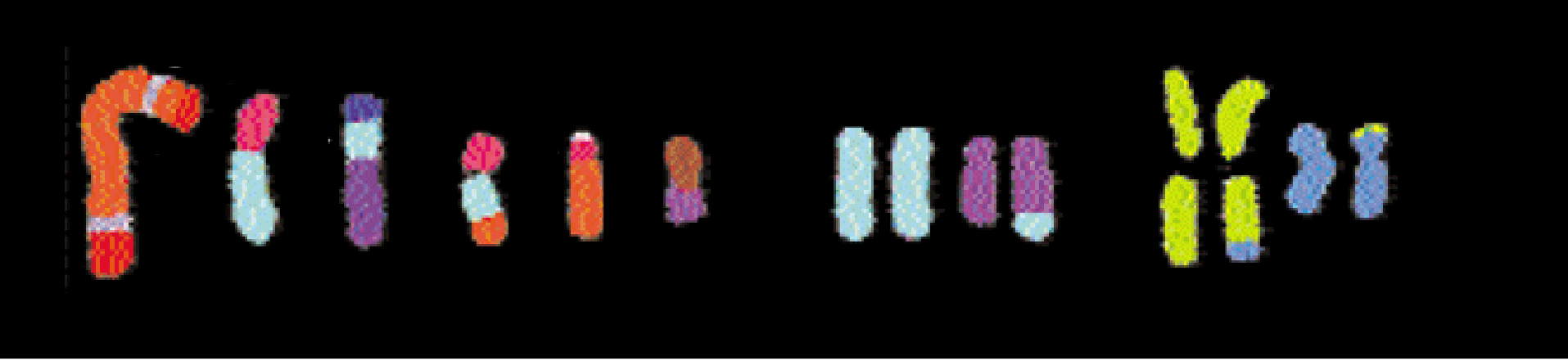


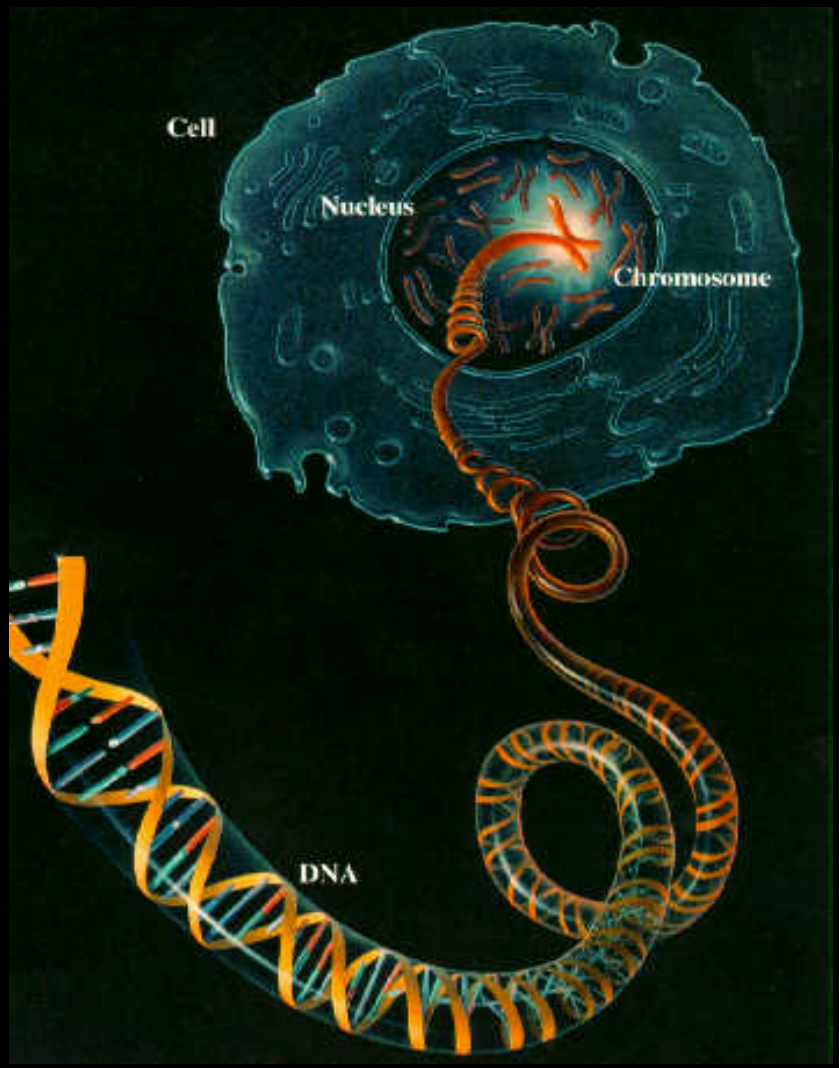
Spatial genome organization in the formation of translocations and in DNA repair

Tom Misteli, Ph.D.

