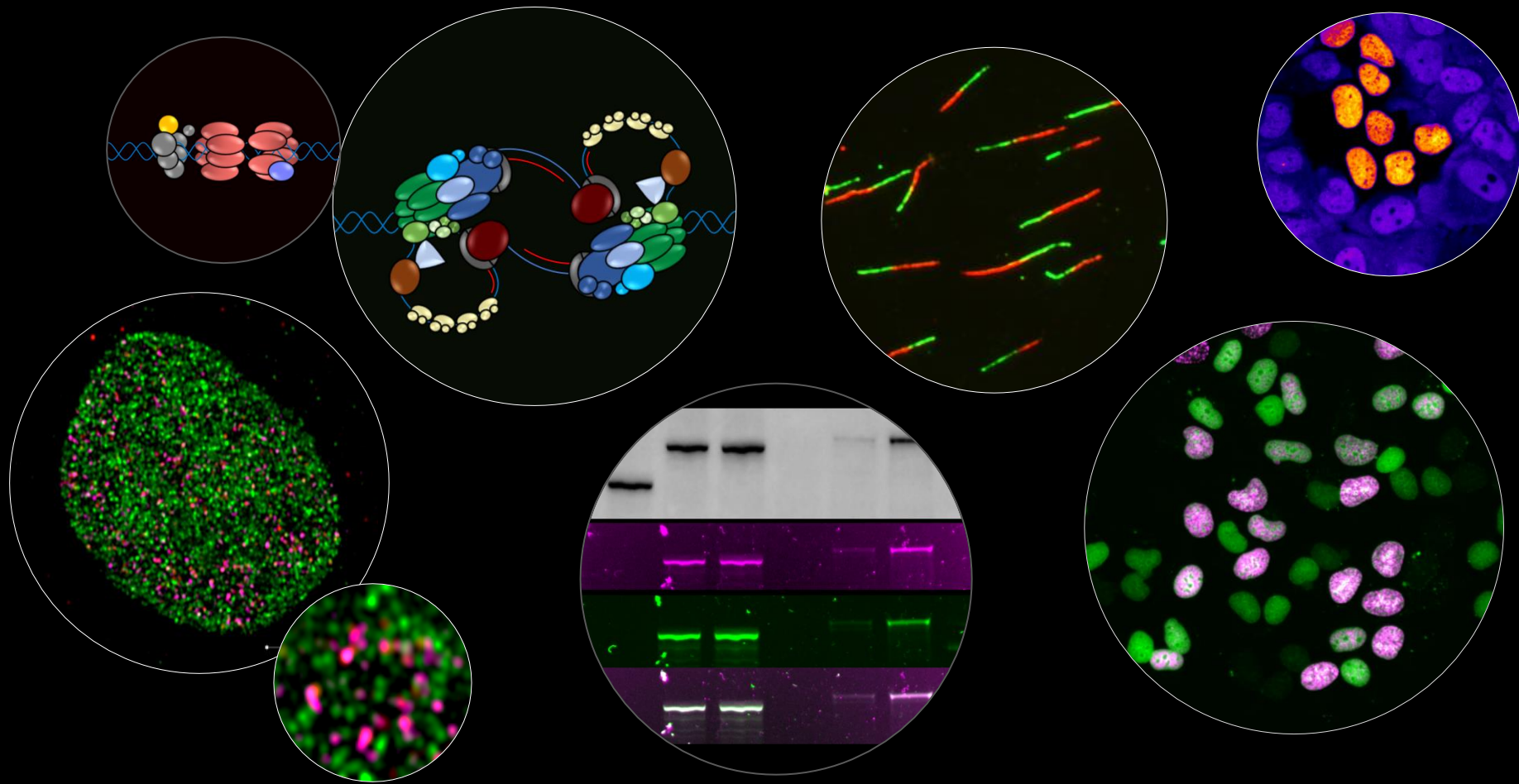
Regulation of DNA Replication in Healthy and Cancer Cells



Hana Polasek-Sedlackova

Bi1077: Introduction to Cell Biology

11th December 2024

SEDLACKOVA LAB

IBP
Institute of Biophysics, Czech Academy of Sciences

Why do you want to do science?

My journey to find the beauty of science..

2008

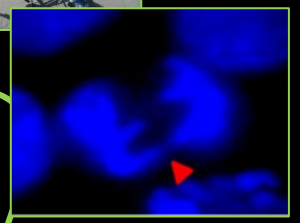
Grammar school student, Hodonin
Arachne: a camp for biologists

2009

Grammar school student
in Laboratory of Dr. Lumir Krejci

2011

Student at
Masaryk
University



2014

BSc in
Biochemistry

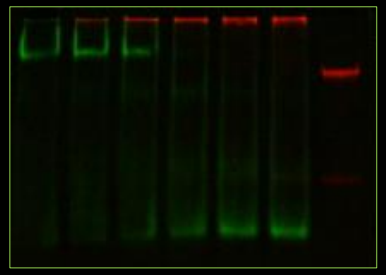
Internship in
the laboratory
of Prof. Ian
Hickson

2016

MSc in
Biochemistry
of Genomes &
Proteomes

Novo Nordisk Foundation
Copenhagen Bioscience
PhD program

PhD student
in Laboratory
of Prof. Jiri
Lukas



RECQ4 helicase preferentially binds branched DNA intermediates.

Sedlackova et al, DNA repair, 2015

Laboratory of DNA Replication
& Genome Stability

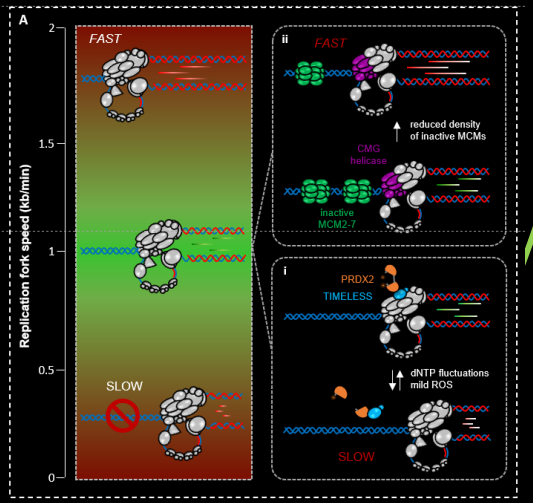
2022

2021

Postdoc in
Lukas lab

2020

PhD in Molecular
Mechanisms of Disease

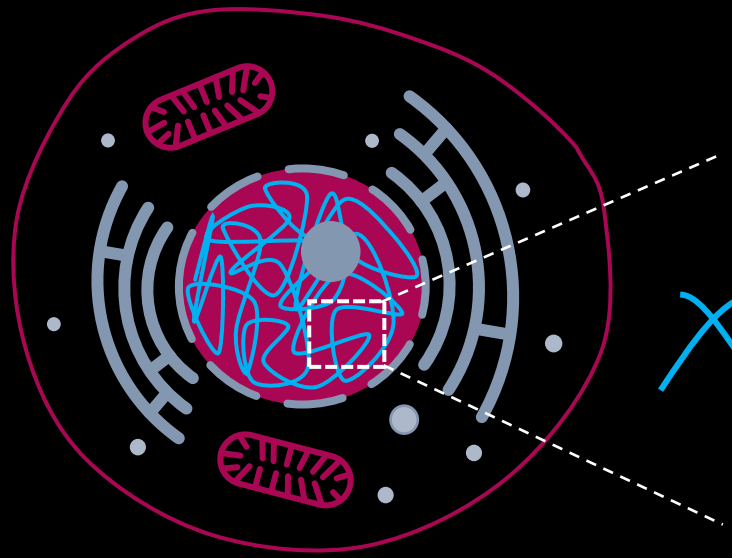


Somyajit et al, Science, 2017
Sedlackova et al, Nature, 2020
Polasek-Sedlackova, Nature Communications, 2022

Cancer is a large group of diseases that can start in almost any organ or tissue of the body when abnormal cells grow uncontrollably, go beyond their usual boundaries to invade adjoining parts of the body, and/or spread to other organs.

