## Simposio

# Nuclear structure and dynamics, through the microscopes

Pavia, 7-8 Luglio 2016
Collegio Alessandro Volta

**Organizzato congiuntamente da**
**Società Italiana Scienze Microscopiche e Società Italiana di Istochimica**

### TRANSCRIPTION TIME-WINDOW APPLIED TO RNA MODIFICATIONS IN HELA CELLS

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We have applied a new method which combines the high spatial resolution of transmission electron microscopy with information on the dynamics of transcription. The incorporation of two different RNA precursors, iodo- and chlorouridine, was used to define a Time Transcription Window on cultured cells treated with hypometabolising peptides which are known to modulate transcription [1,2]. This procedure allows to detect a single fibril of newly synthesized RNA in the time range in which it is transcribed. The same fibril is finally visualized by a selective staining for RNA with terbium citrate[3]. Briefly, D-Ala2-D-Leu5 enkephalin (DADLE) is a synthetic peptide, capable of mimicking the hypometabolising action of the hibernation induction trigger, an opioid δ-receptor agonist occurring in the blood of lethargic hibernators[4] and able to induce lethargy when injected in summer active animals[5]. Similarly to the hibernation induction trigger, DADLE injection can make summer active hibernators enter lethargy[5]; moreover, once injected, this molecule can determine a transient torpor state in rats[6] and, finally, it can induce a hibernation-like state in cultured cells.[2,7] Differently from DADLE, its isomer DALE (D-Ala2-Leu5-enkephalin) is not degradable by enkephalinases; however, data on the hypometabolising effects of DALE are still preliminary[8]. These treatments represent a good model to investigate the possible role of 5mC-containing RNA, whose function is still unknown.

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### DOSE-DEPENDENT TYPES OF NUCLEAR ALTERATIONS INDUCED BY ETOPOSIDE ON HUMAN LEUKEMIA CELLS

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Cytotoxic anticancer drugs at low doses (metronomic therapy) perform efficacious anti-angiogenetic activity; moreover, they exert additional direct actions against tumor cells, which are less explored. Here, we investigate the molecular nature of such actions. We performed cytofluorimetric and ultrastructural analyses on the myelocytic tumor cell line U937 treated with low dose (0.5 μM) of the topoisomerase inhibitor etoposide, compared with the cytotoxic (50 μM) dose.

At variance with the high dose, 0.5 μM etoposide promotes deep nuclear invaginations eventually resulting in granulocyte-like differentiation. The differentiation process requires cell cycle arrest via ATM-mediated DNA damage response, indicating that etoposide-induced DNA breaks are the

differentiation mediators.

Caspases are activated also by low dose etoposide; all treated cells eventually dye acquiring all the hallmarks of apoptosis, but surprisingly in a caspase-independent way (unlike 50 μM etoposide). Instead, caspase inhibition reverts differentiation: molecular analyses point to caspase 6-dependent cleavage of cyclin B1 and nuclear lamins as the events that determine granulocytic differentiation by low doses etoposide.

At the morphological level, TEM images of apoptotic cells induced by the two doses show striking differences.

Similar results were obtained in myeloid leukemia HL-60, KG1 and THP-1 cells, indicating general responsiveness. Importantly, 0.5 and 50 μM etoposide possess equal pro-apoptotic efficiency on leukemia cells, whereas it is reported that normal blood precursors are induced to apoptosis only by high doses. This indicates that metronomic doses of etoposide, unlike the cytotoxic ones, display selective killing of tumor cells, promising to successfully eliminate tumor cells with scarce side effects. These results provide for the first time to our knowledge a biological mechanism implying reprogramming (anakoinosis) of leukemia cells towards normal differentiation behavior by low-dose antitumor therapy with etoposide.

In conclusion, we have shown that metronomic doses of etoposide induce granulocytic differentiation via caspase- and DNA-damage response-dependent events, followed by "turnover" (rather that damage-induced) apoptosis, without requiring maturative cell division.

