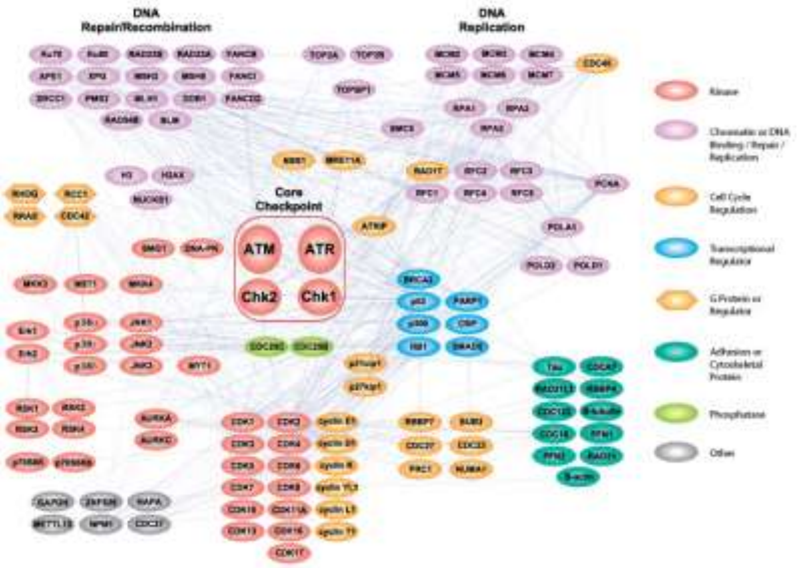
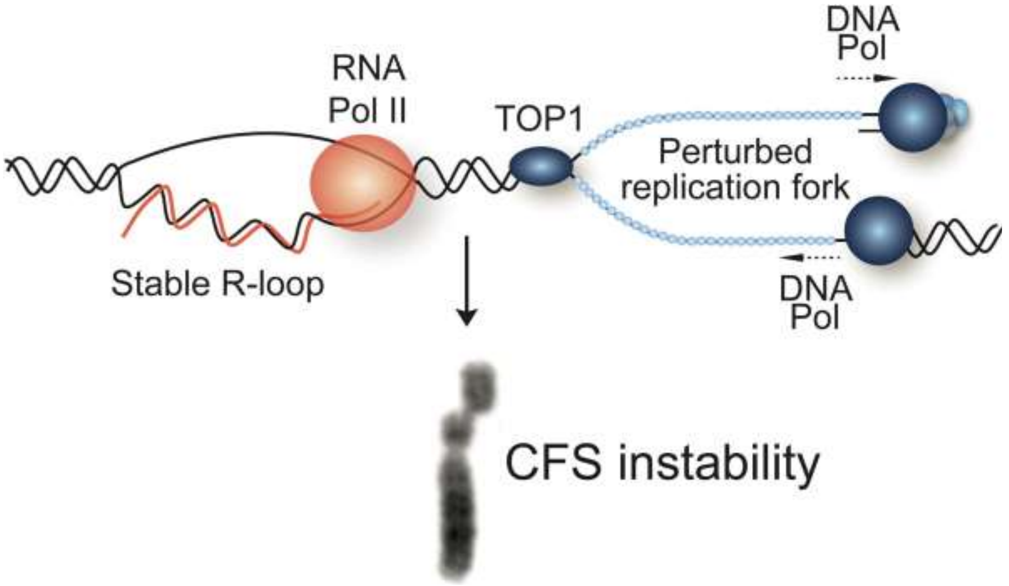
Maintenance of genome integrity:

R-loops – genome in danger?





