Fluorescence microscopy techniques for exploring the cell nucleus structural and functional organization

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Despite the obvious importance of the cell nucleus as the genetic control center regulating cellular growth and differentiation processes, relatively little was known, until recently, about the compartimentalization of gene expression, and how the various steps of gene expression are integrated into nuclear structures. Methodological and technological improvements in the fields of molecular and cell biology, biophysics, microscopy and data processing made it possible to study the 3D arrangement of chromosomes, active and inactive genes, DNA replication factories, transcription and RNA maturation sites.

Research in this field did greatly benefit of new ways and tools for labeling of nucleic acids and proteins, for multi-parametric and multi-dimensional imaging of chromosomal and nuclear structures, for revealing changes in their functional state, for studying their dynamics and for measuring molecular interactions in living cells. The labeling methodologies used include, e.g., the incorporation into nucleic acids of nucleotides directly coupled to fluorochromes, and the expression of fluorescent fusion proteins with different spectral characteristics.

Quantitative analysis of complex statiotemporal processes in live cells is possible by fast multi-color 4-D imaging combined with automated and time-space reconstruction, and by with fluorescence photobleaching techniques. Fluorescence recovery after photobleaching (FRAP) and derived techniques can be used to determine biophysical parameters such as diffusion coefficient and exchange rate of macromolecules. Fluorescence correlation spectroscopy (FCS) makes it possible to calculate the diffusion times and the behavior of different populations of fluorescent molecules by measuring the fluorescence fluctuations in a very small volume. Due to its single-molecule detection sensitivity, FCS is a powerful tool for examining molecular interactions between proteins and between specific fluorescent probes and their targets.

The resurgence in interest in the FRET microscopy (fluorescence resonance energy transfer microscopy) is also largely due to the development of genetically encoded fluorescent protein tags. FRET is the quantum mechanical process involving the radiationless transfer of energy from a donor fluorophore to a nearby acceptor fluorophore. Because the efficiency of energy transfer varies inversely with the sixth power of the distance separating the donor and acceptor, the distance over which FRET can occur is limited to less than 10 nm and FRET can therefore indicate that two proteins are physically associated.

The use of these fluorescence based techniques, in combination with high resolution electron microscopy and X ray microscopy techniques, should soon allow to establish a well grounded structure-function model of the cell nucleus.

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