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ScienceDirect
Methods 39 (2006) 291–308
METHODS
www.elsevier.com/locate/ymeth

Studying nuclear protein import in yeast

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Accepted 15 July 2006

Abstract

The yeast *Saccharomyces cerevisiae* is a common model organism for biological discovery. It has become popularized primarily because it is biochemically and genetically amenable for many fundamental studies on eukaryotic cells. These features, as well as the development of a number of procedures and reagents for isolating protein complexes, and for following macromolecules *in vivo*, have also fueled studies on nucleo-cytoplasmic transport in yeast. One limitation of using yeast to study transport has been the absence of a reconstituted *in vitro* system that yields quantitative data. However, advances in microscopy and data analysis have recently enabled quantitative nuclear import studies, which, when coupled with the significant advantages of yeast, promise to yield new fundamental insights into the mechanisms of nucleo-cytoplasmic transport.

Keywords: Nuclear transport; Karyopherins; Import; Yeast; *Saccharomyces cerevisiae*; Quantification; Transport assay; Importin; Nucleus

1. Introduction

The hallmark feature of eukaryotic cells is the presence of a nucleus, which is defined by the nuclear envelope (NE). Operationally, the NE physically separates nuclear DNA from the cytoplasm; segregating the sites of gene transcription and ribosome biogenesis from the site of protein synthesis. This compartmentalization allows the cell to strictly coordinate numerous key cellular processes, but it also demands that an astonishing number of proteins and RNAs move between the nucleus and cytoplasm. As a result, eukaryotic cell survival is dependent upon bi-directional nucleo-cytoplasmic transport pathways. To understand the mechanisms that drive nucleo-cytoplasmic transport pathways, and how they influence cell growth, numerous studies have focused on unraveling the web of physical interactions that facilitate transport [reviewed in 1–3]. Since the late 1980s our knowledge of the molecular machinery that drives this process and the roles individual components play have increased in parallel with the development of experimental techniques that have permitted the genetic identification, biochemical purification, and visualization of these cellular factors. Collectively, these studies have revealed that, operationally, nuclear transport pathways can be divided into two phases: a stationary phase, comprised of the NE and the macromolecular protein complexes, termed nuclear pore complexes (or NPCs), that are embedded in it; and a soluble (or mobile) phase, which includes nuclear transport receptors, their regulators, and the cargoes they translocate.

NPCs are central to all nucleo-cytoplasmic exchange as they are the conduits through which all communication between the nucleoplasm and cytoplasm occurs. NPCs are highly organized, evolutionarily conserved macromolecular protein assemblies ~45–60 MDa in size, ~100 nm in diameter [4,5], and composed of ~30 distinct proteins, termed nucleoporins or Nups [6,7]. Nups can be divided into three classes [reviewed in detail in [8–11]. Pore membrane proteins (or poms) anchor the NPC to the NE. Non-FG Nups that are thought to primarily provide the positioning scaffold for the third class of nucleoporins, the FG Nups. FG-Nups contain degenerate repeats of the dipeptide

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1046-2023/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ymeth.2006.07.013

phenylalanine–glycine (FG) and, with a few exceptions, are symmetrically distributed on both the cytoplasmic and nuclear faces of the NPC [6,7]. These FG repeat containing Nups are thought to facilitate active transport by providing the binding sites for transport complexes traversing the NPC and, thus, directing the rapid accumulation of proteins and other macromolecules in the nucleus or cytoplasm.

Signal-mediated nuclear transport is dependent on the recognition of signal sequences present in the cargo molecule. Proteins are marked for nuclear import and nuclear export by the presence of nuclear localization signals (NLSs) [12] or nuclear export signals (NESs) [13,14], respectively. These targeting signals are recognized by soluble transport receptors termed karyopherins, or Kaps (they are also known as importins, transportins, and exportins) [reviewed in 1, reviewed in 2]. Eukaryotic cells contain two structurally related families of Kaps: the β-karyopherins (β-Kaps) and the α-karyopherins (α-Kaps). There are 14 β-Kaps in *S. cerevisiae* and more than 20 β-Kaps in higher eukaryotes that can be divided into three categories depending on the direction in which they transport cargo: import β-Kaps, export β-Kaps and β-Kaps that are capable of both importing and exporting cargoes [reviewed in 1, reviewed in 2]. In general, a given β-Kap recognizes and interacts directly with its cargo and facilitates the cargo's translocation across the NE by interacting directly with FG-Nups [reviewed in 1,2, reviewed in [15–19]. The exception to this trend comes from the first import pathway characterized, the Kap β1/Kap α (Kap95p/Kap60p in yeast) transport pathway [20]. In this instance, the α-Kap/Kap60p acts as an adapter, bridging the interaction between the cargo and Kap β1/Kap95p, which mediates the movement of this trimeric import complex through the NPC.

