CELL NUCLEUS AND CHROMATIN STRUCTURE

Michelle Gehringer
Department of Biochemistry and Microbiology, University of Port Elizabeth, South Africa

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Summary

Eukaryotic genomes are found in the nucleus, a double membrane bound structure which serves to isolate the genetic material from the cytoplasm of the cell. The passage of molecules across the membrane is tightly regulated by the nuclear pore. The genome is wrapped round octomers of histone proteins in ordered structures called nucleosomes. These are then condensed with non-histone proteins into chromatin. Normal mammalian cells carry two full sets of chromosomes and are said to be diploid. Haploid cells, such as gamete cells, carry only one set of chromosomes, or a single copy of the genome.

DNA is replicated by DNA polymerases which recognize special origins of replication on the chromosome. Genes are transcribed from the DNA template by one of three RNA polymerases inside the nucleus. Transcription is initiated from a specialized promoter region upstream of the gene. Additional regulation by enhancers or repressor proteins may be required. RNA polymerase II is responsible for transcribing mRNA. It needs special initiation factors to start transcription, as well as elongation and termination factors to make the full length pre-mRNA. This is then modified to remove introns and splice the exons together. The mRNA also gains a 5' cap and a 3' poly-A tail. The mRNA is then exported to the cytoplasm where it is translated into protein. The rRNA and tRNA molecules are also transcribed in the nucleus and exported to the cytoplasm.

It is essential for the cell to tightly regulate both gene expression and genome replication, to ensure the integrity of its hereditary material.

1. The nucleus

The nucleus, ranging from five to seven microns in diameter, is the most prominent feature found within the eukaryotic cell .

1.1 The nuclear membrane

The nucleus is enclosed in a double membrane. This nuclear membrane keeps the nuclear material isolated from the cytoplasm. It is continuous with the endoplasmic reticulum and at times carries ribosomes on its cytoplasmic face. Each membrane layer consists of a phospholipid bilayer and is permeable to small, non-polar compounds.

1.2 The nuclear pore

Nuclear pores serve to connect the interior of the nucleus with the cytoplasm. This allows small polar molecules, ions and macromolecules to move between the two compartments. Molecular entry and exit is regulated in order to maintain the individual biochemistry of the nucleus. It also aids in the regulation of gene expression within this compartment. The outer and inner nuclear membranes usually fuse to form a pore of with an outer diameter of 120 nm. The inner opening carries a transporter core which contains a pore of

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approximately 9 nanometers in diameter. Along these channels synthesized messenger, ribosomal and transfer RNAs are transported to the cytoplasm. They also allow entry of transcriptional and signal transduction proteins, histones, nonhistone proteins and ribosomal proteins into the nucleus.

Molecules can be transported through the nuclear pore in two ways: either passive diffusion or energy dependent transport mechanisms. The aqueous channels which go through the center of the pore allow small molecules of the range of 5000 daltons to pass through unhindered, in either direction. The size limit for molecules passing from the nucleus to the cytoplasm or vice versa is gauged to be nine to ten nanometers. Proteins of ranging from 5 to 50 kD enter the nucleaus at a rate proportional to their size: the larger the molecule, the slower it enters the nucleus. The active transport mechanism recognizes specific proteins and RNAs and selectively transports them across the nuclear membrane. This process is energy dependent and is used for proteins bigger than 50 kD.

1.2.1 Structure of the nuclear pore

The pore consists of eight protein spokes arranged in a circle across the nuclear membranes. These spokes are anchored inside the pore at the site of inner and outer nuclear membrane fusion. Each spoke is attached to a ring protein either on the nuclear or on the cytoplasmic face of the nuclear pore. Both the cytoplasmic and nuclear rings are anchored to the cytoplasmic filaments and the nuclear lamina filaments respectively. These filaments form basket-like structures on each face of the pore and serve to direct the transfer of molecules across the nuclear membrane. The central diameter of the spoke structure is about 40 nm which is more than large enough to allow uncontrolled transport of molecules such as RNA across the membrane. The transporter is situated within the center of the nuclear pore, resembling a plug, and is thought to intrinsically control transport across the pore complex. The spoke ring complex also forms eight small channels of about 9 nm in diameter through which small molecules may diffuse.