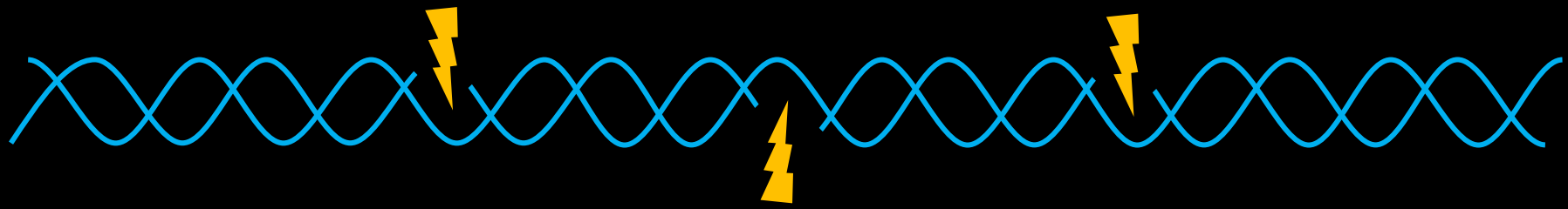
Cancer disease is one of the most common causes of death worldwide.

World Health Organization



eukaryotic cell

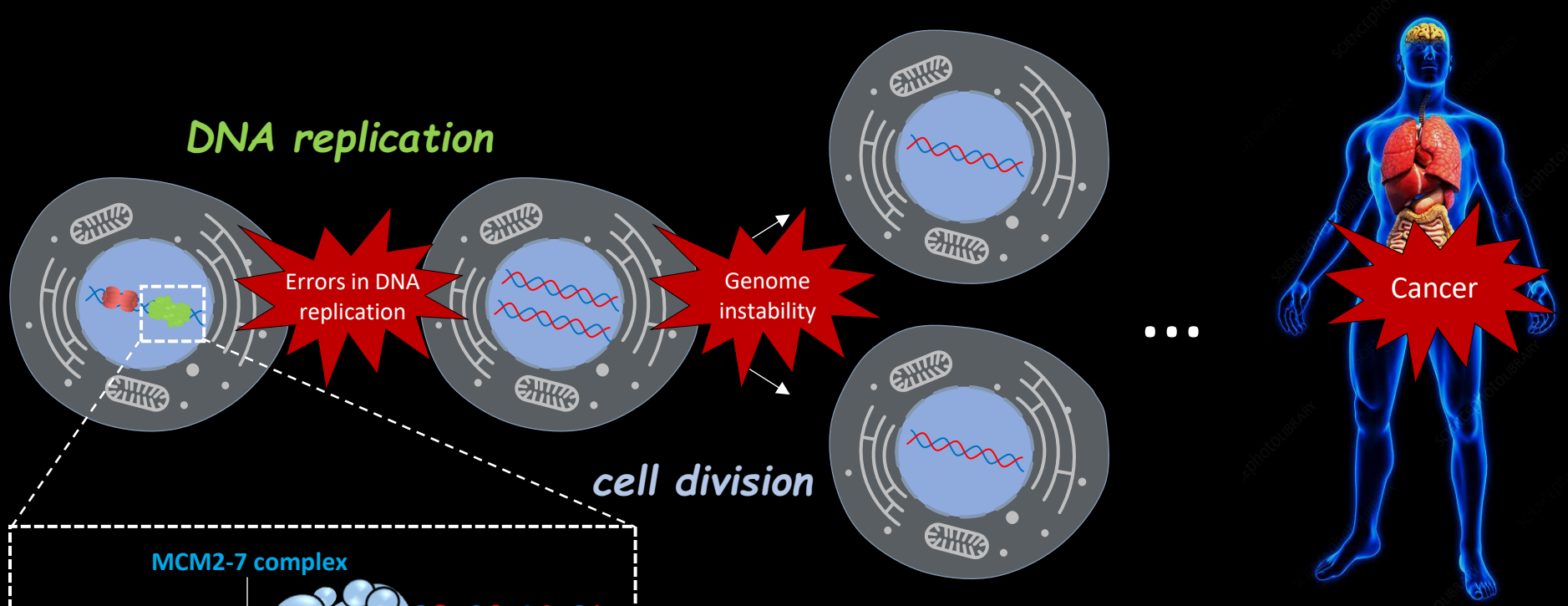
DNA damage causing cancer

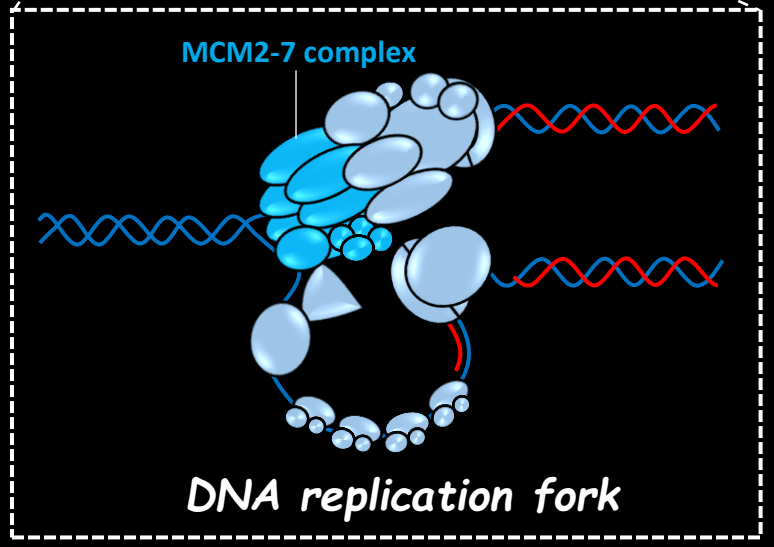


Two-thirds of all cancers arise from DNA replication errors.