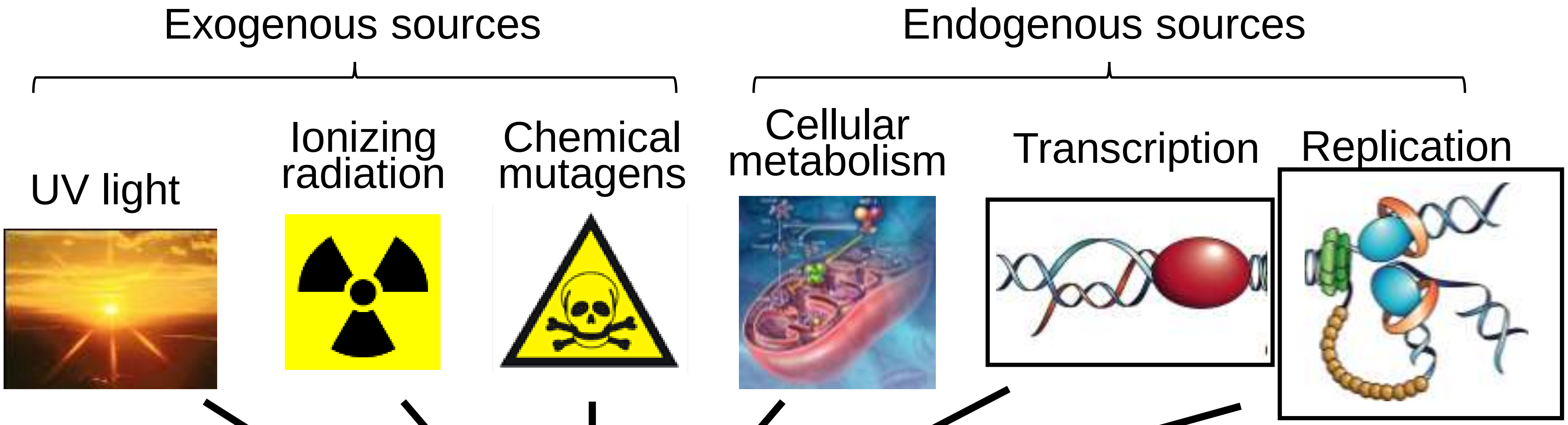
Jana Dobrovolná

Laboratory of genome integrity

IMG

Institute of Molecular Genetics of the ASCR, v. v. i.