## FROM MICROSCOPY TO CYTOMETRY THROUGH FLUORESCENCE: CELLULAR EVENTS AT THE NUCLEAR LEVEL

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Confocal Laser Scanning Microscopy constitutes a powerful tool to analyse the intracellular location of various components through the high-resolution optical imaging and the three-dimensional reconstruction. There are a large number of DNA-specific fluorescent dyes, and each of these has individual characteristics (binding affinity, specificity for DNA or other macromolecules and wavelength of excitation and emission). Some nucleic acid fluorescent probes are able to enter live cells to visualize nucleus and DNA-containing organelles, and can be used as vital stains[1,2]. Other DNA stains diffuse through the cell membrane as a marker to identify the double-stranded DNA breaks in dead cells[3].

Flow cytometry (FC) is a biophysical technology employed in cell counting, cell sorting, biomarker detection and protein engineering and can be easily used to analyse nucleic acids and chromatin compaction, important elements in cell differentiation and pyknosis of nuclei. Furthermore, one of the most useful cytometric approach is the evaluation of DNA content and cell cycle analyses, by means of several probes[4]. Nuclear fluorochromes can be applied to indirectly measure ROS levels and membrane permeability[5]. Finally, FC offers the possibility of rapid enumeration of parasitemia. It relies on staining the parasite DNA to distinguish between infected and non-infected red blood cell populations. In biological research and medical diagnosis, volume, shape, DNA content, and chromatin pattern of nuclei it can be important for the diagnosis and prognostic impact of many cancers. Some models are here presented to demonstrate the importance of microscopy and flow cytometry in highlighting nuclear domains.

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## EFFECT OF ADAPTED PHYSICAL EXERCISE ON THE FINE DISTRIBUTION OF PAX7 AND MYOD IN MYONUCLEI OF OLD MICE

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Satellite cells (SCs) are mitotically quiescent cells located between the basal lamina and the sarcolemma of myofibers[1]. Following injury, muscle tissue may regenerate through the activation of SCs that first proliferate and then differentiate into myocytes which fuse either together or with pre-existing myofibers. Activation, proliferation and differentiation of SCs may undergo alteration during ageing[2,3] with a consequent reduction in

the efficacy of muscle renewal. In fact, during ageing a progressive decline of muscle mass, strength and quality take place, a condition termed sarcopenia[4]. Although no specific therapy is presently available to counteract the onset and progression of sarcopenia, it has been demonstrated that physical exercise may efficiently mitigate the age-related muscle atrophy. In particular, we demonstrated that an adapted aerobic physical exercise (treadmill running) leads to the reactivation of nuclear activity, increasing transcriptional and post-transcriptional processes in both myofibers and SCs of old skeletal muscles[5,6].

The process of SC activation is finely regulated by the expression of specific factors, among which the paired box protein 7 (Pax7) and the myogenic differentiation factor D (MyoD). Although largely investigated in SC nuclei, the distribution and function of Pax7 and MyoD in myonuclei are poorly known. We have therefore focused our attention on the possible effects that age-related atrophy as well as exercise-related nuclear reactivation may induce on the fine distribution and relative amounts of Pax7 and MyoD in myonuclei of old mice. Our results shed light on the possible functional role played by Pax7 and MyoD in the myonuclear response to physical exercise and, more generally, in skeletal myofiber regeneration.

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## HISTOCHEMICAL STUDY OF MYOCARDIAL ISCHAEMIC TISSUE IN ADVANCED PUTREFACTION: PRELIMINARY RESULTS


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Histologic diagnosis in forensic context it's often hampered by autolysis and putrefaction. Specimens from autopsies performed on exhumed cadavers or on bodies in advanced state of decomposition render recognition of pathological alterations quite difficult. Moreover tissue decomposition can sometimes simulates non-existent histopathological processes[1]. In forensic pathology practice it is common to encounter heavily putrefied bodies. In these cases to prove the possible presence of an acute myocardial infarction as the cause of death is very important. Moreover, the appearance of myocardial ischemia can be masked or even imitated by autolysis and putrefaction[2].