Tomasetti & Vogelstein, Science, 2015

DNA replication: a fundamental process of life

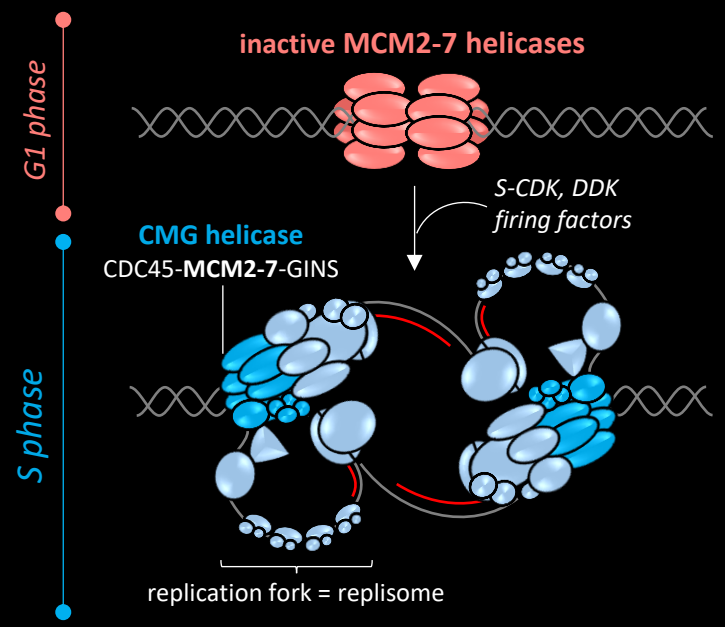


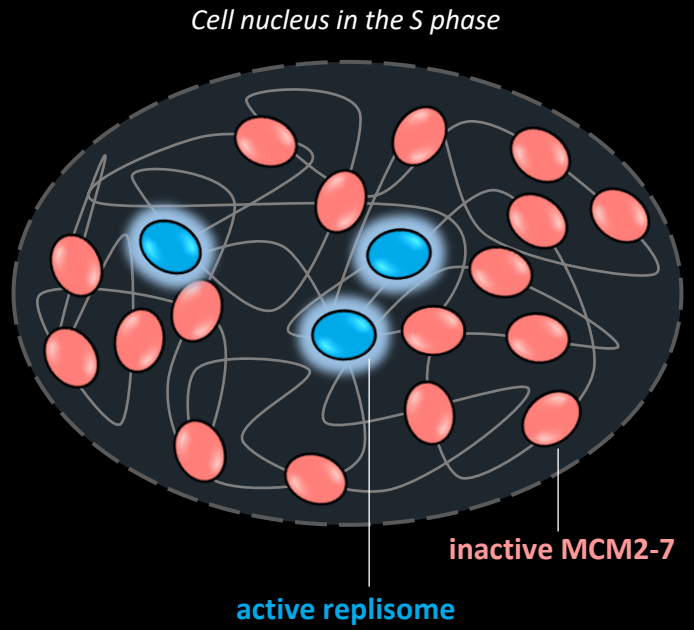


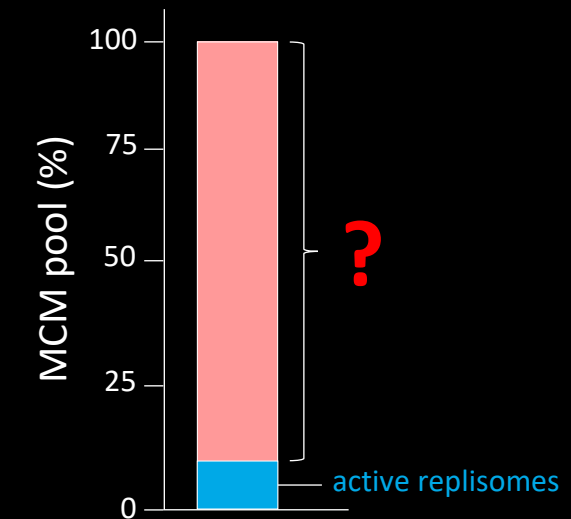
AIM:

How is DNA replication regulated in human cells to achieve high fidelity of copying genomes?

MCM2-7 protein complex: *the heart of every replication fork*



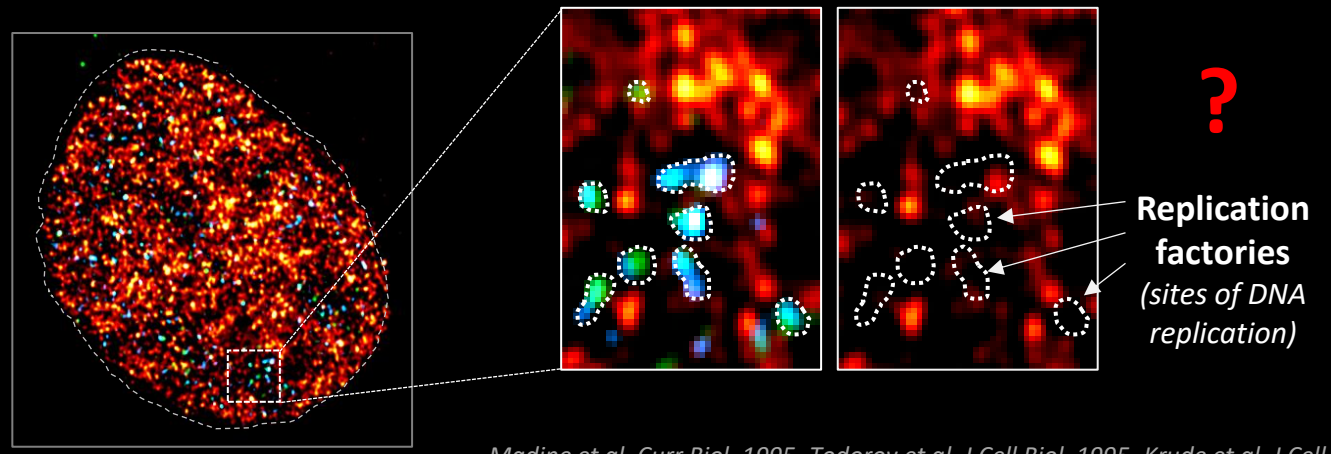




MCM paradox

I. Why do cells carry a huge excess of MCMs if only 5-10% is used as active CMG helicases?

II. Why active replicative helicases are not detected at DNA replication sites by immunofluorescence?



Recently reviewed in Yadav & Polasek-Sedlackova, Communications Biology, 2024

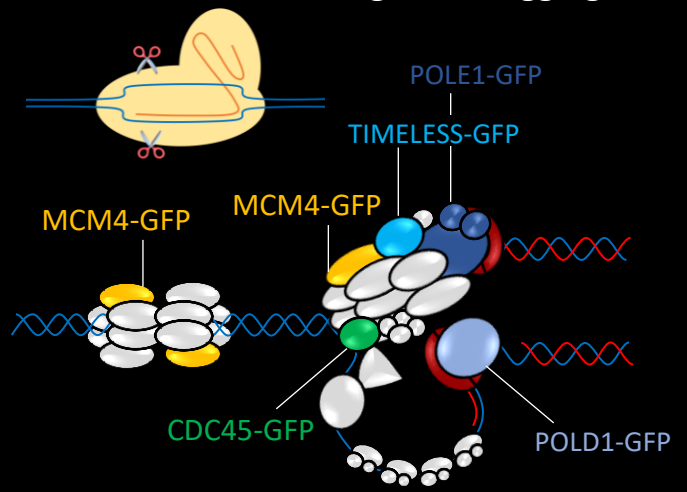
Madine et al, Curr Biol, 1995, Todorov et al, J Cell Biol, 1995, Krude et al, J Cell Sci, 1996, Dimitrova et al, J Cell Biol, 1999, Walter J. & Newport JW., Science, 1997, Ibarra A. et al,PNAS, 2008, Hyrien O. et al, Bioessayes, 2003 and others