Our DNA encounters with damaging agents every second



Exogenous sources

Endogenous sources

UV light

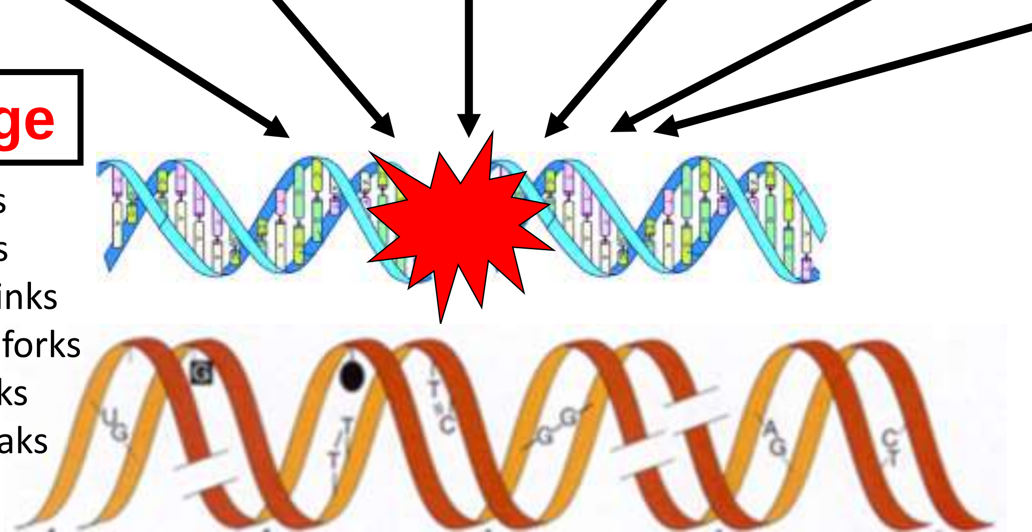
Ionizing radiation

Chemical mutagens

Cellular metabolism

Transcription

Replication



DNA damage

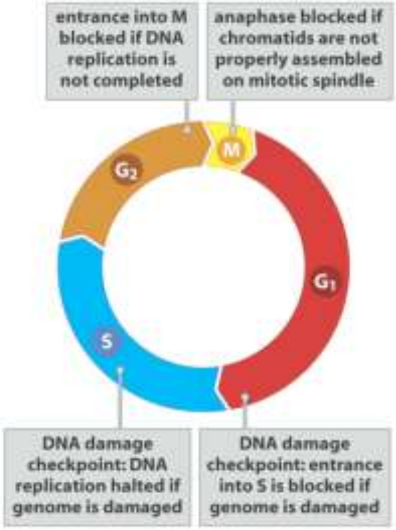
- Base modifications
- Bulky DNA adducts
- Inter-strand crosslinks
- Stalled replication forks
- Single-strand breaks
- Double-strand breaks

Replication stress !!!

The fate of cells with damaged DNA

DNA damage

Check-point activation
Transient cell cycle arrest



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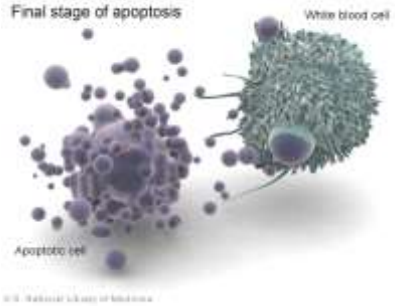
Repair

