4.6. Pathobiochemistry of the Nucleus

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The nucleus is at the focal-point of cellular life in eukaryotic organisms, featuring an extended portfolio of various cellular events far beyond the replication and transcription of DNA. Due to the strategic position of nuclear structures, many nucleus-related pathobiochemical features have already been described in previous chapters on signal transduction, metabolism and individual pathological states. The current chapter therefore focuses on the relationship of the cell nucleus to other cellular compartments (mostly the cytoplasm) and summarizes the changes occurring in various pathological conditions.

Structure of the Cell Nucleus and its Pathology

In the evolution of advanced monocellular organisms, became separated genetic material, that is, DNA, by a complex protein-membrane structure approximately 1.5 billion years ago. This had a number of advantages: Since DNA was protected by a separate compartment, (1) DNA-length could be extended, and the increasing danger of DNA breaks by cytoskeletal movements could thus be avoided. An extra level of DNA protection could be achieved by lowering the redox potential of the nuclear compartment below the already reduced cytoplasm.

(2) The separation of RNA and protein synthesis provided additional options for regulation. These extra regulatory events are the 'maturation' of primary RNA transcripts, their transport rate, the spatial and temporal organization of their nuclear transport, and of that of regulatory proteins. Concentration gradients of various ions and small molecules provided an additional layer of regulatory complexity. Moreover, the complex structure of the nuclear pore complex (described below) serves as an 'organization center' during mitosis, whe the whole nucleus has to be disassembled and reassembled again.

(A) The major structural elements of the cell nucleus are shown on *Fig. 1*. The following characteristics of nuclear structure should be highlighted:

(1) *The nucleus is not separated from the rest of the cellular organelles*. The double membrane separating the nucleus from the cytoplasm is connected to the membrane of the endoplasmic reticulum, and the cell nucleus is also tightly connected to the cytoskeletal structure.

(2) *The cell nucleus is not uniform*. It consists of a nucleolus, as well as euchromatin and heterochromatin structures, consisting of DNA organized into a looser, active or a tighter, inactive form, respectively. The nucleus also contains a large number of 'centers' and 'granules'. The exact nature, function, dynamics and connections of these centers and granules are the subject of intensive research. Many enzymes, lipids and small species, such as calcium ions, are distributed in a highly uneven fashion in the cell nucleus.

(3) *The nucleus*, contrary to the figure shown below, *is not static*. During replication and transcription processes the structure of the nucleus changes continuously. Core histones, for example, have a residence half-life of only a few minutes in the nucleosomal structure. Moreover, a complete disassembly-reassembly cycle is performed at each mitotical event. These are remarkable features given thatthe nucleus has an extremely compact structure in which the accomplishment of such gross rearrangements is difficult.

(B) The structure of the cell nucleus is grossly rearranged in malignant cells. As special features the polymorphism of the chromatin structure (hyperchromasia) and the proliferation of the nucleolar volume have all been observed during tumor development. The extension of nucleolar volume can explained by the fact that ribosomal RNA synthesis and

Table 1. *Damage of nuclear proteins in various diseases*

Damaged Nuclear Protein	*Disease*
Lamin A, C	*Emery-Dreifuss muscular dystrophy, autosomal recessive mandibuloacral dysplasia, cardiac and skeletal muscle diseases, lipodystrophy and premature ageing*
Emerin (nuclar membrane protein)	*Emery-Dreifuss muscular dystrophy*
LBR (lamin B binding protein)	*Pelger-Huet anomaly, Greenberg dysplasia*
SMN ("survival motor neuron" protein)	*Spinal muscular atrophy*
SIX5 (homeobox gene protein)	*Myotonic dystrophy*
Calpain 3 protease enzyme	*Limb-girdle muscular dystrophy, type 2A*
PABP2 (poly(A) binding protein 2)	*Oculopharyngeal muscular dystrophy*
Androgen receptor	*Spinal and bulbar muscular atrophy*
Ataxin	*Hereditary ataxias*

maturation take place in the nucleolus, and malignant cells need a much larger number of ribosomes to help their expanded protein synthesis. The nucleolus of cancer tissue cells is enriched in nucleolin- and numatrin-related silver-stainable granular structures (silver-staining nucleolar organizer regions, AgNORs). The expansion of these granules can be explained by the key function of nucleolin and numatrin in rRNA maturation. However, the exact function of AgNOR regions has not been completely elucidated.

Changes in the structure of malignant cell nuclei are related to the genetic instability of tumor cells. However, the background of these changes also involves an increase in the ratio of lamin B to lamin A. Lamin A is the major constituent of the nuclear structure of differentiated cells. Mutations of lamin A and other proteins have been observed in various dystrophies and other diseases. A summary of these changes is given in *Table 1*.

Nuclear structure becomes damaged in several pathological states to such an extent, that various elements of the cell nucleus escape from the cell. (As an alternative explanation, the dynamics of the cellular membrane structure are increased to such an extent, that traffic-control mechanisms become overridden.) Under such pathological conditions the immune system may recognize these normally well-packed proteins as 'danger-signals', and may launch a massive autoimmune attack against them. Thus anti-nuclear antibodies have been observed in systemic lupus erythematosus, rheumatoid arthritis and *sclerosis multiplex* v. systemic scleroderma.