Our approach to study human DNA replication

LUKAS LAB

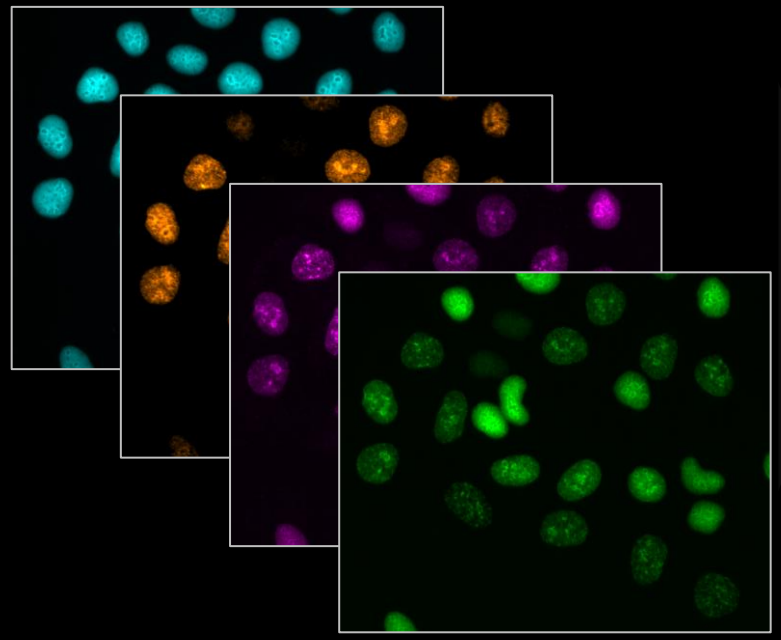
UNIVERSITY OF COPENHAGEN

CRISPR-Cas9 endogenous tagging

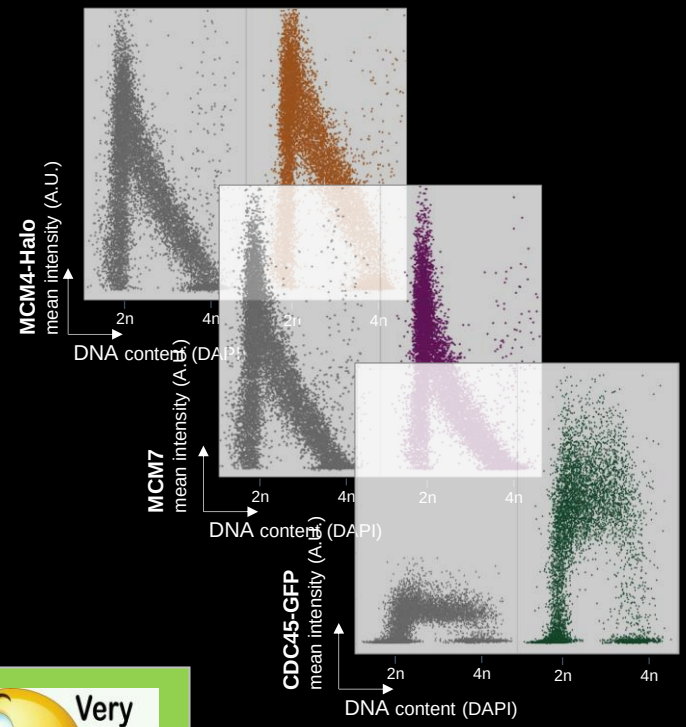


Olympus ScanR High-Content Screening Station

Automated Image Acquisition using ScanR acquisition software

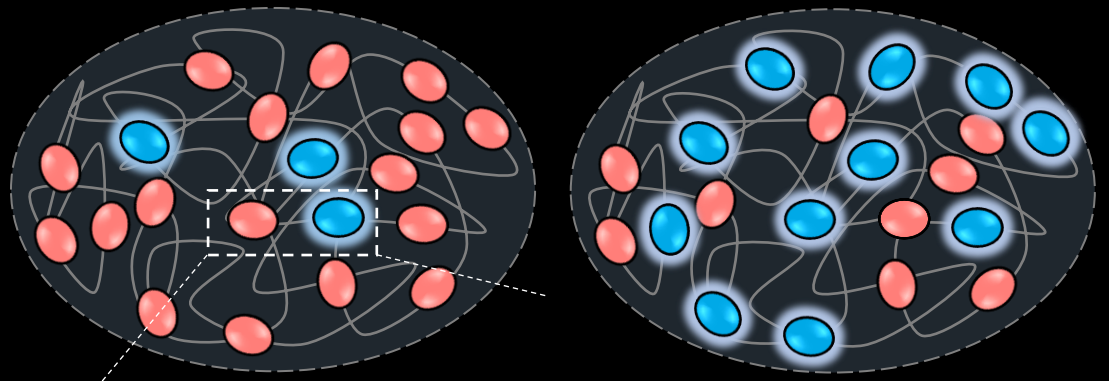


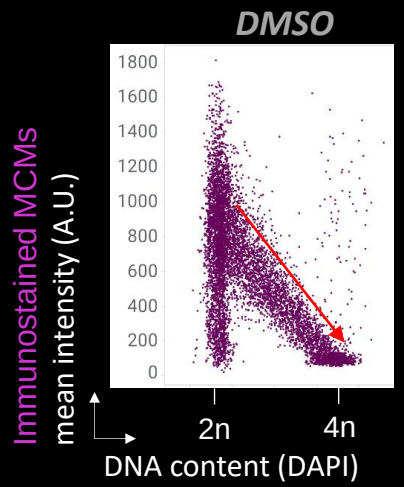
Automated Multiparameter Image Analysis by ScanR analysis & Spotfire softwares

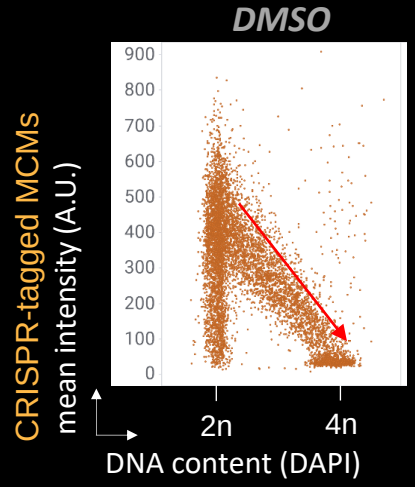


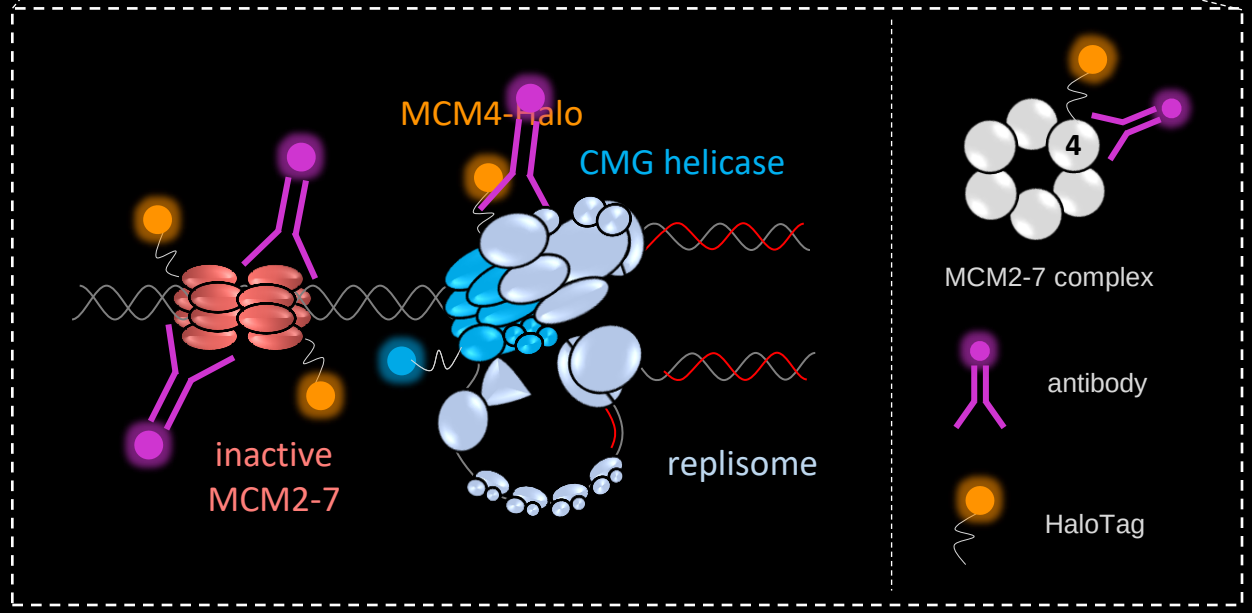
- *fully automated, unbiased, quantitative approach*
- *acquisition of 10 000 cells per 10 min*

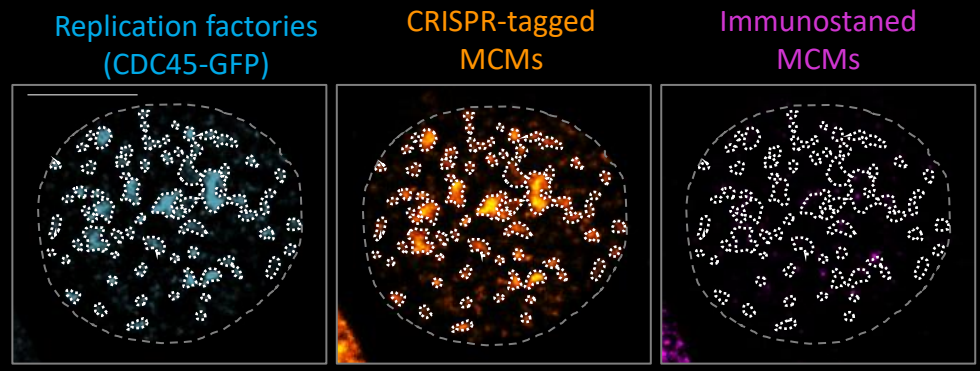
I. Why active replicative helicases are not detected at DNA replication sites by IF?