The use of hematoxylin-eosin (HE) stain is not sufficient to investigate decomposed myocardial tissue[3]. The use of histochemical techniques could help in the histopathological analysis of this type of material to increase the diagnostic specificity[4]. The purpose of this research is the evaluation of the efficacy of histochemical stains to identify ischemic areas in the putrefied myocardial tissue.

Heart tissue specimens was taken from eight cases of macroscopically evident acute myocardial infarction (AMI) during diagnostic autopsies. Specimens was obtained from an area containing ischemic and non-ischemic myocardium. One tissue fragment was immediately fixed in a 10% buffered formalin and used as control. Specimens from AMI were placed in an open case and stored at controlled room temperature, ranging between 16 °C and 20 °C. At time interval of 15 and 30 days of putrefaction the samples of AMI tissues were fixed 42 hours in 10% formalin, processed and embedded in paraffin. Sections of 4µ were cut from paraffin blocks and stained with standard HE and Mallory trichrome stain.

Preliminary results showed in all cases: after 15 days of putrefaction HE stains was no longer able to detect AMI areas. Instead, after 30 days of decomposition, Mallory trichrome showed strongly positive staining of non-ischemic cardiac fibers (red colored), while ischemic myocardium was very less intense.

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## HIGHLIGHTING THE APOPTOTIC NUCLEUS: THE PRECIOUS CONTRIBUTION OF THE ELECTRON MICROSCOPE

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Apoptosis is a peculiar form of physiological, genetically controlled cell death, characterized by an intriguing and exclusive cell behavior.(1) Apoptotic chromatin investigations took great advantage by freeze-fracture techniques, which allowed the identification of novel types of chromatin fibers in the complex scenario of apoptotic nucleus.(2) On the other hand, cytochemical approaches revealed the correlation between nuclear domain changes and DNA role. When analysed at TEM, apoptotic chromatin clusters at nuclear periphery, forming cup-shaped dense masses and determining a characteristic nuclear pore translocation.(3) Micronuclei progressively appear throughout the cytoplasm and are finally released into the extracellular space. Secondary necrosis represents a frequent final fate or, when possible, apoptotic cells are engulfed by circulating macrophages. The underlying machinery is not yet completely understood, even if an extrinsic pathway ,or, more frequently, an intrinsic, mitochondria-mediated one , are commonly accepted. DNA cleavage, in oligo-nucleosomic or larger fragments(4) occurs. This has been widely demonstrated *in vitro*, by electrophoretic techniques and *in situ*, by TUNEL reaction, successively more detailed by TUNEL/TEM, associating gold particles to DNA breaks.(5) These patterns have been demonstrated in a variety of cell models, even long considered resistant, in response to different stimuli.(6,7) *In vitro* models of skeletal muscle cells, human chondrocytes and keratinocytes have been studied by a multiple approach(8), and a common project in apoptotic cell death can be identified, which could be then considered also a target for potential anti-apoptotic agents.(9,10)

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## DYNAMICS AND REGULATORY MECHANISMS INVOLVED IN IMMATURE OOCYTE CHROMATIN REMODELING