NATIONAL® CANCER INSTITUTE

Center of Excellence in CHROMOSOME BIOLOGY

Genomes exist in the cell nucleus



3 billion bp

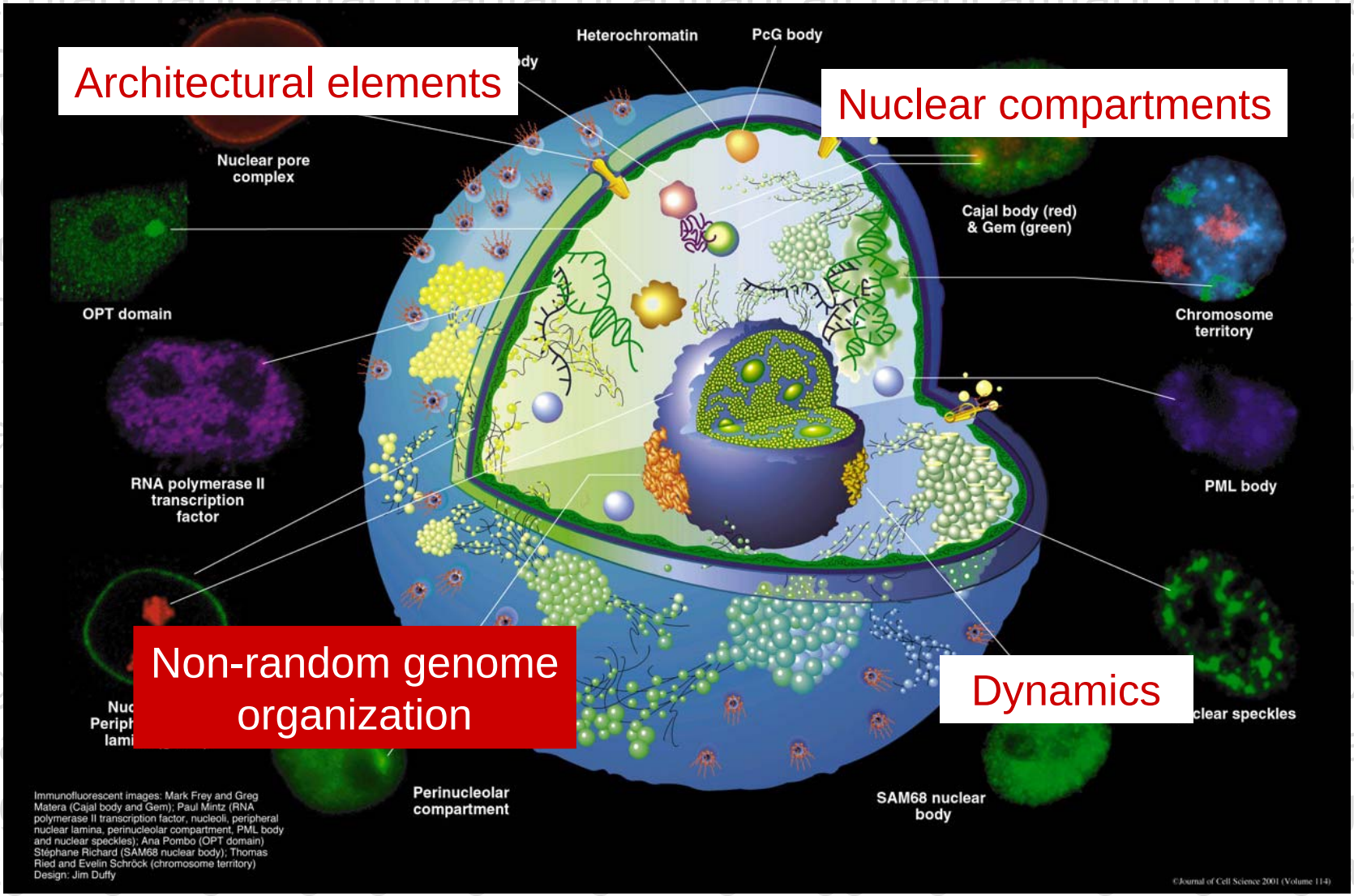
10 um nucleus

2m DNA

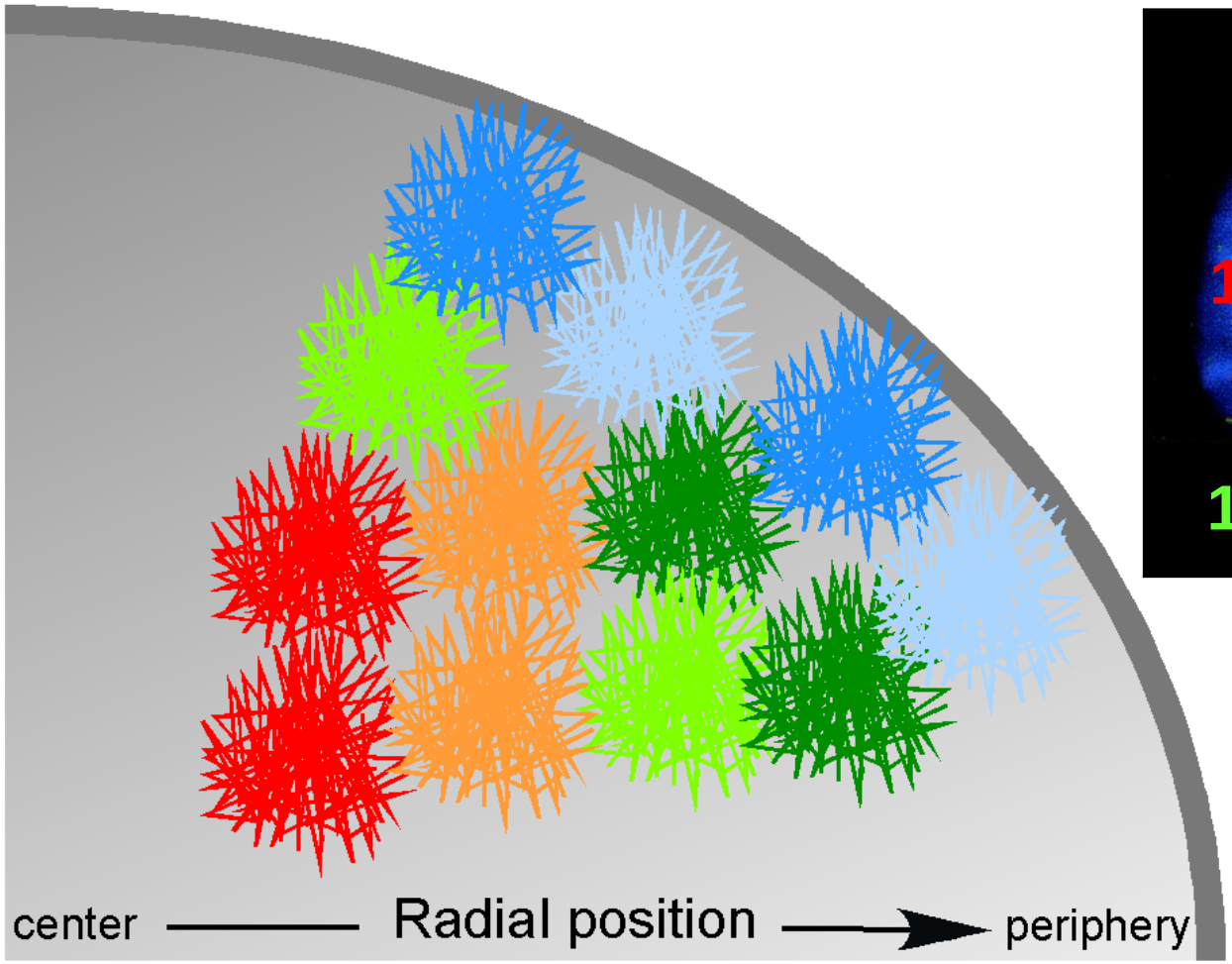
$5x10^{12}$ cells/person
10^{13} m DNA/person