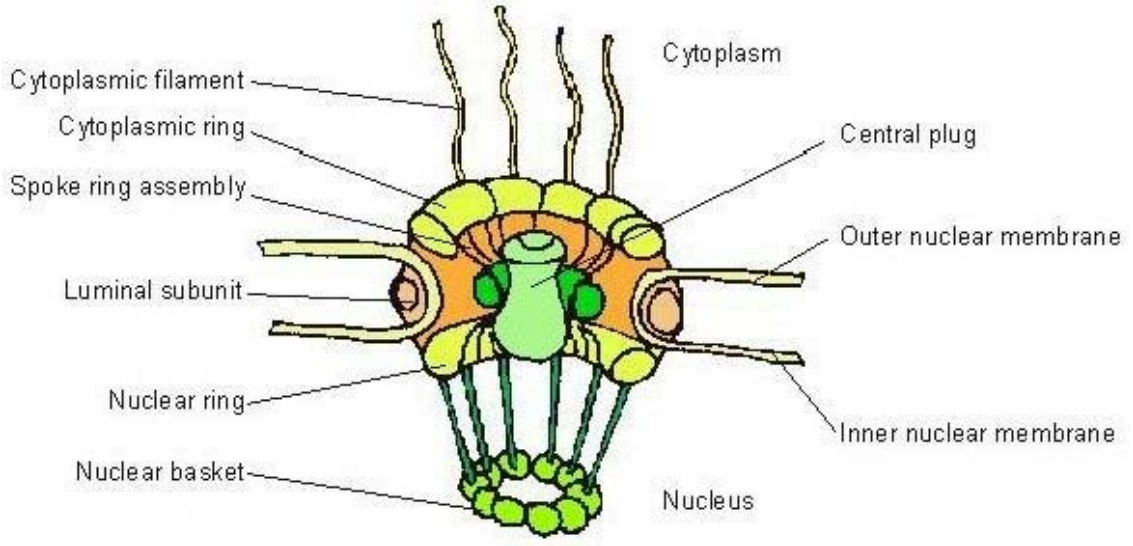

Figure 1. The structure of a nuclear pore.

1.2.2 Function of the nuclear pore

RNA export from the nucleus

Most RNAs require translocation through the nuclear pore into the cytoplasm. This is an energy dependent process that makes use of the Ran-GTP-binding protein. It appears that RNAs are transferred as RNA-protein complexes, but the precise mechanism for translocation has not been solved. The signals for transfer of the RNA across the nuclear pore may reside intrinsically within its molecular structure, or in the proteins associated it.

Pre-mRNA molecules are associated with heterogenous ribonuclear proteins (hnRNPs) which process the pre-mRNA into mRNA. One of these hnRNPs is known to be a shuttling protein and involved in export of the mRNA across the nuclear pore.

Ribosomal RNAs associate with their ribosomal proteins within the nucleus. Export signals on these ribosomal proteins target them for export. The mechanism of tRNA export remains unknown.

Protein uptake into the nucleus

Proteins to be targeted to the nucleus after synthesis usually carry a nuclear localization signal. This directs the protein to the nuclear pore complex for transfer across the nuclear membrane. The signal to transport the protein to the nucleus is bipartite, in other words, it consists of two protein sequence signals. These are usually stretches of basic amino acids such as lysine and arginine.

The first mechanism of protein translocation requires two cytoplasmic proteins: importin α and β. The first sub-unit, importin α, binds the nuclear localization signal on the newly synthesized proteins. Importin β then facilitates the binding of importin α to the nuclear pore complex. Energy is required in the form of ATP or GTP in order to translocate the protein through the pore complex and into the nucleus. Importin α remains associated with the protein during the translocation process whereas, importin β remains behind in the cytoplasm. It dissociates from the pore complex once the protein is translocated.

It now appears that a small protein, Ran, is involved in the translocation process. It is a small GTP-binding protein that belongs to the Ras family of cell signaling molecules. Its exact role in the protein translocation process remains to be clarified.

Several proteins that carry nuclear targeting signals are transcription activators and are only required at certain times within the nucleus. In order to regulate transcription within the nucleus, it is necessary to control the import of these activators into the nucleus. These transactivators are often associated with specific proteins which block their nuclear localization signal. Only once the gene it regulates needs to be transcribed, is the nuclear targeting signal revealed. The masking protein is then removed, thereby enabling the transctivator to bind importin and be imported into the nucleus.

1.3 The lamina

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The nuclear lamina is a fibrous network of intermediate fibers found in the inner side of the nuclear membrane, for which it provides structural support.

Lamins are proteins of 60 to 80 daltons which are classified with the intermediate fibers of the cytoskeleton. Mammalian cells contain four types, A, B_1, B_2 and C which combine together to form filaments. A pair of lamins form a helical coil, which then associates with other coiled lamin pairs to form a complete filament. These nuclear filaments also bind to integral proteins found within the nuclear membrane, thereby attaching the meshwork to the inner nuclear membrane and stabilizing the structure of the nucleus. A further function of the lamina network is to bind chromatin and position it within the nucleus.

