Structure and function of the cell nucleus

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During the Wilhelm Bernhard Workshop, the 18th International Workshop on the Cell Nucleus, held in Pavia last September, the idea was born to ask some of the participants for sending a paper for a special issue on the cell nucleus of the European Journal of Histochemistry, to celebrate the 50th anniversary of its foundation. In the following pages, the contributions of several scientists working in this field have been collected, each article having a special look and attention to a particular aspect of nuclear biology.

As it was pointed out during the opening lecture of the Workshop, we had several anniversaries in 2003, which are concerned with the cell nucleus. The 50 years of the double helix paper have been celebrated all over the world, but two others are worth mentioning: the paper by Cogliati and Gautier on the synthesis of osmium ammine for the specific staining of DNA at electron microscopy was published exactly 30 years ago, and 25 years ago Dr. Wilhelm Bernhard died. Bernhard's contributions to the study of the cell nucleus have been significantly important, and his will to create a meeting where people could freely exchange data and opinions is still living on.

It is not easy to write an introductory note to this issue: the names of the scientists speak for themselves. Therefore, a short description of the contents will be only given, as the researches on the cell nucleus are so rich, heterogeneous and numerous that is positively impossible to even cover the literature of just one year.

In 2003, at the congress of the Italian Society of Histochemistry, in Rapallo, Dr. Stan Fakan was awarded the Maffo Vialli International Award for Histochemistry; later on and independently, the Organizing Committee of the Wilhelm Bernhard Workshop awarded him the prestigious Wilhelm Bernhard Lecture which traditionally opens the Workshops. The content of both lectures is presented here, in his paper on the ultrastructural analysis of the nuclear functional architecture. It is a fresh, clear presentation of data and conclusions gathered over more than 30 years of intense activity in the field, which are here collected and presented without emphasis, with a final view over a wide horizon. It is likely that this paper will become a reference for the biology of the cell nucleus.

Histones are back again on the stage, after having changed in 1973 the face of chromatin structure, allowing for hidden dreams of compaction and for visions of an ordered, established, small but supple universe. Now, histones play a different role, acting as movie directors, not only as actors (or as sometime they have been considered, dummies). In the paper by Cremer and co-workers the chemical modification of histones is shown to represent an important epigenetic mechanism for the organization of higher order chromatin structure and gene regulation. Methylation of position-specific lysine residues in the histone H3 and H4 amino termini has been linked with the formation of constitutive and facultative heterochromatin as well as with specifically repressed single gene loci. These methylation patterns are possibly causally involved in the formation of cell type specific heterochromatin compartments, composed of (peri)centromeric regions and chromosomal subregions from neighboring chromosome territories, which contain silent genes.

Lots of genes do their work in silence, but are not silenced. Some of them, many indeed, work and are not acknowledged until it is too late. This seems to be the case for some genes located on fragile sites which act as tumor suppressors. In their paper, Huebener and coworkers discuss the features of two of these genes and suggest clues to understand the relationship between genomic instability and tumor biology. The most important issue is that the identification of the basis of instability at fragile sites and the related genes provides an *entrée* for understanding important aspects of chromosomal instability, a prominent feature of neoplastic genomes.

Along the same line goes the work by Bottiroli *et al.*: the search for time-dependent relationship between cell cycle phase and activation of early expressed genes and possibly for the earliest markers of the switching-on of these genes is an important challenge. FRET analysis is used in this paper on human fibroblasts stimulated to proceed from G_0 to G_1. As early as 1 hour after the stimulus, FRET is capable of detecting the subtle changes which indicate that the cell is entering G_1; interestingly, these data in fact make another marker, Ki-67, almost obsolete in the sense that the expression of this protein comes only after 24 hours.

Where a single gene product is located is the topic of the following review. A different histochemical approach is reviewed by Thoru Pederson to study RNA in unfixed, living cells. Microinjection of fluorescent RNA into cells allows to reveal intracellular sites at which a given RNA resides, or will act as a tracer to allow movements of a dynamically translocating RNA to be followed in living cells. This in fact is the major point which can actually make this technique an extremely interesting tool to gain information while information is actually created inside the cell. It is then possible to follow either the dynamic behaviour of RNA or its location. An interesting possibility is represented, for instance, by the labelling of small RNAs or snoRNAs with the possibility to follow their movements from the region where they are transcribed.

