CHAPTER 12

Nuclear Import of DNA

David A. Dean and Kerimi E. Gokay

Nuclear trafficking of macromolecules usually brings to mind the nuclear import of NLS-containing proteins and certain RNAs and the export of NES-containing proteins and mRNAs. One macromolecule whose nuclear import is often overlooked and under-appreciated is exogenously administered DNA. While extrachromosomal DNA may not be a "normal" species in the cell, its nuclear localization is integral to the life cycles of many pathogens and necessary for the success of transfections in the laboratory and gene therapy in the clinic. Moreover, the movement of DNA from the cytoplasm to the nucleus remains one of the major barriers to efficient gene transfer and expression (Fig. 1). Without localization of DNA to the nucleus, no transcription, replication, integration, maintenance, or "gene therapy" can take place. Surprisingly, there has been relatively little attention directed toward either discovering or exploiting the mechanisms used by the cell to direct DNA to the nucleus, despite its importance in gene therapy. The discussion that follows will highlight our working knowledge of the mechanisms of DNA nuclear import in both non-viral and viral systems.

The Nuclear Envelope Is a Barrier to Gene Delivery

That the nuclear envelope presents a major barrier to gene transfer and viral infections was realized over 20 years ago in seminal experiments by Capecchi and others in which plasmids that had been microinjected into the cytoplasm were found to be virtually incapable of directing gene expression while those injected into the nucleus were highly proficient for gene expression.[8] Using similar microinjection strategies, Graessman demonstrated that when 1000 to 2000 copies of a plasmid were injected into the cytoplasm, less than 3% of the expression was seen as compared to cells injected in the nucleus with the same number of plasmids.[33] Other experiments in a variety of mammalian cell types,[63,84] as well as in *Xenopus* oocytes[104] has confirmed that plasmids injected into the cytoplasm are much less capable of directing gene expression than those injected into the nucleus. The same is true for many viral genomes: direct nuclear injection, instead of cytoplasmic injection, of the DNA genome from SV40 or a reverse-transcribed retroviral genome resulted in 10 to 100-fold more infectious virus particles in a given time.[32,49,64]

During mitosis, the nuclear envelope breaks down, eliminating a major barrier to gene transfer. If plasmids or viral DNA genomes are present in the cytoplasm, they have unencumbered access to the nuclear compartment during this stage of the cell cycle. By contrast, in non-dividing cells, the nuclear envelope provides a substantial barrier to the DNA (see above). Indeed, it has been demonstrated that retroviruses cannot productively infect non-dividing cells due to the fact that their reverse-transcribed viral genomes (rtDNA) cannot traverse the nuclear envelope to gain access to the nucleus. However, when the cells undergo mitosis, rtDNA can localize to the nucleus, integrate, and lead to new rounds of replication.[54,62] Similarly, it is greatly appreciated by researchers the world over that non-dividing cells (growth-arrested cells, confluent cells, primary

Nuclear Import and Export in Plants and Animals, edited by Tzvi Tzfira and Vitaly Citovsky.

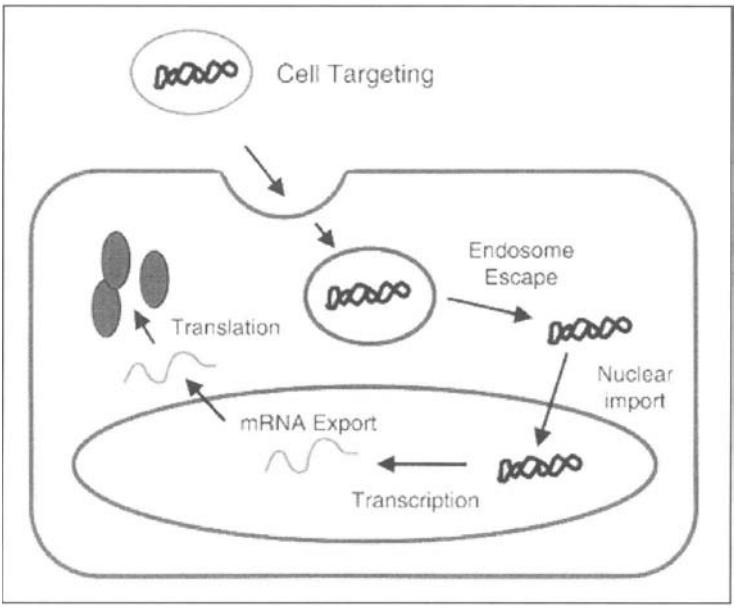

Figure 1. Cellular barriers to gene delivery. Extracellular DNA, delivered to cells in either viral particles, liposomes, or other vehicles, must traverse the plasma, endosomal, and nuclear membranes before any transcription, replication, or integration can occur.

cells, etc.) are exceedingly difficult to transfect. Again, the main reason for this is that the nuclear envelope restricts access of exogenous DNA to the site of transcription. In at least a number of the cells of an actively dividing population, DNA is able to enter the nucleus and express itself. Using primary human airway epithelial cells, it was shown that actively dividing cells, identified by BrdU incorporation, were ten times more likely to express a transferred gene product than BrdU-negative cells.[27] In a more recent study, synchronized cells transfected by a variety of techniques in the G2 or G2-M stage expressed 50- to 300-fold more gene product than those transfected in G1.[6] Thus, nuclear import of DNA is crucial to gene transfer reactions.

During transfections, large numbers of plasmids are delivered into the cytoplasm of cells, but only a fraction of these plasmids make it to the nucleus for gene expression. In one recent study, HeLa and CV1 cells (1-2 x 10^5 cells) were transfected with fluorescently labeled plasmids (1.25 μg) using liposomes, and the amount of DNA in the cytoplasm and nucleus was quantified by flow cytometry.[43] Within 2 hours, approximately 2000 copies of plasmid were found to be intracellular in either cell type, and by 24 hours, the number had increased only slightly. These numbers are in close agreement with previous studies.[14,85] However, when the amount of DNA in the nucleus was measured, more than 60% of the plasmids were in the nuclei of HeLa cells, versus 30% for CV1 cells.[43] One interpretation of these results is that different cell types may be more or less efficient at nuclear targeting of DNA than others. Alternatively, differences in cell division rates between the cells or differences in rates of cytoplasmic degradation of plasmids[18,52] could also account for the differences. Nonetheless, relatively few plasmids enter the nuclei of dividing cells, let alone those of non-dividing cells. In studies from our laboratory, it took approximately 30 to 100-times more plasmid injected into the cytoplasm of dividing cells to observe the same level of gene expression as compared to nuclear-injected DNAs, again illustrating that nuclear import is a relatively inefficient process, even when the nuclear envelope breaks down.[20]