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During meiotic arrest, and particularly during the oocyte growth phase leading to the formation of fully-grown oocyte, the chromatin enclosed within the oocyte nucleus, also known as Germinal Vesicle (GV), is subjected to several levels of regulation controlling both its structure and function. These events include mechanisms acting both locally, on specific loci, and on a large scale to remodel wide portions of the oocyte genome. Morphologically, the chromosomes lose their individuality as well as their characteristic appearance and form a loose chromatin mass, which in turn undergoes profound and dynamic rearrangements within the GV before the meiotic resumption. These 'large-scale chromatin configuration changes' are temporally correlated with the process of transcriptional silencing in the oocyte nucleus as well as with epigenetic modifications such as histone tail modifications and changes in the global level of DNA methylation. Moreover, chromatin configuration rearrangements are tightly associated with the acquisition of meiotic and developmental competence. The molecular mechanisms governing changes in large-scale chromatin configuration still remain largely unknown. Most likely, strategies set in place for the control and coordination of these events are part of a complex physiological process that ultimately confers the oocyte with meiotic and developmental competence. Here, we summarize some studies intended to explain the mechanism(s) regulating this complex process.

## BARRIER-TO-AUTOINTEGRATION FACTOR INVOLVEMENT IN PRELAMIN A-RELATED CHROMATIN REMODELING

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Defects in lamin A precursor maturation pathway cause nuclear morphological changes and chromatin remodeling. It has been previously described that the accumulation of lamin A precursors by pharmacological treatments or by transfection with prelamin A uncleavable constructs, induces heterochromatin markers redistribution within the nucleus and nuclear size increase(1) Similar nuclear defects were observed in a group of rare diseases named laminopathies which arise from mutations of the nuclear lamina or the nuclear envelope proteins. Among laminopathies, chromatin structure alterations seem to be more severe in those forms showing prelamin A accumulation. Recently we demonstrated a molecular link between prelamin A and Barrier-to-Autointegration Factor (BAF) a 10 kDa chromatin-interacting protein identified as a component of chromatin dynamics-related molecular complexe.(2,3) Since this finding suggested a possible BAF role in prelamin A chromatin remodeling function, we wondered if prelamin A effects on chromatin could be due to prelamin A-BAF interaction. In particular, we took advantage of the study of the heterochromatin marker H3K9me3 distribution in Néstor Guillermo Progeria Syndrome (NGPS) cells, treated with mevinolin, a prelamin A processing interfering drug. We observed that the BAF mutant (Ala12Thr) identified in this rare disease prevented H3K9 intranuclear localization in response to prelamin A accumulation. In order to confirm this finding we performed additional experiments in HEK-293 cells induced to accumulate lamin A or prelamin A in combination with wild type BAF (WT-BAF) or mutated BAF forms (BAF-A12T, BAF-G47E). Interestingly, we observed that when prelamin A-BAF interaction could not occur properly prelamin A was unable to modify the intranuclear localization of chromatin-related proteins HP1a and LAP2a or to affect chromatin ultrastructural organization. Similar results were obtained in HEK293 cell BAF depleted by siRNA treatment transfected with prelamin A constructs. Finally we confirmed BAF involvement in prelamin A related chromatin organization effects in Hutchinson Gilford Progeria cells. In particular, we were able to rescue the proper intranuclear localization of chromatin-related protein LAP2a affecting progerin-BAF interaction.(4) Our results demonstrate that BAF permits prelamin A chromatin remodeling functions and that the perturbation of this mechanism due to BAF mutations, or its permanent activation by impairment of lamin A precursor processing, could be involved in the pathophysiological mechanism of progeroid syndromes linked with alterations of nuclear lamina proteins.

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## MOLECULAR COMPOSITION AND DYNAMICS OF NUCLEAR FOCI IN MYOTONIC DYSTROPHY

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Myotonic dystrophies (DM) are genetically heterogeneous neuromuscular disorders with autosomal dominant inheritance: DM type 1 (DM1) is caused by a CTG repeat expansion in the 3' UTR region of the DMPK gene, while DM type 2 (DM2) is linked to the instability of a CCTG repeat in the CNBP (also known as ZNF9) gene. Both DMs are characterized by multisystemic pathologic features including myotonia, muscular dystrophy, dilated cardiomyopathy, cardiac conduction defects, insulin resistance.