100x distance from Earth-Sun

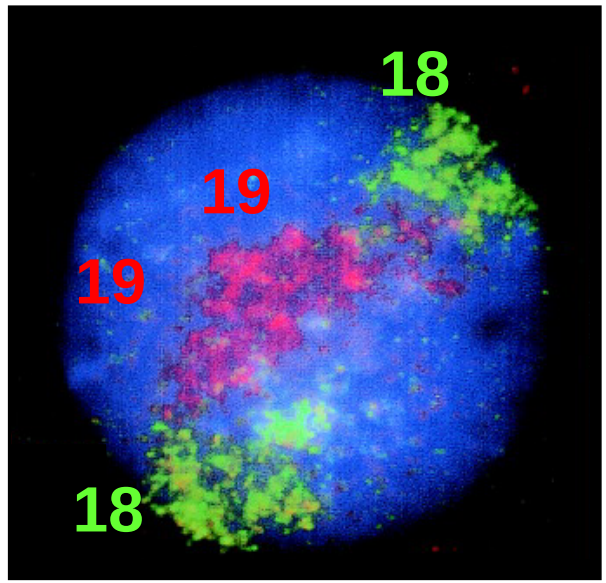
The complex cell nucleus



Non-random spatial genome organization

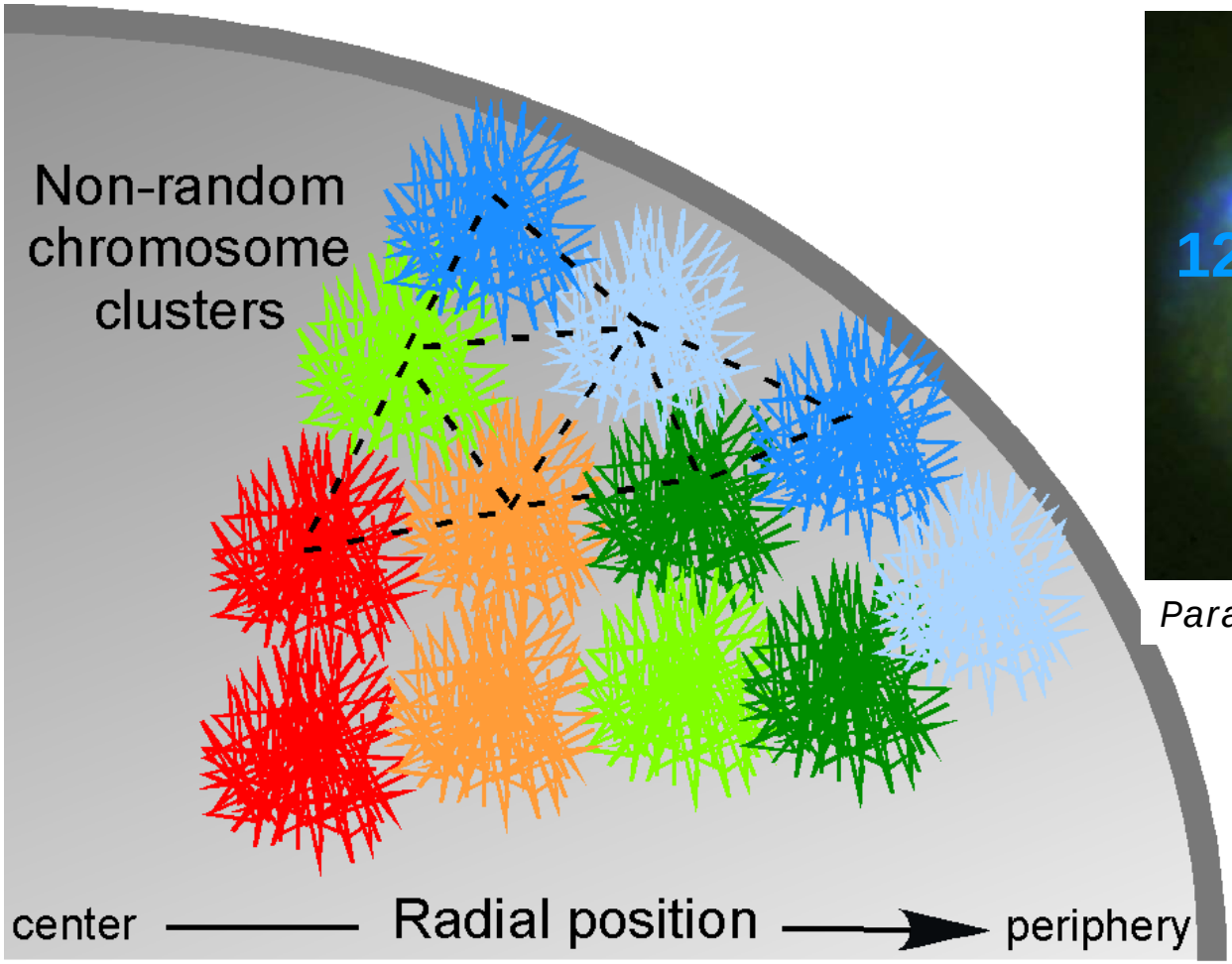


Meaburn & Misteli, Nature, 2007

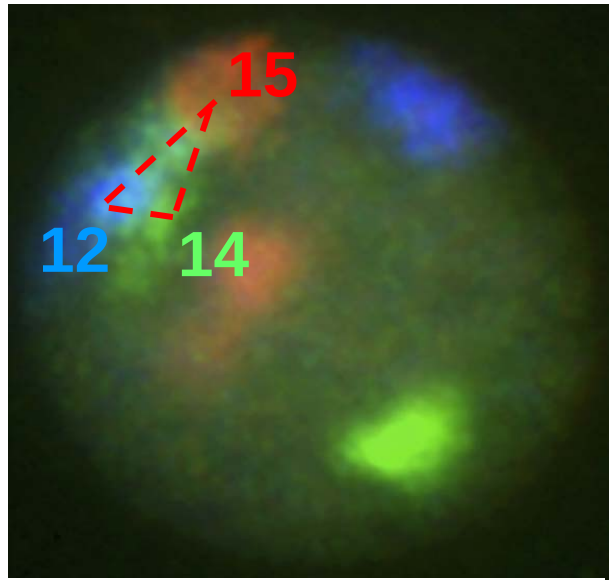


Croft et al., JCB, 1999

Non-random spatial genome organization

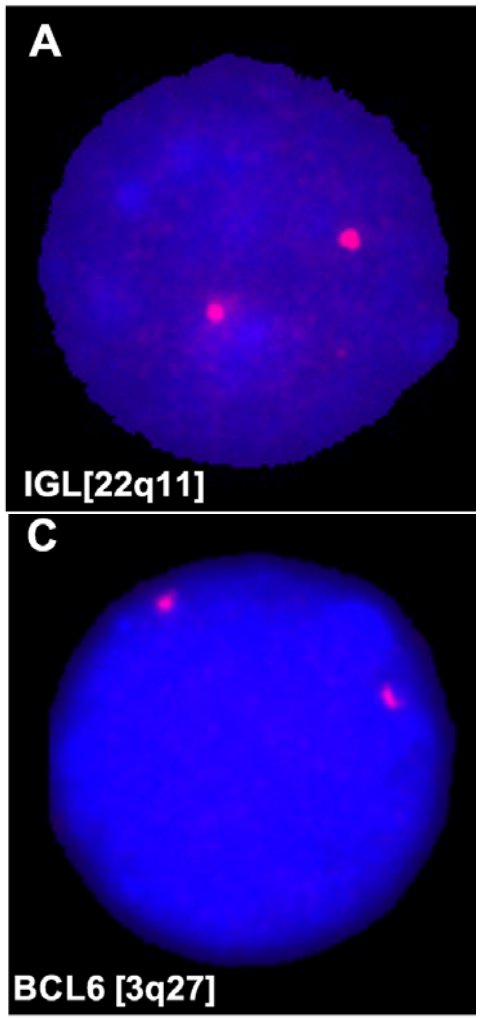


Meaburn & Misteli, Nature, 2007

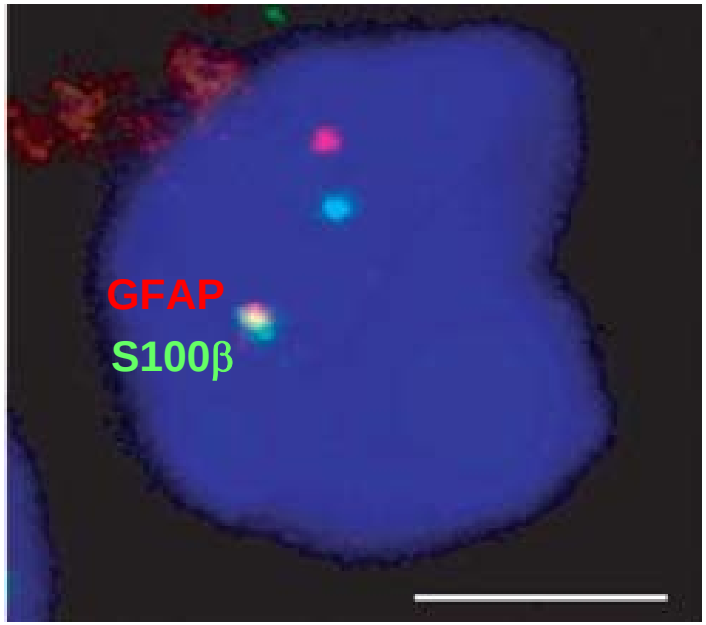