The small GTPase Ran is also a key regulator of transport that provides directionality to nucleo-cytoplasmic transport, in part by regulating the formation of Kap-cargo complexes [2,10,11, reviewed in [18,21–24]. In eukaryotic cells, Ran cycles between a GTP- and GDP-bound state. The cytoplasmic localization of RanGTPase-activating protein [25,26] and the nuclear localization of it's guanosine nucleotide exchange factor (RanGEF) [27,28], generates a gradient of RanGTP across the NE. This gradient is thought to control the association and dissociation of Kap-cargo complexes [24,29]. Accordingly, import complexes form in the cytoplasm where the concentration of RanGTP is low. Once on the nucleoplasmic face of the NPC, import complexes encounter an environment rich in RanGTP. Here, RanGTP binds the import β-Kap, stimulating Kap-cargo complex dissociation and terminating the import cycle. Conversely, export karyopherins bind their cargoes cooperatively with Ran-GTP in the nucleus. These nuclear export complexes dissociate once they reach the cytoplasm, where RanGAP induces GTP hydrolysis [reviewed in 2,10,11, reviewed in 18,23,24].

Now that the roles of many of the soluble transport factors have been defined, the remaining questions about nucleo-cytoplasmic transport are rather more subtle, concerning how exactly individual transport reactions proceed, how they determine transport rates and how they are regulated. Biochemical methods have generated much useful data using purified proteins with *in vitro* solution binding assays [reviewed in [8–11]. Hypotheses based on these biochemical findings have been tested in *in vitro* nuclear import assays. These assays permeabilize the plasma membrane of tissue culture cells with digitonin [30], then re-supply the system with purified transport factors to reconstitute nucleo-cytoplasmic transport in a semi-cell-free environment. Using this technique, a number of the key soluble cellular factors and metabolites required for nuclear transport *in vitro* were characterized [21,31–42]. This *in vitro* system has also been used to measure the import rates of transport factors with or without their cargoes [43,44], and has been coupled with single molecule microscopy methods to measure the millisecond dwell times of NTF2 [45] and Kap-cargo import complexes at the NPC [44]. *In vitro* techniques have also been employed to study import in *Xenopus* oocytes, either using intact nuclei [46] or by adhering nuclear envelopes over microscopic wells, such that import into these wells recorded the transport of single NPCs [47–49]. These studies have provided information about the rates of individual transport reactions, but it is unclear whether these transport rates relate to the situation *in vivo*, where the import of a particular cargo likely competes with that of many other cargoes and transport pathways. Microinjection of labeled cargoes into intact tissue culture cells [48,95,94] or *Xenopus* oocytes [98] have thus provided more physiologically relevant import rates, that often differ from their *in vitro* counterparts.

While the techniques described above were developed to study nucleo-cytoplasmic transport in metazoan cells, *S. cerevisiae* is the most genetically tractable nucleated model organism, in which it is possible to make systematic alterations to components of its nuclear translocation machinery. Many of the techniques available to perform such manipulations in yeast are either completely unavailable or prohibitively time-consuming in metazoan cells. In yeast, proteins of interest can be expressed from either genomically integrated cassettes or from autonomously replicating plasmids [50], often as fluorescent fusion protein chimeras (FPs) attached to the Green Fluorescent Protein (GFP) from *Aequorea victoria*, or one of its derivatives [99,100]. The distribution of these proteins can be analyzed in batteries of systematic knockout or temperature-sensitive (*ts*) strains. Directed hypotheses about nucleo-cytoplasmic transport can, therefore, be tested by studying the effects that these genomic modifications have on both cell viability and gross nuclear transport [reviewed in 1–3]. Until recently however, our ability to study the more subtle aspects of nucleo-cytoplasmic transport in yeast were restricted by the experimental tools available, which were relatively limited compared to those available for use with metazoan cells. This was because yeast cells are much smaller than mammalian tissue culture cells or *Xenopus*