2. The genome

The total amount of DNA found within a cell is referred to as the genome of that organism.

The sizes of the genomes of organisms vary greatly and there is no obvious correlation between the complexity of an organism and the size of its genome. The human genome is about one thousand times longer than that of a typical bacterium, and yet some amphibians contain ten times as much DNA than humans.

If all the DNA contained within the human chromosomes were to be unwound, it would be two meters in length, of which only about 1% of the genetic information is actually used in the normal life cycle of the cell. Some of the additional DNA is involved in regulation of gene expression and some contain signals for folding and condensing into chromosomes. It is possible that a large amount of the extraneous DNA is evolutionary waste which once encoded necessary information for survival, or possibly is the genetic fingerprint of previous invading microorganisms.

Most eukaryotic genes are interrupted by introns. These are regions of DNA that do not encode any proteins and are usually spliced from the messenger RNA before translation. Eukaryotes also carry several copies of the same gene to allow for slight variants of a protein.

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Bibliography

Books

Alberts B., Bray D., Johnson A., Lewis J., Raff. M., Roberts K. and Walter P. (1998) Chapt. 8: *Chromosomes and gene regulation* in Essential Cell Biology, 481. Garland Publishing, NY, US. [This is an

excellent introductory text on the structure of eukaryotic chromosomes and gene regulation.]

Lewin B. (2000) Genes VII Oxford University Press, New York, US. [This text offers the reader an excellent detailed summary of the complex processes of the molecular structure, organization and regulation of cells.]

Mathews C. K., van Holde K. E. and Ahern, K. G. (2000) Capt. 28: *Eukaryotic genes and their expression* in Biochemistry, third edition, 1068. Addison Wesley Longman, San Francisco, US. [This chapter is an excellent, informative summary of eukaryotic nuclear structure and gene expression. The book is a comprehensive text on the biochemistry of the cell.]

Reviews

Blackburn E. H. (1984) The molecular structure of centromeres and telomeres. *Ann. Rev. Biochem*. **53**, 163-194.

Goffeau A. *et al.* (1996) Life with 6000 genes. *Science* **274**, 546-567. [The entire sequence and genomic structure of the yeast chromosome revealed.]

Kornberg R. D. (1977) Structure of chromatin. *Ann Rev. Biochem*. **46**, 931-954. [An historic look at the discovery of chromatin structure.]

Kornberg R. D. and Lorch Y. (1992) Chromatin structure and transcription. *Ann. Rev. Cell Biol*. **8**,563-587.

Lamond A. I. and Earnshaw W. C. (1998) Structure and function in the nucleus. *Science* **280**, 547-563.

Luger K. *et al*. (1997) Crystal structure of the nucleosome core particle at 2.8Å resolution. *Nature* **389**, 251-260.

Mitchell P. and Tjian R. (1989) Transcriptional regulation in mammalian cells by sequence-specific DNA-binding proteins. *Science* **245**, 371-378.

Nikolov D. B. (1997) RNA polymerase II transcription initiation: a structural view. *Proc. Natl. Acad. Sci. USA* **94**, 15-22.

Platt T. (1986) Transcription termination and the regulation of gene expression. *Ann. Rev. Biochem.* **55**, 339-372.

Roeder, R. G. (1996) The role of general initiation factors in transcription by RNA polymerase II. *Trends in Biochem. Sci*. **21**, 327-335. [This excellent edition of *TIBS* is solely on eukaryotic transcription.]

Sharp P. A. (1985) On the origin of RNA splicing and introns. *Cell* **42**, 397-400.

Biographical Sketch

Michelle Gehringer is a visiting scientist at the School of Biotechnology and Biomolecular Sciences of the University of New South Wales in Sydney, Australia. She is continuing her work on the toxic effects of the cyanobacterial toxins, microcystin and cylindrospermopsin, on humans and animals that accidently ingest them from contaminated drinking water sources. This research has provided insight into the way the body deals with the toxin as well as potential means of offering dietary protection to potential victims.

Dr Gehringer has several years of lecturing experience form the University of Port Elizabeth, South Africa, where she was actively involved in introducing the topics of Biochemistry and Microbiology to the general public and school goers. Her MSc was obtained at the University of Cape Town, South Africa where she worked on means to control Cucumber Mosaic Virus infections of crop plants.

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