Parada et al., Cur. Biol., 2002

Non-random gene positioning

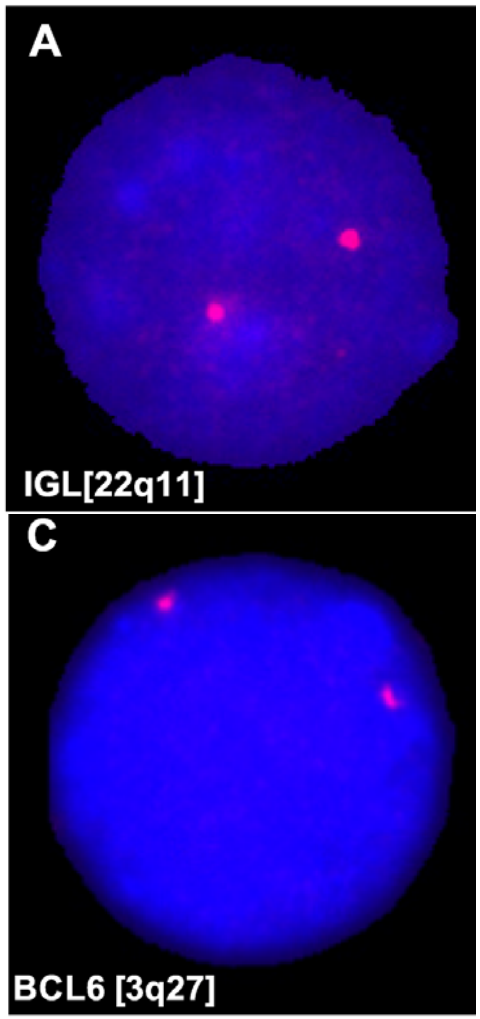


Roix & Misteli, Nat Gen., 2003



Takizawa and Misteli, G&D, 2008

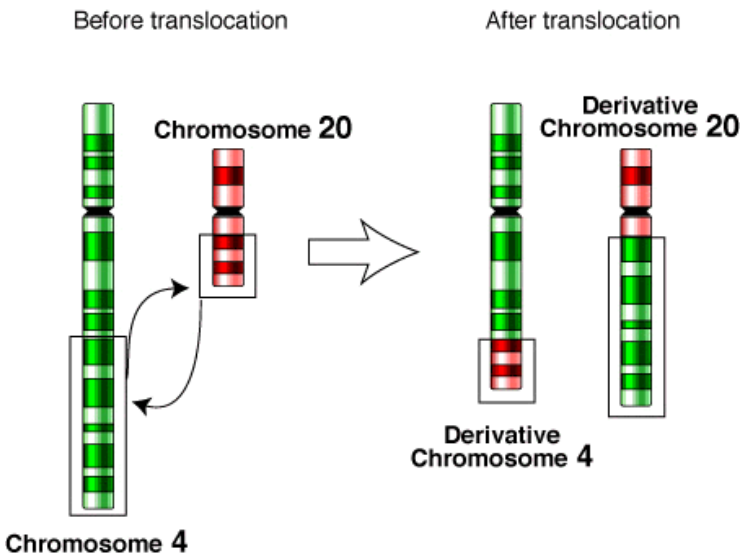
Non-random gene positioning



Roix & Misteli, Nat Gen., 2003

- **Activity-dependent**
- **Cell type-specific**
- **Tissue-specific**
- **Evolutionarily conserved**
- **Differentiation**
- **Development**
