Binder Innovationspreis für M. Cristina Cardoso

Wissenschaftliche Laufbahn:

1981-1986	M.S., Department of Biology, School of Sciences, University of Lisbon, Portugal
1986-1990	Ph.D. in Biology-Molecular Biology, New University of Lisbon, Portugal; doctoral work on bacteriophage lysogeny at the Gulbenkian Institute of Science (Oeiras-Portugal) and at the Max Plank Institute for Molecular Genetics (Berlin-Germany)
1991-1994	Post-doctoral work on cell cycle regulation during terminal differentiation and functional organization of the nucleus in the group of Dr. B. Nadal-Ginard at the Harvard Medical School (Boston-USA)
1995-1997	Group leader at the Franz Volhard Clinic (Berlin-Germany)
since 1997	Group leader at the Max Delbrueck Center for Molecular Medicine (Berlin-Germany)

Fellowships:

1987-1990	Ministry of Science and Education (JNICT) pre-doctoral fellowship
1991	Luso-American Foundation for the Development fellowship
1991-1992	Ministry of Science and Education (JNICT) post-doctoral fellowship
1992-1994	Howard Hughes Medical Institute post-doctoral fellowship
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A Look at Genome Duplication in Living Cells and the Development of Cell Cycle Markers

The eukaryotic nucleus is the most conspicuous subcellular structure and has caught the attention of cell biologists since centuries. Nevertheless, very little is known of how major nuclear functions (e.g., DNA and RNA synthesis) are organized in the context of a living cell. In eukaryotic cells, the genetic information is packed into chromatin and needs to be accurately replicated every time a cell divides. This duplication takes place during a particular stage of the cell cycle designated S phase. All other cellular components are coordinately doubled throughout the interphase and several regulatory activities are needed to ensure that the daughter cells receive the entire complement of cellular components.

We are interested in the coordination of the multiple enzymatic activities involved in the duplication of the genome at every cell division cycle. Replication of the mammalian genome starts at tens of thousands of origins that are activated at specific times during S phase. Using initially antibodies and later translational fusions to green or red fluorescent proteins, we found that different replication proteins as well as cell cycle and DNA methylation factors, associate with replication sites within the nucleus. This indicates a direct coupling of cellular activities involved in cell cycle regulation and the maintenance of epigenetic information with the replication machinery in vivo. Time-lapse microscopy revealed that the replication sites form subnuclear patterns, which gradually change in a characteristic sequence throughout S phase. This spatio-temporal program of DNA replication is inherited through consecutive cell division cycles.

To investigate the progression from earlier to later replicons throughout S phase, we employed fluorescence photobleaching analyses as well as in situ biochemical extractions. The results show-ed that this transition occurs by disassembly of the replication machinery into a nucleoplasmic pool of rapidly diffusing subcomponents and reassembly at newly activated sites. Surprisingly, the individual components of this replication machinery exhibit very different exchange dynamics at replication sites, which was unforeseen from the available wealth of biochemical data obtained in vitro. To examine the temporal and spatial assembly of new replication machines we overlayed the images collected at consecutive times. We found that new replication foci were assembled in close proximity to earlier ones suggesting that activation of neighboring origins may occur by a domino effect possibly involving local changes in chromatin structure and accessibility.

These studies on the replication of the genome in living cells using fluorescent replication proteins also provided us with a simple and direct method to visualize and follow the cell cycle. By light microscopy, one can only distinguish mitotic cells and interphase cells. Further subdivision of interphase in living cells was not possible due to the lack of suitable markers. The differential subcellular distribution of the fluorescent replication factors over the cell cycle, provides a direct indication of the cell cycle stage, i.e., G1, S and G2. Moreover, the characteristic patterns of replication foci formed in the course of S phase, not only subdivide further early, mid and late S but also provide a direct localization of euchromatin versus heterochromatin. In view of the important role of different chromatin states in the epigenetic regulation of gene expression and in genome stability, this additional information will be very useful to directly study the association of factors to eu/heterochromatin in living cells, in real time. In summary, using this simple strategy one can unequivocally identify in situ all the cell cycle stages in live cells. These cell cycle markers allow now new experimental approaches to directly study cell cycle dependent processes like changes in protein dynamics or localization as well as the effect of drugs and mutations on cell cycle progression.

Selected Publications:

Cardoso, M.C., Leonhardt, H. and Nadal-Ginard, B. (1993). Reversal of terminal differentiation and control of DNA replication: cyclin A and cdk2 specifically localise at subnuclear sites of DNA replication. *Cell* **74**, 979-992.

McDermott, J.C., Cardoso, M.C., Yu, Y.-T., Andres, V., Leifer, D., Krainc, D., Lipton, S.A. and Nadal-Ginard, B. (1993). hMEF2C gene encodes skeletal muscle- and brain-specific transcription factors. *Mol. Cell. Biol.* **13**, 2567-2577.

Cardoso, M.C., Joseph, C., Rahn, H.-P., Reusch, R., Nadal-Ginard, B. and Leonhardt, H. (1997). Mapping and use of a sequence that targets DNA ligase I to sites of DNA replication in vivo. *J. Cell Biol.* **139**, 579-587.

Cardoso, M.C. and Leonhardt, H. (1999). DNA methyltransferase is actively retained in the cytoplasm during early development. *J. Cell Biol.* **147**, 25-32.

Leonhardt, H., Rahn, H.-P., Weinzierl, P., Sporbert, A., Cremer, T., Zink, D. and Cardoso, M. C. (2000). Dynamics of DNA replication factories in living cells. *J. Cell Biol.* **149**, 271-280.

Derer, W., Easwaran, H. P., Leonhardt, H., and Cardoso, M. C. (2002). A novel approach to induce cell cycle reentry in terminally differentiated muscle cells. *The FASEB J.* **16**: 132-133.

Sporbert, A., Gahl, A., Ankerhold, R., Leonhardt, H. and Cardoso, M. C. (2002). DNA polymerase clamp shows little turnover at established replication sites but sequential de novo assembly at adjacent origin clusters. *Mol. Cell*, **10**: 1355-1365.