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Deadline: March 1st, 2024

EUR CARe PhD program pre-proposal

(2 pages maximum)

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Research project title: Decoding the RNA Polymerase II Citrullination Code in Biomolecular Condensate in Cancer**.**

Research program abstract (max 500 words):

Eukaryotic transcription regulation is crucial for establishing how cells respond to various signals and maintain proper gene expression patterns. Notably, the eukaryotic nucleus contains several phase-separated transcriptional condensates that compartmentalize and concentrate biomolecules with distinct physicochemical properties. Liquid-liquid phase separation (LLPS) is known as the predominant phenomenon to compartmentalize nucleic acids and proteins in membrane-less organelles, also termed biomolecular condensates (**1-2**). Importantly, the formation of biomolecular condensates by the LLPS has emerged as a regulatory mechanism for the spatiotemporal coordination of cellular events and the initiation and/or evolution of cancer and could contribute to modulating the tumor microenvironment (**3-6).** In this regard, arginine residues in low-complexity proteins are observed to be a driving force in the maintenance of the LLPS principle in cells (**7**). However, the molecular mechanisms by which arginine residues could govern the physical principle of phase separation in cells and contribute to oncogenesis processes have not been understood.

Consistent with this idea, it is essential to highlight that arginine residues could be post-translationally converted to the non-coded citrulline amino acid by a family of enzymes known as peptidyl arginine deiminase (PADI) (**8**). During citrullination, there is a loss of a positive charge and an increase in the hydrophobicity of proteins, which can potentially affect protein structure, protein-protein interactions, and protein-nucleic acid interactions (**8**). Given the significance of these interactions for essential cellular functions, citrullination may uniquely modulate critical pathophysiological processes involved in cancer progression. Our work spotlights that PADI2 citrullinates the arginine at position 1810 (R1810) present in the large subunit of RNA polymerase II (RNAPII) (**9-10**). Our

observation that the procuration of Cit1810 of RNAPII skews the transcriptional regulation to promote cell proliferation has led us to hypothesize that Cit1810 could direct LLPS-derived chromatin organization and transcriptional plasticity in aggressive breast cancer patients. Importantly, low-complexity domains are often a hallmark of proteins present in membrane-less cellular compartments and display LLPS properties (**11-12**). Our preliminary *in vitro* data showed that Cit1810 had a higher potential for phase separation than R1810 at RNAP II. Therefore, the overall aim of the project is **to explore the first essential step of how the Cit1810 switch at RNAP II controls the distinct formation of biomolecule condensate,** which plays **a significant role in cellular events contributing to cancer progression**. We will adapt state of art molecular, cell biology, and biophysics methods along with single particle tracking and machine learning tools to document the function of RNAP II Cit1810 condensates. This will help to gain insights for future work to investigate its implication in new therapeutic approaches for resistance to aggressive breast cancer.

References Pls references in this project highlighted

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5. Mehta S, et al. (**2022**). ***Nat Rev Cancer***. doi: 10.1038/s41568-022-00444-7
6. Ahn JH, et al. (**2021**). ***Nature.*** doi:10.1038/s41586-021-03662-5
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9. Sharma P, et al., (**2019**). ***Molecular Cell***. doi: 10.1016/j.molcel.2018.10.016
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11. Hnisz D, et al. (**2017**). ***Cell***, 169: 13-23. doi: 10.1016/j.cell.2017.02.007
12. Boehning M, et al. (**2018**). **NSMB.** 25: 833-840. doi:10.1038/s41594-018-0112-y

Describe in 50 words max for each how this project fits the 3 defining criteria of the CARe graduate programme:

1) Relation to CARe topics of Cancer, Ageing and/or Rejuvenation

The proposed project is focused on gaining functional insights into the transcriptional biomolecular condensate in oncogenic processes is the main goal of the porposed project. We aim to investigate the role of RNAPII citrullination in biomolecular condensate formation and oncogenesis processes in aggressive breast cancer to gain potential insights for effective therapeutic designs for patients.

2) Multidisciplinary aspect

The selected candidate would conduct the suggested research to characterize the transcription condensates in cells using a variety of molecular biology techniques, high-resolution imaging, and machine learning tools. In Barcelona, Spain, the applicant will work with X. Salvetella's group to identify the transcriptional condensates by learning and applying sophisticated biophysical techniques.

3) International and/or industrial aspect(s)

The proposed project will be carried out under the supervision of Dr. P. Sharma (IPBS, Toulouse, France) and Prof. X. Salvetella, Biomedical Research Institute (IRB), Barcelona, Spain.

5 keywords in line with EUR CARe

Multidisciplinary Approaches, Interface of Biology and Biophysics, Cancer

5 references of the teams, highlighting the co-signatory students:

(Last 5 years)

José Luis Villanueva-Cañas, Narcis Fernandez-Fuentes, Catherine Teyssier, Malgorzata Ewa Rogalska, Ferran Pegenaute Pérez, Baldomero Oliva, Cedric Notredame, Miguel Beato, **Priyanka Sharma.** Evolutionary analysis reveals the role of a non-catalytic domain of peptidyl arginine deiminase 2 in transcriptional regulation. bioRxiv 2023; https://doi.org/10.1101/2022.09.19.508513 (*Under revision in iScience*).