- **Disease**

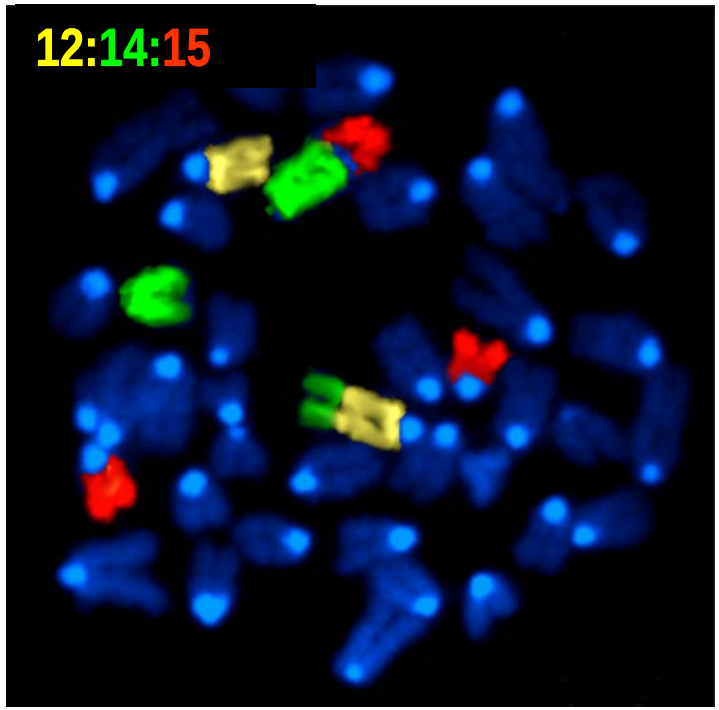
Chromosome translocations



- Hallmark of cancer cells
  - Formation of fusion proteins
  - Gene misregulation
- Can be causal in tumor
- Form by illegitimate joining of broken chromosomes

Fundamentally a spatial problem:
Translocations require physical interaction of partners

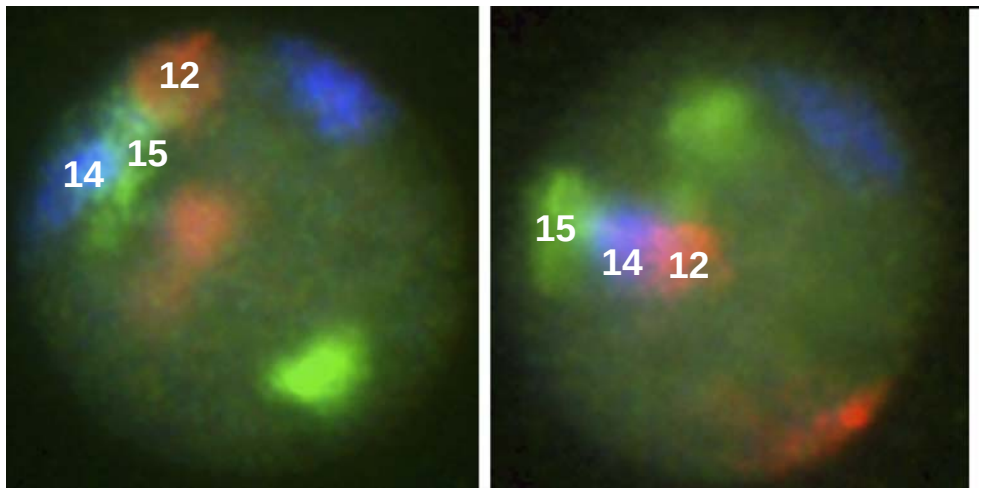
Spatial positioning of translocation partners

