CHAPTER 3

Subnuclear Trafficking and the Nuclear Matrix

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The nuclear matrix is the nuclear substructure that remains after the majority of DNA and soluble and chromatin-bound proteins have been removed from the nucleus.[1-3] Electron micrographs show that the animal nuclear matrix consists of the nuclear pore complexes embedded in the nuclear lamina, and a network of internal 10 nm filaments, into which granular structures and the nucleoli are embedded.[4,5] In two-dimensional protein gels of nuclear matrix preparations, more than 200 polypeptides can be distinguished, but only few components of the nuclear matrix have been cloned.[6,7] Best studied from animal systems are the nuclear lamins, a group of intermediate filament proteins that form the lamina, a filamentous protein meshwork that lines the nuclear envelope and is connected to the nuclear pore complexes. The nuclear lamins are attached to the inner envelope membrane by farnesylation and interactions with membrane-associated proteins.[8-10]

The nuclear matrix specifically binds to DNA fragments called matrix attachment regions (MARs). MARs are large, AT-rich DNA fragments with little sequence similarity, and are predicted to form the bases of chromatin loops attached to the nuclear matrix during interphase.[11] MARs bind to nuclear matrix preparations across species borders, and have been implicated in reducing position effects and increasing expression of transgenes in animals and plants.[12-14] Several MAR-binding proteins have been identified, which are components of the nuclear matrix.[15-22] In addition, proteins involved in transcription, splicing and RNA processing have been found to be associated with the nuclear matrix,[23-25] and significantly the respective processes have been shown to take place at specific sites of an isolated nuclear matrix fraction.[26-28] Together, the available data suggest that the nuclear matrix represents a core nuclear structure that is involved in chromatin organization and in different aspects of nucleic acid metabolism.

However, the nuclear matrix as a static, cytoskeleton-like structure is still an issue of intense debate (see for example, refs. 29, 30). The major objections are (1) that the procedures used to isolate the matrix might cause precipitation artifacts that we view as nuclear matrix fibers and (2) that proteins forming the interior matrix (as opposed to the lamins that form the outer shell) remain to be identified. It is probably equally possible that the observed specific subnuclear organization and the spatial "addresses" of chromatin domains, proteins, and protein complexes are caused by dynamic soluble interactions or by the association with a (either dynamic or static) solid-state structure. In any case, the information for specific subnuclear positioning exists, and it will be well worth addressing to what degree this information contributes to the biological functions of the respective molecules.

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This Chapter does not focus on the association of DNA with the nuclear matrix, the function of nuclear matrix attachment regions, or the proteins binding to MARs. Instead, it investigates the information presently available about signals involved in the intranuclear targeting of proteins, either to the nuclear matrix or to specific subnuclear domains.

Of the several arguments that can be made for the functional importance of specific subnuclear protein targeting—and a role for the nuclear matrix in that targeting—three points will be discussed here. First, several proteins have been found to contain specific signals for their association with the nuclear matrix or for their targeting to specific subnuclear sites. These nuclear matrix-targeting signals (NMTSs) sometimes overlap with other functional domains, like DNA-binding domains or nuclear localization signals (NLSs). However, at least in some cases they could be separated from these functions, showing that nuclear matrix association is independent from DNA binding and that targeting to the nuclear matrix requires a signal in addition to the nuclear import signal. In some cases, the NMTS can confer nuclear matrix targeting to a heterologous protein, and in at least one case it aids to the activity of a heterologous transcription factor, thereby suggesting a functional significance associated with the subnuclear targeting of the protein.

Second, the association of proteins with the nuclear matrix and with specific subnuclear sites has been found to be a regulated process in at least some cases, indicating that it is likely of biological significance beyond a simple "sticking together" of cellular components. And finally, the disruption of the specific subnuclear targeting of some nuclear proteins has been found associated with human diseases caused by chromosome translocations. These are strong indications that spatial information is required for the proper functioning of the respective proteins and that subnuclear mislocalization can have a severe impact on their function.

Nuclear Matrix Targeting Signals

For a number of nuclear proteins that have been found either associated with the nuclear matrix, or localized in specific subnuclear domains, amino acid sequences have been identified that are necessary and sufficient for this localization. They range from small peptide motifs capable to confer nuclear matrix localization to a heterologous protein to larger portions of the protein, which in an additive or synergistic way contribute to nuclear matrix association. The following gives an overview over the motifs mapped in different nuclear proteins, including transcription factors, DNA- and RNA-binding proteins, viral proteins, kinases, and kinase adapters. Figure 1 shows a compilation of the locations and sequences of these motifs. As discussed below, there is presently no consensus sequence that can be derived from their comparison.

Steroid Receptors

Steroid receptor binding to the nuclear matrix was first described in the 1980s and was an early realization of a potential functional association of a regulatory protein with a structural component of the nucleus.[29,30] The domain necessary for association with the nuclear matrix has been narrowed down in the human glucocorticoid receptor (hGR) and the human androgen receptor (hAR).[31] Both proteins consist of an N-terminal domain involved in activation, a central DNA-binding domain (DBD) followed by a tau2 transactivation domain, and a C-terminal steroid-binding domain. While in these early studies the domain required for nuclear matrix attachment was localized to the C-terminal steroid binding domain in hAR, both the DNA-binding domain and the C-terminal domain of hGR were found to be required.

A more detailed mapping of the NMTS of the hGR was performed by Tang et al,[32] who showed that the DBD in combination with the C-terminal tau2 transactivation domain constitute an NMTS of the hGR. Neither the DBD nor the tau2 domain alone was sufficient for nuclear matrix binding and the tau2 domain alone could not confer nuclear matrix binding to the heterologous GAL4 DNA-binding domain. Transactivation and nuclear matrix binding