Pathological Nuclear Transport Processes

The double membrane of the cell nucleus contains 2 60 nuclear pores per square micron. Initial experiments on nuclear transport approximately 30 years ago gave rise to the starting concept that these nuclear pores behave as molecular sieves allowing the free diffusion of all molecules below 20–40 kDa. This concept excluded the development of concentration gradients of small species, such as ions,between the two sides of the nuclear membrane. However, in later years many research groups showed the existence of such concentration gradients for calcium ions and other small molecules. These gradients showed abrupt and concentration-dependent changes on a large variety of stimuli, providing yet another exciting mode of nuclear signal transduction. These findings showed that the nuclear membrane does not allow the diffusion of all small species, and that nuclear membranes (similarly to all other cellular membranes) may contain a large number of active ATP-driven transport mechanisms. Another discriminative feature of the cell nucleus is its more reduced environment as compared to the cytoplasm or any other cellular compartment. This redox balance often becomes damaged in various diseases, such as Alzheimer's disease, leading to increased oxidative damage of DNA.

The nuclear pore complex contains hundreds of proteins. One of them, called ALADIN, is transported from the nucleus to the cytoplasm in achalasia-alacrymia-ACTH-insensitivity ("triple-A")

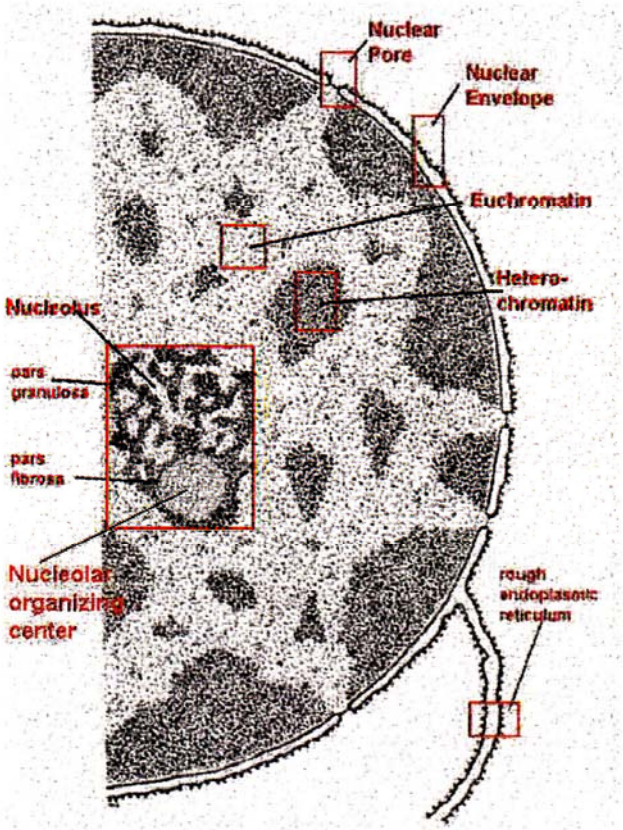

Figure 1. The major structural elements of the cell nucleus.
Draft structure of the cell nucleus. The figure shows the sketch of the nuclear structure after http://www.cytochemistry.net/Cell-biology/nucleus.htm. The nucleus is surrounded by the double nuclear membrane (nuclear envelope), which is in a direct contact with the rough endoplasmic reticulum. The membrane is punctuated by nuclear pore complexes, which are the places of nuclear transport processes. In the electron microscopic image euchromatin, heterochromatin and the nucleolus can all be separated. The sub-structures of the nucleolus playing key roles in the synthesis, maturation and packing of ribosomal RNA are the following: nucleolar organizing center, pars granulosa and pars fibrosa

syndrome. Since the ALADIN protein is an important player in nuclear transport, this dislocation inhibits a large number of nuclear transport processes. The cytoplasmic dislocation of X-linked cyclin-dependent kinase-like 5 (CDKL5) causes severe developmental delay apparent within the first months of life and is characterised by hypotony, early infantile spasms and autistic features.

Pathological nuclear transport has a profound effect on many tumors. Perhaps the best known example of pathological nuclear dislocation is the case of Bcr-Abl tyrosine kinase, which is a chimeric protein of the Bcr and Abl proteins in several leukemias. In healthy cells, the Abl tyrosine kinase is localized in the nucleus. In contrast, the malignant Bcr-Abl product is localized mostly in the cytoplasm, where it erroneously and unconditionally activates several signaling pathways. Survivin is an apoptosis inhibitor and a regulator of cell division during development and tumorigenesis. This protein can only exert its effect when exported from the cell nucleus; this is prevented in many types of colorectal cancer showing a good prognosis for survival.

The Pin1 protein is also depleted in the cell nucleus in various pathological conditions. The Pin1 protein is a peptidyl-prolyl cis-trans isomerase, which binds to phosphorylated proteins in the cell nucleus, and helps the development of their correct conformation and activity. In Alzheimer's disease the hyperphosphorylated tau proteins of cytoplasmic protein aggregates and egregate the Pin1 protein to the cytoplasm, which significantly damages the cell nucleus and contributes to the death of the hosting neuron.

Many viruses replicate in the cell nucleus. In order to replicate, the virus has to 'borrow' the cellular transport processes, including nuclear transport. Changes in the nuclear transport induce changes in the nuclear pore complexes and the structure of lamin A, B and C. This generates an avalanche of secondary changes in nuclear functions. As an interesting secondary effect, alpha-herpesvirus induces the formation of nuclear actin filaments in affected neurons.

Not all nuclear rearrangements are in direct causal contact with the underlying disease pathology. As an example, disjoint nuclear localization is the causative agent of Huntington's disease, in which the poly-glutamin protein appears in the cell nucleus. In conflict with the initial idea, the nuclear localization of the poly-Gln protein is not a major cause of protein-toxicity but only a secondary effect of the toxic tubulin depolymerization in the cytoplasm.

As a closing remark, I would like to add that localization inside the cell nucleus may be an equally important property as overall nuclear localization itself. As an example, a significant change of the exact nuclear localization of the androgen receptor inside the cell nucleus is a major accompanying event in spinal and bulbar muscular atrophy, yet it also occurs in prostate cancer and androgen insensitivity.

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