2023-12-19

International PhD Program IC-PhD

PhD thesis project

2024 Call for application

Genome plasticity from evolution to cancer: an inducible system to understand the genesis and stability of neocentromeres

General information

Call	2024
Reference	2024-04-FACHINETTI
Keyword(s)	Cancer; evolution; genome instability; epigenetics; cell division

Director(s) and team

Thesis director(s)	Daniele Fachinetti
Research team	Molecular Mechanisms of Chromosome Dynamics
Research department	UMR 144 – Cell Biology and Cancer

Description of the PhD thesis project

Abstract

Centromeres are the chromosomal structures driving kinetochore assembly for faithful chromosomes segregation. Centromere dysfunctions can lead to aneuploidy, often observed in cancer. Despite the typical association of centromeres with DNA repeats, de novo centromeres (neocentromeres) can rarely form on single-copy loci, proving that centromeric function does not strictly require the presence of repeats. However, paradoxically, the abundance of repetitive DNA is associated with higher segregation fidelity and repeats exist at all human and in most mammalian centromeres: the untangling of this contradiction requires a deeper understanding of neocentromeres, whose formation mechanisms are largely unknown. Open questions remain regarding the factors triggering their establishment, the determination of the formation site, the associated epigenetic changes and the effect on segregation. In addition, human neocentromeres are mainly associated to diseases, including cancer, highlighting that their formation is linked to an increase in genome instability. The main goal of this project is to uncover the genesis of neocentromeres by inducing their formation in real time in different cellular model systems. We will characterize neocentromeres on the genetic and epigenetics level and identify novel factors involved in the determination of centromere identity. Altogether, we will shed light on the role of neocentromeres in cancer, evolution, genetic disease and genome instability.

Background

Centromeres are crucial in mediating correct chromosome inheritance during both mitosis and meiosis. Defects in centromere formation or function lead to numerical (aneuploidy) and structural chromosome alterations, common features of cancer cells and developmental disorders.

Centromeres are unique chromosomal structures composed of specialised protein complexes built on long tandem DNA repeats (named alpha-satellites), and are defined by a unique histone H3 variant, CENP-A, the

epigenetic mark that identifies and maintains centromeric position throughout the cell cycle. One of the strongest indications that centromeres are epigenetically inherited come from the discovery of neocentromeres. These are centromeres that form de novo at non-centromeric DNA sequences. Human neocentromeres are mainly associated with chromosomal rearrangements, entailing the partial or total excision of the original CenDNA, and they are found in developmental diseases and some types of tumors, including liposarcoma. Neocentromere formation are also part of an evolutionary phenomenon called centromere repositioning – observed in different mammalian lineages such as equids, chicken and primates – leading to the formation of an Evolutionary New Centromere (ENC). The mechanisms of neocentromere formation are still unknown and the existence of satellite-less neocentromeres poses questions regarding how they maintain their identity and function (preventing aneuploidy) in absence of satellite DNA.

Objectives

Although neocentromere existence has been known for decades, the genesis of their formation and their impact on chromosome segregation and genome stability are still obscure phenomena, as their study has so-far been limited to isolated cases that failed to provide a comprehensive analysis. The main goal is to decipher the neocentromere conundrum by performing a large scale, in-depth investigation of several induced neocentromeres, with a focus on the different cellular contexts in which they arise, and providing insights on their role in cancer, evolution, and genome instability. Specifically, this project aim to:

1. *Induce neocentromere formation in different cellular contexts.*
2. *Identify the universal or system-specific genetic and epigenetic factors that promote de novo centromere formation.*
3. *Decipher the segregation fidelity of neocentromere-carrying chromosomes.*
4. *Evaluate the consequences of genome instability on centromere integrity and elucidate how centromeres are inactivated.*

Experimental approaches

Beside molecular and cellular tools commonly available in the laboratory (e.g. state-of the art microscopy, live cell imaging, cytogenetic and FISH approaches, ..) the PhD candidate will use:

CUT&RUN and DiMeLo: recently established methods for mapping protein–DNA interactions genome-wide using Nanopore or Illumina sequencing such as Cleavage Under Targets and Release Using Nuclease (CUT&RUN) and DiMeLo mapping.