Apoptosis

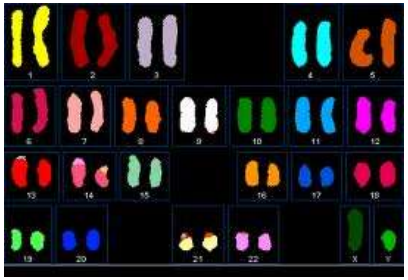


U.S. National Library of Medicine

Senescence

Genome instability → CANCER

Normal karyotype



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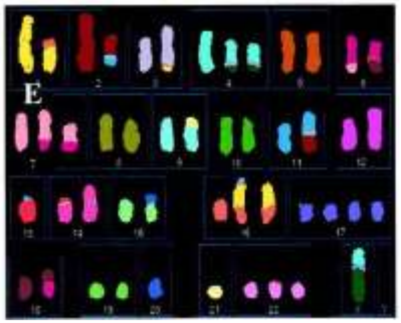
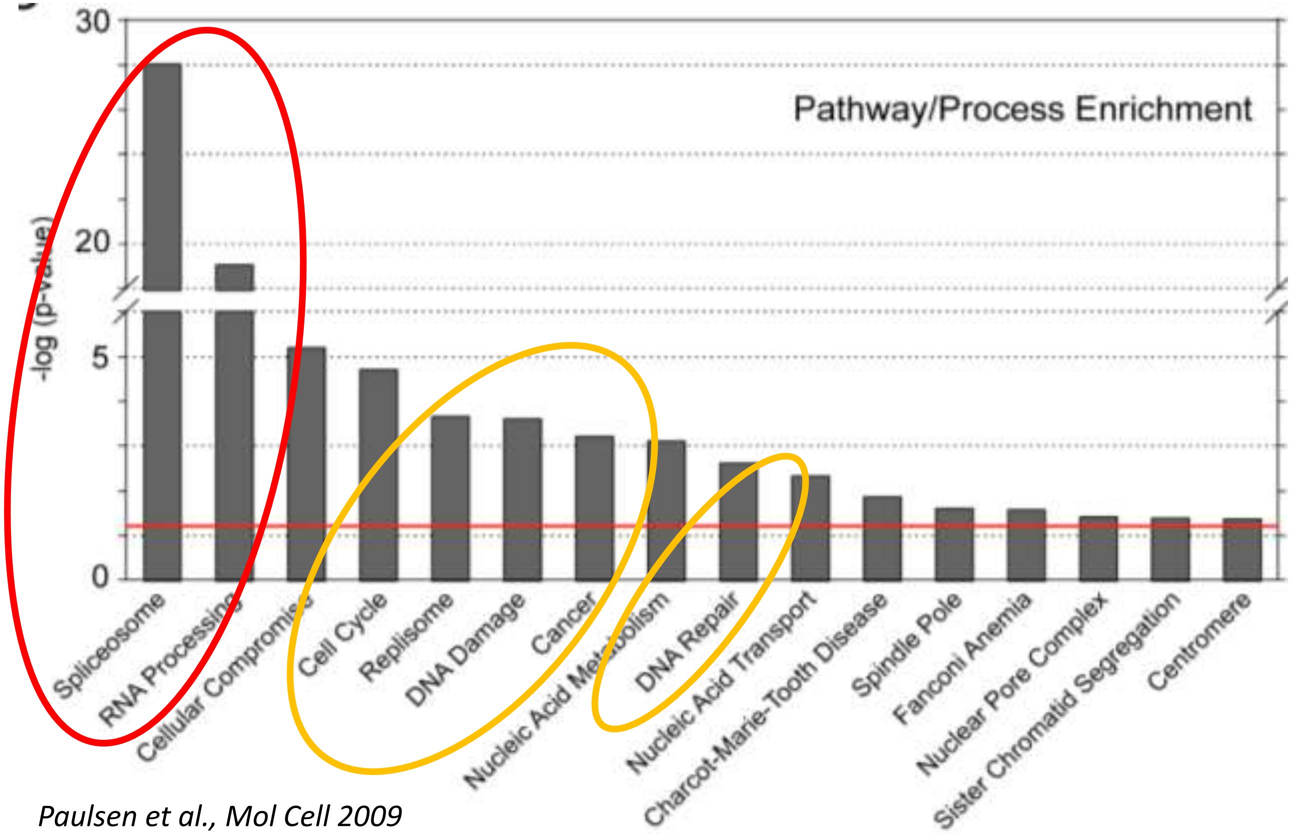
Cancer karyotype



Transcription associated genomic instability

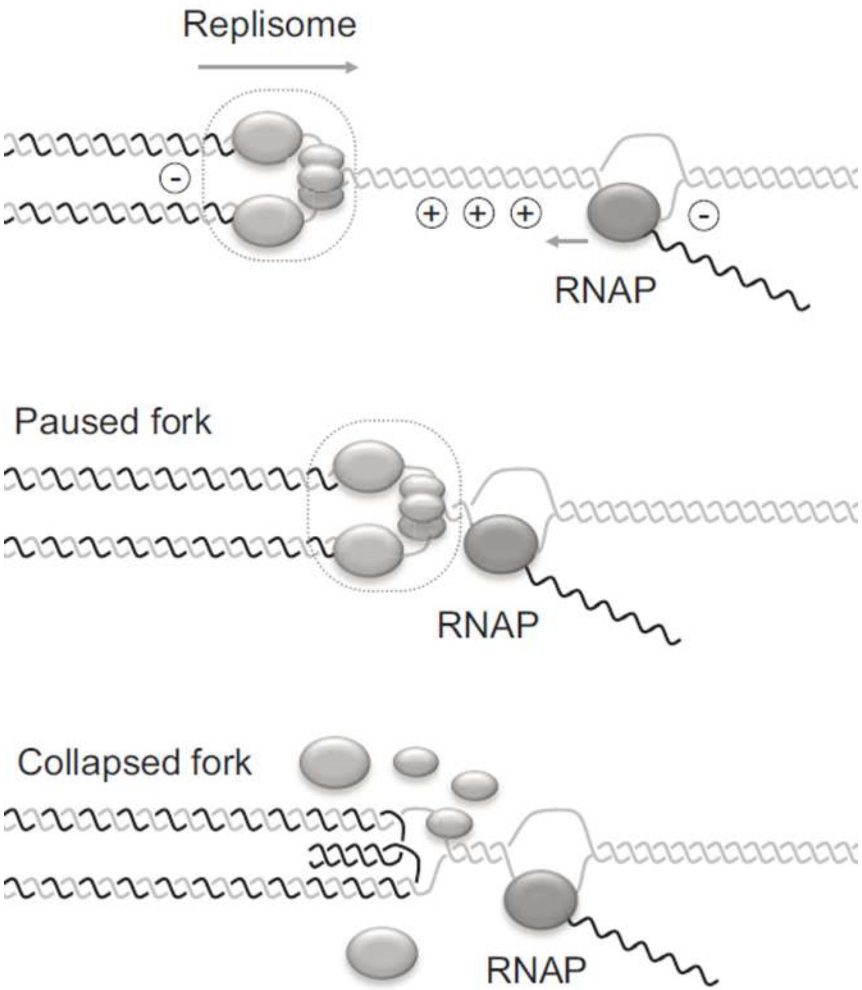
Genome-wide siRNA screen for factors involved in genome stabilization