Combining biomolecular and cytochemical techniques, it has been demonstrated that the

basic mechanisms of both DMs reside in the nuclear sequestration of the expanded RNAs: CUG- and CCUG-containing transcripts accumulate in intranuclear foci of DM1 and DM2 cells respectively, and alter the regulation and intranuclear localization of the RNA-binding proteins CUGBP/Elav-like family member 1 and muscleblind like 1[1]. It has been found that DM foci also sequester snRNPs and hnRNPs, splicing factors involved in the early phases of transcript processing[2]. This likely causes a general alteration of the pre-mRNA post-transcriptional pathway in DM-affected cells. Accordingly, splicing and cleavage factors have been found to accumulate in skeletal muscle myonuclei of DM1 and DM2 patients, indicating an impairment of pre-mRNA processing[3]. Finally, by analyzing the dynamics of the DM-specific intranuclear foci, it has been demonstrated that, in cycling cells, they undergo disassembly to be reformed in the nucleus at each mitosis whereas, in non-cycling cells, they progressively increase in size[4], likely leading to the worsening of the pathological traits.


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## THE NUCLEAR LAMINA AS A CHROMATIN ORGANIZER


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The nuclear lamina (NL) has been evolutionary expressed in metazoan, and the V-type intermediate filaments, the lamins B and A/C, which undergo polymerization/depolymerization phases, determine the NE assembly/disaggregation along the cell cycle. The role of nuclear lamins in tissue-specific cell differentiation has been highlighted by studies on the pathogenic mechanism leading to a variety of human diseases, collectively referred to as laminopathies[1]. Laminopathic cells are characterized by the expression of immature forms of lamin A (prelamin A), whose accumulation in the nucleus determines loss of peripheral heterochromatin, as revealed by the reduction of typical heterochromatin markes (tri-H3K9) and by electron microscopy[2]. These nuclear alterations and the transcriptional activity can be recovered by drugs that interfere with prelamin A accumulation[3]. The NL and mainly lamin A/C determine stable interactions with particular districts of the genome, characterized by a high histone H3 dimethylation and block of transcriptions. These lamin A/chromatin interactions, in correspondence with a thousands of LADs (Lamin-Associated-Domains) are mediated by epigenetic repressors such as HADCs, and by chromatin-associated proteins including HP1 and BAF[4]. Among laminopathic phenotypes, the most severe is represented by the Hutchinson-Gilford progeric syndrome (HGPS) in which tissues degenerate undergoing premature senescence. Animal models, and cell from laminopathic patients have been treated with drugs that interfere with prelamin A accumulation; the results obtained encouraged the applications of analogous treatments in three international clinical trials. These studies suggested that accumulation of prelamin A could also affect normal cell and organism aging. We obtained further evidence on the crucial role on chromatin organization and gene expression of prelamin A processing and on the particular prelamin A expression that characterizes a special group of aged persons, the centenarians, which present a nuclear arrangement which is closest to young than aged people[5].


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## ULTRASTRUCTURAL ANALYSIS OF NUCLEIC ACID METHYLATION


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Transmission electron microscope analysis of 5-methylcitosine (5 mC) distribution represents a different approach to study epigenetic modifications on nucleic acids. Moreover, this allows to specifically identify RNA fibrils carrying these epigenetic markers. We have investigated this distribution on HeLa cells, due to their high transcrip-

tion rate, while rat liver were considered as a control cell model.

We found 5mC labelling in the perichromatin region, where transcription by RNA polymerase II occurs[1]. The colocalization of 5mC and fluoro-uridine, an RNA precursor, together with specific staining methods confirmed the presence of this modified base on RNA fibrils. Since data in the literature indicate that lncRNAs are localized at the periphery of condensed chromatin region to regulate genome activity[2], we have used several markers to elucidate the 5mC presence on mRNA. This mRNA modification is indeed not clearly understood[3]. We identified labelling for 5mC near ribosomes in the cytoplasm, both alone and in combination with labelling for poly(A) tail. Furthermore, our EM data show that RNA methylation occurs cotranscriptionally and is stable during RNA life until its translation. We are setting up a panel of antibodies for discriminating the different types of RNA in the perichromatin region.