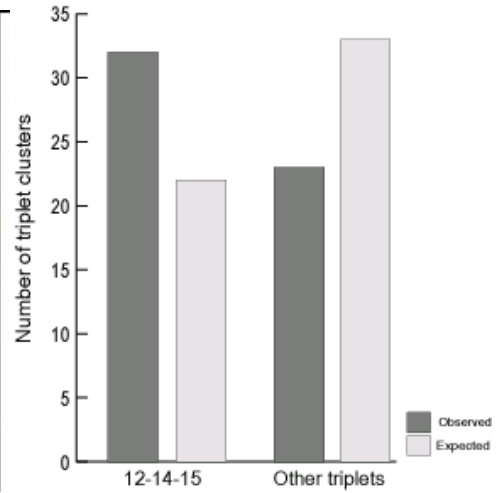


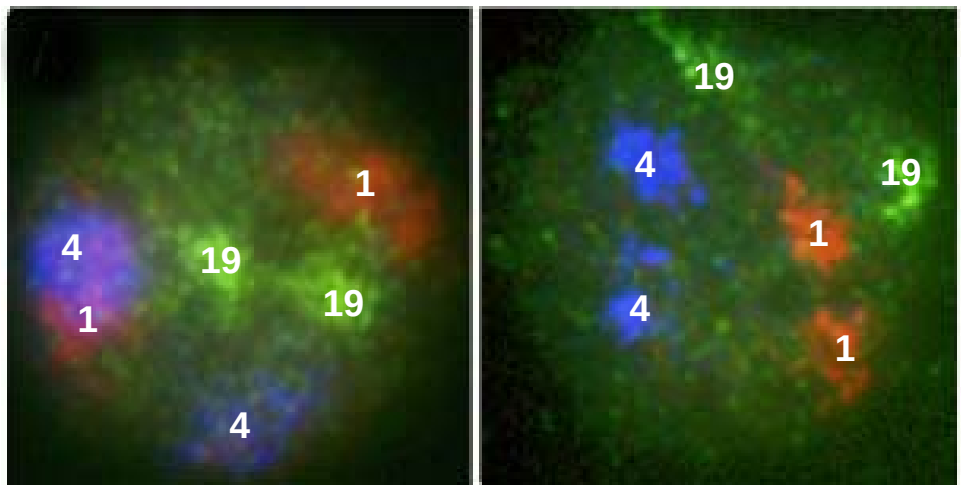
80% of lymphomas contain translocations involving combinations of 12/14/15

