it has (i) uncovered a new cellular transdifferentiation mechanism of vascular cells giving rise to myogenic cells *in situ*/*in vivo* [20], a finding that has led to efficient cell therapy

* Corresponding author. Tel.: +49 391 6117175; fax: +49 391 6117176.
E-mail address: walter.schubert@med.ovgu.de (W. Schubert).

0167-4889/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2008.07.019

models of muscle disorders [21–24]; (ii) discovered a new target protein in sporadic amyotrophic lateral sclerosis by hierarchical protein network analysis [25], a finding that has been confirmed by a mouse knock out model [26], (iii) uncovered a lead target protein in tumour cells that controls cell polarization as a mechanism that is fundamental for migration and metastasis formation [43] and (iv) found new functional territories in the CNS defined by high-dimensional synaptic protein clusters [27,28].

The MELC/TIS technology is unparalleled when it comes to the colocalization of a very large number of proteins using photonic microscopy. The ability to colocalize proteins on a large scale is regarded as a bottleneck in the drive to understand how proteomes are organized in individual cells and how interlocked clusters of proteins exert control over cellular functions. In the present study we have chosen a primary human hepatocyte undergoing apoptosis to construct a toponome reference map. It allows the investigator to study simultaneously in one cell changes in the cell nucleus and in the cytoplasm. We regard this mapping procedure as a new principal towards the analysis of molecular and structural changes during apoptosis. The map does not focus on specific molecular clusters in the nuclear interior but rather on the feasibility to localize simultaneously 15 different MCC as specific markers of cellular organelles, substructures of the cell nucleus and molecular events taking place in apoptosis. Together we consider this map as a reference map (a pan-cellular molecular 'landscape') that can be used in future studies to construct toponome cybermaps of the cell nucleus, in which hundreds of additional MCC can be colocalized relative to the apoptosis-toponome structures presented in this study.

2. Material and methods

2.1. Tag library

The tags used to label proteins and ligands, assembled as a toponome mapping library, are summarized in Table 1. The molecules selected for the toponome imaging procedures in this study are all reference markers of subcellular organelles or molecular systems in the cell undergoing substantial changes during apoptosis or liver disease [7,8,29–43].

Table 1
Summary of molecules labelled by MELC/TIS and corresponding locus link annotations

Molecule/moiety recognized	Official symbol	Name
c-*fos*	[FOS]	v-*fos* FBJ murine osteosarcoma viral oncogene homolog [Homo sapiens]
CD49f	[ITGA6]	Integrin, alpha 6 [Homo sapiens]
CD138	[sdc1]	Syndecan[Homo sapiens]
ConA lig.	–	Alpha-man, alpha-glc
Cytochrome C	[CYC1]	Cytochrome c-1 [Homo sapiens]
Cytokeratin	[KRT8], [KRT18]	Keratin 8 [Homo sapiens] and keratin 18 [Homo sapiens]
Golgi complex Ab-1	–	Golgi complex-specific antigen
Hoechst 3328 lig.	–	Nucleic acids
Lamp1	[LAMP1]	Lysosomal-associated membrane protein 1 [Homo sapiens]
Membrin	[GOSR2]	Golgi SNAP receptor complex membrane 2 [Homo sapiens]
Mitochondria Ab-2	–	Mitochondria specific 60 kDA nonglycosylated protein
p53	[TP53]	Tumor protein p53 (Li-Fraumeni syndrome) [Homo sapiens]
p170	[ITGAM]	Integrin, alpha M (complement component receptor 3, alpha; also known as CD11b (p170), macrophage antigen alpha polypeptide) [Homo sapiens]
Prop lig.	–	Nucleic acids
WGA lig.	–	NeuAc, $(glcNac)_2$, $(glcNac)_3$

2.2. Cells

Primary human hepatocytes were cultured on glass cover slips as described [5]. Before use for toponome mapping procedures cells were washed with 10% DMSO in PBS, fixed in fresh 4% paraformaldehyde, and washed in PBS. Cells were then air dried and snap frozen in liquid nitrogen via pre-cooled isopentane. Before use samples were rehydrated in PBS at 20 °C, incubated with normal goat serum for 30 min, and washed again in PBS, as described [5].

2.3. MELC/TIS technology for toponomics

Multi-Epitope-Ligand-Cartography (MELC) [4,9,14], recently further developed as Toponome Imaging System (TIS) [5,44], and now termed MELC/TIS technology, and its use in biology and medicine has been described previously in detail [4,5]. It is a multidimensional microscopic robot technology which runs cycles of fluorescence tagging, imaging and bleaching *in situ*. This technology combines three advances: a technique capable of mapping hundreds of different proteins at light microscopic resolution in one tissue section or cell sample; a method for selecting the most prominent combinatorial molecular patterns by representing the resulting data as binary vectors; and a system for imaging the distribution of these protein groupings in a 'toponome map'. We have shown that this approach reveals new hierarchical properties of protein network organisation [4], in which the frequency distribution of different protein groupings obeys Zipf's law [4,45].

To map a large number of proteins/ligands simultaneously in a sample, a slide with the sample is placed on the stage of an inverted wide-field fluorescence microscope equipped with fluorescence filters for FITC [5]. Fluorochrome-labelled tags and wash solutions are added and removed robotically under temperature control, avoiding any displacement of the sample and objective. In each cycle, a tag is added; phase contrast and fluorescence images are acquired by a high-sensitivity cooled CCD camera; the sample is washed with PBS and bleached at the excitation wavelengths; and post-bleaching phase contrast and fluorescence images are acquired. Usually (but not exclusively) data are acquired by using a 63x oil objective (1.4 aperture) yielding a pixel dimension of 216×216 nm (0.0467 μm^2) as outlined in detail in our earlier study [5]. Data acquisition is fully automated using home made software. By this approach, the technology overcomes the spectral limitation of traditional fluorescence microscopy [46].

2.4. Construction of 2D toponome maps

Fluorescence images produced by each tag are aligned pixel-wise using the phase contrast images, with an in- register accuracy of ±1 pixel. Background and illumination faults are then removed by flat-field correction. Fluorescent pixels are then parsed by regarding the list of fluorescence intensities I_1, I_2, I_3…I_n for proteins 1, 2, 3…n in any particular pixel or voxel as the values of an n-dimensional vector associated with that pixel. This vector can then be binarized by selecting thresholds T_1, T_2, T_3…T_n for proteins 1, 2, 3…n, and setting the vector values for any protein m to zero if $I_m < T_m$ and to 1 if not, using thresholds manually set by human experts from within an automatically generated range. The binarized images are then combined to form a list of combinatorial molecular phenotypes (CMPs) representing the proteins/molecules expressed in each pixel, or groups of CMPs, representing regions of interest. Given CMPs (protein clusters) or groups of CMPs are visualized in false color at their location. This imaging procedure can be performed in 2D or 3D [4].

A rapid method to map a large number of different CMPs in one sample is to introduce a threshold grey value for each fluorescence signal (representing the location of a labeled molecule). Each signal