Finally, in order to deeper investigate the role of this early and stable epigenetic modification on mRNAs, we propose an analysis on exosomes where the presence of miRNA and mRNA has been described[4]. Terbium positive molecules were found at the periphery of the exosome near the membrane and 5mC was also detected.

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## A PUTATIVE 2N=26 ROBERTONIAN MOUSE EMBRYONIC STEM CELL LINE MAINTAINS ITS KARYOTYPE STABILITY DURING CULTURE


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The potential use of mouse embryonic stem cell (mESC) for investigating *in vitro* early embryonic development or for screening the effects of new drugs or xenobiotics depends on capability to maintain their genome integrity during prolonged culture and differentiation. Culture-induced variations of specific chromosomes or genes are almost all unpredictable and, as a whole, differ among independent cell lines. They may arise at different culture passages, suggesting the absence of a safe passage number maintaining genome integrity and rendering the control of genomic stability mandatory since the very early culture passages.

To date, all mESC lines available have been obtained from *Mus musculus* laboratory stains with the 2n=40 all telocentric standard karyotype. These inbreed laboratory strains posses a mixed genome background, a condition that can influence the genome stability.

We have recently derived the first wild-type putative mESC line from mice with a reduced chromosome number (2n=26) for the presence of 8 Robertsonian (Rb) metacentric chromosomes. This cell line expresses pluripotency markers and it is able to form embryoid bodies and the three germ layers.

Putative Rb mESCs were cultured for a period of up three months, monitoring, at regular time intervals, the chromosome complement and the appearance of chromosome abnormalities.

Rb mESCs were cytogenetically analysed between passages 2 and 28 and, on average, 50 metaphase spreads were scored for each passage. This mESC line displayed a stable diploid Robertsonian karyotype during culture (ranging from 84 to 100%), with a very low percentage of numerical chromosomal aberrations (subtetraploid and tetraploid populations). Also, after freezing/thawing procedure, the karyotype stability was maintained.

At each passage, DAPI banding of 5 randomly chosen metaphases showed normal chromosome complement. In just one metaphase at passage 10, although numerically euploid, a chromosome 1.3 trisomy coupled with a chromosome 7 unisomy was found. This aberration was not recovered in the subsequent passages, suggesting that cells with abnormal karyotypes were negatively selected during culture.

This line, characterized by a metacentric phenotype of the chromosomes and reduced chromosome number, represents a precious resource for the study of the centromere function and of the mechanisms involved in the onset of both numerical and structural chromosome abnormalites.

## NUCLEAR DIFFUSION ASSAYS FOR THE STUDY OF DNA DAMAGE AT THE SINGLE CELL LEVEL

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The need for express screening of the DNA damaging potential of chemicals has progressively increased over the past twenty years due to the wide number of new synthetic molecules to be evaluated, the adoption of more stringent chemicals regulations such as the EU REACH[1] and risk reduction politics. To this regard, DNA diffusion assays such as the microelectrophoretic comet assay paved the way to a new concept of rapid genotoxicity testing, but a further and more significant simplification and speeding up of the experimental processes was achieved with the fast halo assay[2] (FHA). This assay operates at the single cell level and relies on the radial dispersion of the fragments of damaged DNA from intact nuclear DNA: fragmented DNA is separated by virtue of diffusion in alkaline solvent, stained, visualized at the microscope, and finally quantified using appropriate computer-assisted image analysis programs to assess the extent of DNA breakage caused by different types of DNA lesions. FHA has proven to be sensitive, reliable and flexible. To our best knowledge, FHA is currently the simplest, cheapest and quickest assay for studying DNA damage and repair in living cells. The assay (the preparation of samples ready to be visualized at the microscope) can be performed in about forty min, i.e. one fourth of the mean time required to execute the comet assay and it is very cheap since it requires no electrophoretic apparatus nor expensive reagents. Moreover, recent modifications further implemented FHA allowing a full exploitation of its analytical potential as a technique for large-scale, rapid genotoxicity screening.