Sequencing and centromere mapping: 1) Illumina reads and well-established bioinformatic pipeline to faithfully align centromeric reads on these reference sequences, allowing to assign reads to specific centromeres; 2) Oxford Nanopore sequencing system. With a read length of several kilobases, it allows detection of other types of sequence variations (e.g. DNA methylation) that may affect CENP-A binding competence to centromeric DNA.

Centromere DNA enrichment: Our lab developed a technique (CenRICH) based on enzyme digestion and sucrose gradient purification to enrich for human CenDNA from live cells. The combination of this digestion protocol with size-selection result in a sample that is enriched in CenDNA by at least 20-fold compared to unselected genomic DNA.

International, interdisciplinary & intersectoral aspects of the project

International collaborations

- John Maciejowski (Sloan Kettering Institute, USA) for sharing the Chromothripsis-derived neocentromeres.
- Elena Giulotto (University of Pavia, Italy) for sharing of equid-derived evolutionary neocentromeres.
- Leonid Mirny (Institut Curie & MIT, US) for the study of 3D genome architecture.
- Nicolas Altemose (Stanford, USA) to assist in the Nanopore-mediated centromere mapping and DiMeLo mapping.

- Joshua Waterfall (Institut Curie), who has expertise in WD/DDLPS, for the study of the proprieties of the Cancer-Neo system and future studies on centromere abnormalities in cancer.
- Chunlong Chen and Marc-Henry Stern (Institut Curie) for future development of an optical mapping pipeline suitable for human centromeric DNA.

Intersectoral collaborations
Possible development of optical mapping with Bionano (https://bionano.com/) to perform optical mapping suitable for human centromeric DNA.

Recent publications

1. Keizer, V., Grosse-Holz, S., Woringer, M., Zambon, L., Aizel, K., Bongaerts, M., Kolar-Znika, L., Scolari, V., Hoffmann, S., Banigan, E., Mirny, L.A., Dahan, M., **Fachinetti, D.*** and Coulon, A. * (*Co-corresponding authors). Live-cell micromanipulation of a genomic locus reveals interphase chromatin mechanics. **Science** Jul 29;377(6605):489-495.

2. Chardon F, Japaridze A, Witt H, Velikovsky L, Chakraborty C, Wilhelm T, Dumont M, Yang W, Kikuti C, Gangnard S, Mace AS, Wuite G, Dekker C, **Fachinetti D.** CENP-B-mediated DNA loops regulate activity and stability of human centromeres. (**2022**) **Mol Cell**, May 5;82(9):1751-1767.e8

3. Giunta, S.*,#, Herve, S.*, White, R., Wilhelm, T., Dumont, M., Scelfo, A., Gamba, R., Wong, C., Rancati, G., Smogorzewska, A., Funabiki, H.# and **Fachinetti, D.#** (#Co-corresponding authors). CENP-A chromatin prevents replication stress at centromeres to avoid structural aneuploidy (**2021**) **PNAS** Mar 9;118(10):e2015634118. highlighted in preLights.

4. Hoffmann, S., Izquierdo, H., Gamba, R., Chardon, F., Keizer, V., Dumont, M., Herve, S., McNulty, S., Sullivan B., Manel, N. and **Fachinetti, D**. A genetic memory initiates the epigenetic loop necessary to preserve centromere position. (**2020**) **EMBO Journal,** *Sep 18:e105505*.
   "News and Views" section by Van de berg S.J.W. and Jansen L.E.T.

5. Dumont, M.*, Gamba, R.*, Gestraud, P., Klaasen, S., Worrall, J.T., De Vries, S.G., Boudreau, V.,Salinas-Luypaert, C., Maddox, P.S., Lens, S.M.A., Kops, G.J.P.L., McClelland, S. E., Miga, K.H. & **Fachinetti, D.** (**2020**). Human chromosome-specific aneuploidy is driven by DNA-dependent centromeric features. **EMBO Journal,** *Jan 15;39(2):e102924*.

Expected profile of the candidate

Training and Skills required
Applicants should have a master degree in biology and a strong interest in the fundamental mechanisms of chromosome dynamics and the desire to do multidisciplinary research in biology, biophysics and bioinformatics.
Background in molecular biology, knowledge in cell biology approaches using human cells and bioinformatics skills oriented to solve biological problem will be extremely valuable.
Abilities
The ideal candidate should show solid capacity for independent and creative thinking, and to work multitasking.
The applicant should feel comfortable working in an interdisciplinary and international environment.