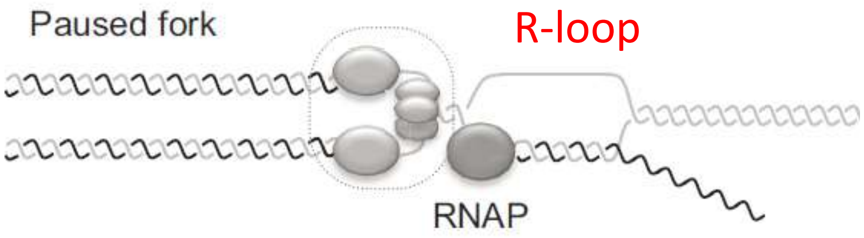


Paulsen et al., Mol Cell 2009

→A wide-spread role for mRNA-processing factors in preventing DNA damage

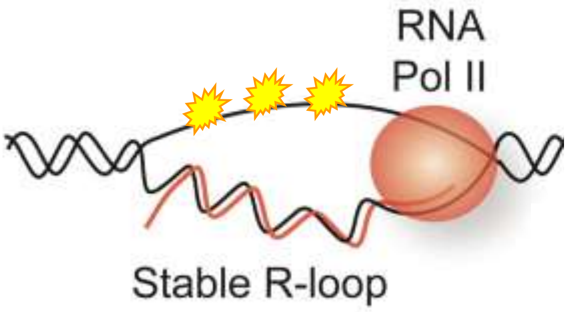
Transcription-replication collision





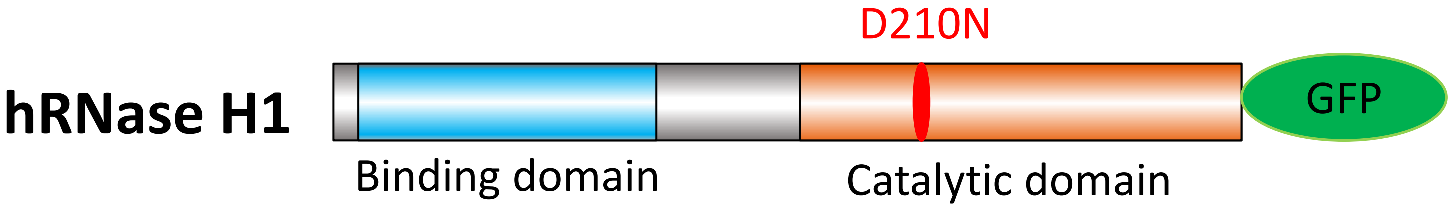
R-loops

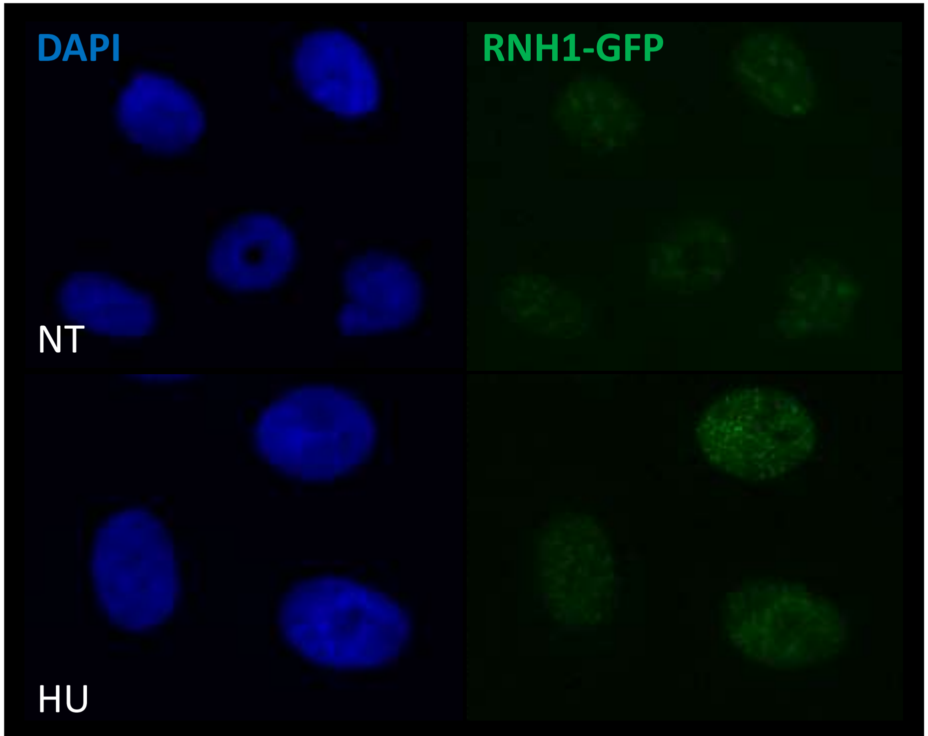
- RNA:DNA hybrids – nascent RNA anneals to transcribed DNA strand and displaces complementary DNA strand
- Unscheduled formation and stabilization of R-loops has highly genotoxic effects
- DNA breakage at common fragile sites of very long genes and at early replicating fragile sites was shown to be dependent on R-loop formation
- Result of impaired RNA elongation, splicing or export
- Cause and/or consequence of collision between replication and transcription machinery
- Bound and degraded by endonuclease RNaseH1

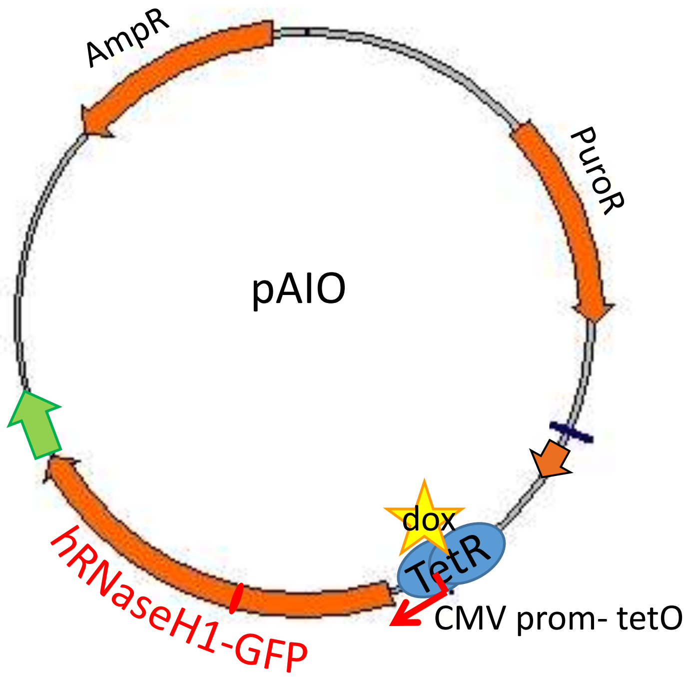


Tool for R-loop isolation

Stable tetracyclin-inducible cell line producing mutated RNase H1, which is binding R-loops but not degrading them = U2OS-T-REx/mutRNaseH1-GFP