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## NUCLEAR DUALISM AND GENOME ORGANIZATION IN CILIATES

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In multicellular organisms, the germline and somatic genomes are physically and functionally separated during the development into distinct cell types. In contrast, the ciliates, a group of evolutionarily very successful unicellular eukaryotes, are unique to carry both their germline and somatic genomes enclosed in the same cytoplasm. The germline genome, transcriptionally silent, resides inside spherical micronuclei that divide mitotically and undergo meiosis to generate gamete-nuclei during every sexual event. The somatic genome, transcriptionally active, resides inside larger and variously shaped macronuclei that divide amitotically and are destroyed and built ex-novo starting from mitotic products of the synkaryon formed by fertilization of the gamete-nuclei. These two genomes have remarkably diversified organizations[1]. While the DNA of the micronuclear genome is organized as conventional chromosomes, the DNA of the macronuclear genome forms unconventional nano-chromosomes, or linear 'gene-sized' molecules which range in size from 500 to 20000 bp and usually contain a single coding region[2]. The generation of the gene-sized genome from the chromosomic genome involves impressive DNA rearrangements including chromosome polytenization, fragmentation and elimination of up to 90% of the original DNA sequences[3,4]. The conclusive developmental step is the amplification of the relatively low number of genes that are essential for the cell life into hundreds, or thousands of copies[5].

This contribution will focus on the sophisticated epigenetic mechanisms that, driven by RNA molecules as mediators, are responsible for the complex but precise rearrangements that transform the germinal transcriptionally silent micronuclear genome into the somatic transcriptionally active macronuclear genome.

### References

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## TIME-LAPSE IMAGING OF CHROMATIN AND CYTOPLASMIC MOVEMENTS OCCURRING DURING THE GV-TO-MII TRANSITION: IN SEARCH FOR MARKERS OF MOUSE OOCYTES DEVELOPMENTAL COMPETENCE

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The transition from the germinal vesicle to the metaphase II stage (GV-to-MII transition) is crucial to the acquisition of the oocyte developmental competence. Here, using live, time-lapse, imaging we describe the movements occurring during the GV-to-MII transition to the chromatin (CHR-MOV) and to the cytoplasm (CYTO-MOV) of mouse oocytes of known developmental competence or incompetence.

Fully-grown cumulus-oocyte-complexes were punctured from the ovarian surface, the GV oocytes isolated and stained with the supravital Hoechst 33342 fluorochrome (Ho) which allowed the identification of gametes whose nucleolus is surrounded by a ring of Ho-positive chromatin (surrounded nucleolus, SN, oocytes) from those that lack this ring (not surrounded nucleolus, NSN, oocytes). Importantly, when *in vitro* cultured to MII and inseminated with sperm, whilst SN oocytes may develop to term, NSN oocytes arrest development at the 2-cell stage.

The time-lapse observation of CHR-MOV describe distinct chromatin changes in NSN compared to SN oocytes, with a longer GV-to-MII transition in NSN oocytes that reach the M-phase without the gathering of heterochromatin regions around the nucleolus.

Furthermore, by coupling bright-field time-lapse observations with the Particle Image Velocimetry method, we analysed the CYTO-MOV of these two types of oocytes. We showed that SN and NSN oocytes exhibit distinct profiles and, at four main time-frame intervals, their CYTO-MOV velocity is significantly different. In addition, we integrated the information of the CYTO-MOV profile of each single oocyte with an artificial neural network analysis that blindly identified the oocyte as SN or NSN with a robust probability.

The presence of SN and NSN oocytes in all mammals, including humans, extends the interest of these results to the field of assisted reproductive technologies (ART).