

Phd THESIS DEFENSE: Quantitative Fluorescence Imaging of Spatiotemporal Dynamics of DNA Damage and DNA Replication in Health and Disease

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11:00

ICFO Auditorium and Online (Teams)

Genomic instability, caused by DNA damage, is the main determinant for cancer and aging. To safeguard genomic integrity, cells evolved complex mechanisms to ensure error-free DNA replication and DNA damage repair. However, cells are not always able to repair DNA damage, and have to halt proliferation in a state of senescence, or perform the programmed cell death, apoptosis, to prevent giving rise to tumors and to protect the organism. Yet, this loss of proliferative potential ultimately leads to aging of the organism.

The significance of DNA damage repair is underlined by mutations in genes encoding DNA

repair proteins, which lead to premature aging diseases associated with a wide spectrum of early-onset age-related diseases. Notably, Hutchinson-Gildford progeria syndrome (HGPS), the most severe premature aging disease, is not caused by mutations in a DNA repair protein, but in the nuclear intermediate filament protein lamin A. Nonetheless, DNA damage is considered a main driver of this disease.

The affected protein lamin A is a main component of the nuclear lamina, which is an intermediate filament meshwork and one of the layers of the nuclear envelope which surrounds the nucleus. To date, the pathological mechanism how the mutant form of lamin A leads to DNA damage in HGPS is poorly understood.

Here, we propose our hypothesis that this mutations disturbs the interactions between the nuclear lamina and peripheral DNA in a manner that mechanically interferes with the local progression of DNA replication sites. Consequently, our hypothesis predicts that DNA damage predominantly arises during DNA replication of peripheral DNA in close proximity to the nuclear lamina. This creates a spatial correlation between the occurrence of DNA damage and the nuclear periphery, as well as a temporal correlation with DNA replication of peripheral DNA which occurs during late S-phase of the cell cycle.

Hence, in Chapter 3, we present our approach to characterize the spatiotemporal dynamics of DNA damage throughout the cell cycle. This approach employs simple reporter cell models of DNA damage and DNA replication, along with long-term multi-color fluorescence live cell imaging microcopy, and a quantitative analysis pipeline. This analysis pipeline monitors and follows cells over multiple days and quantifies DNA damage foci formed by fluorescent DNA damage repair

proteins, and employs machine learning-based algorithms to classify distribution patterns of the DNA replication protein PCNA to perform post hoc in silico synchronization of cell cycles.

In Chapter 4, we describe how we employed this approach to quantify DNA damage foci and to characterize their distributions throughout the cell cycle in cell models of HGPS. We conducted these experiments under different conditions and with different cell lines, however we could not detect differences between HGPS cell models and healthy controls. Finally, we discuss our findings as well as technical and biological aspects of our approach in the context of literature.

In Chapter 5, we present an approach which we developed to study the influence of the mutant form of lamin A on the mobility of DNA replication sites, and thus to test the mechanical aspects of our hypothesis. This approach is based on single molecule tracking of the DNA replication protein PCNA. While we could not detect differences between HGPS cell models and healthy controls with this approach, we revealed two slow mobility states of PCNA within DNA replication sites. These two mobility states are consistent the the PCNA meshwork model proposed by Boehm et al. in 2016 and may represent DNA replication condensates.

Finally, in Chapter 6, we summarize the main results of this thesis and discuss future and

potential applications of our approaches to advance our understanding of the cell cycle-dependent dynamics of genome maintenance, and the structural organization of DNA replication sites.

Thursday June 03, 11:00 h. ICFO Auditorium and Online (Teams)
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Hosted by: Prof. Dr. Maria Garcia-Parajo and Academic Affairs