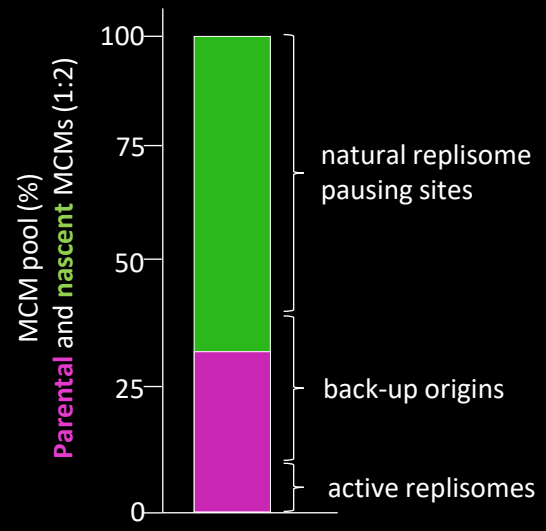


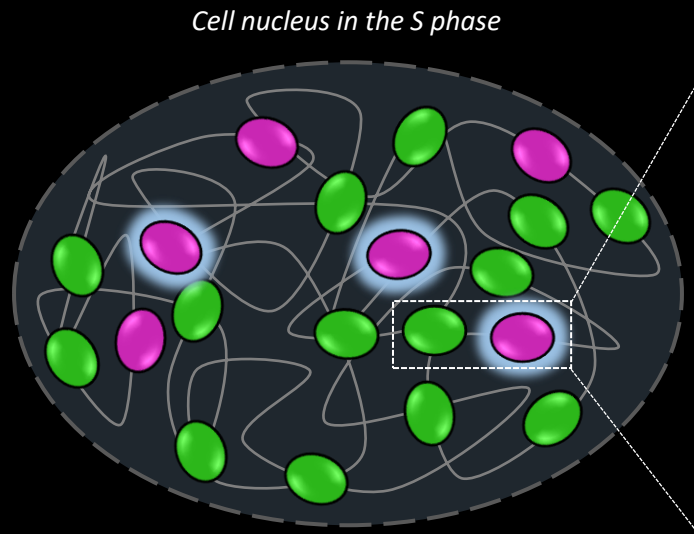


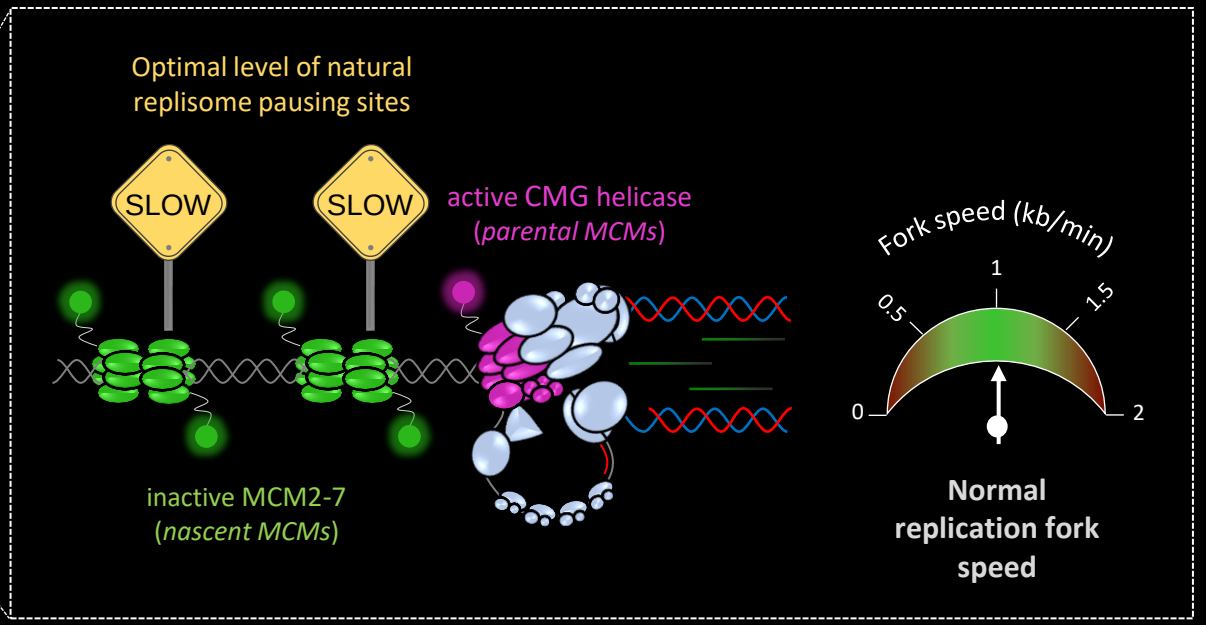
Late stage of the S phase, ATRi treatment

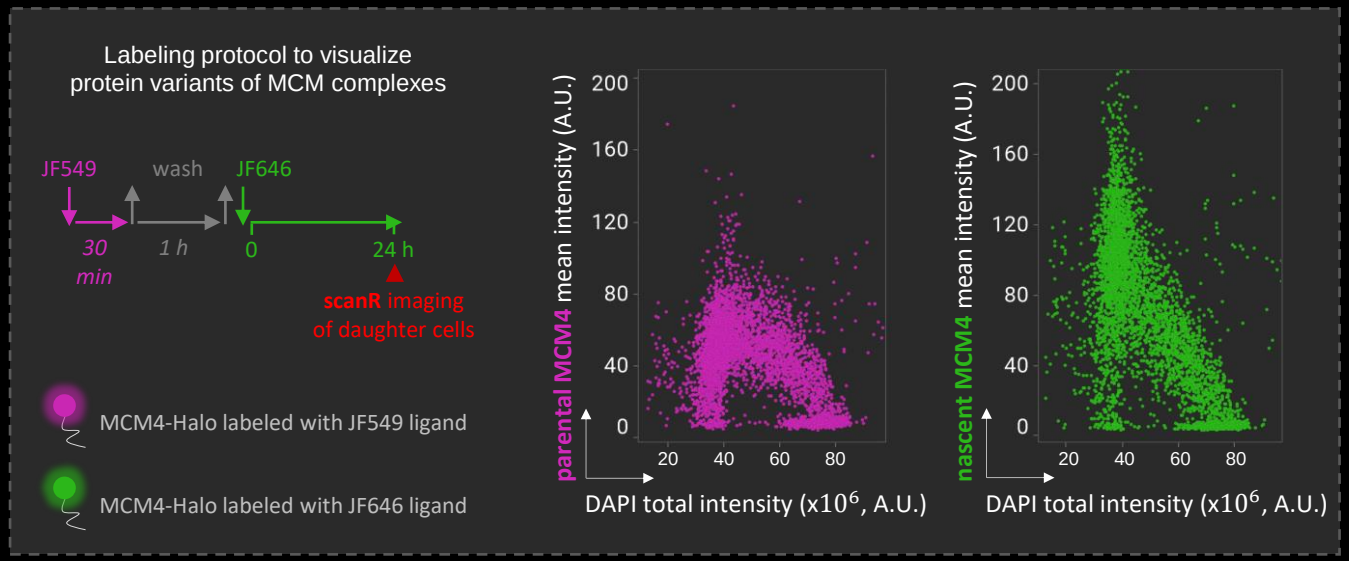
Polasek-Sedlackova et al., Nature Communications, 2022

