ABSTRACT

The eukaryotic cell is compartmentalized into different organelles which are functionally distinct. The precise sorting and translocation of proteins from the site of their synthesis to their proper destination is crucial for the cell. The nucleus, where the genetic material is stored, transcribed and replicated, is separated from the cytoplasm by the nuclear envelope, which plays an important role in the regulation of macromolecular transport, from and into the nucleus. The nuclear envelope consists of two lipid bilayers, the inner and outer nuclear membranes, separated by a cisternal space. The outer nuclear membrane is continuous with the endoplasmic reticulum whereas the inner nuclear membrane is associated with a fibrous meshwork of proteins called the nuclear lamina. The nuclear envelope is perforated by the pore complexes which are large proteinaceous assemblies.

Nuclear pores are thought to be the major route of macromolecular traffic across the nuclear envelope. Protein transport into the nucleus is highly selective and energy dependent. Many nuclear proteins have been shown to have, in their mature structure, a stretch of 7-15 amino acids, which is necessary and sufficient for their nuclear localization and is referred to as the nuclear localization signal (NLS). But the exact mode of action of the NLS in transport is not known. Most of the proteins associated with nuclear membranes or the pore complex have not been well-characterized. Studies on the changes in protein composition of the nuclear envelope during embryonic development, and the role of different proteins in the assembly and disassembly of the nuclear envelope during the cell cycle have been initiated only recently.

The work described in this thesis was undertaken in order to, firstly, characterize the nuclear envelope in more detail and study the lamina composi-
tion and organization during embryonic development. Secondly, the binding of a prototype NLS to the nuclear pore complex has been characterized using photo-labelled synthetic NLS peptides. Finally, monoclonal antibodies have been produced against nuclear envelope proteins in order to study the functional importance of different proteins in nuclear transport.

The thesis is divided into six chapters and the contents of each chapter are given briefly below.

Chapter I is the introductory chapter where a brief history of the progress of studies on the nuclear envelope, details of the structure of the nuclear envelope, its role in the control of macromolecular traffic and the nature of the NLS are described.

Chapter II gives the source of chemicals and other materials, and describes the methods used in this study. Procedures of subcellular fractionation, enzyme assays, biochemical estimations of proteins, nucleic acids and phospholipids, electrophoretic procedures, microscopic techniques, methods of cell culture, radiolabelling of proteins, and synthesis of amino acid derivatives are described in this chapter.

In Chapter III, a detailed account of the isolation and characterization of nuclear envelopes is given, followed by studies on the developmental changes in the organization of the lamina. Nuclear envelopes were isolated from mouse or rat liver nuclei by nuclease digestion followed by salt extractions. Purified nuclear envelopes were characterized morphologically by phase contrast and electron microscopy and were seen as intact, double-layered, empty vesicles. Using marker enzyme assays, nuclear envelopes were shown to be free of cytosolic, mitochondrial and plasma membrane contamination. SDS-polyacrylamide gel
Electrophoresis confirmed the absence of histones (major intranuclear proteins) in purified nuclear envelopes. Using an immobilized water-insoluble iodinating reagent (Iodogen), surface specific radioiodination of purified nuclei was carried out and nuclear envelopes were isolated from labelled nuclei. Proteins labelled in this way were analysed by SDS-PAGE and autoradiography. In all, 13 polypeptides of molecular masses 145, 115, 98, 85, 75, 70, 65, 54, 50, 45, 40, 38, and 36 kDa were identified and were shown to be integral membrane proteins or part of the pore complex by biochemical fractionation procedures.

Lamin A, B and C are the three proteins involved in the formation of the nuclear lamina in mammalian cells. Polyclonal antibodies were raised against purified lamin B and lamins A/C. The specificity of the antibodies was determined and they were used in the study of the lamina composition of adult and fetal mouse liver nuclei. Lamin B was found to be the only lamin present in fetal nuclear lamina. Using a blot binding technique, the association of lamin B with nuclear envelope and lamina proteins was studied. It was observed that lamin B binds to at least three membrane proteins (96, 54 and 34 kDa) and to lamin A and C in adult nuclear envelopes, but only to the 54 and 34 kDa proteins and lamin B itself in fetal nuclear envelopes, where lamin B appears to be hyper-phosphorylated.

In Chapter IV, photolabelling studies on the binding of a prototype NLS to nuclear envelope proteins are described. Recent observations have suggested that transport of proteins into the nucleus occurs in at least two steps, the first step being the binding of the nuclear protein at the nuclear envelope, followed by the energy-dependent translocation of the protein into the nucleus. Based on these findings and the nature of the NLS, it was postulated in this study that some component of the nuclear envelope in or near the pore complex, could be involved in the recognition of the NLS of nuclear proteins, and may be playing an
important role in selective transport. The NLS of SV40 large T antigen was chosen to study the interaction of NLS sequences with nuclear envelopes, as it is the prototype NLS and has been well-characterized. Both native and mutant (nonfunctional) NLS peptides were synthesized by solid phase peptide synthesis. The strategy of photoaffinity crosslinking was used to identify NLS-binding proteins. A photoactivatable group (azidosalicylic acid) was attached to the amino terminus of the peptide and the photolabelled peptide was radioiodinated. This peptide was photoactivated in presence of nuclei, nuclear envelopes and other cellular fractions. It was observed that the native NLS but not the mutant NLS binds specifically, and in a saturable manner to a 60 kDa protein of nuclei and four proteins (67, 60, 53, and 47 kDa) of nuclear envelopes. The 60 and 67 kDa polypeptides were localized to the pore complex and the 53 and 47 kDa to the periphery of the envelope.

In Chapter V, the production of monoclonal antibodies against total nuclear envelope proteins, characterization of these monoclonal antibodies, and the effect of some of these antibodies on NLS-binding to nuclear envelope proteins are described. Splenocytes from mice immunized with purified rat liver nuclear envelopes were fused with SP2/O myeloma cells. Successful hybridomas, selected in HAT medium, were screened by an enzyme-linked immune assay (ELISA) for antibodies produced against nuclear envelopes. Nine positive clones were obtained and the antibodies were further characterized by Western blot analysis, immunolocalization assays and effect on NLS-binding assay. Two antibodies specifically interfered with the photolabelling of NLS-binding proteins.

In Chapter VI, the results obtained in Chapters III, IV and V are discussed in light of the present status of the work in the field.
LIST OF PUBLICATIONS


