NUCLEAR TRAFFICKING

Edited by

Carl M. Feldherr

CELL BIOLOGY

A Series of Monographs

Nuclear Trafficking

Edited by

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Preface

The translocation of macromolecules across intracellular membranes is a fundamental cellular process that has been actively investigated since the mid-1950s. These studies have centered primarily on cotranslational and posttranslational uptake of proteins into the endoplasmic reticulum, mitochondria, and chloroplasts. Despite its obvious potential for regulating nucleic acid and protein synthesis, transport across the nuclear envelope has not been investigated at the same pace as other membrane systems. One factor that contributed to an initial lack of interest in this problem was the view that passive diffusion, with some qualifications, could account for the bulk of nucleocytoplasmic trafficking, especially protein uptake. The lack of specificity associated with simple diffusion suggested a limited regulatory role for transport across the nuclear envelope. This perception changed dramatically in the early 1980s when it was demonstrated that the nuclear uptake of karyophilic proteins is a highly selective, signal-mediated process. Since then, molecular and immunological techniques have made it possible not only to characterize targeting sequences but also to identify pore complex proteins involved in the transport process. Advances in cryoelectron microscopy and procedures for analyzing transport in isolated nuclei have further facilitated research in this area.

The object of this volume is to summarize the current state of knowledge in this rapidly expanding field. The first two chapters provide an overview of diffusion and signal-mediated transport through the pores and should be especially useful to people new to the field. The subsequent sections contain detailed accounts of pore structure and composition, nuclear localization signals, signal binding proteins, RNA efflux, and biochemical factors influencing nucleocytoplasmic exchange.

I would like especially to thank the contributing authors. The positive impact this volume has on the field is primarily due to their efforts. I am also indebted to Debra Akin for her assistance in indexing and to the editorial staff of Academic Press.

Carl M. Feldherr

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Molecular Exchanges across the Nuclear Envelope

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Diffusion between Nucleus and Cytoplasm

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I. INTRODUCTION

Eukaryotic form necessitates continual trafficking of molecules, both within the cytoplasm and the nucleus and across the nuclear envelope. Diffusion, the most fundamental mode of intracellular transport, results from the thermal motions of solutes and their continual random collisions with surrounding water molecules. Diffusion effects directed movements of all molecular species down their chemical activity gradients. Because nucleocytoplasmic movements of specific macromolecules take place in the absence of (perhaps even against) such gradients and across the nuclear envelope, cells must employ more complex mechanisms, in addition to simple diffusion, to accomplish nucleocytoplasmic transport.

For example, nucleophilic proteins (N-proteins) are synthesized in the cytoplasm like all other proteins, but are distinguishable by two characteristics:

(1) they accumulate markedly in the nucleus — some reaching nucleus to cytoplasm concentration ratios >100:1 — and (2) if isolated and microinjected into the cytoplasm, they reconcentrate in the nucleus at rates much greater than expected of diffusional transport through the nuclear envelope pores. Furthermore, some N-proteins seem to be physically larger than the available patent diameter of the pore complex. Hence, specific transport mechanisms in addition to diffusion are strongly implicated in the cytoplasm → nucleus movement of N-proteins. Throughout this chapter, discussion of diffusion *vis-à-vis* other transport mechanisms focuses on the well-studied behavior of N-proteins; however, analogous considerations apply to the nucleocytoplasmic trafficking of specific ribonucleoproteins (see Chapters 9 and 10).

What is the role of diffusion in nucleocytoplasmic trafficking? What additional roles are played by specific nondiffusional transport mechanisms? The answers essential to a full understanding of eukaryotic interphase functions and cell division are ultimately determined by the physicochemical characteristics of (1) the cytoplasm, (2) the nuclear envelope, (3) the intranuclear compartment, and (4) the transported materials themselves. Knowledge of these four parameters is presently insufficient to permit us to fully resolve diffusional from nondiffusional transport mechanisms and to measure their individual contributions, because each parameter is highly dependent on the steady-state intracellular environment and undergoes drastic irreversible alterations during typical biochemical procedures. Fortunately, *in vivo* experimental approaches using intact cells have begun to supplement biochemical approaches uniquely and soon should permit more accurate resolution and measurement of the transport mechanisms.

Systematic analysis of nucleocytoplasmic trafficking properly begins with consideration of simple diffusion in the living cell. One must characterize intracellular diffusion adequately to identify and measure other transport mechanisms. When nondiffusional mechanisms are employed, they do not supplant the diffusional processes that inexorably occur; rather, they necessarily are superimposed on diffusion. Of the four parameters listed earlier, the influence of the nuclear envelope on diffusion has been characterized best *in vivo*. Knowledge of the *in vivo* nuclear and cytoplasmic compartments is comparatively less complete, and understanding of the *in vivo* physicochemical characteristics of molecules transported between nucleus and cytoplasm is relatively rudimentary. In this chapter, the influence of the nuclear envelope on diffusion and the resulting implications for nucleocytoplasmic transport mechanisms are reviewed; the closely related topic of the physicochemical nature of transported materials is also discussed. Nondiffusional envelope transport mechanisms, as well as the roles of the cytoplasmic and intranuclear compartments in nucleocytoplasmic trafficking, are discussed in subsequent chapters in this volume.