II. Why do cells carry huge excess of MCMs if only 5-10% is used as active replisomes?







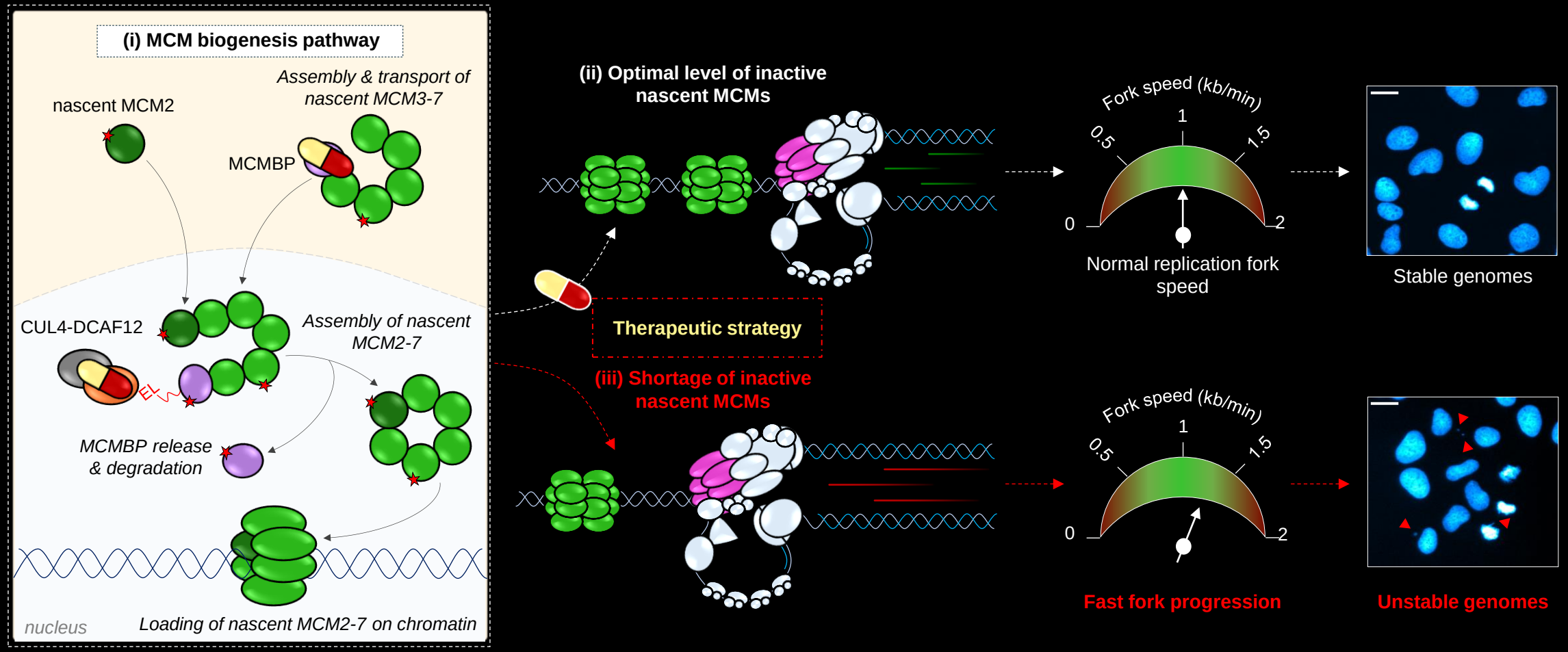


What are the biochemical properties of parental and nascent MCMs?

What is the molecular mechanism(s) behind MCM biogenesis and recycling pathways?

Sedlackova et al., Nature, 2020

Specific biogenesis pathway regulates the optimal level of nascent MCMs



Sedlackova lab

Cermak lab

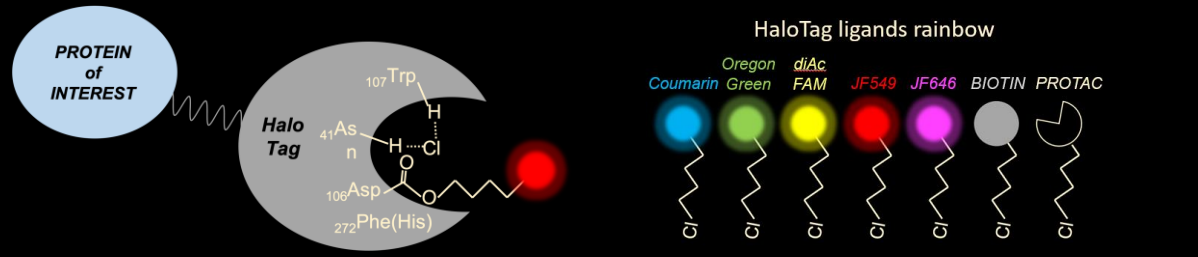
CRL4^{DCAF12} regulation of MCMBP ensures optimal licensing of DNA replication

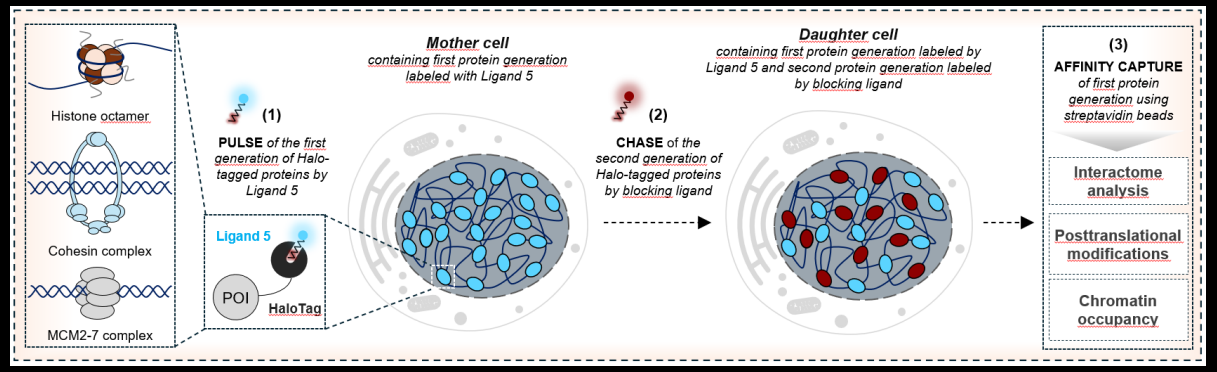
Yadav A. K., Abdirov A., Ondruskova K., Negi S., Kolarova K., Dibus N., Krejci J., Polasek-Sedlackova H., Cermak L. bioRxiv, 2022

Developing new imaging tools and workflows

Development of a cell-permeable Biotin-HaloTag ligand to explore functional differences between protein variants across cellular generation

Yadav A. K., Jadhav A. S., Szczepanik P., Fagherazzi P., Kabelka I., Vacha R., Svenda J., Polasek-Sedlackova H.,





Driven by Sedlackova & Svenda laboratory

Thank you!

SEDLACKOVA LAB

Sedlackova group

Hana Polasek-Sedlackova
Jana Krejci
Paolo Fagherazzi
Anoop Kumar Yadav
Simran Negi
Klara Janjic *(guest researcher)*

ScanR technical support & maintenance

Jiri Polasek
Tomas Pop (Evident)
Tomas Jendrulek (SVEN Biolabs)