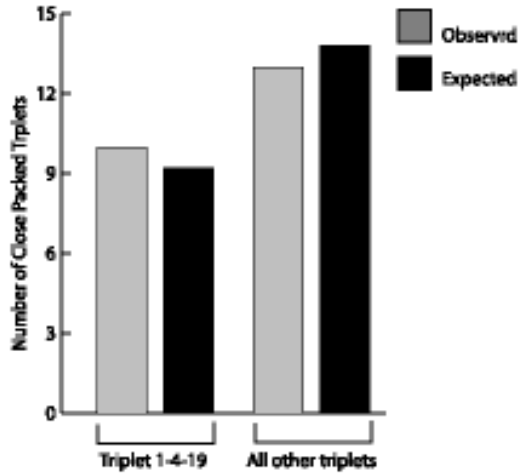
Liyanage et al., Blood, 2000

A cluster of chromosomes 12/14/15 in lymphocytes



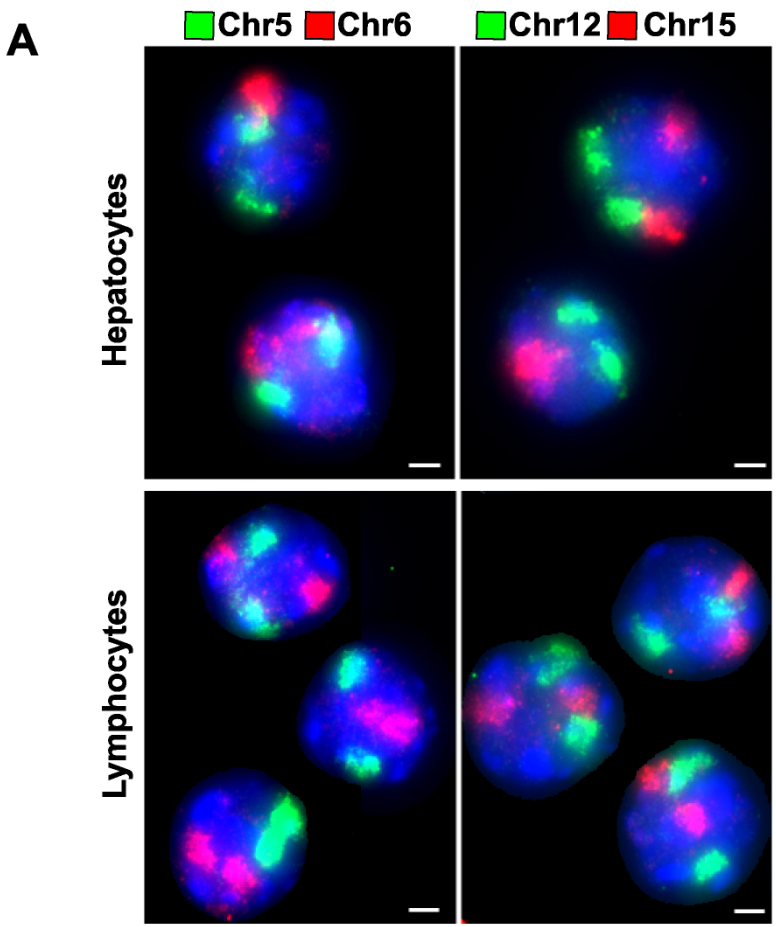


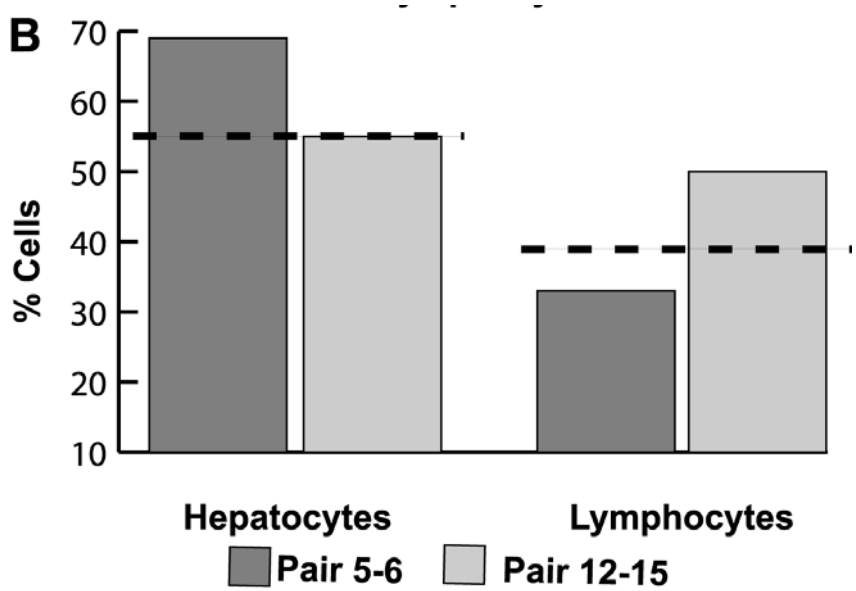




Parada et al., Current Biology, 2002

Tissue-specific translocations and tissue-specific positions





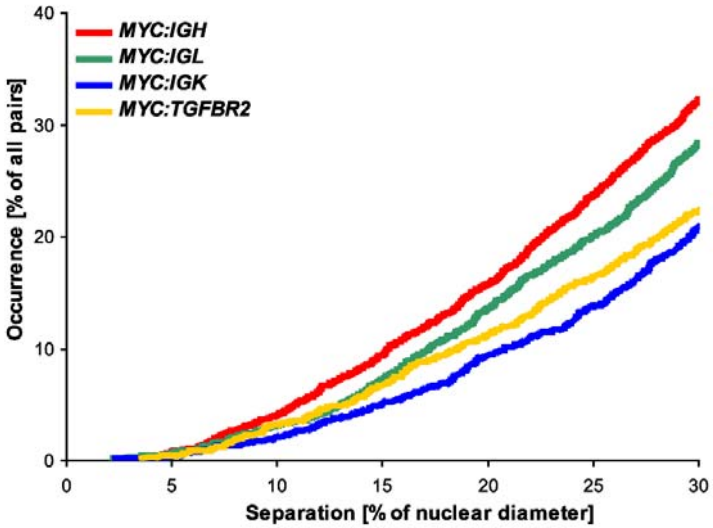
Parada et al., Genome Biology, 2004

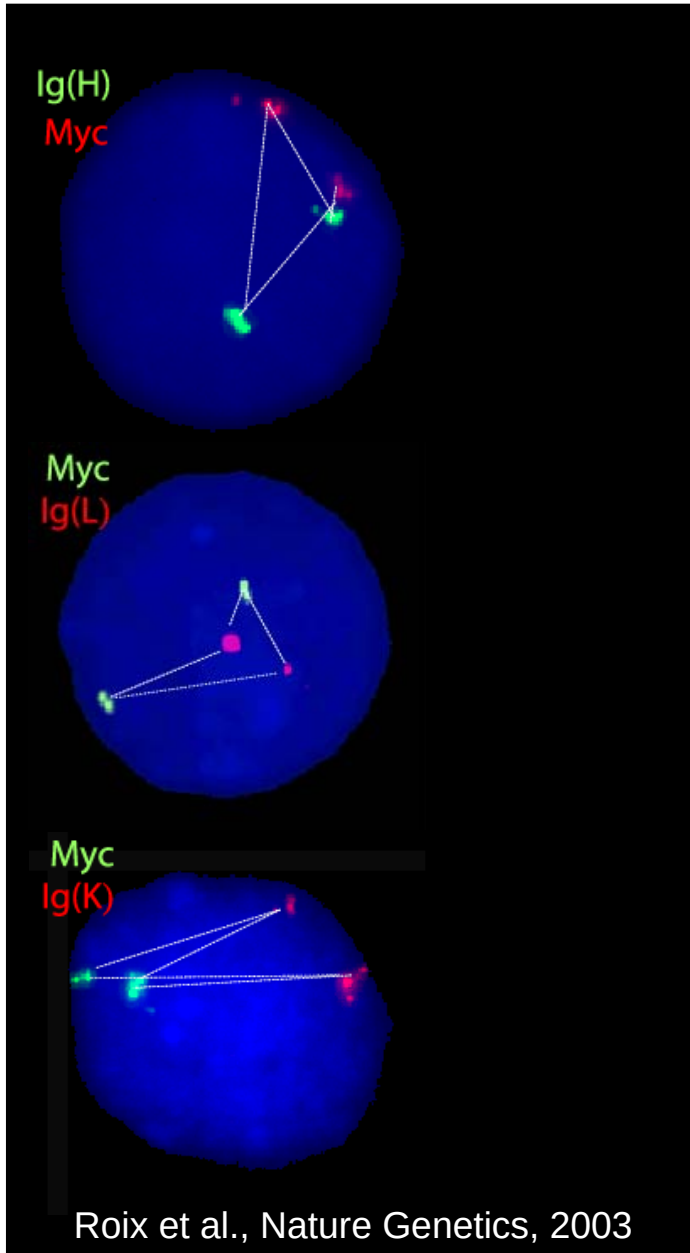
Proximity of translocation partners in Burkitt's lymphoma