It is then important, both from basic cell biology and in the attempt to understand the bases of pathogenesis, to find out additional tools capable of indicating that the earliest steps toward transcription are taking place at the perichromatin region. Cerná *et al.* describe in their paper the possibility that the distribution of Z-DNA stretches signals the sites related to nuclear transcription. In fact, Z-DNA forms transiently behind the active RNA polymerases, because of the mechanical torsional stress produced during transcription. The Z-containing structures nearly disappeared when non-nucleolar RNA polymerase II-dependent transcription had previously been abolished by the adenosine analogue, DRB. They propose to use the *in situ* immunodetection of Z-DNA as a marker of the transcription level in both nucleolus and non-peripheral nucleoplasmic regions of nuclei.

RNA is also the main character playing in the paper by Stein and coworkers. The authors show the functional interrelationships between the intranuclear organization of nucleic acids and regulatory proteins since these are obligatory for the fidelity of transcriptional activation and repression to be achieved. Microenvironment within the cell nucleus becomes the *condicio sine qua non* to obtain the correct relationship among the different players of the transcriptional activity. This microenvironment, in addition, is required to be dynamic and highly adaptable to the needs of the cell nucleus.

Among new functions discovered in the cell nucleus, the last 15 years have pointed to the capability of this organelle to have an autonomous signalling system. Numerous laboratories (and among them, the group in Bologna has been pioneering), have demonstrated a lipid based signalling, which is reviewed in this special issue. There is compelling evidence that phospholipids are present in the nucleus, and among them the inositol lipids in particular. For example PI-PLC $\beta 1$ is partly in the nucleus, and that after growth-factor stimulation of quiescent cells, or at distinct points in the cell cycle, its activity is stimulated to generate DAG, which in turn attracts PKC isoforms. The activation of PI-PLC $\beta 1$ will also generate Ins(1,4,5)P3,which has the potential to control the nuclear Ca^{2+} concentration. Other phospholipids are also clearly present, and it seems impossible to believe that PtdIns(4,5)P2 will confine itself to merely being a PI-PLC $\beta 1$ substrate and some promising hints at other functions are emerging. PtdCho in particular, is present in quantities to be credible component of nuclear structure and origin for DAG.

The complexity of lipid signaling proved to be surprisingly rich, with the number of potential signalling molecules increasing. In the papers by Tu-Sekine and Raben, by Cocco *et al.* and by Martelli *et al.*, the pathways linking the lipid signalling to nuclear functions and to cell death are elucidated, and it is discussed how this signalling system achieves its important role within the complexity of functions of the cell nucleus. In comparison to the longstanding field of investigation related to both the structure of the nucleus and the biochemistry of nucleic acids, the nuclear lipid signalling is a baby-born one. We are keen to quote a line by Robin Irvine, FRS to signify this aspect and to underline the great potential of this new field: "... the people who work on nuclear functions regard phospholipids as something vaguely unpleasant, to be removed at the first possible opportunity and certainly not worth much serious thought. However,

before the 1980s, this was exactly the attitude of most biochemists to phospholipids in general. If we look at how phospholipids and their signalling and regulatory functions are now appreciated as being central to almost every aspect of cell biology, it could be that a similar revolution in attitude to nuclear lipids might now be due''.

We do believe that the reviews in this special issue will serve to the purpose of giving useful tools for a better understanding of the relationship between nuclear structure and function, including nuclear signalling. Maybe, the revolution is on the move, since in the next year a new Gordon Research Conference on ''Signal Transduction within the Nucleus'', will be held at the Rancho Santa Barbara Marriott, Buellton, CA, USA, on February 6-11, chaired by L. Cocco and D. Raben.

Finally, this special issue offers an unique possibility to celebrate another important event. Besides being, as we already mentioned, the 50th anniversary of the European Journal of Histochemistry, 2004 coincides with the 80th birthday of our Editor-in-Chief, Maria Gabriella Manfredi Romanini who is leading the journal with passion and incomparable skilfulness: we are especially glad that this special issue deals with the very field in which she has spent so much time and devoted a lot of energy achieving remarkable results since the late fifties of the last century.