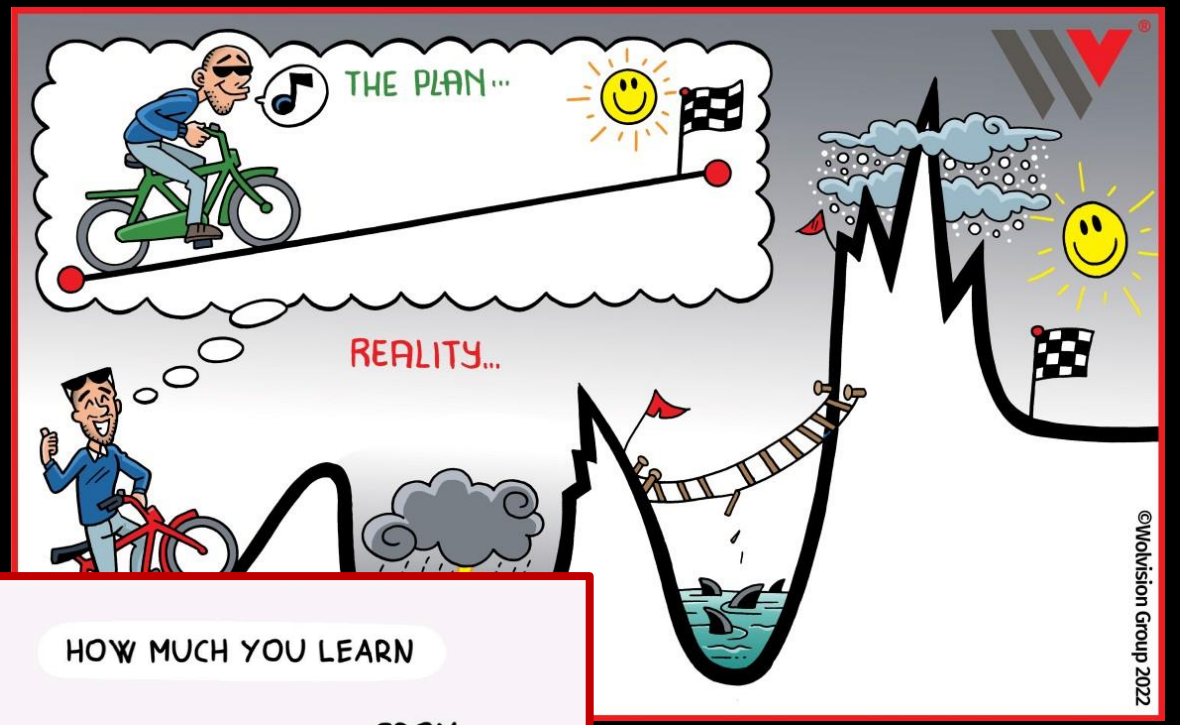
Collaborations

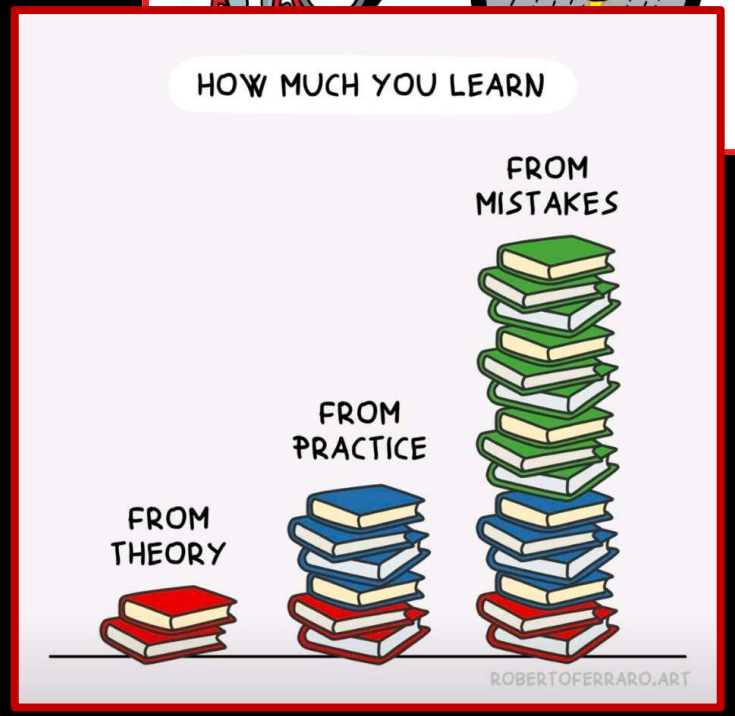
Vincenzo Costanzo
IFOM, Italy
Dipanjan Choudhury
Dana-Farber Cancer Institute, Harvard Medical School, USA
Timo Diekmann
Evident, Germany
Kumar Somyajit
University of Southern Denmark, Denmark
Hasan Yardimci
Francis Crick institute, UK
Lukas Cermak
Institute of Molecular Genetics, Czech Republic
Lumir Krejci
Masaryk University, Czech Republic Republic
Karel Soucek
Institute of Biophysics, CAS, Czech Republic
Jakub Svenda
Masaryk University, Czech Republic

GAČR CZECH SCIENCE FOUNDATION JUNIOR STAR
IBP Department of Cell Biology and Epigenetics
Funded by the European Union
jcmm EMBO CSMS

#WE ♥ SCANR

What does a scientific journey look like?

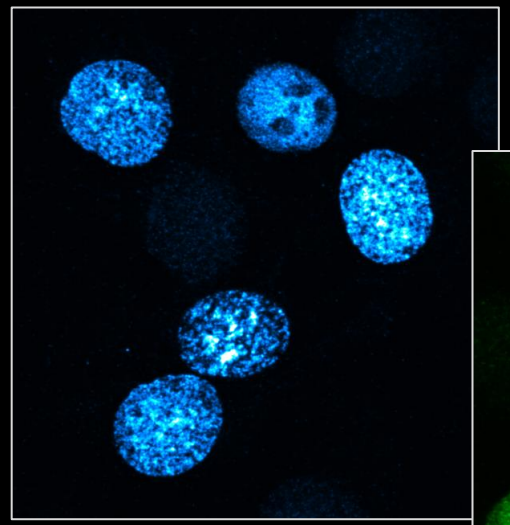




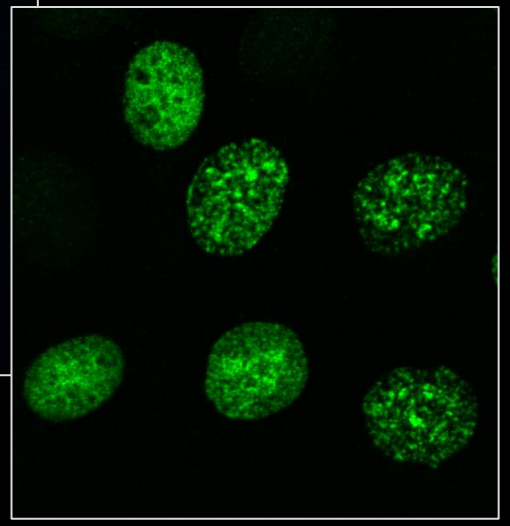
Don't be afraid of mistakes.

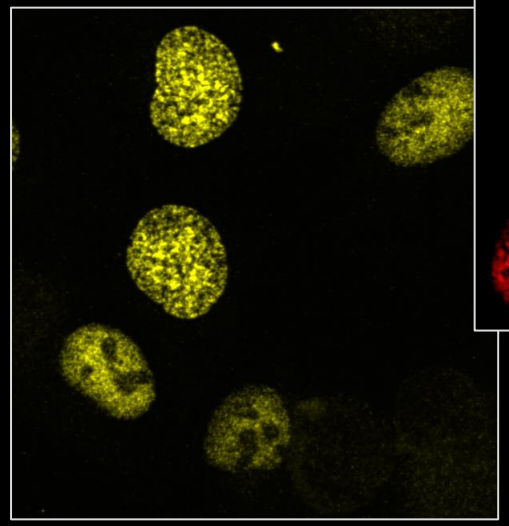
Some tips…

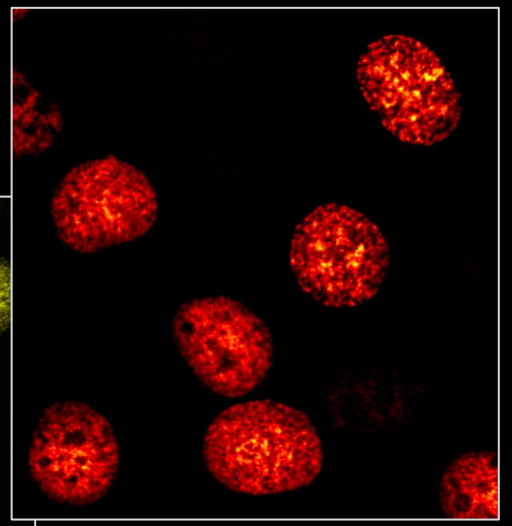
Motivation

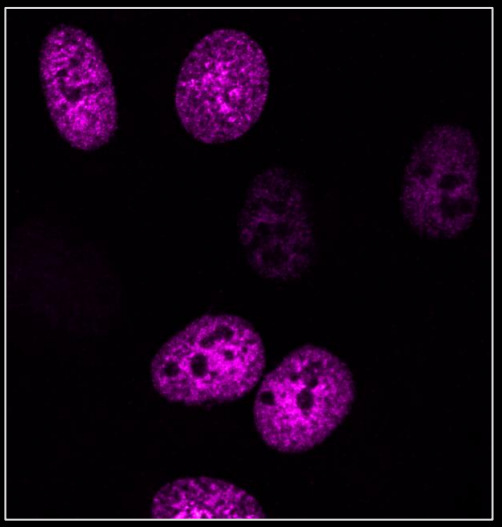


To see things that no one in the world has seen before you.