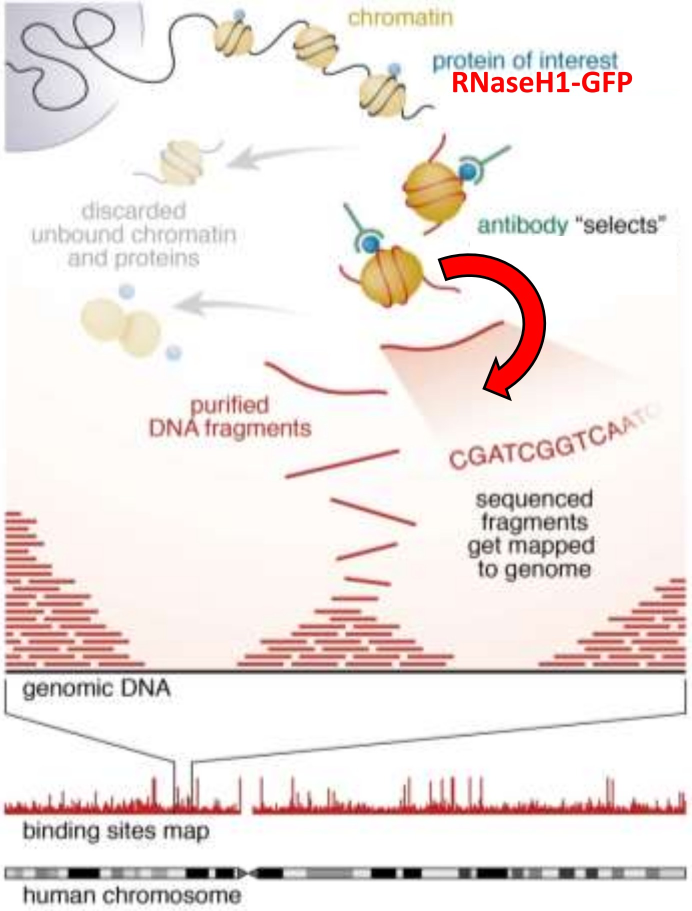






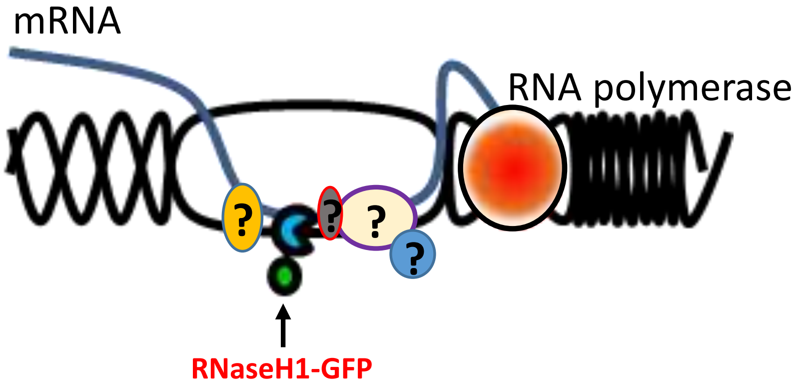
Aims

Chromatin immunoprecipitation followed by massively parallel sequencing (ChIP-Seq)



→ Identify the loci prone to R-loop formation

Chromatin immunoprecipitation followed by mass spectrometry analysis (ChIP-MS)



→ Identify the proteins associated with R-loops

→ Validate role of identified protein in prevention and resolution of collisions between transcription and replication

Thank you for your attention

Diploma and PhD student positions are available ☺ Contact: jana.dobrovolna@img.cas.cz

Laboratory of Genome Integrity

http://www.img.cas.cz/vyzkum/jiri-bartek/

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