Find something that will motivate you to go to the lab every day.

Networking

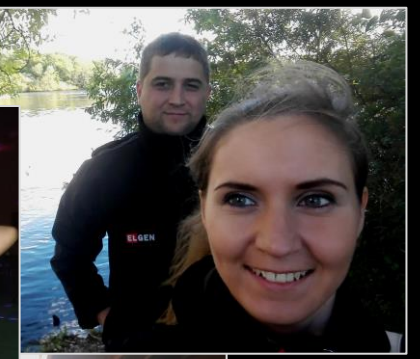
Surround yourself with people, who..

..will stay behind even in a strong storm during your career.

..will always help you no matter what.

..but don‘t forget to listen to their advice!

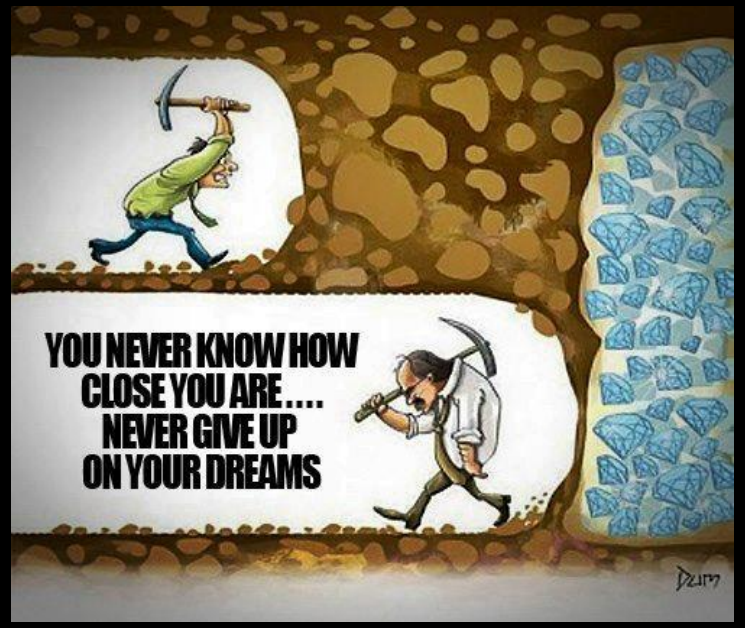
..will always support you in your career.



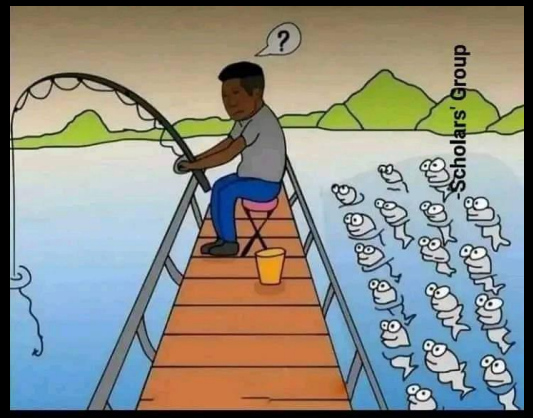
..with whom you can share your ups and downs.

Some tips…

Never give up!



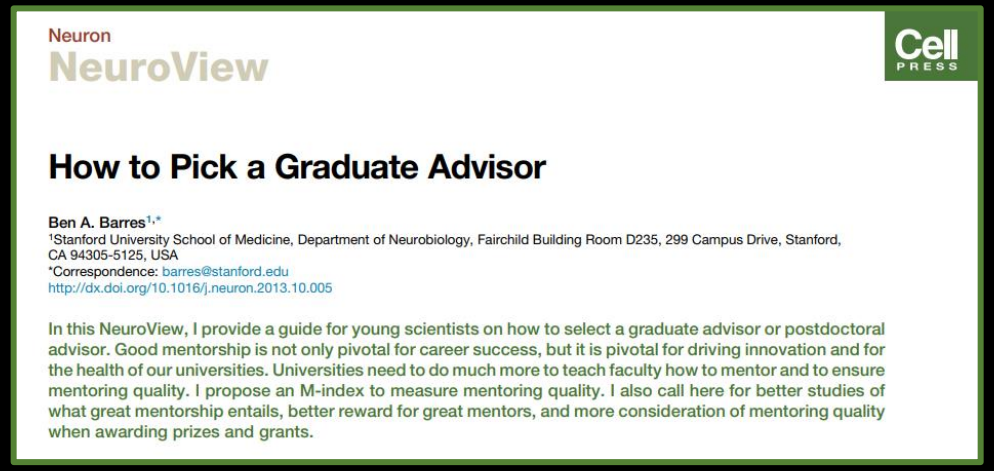
Always have a plan B!



Sometimes you need to change the direction.
Patience is not always the optional way.

JUST
Smile
and be
HAPPY

How to pick a supervisor?



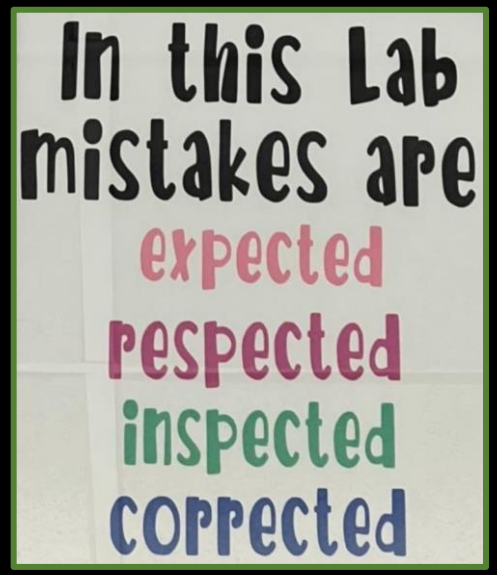
Neuron
NeuroView

Cell PRESS

How to Pick a Graduate Advisor

Ben A. Barres[1,*]
[1]Stanford University School of Medicine, Department of Neurobiology, Fairchild Building Room D235, 299 Campus Drive, Stanford, CA 94305-5125, USA
*Correspondence: barres@stanford.edu
http://dx.doi.org/10.1016/j.neuron.2013.10.005

In this NeuroView, I provide a guide for young scientists on how to select a graduate advisor or postdoctoral advisor. Good mentorship is not only pivotal for career success, but it is pivotal for driving innovation and for the health of our universities. Universities need to do much more to teach faculty how to mentor and to ensure mentoring quality. I propose an M-index to measure mentoring quality. I also call here for better studies of what great mentorship entails, better reward for great mentors, and more consideration of mentoring quality when awarding prizes and grants.



Mentorship ability

Is the potential advisor a good mentor?

A good mentor does not put his/her student on a scientifically trivial question.

Good mentors spend enormous amounts of time with each of their students discussing science, how to design good experiments and interpret and analyze data, how to write research papers and grants, how to read and review papers, practicing talks, and providing career guidance.

Good mentors encourage their trainees to take time away from their research to ***do other activities*** *(work-life balance, parental leave tec.)*

Scientific ability

How to identify an advisor who is a good scientist?

Is the potential advisor asking important scientific questions?

How to evaluate the quality of research work? (papers in top journals, acquired funding, awards)

Passion

Is the potential advisor passionate about his/her work (work performed by his/her students or postdocs)?


thank you