T(8;14) myc-Ig(H) 85% of patients

T(8;22) myc-Ig(λ) 10% of patients

T(8;2) myc-Ig(κ) <5% of patients



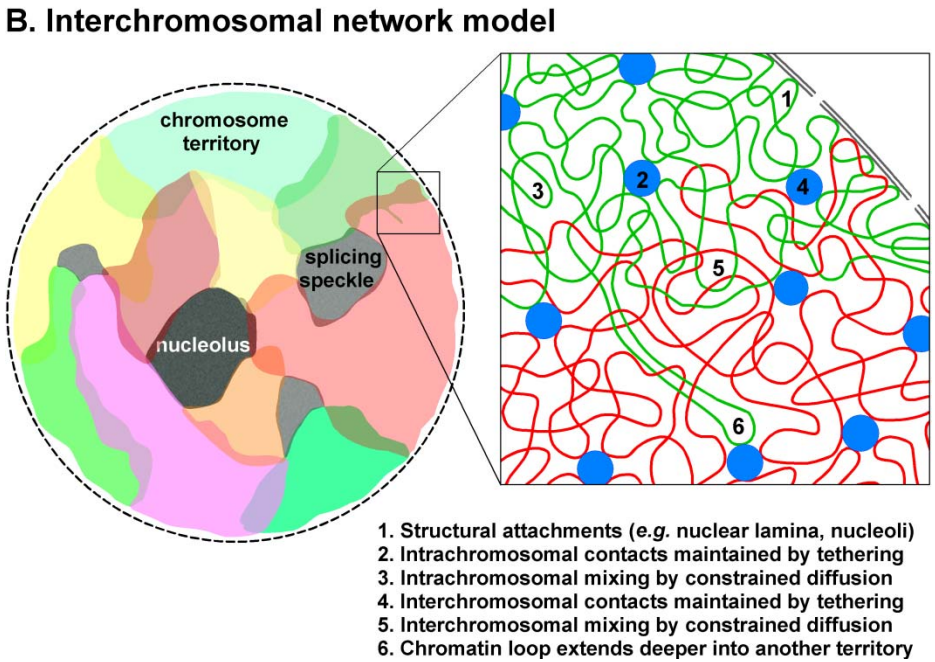


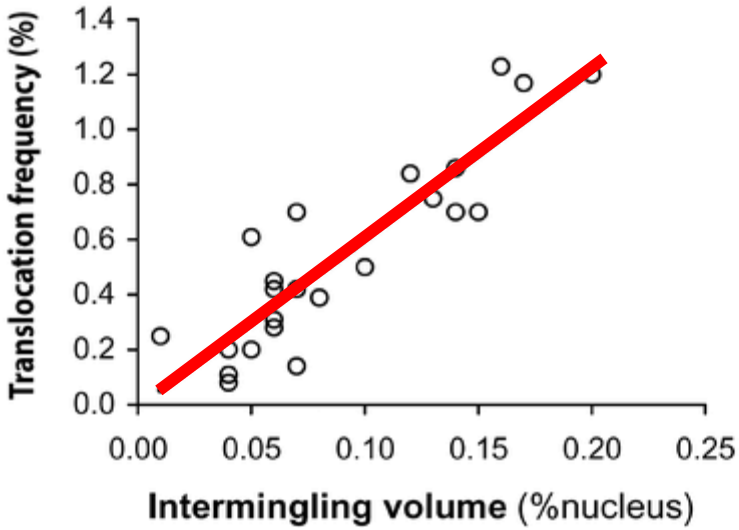
Roix et al., Nature Genetics, 2003

Proximity of translocation-prone partners

<u>Human</u>	
Burkitt's lymphoma	**multiple partners**
Chronic lymphocytic lymphoma	**multiple partners**
Chronic myeloid leukemia	**BCR – ABL**
Promyelocytic leukemia	**PML – RAR**
Papillary thyroid cancer	**RET - H4**
Ewing sarcoma	**EWSR1 - FLI1**
Anaplastic large cell lymphoma	**multiple partners**
<u>Mouse</u>	
Lymphoma	**12:14:15**
Hepatoma	**5:6**

Chromosome intermingling and tranlsocations

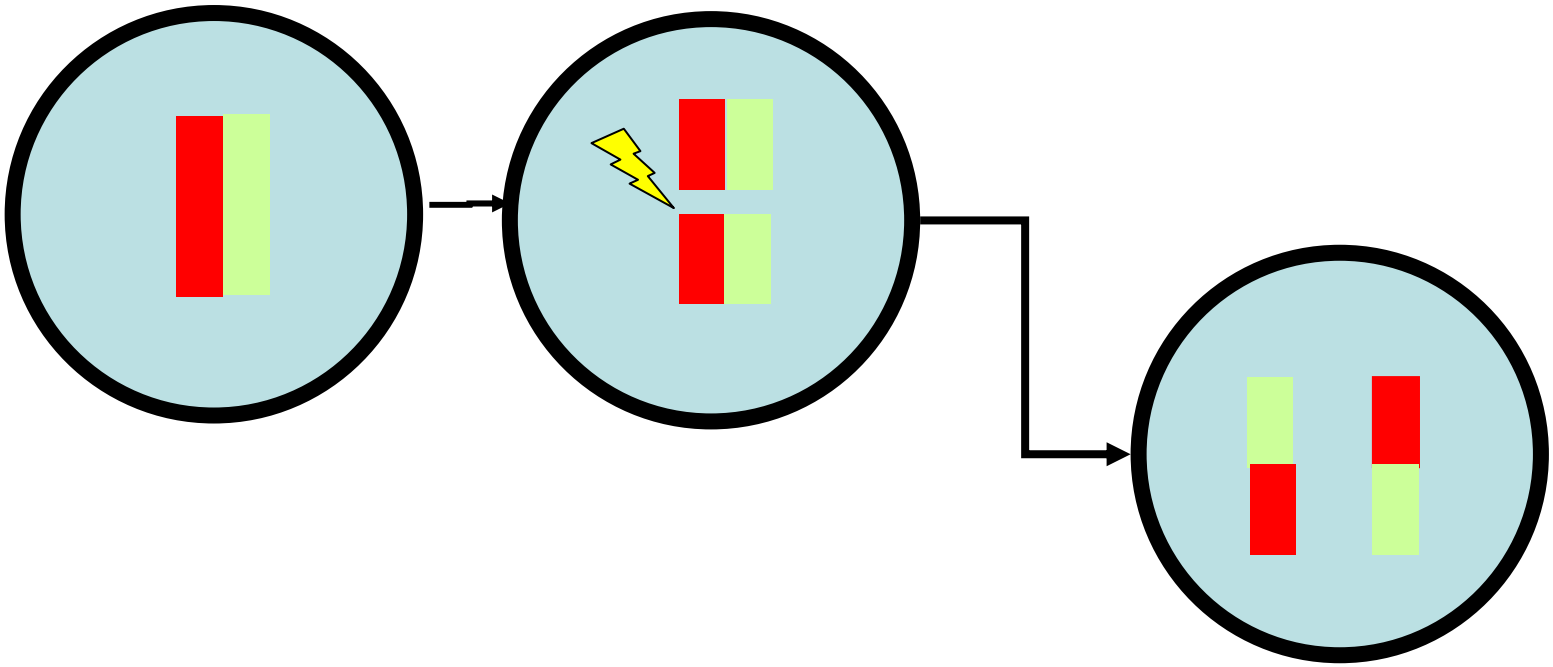




From Branco & Pombo, PLoS Biology, 2006

Formation of chromosome translocations

Contact first



Formation of chromosome translocations

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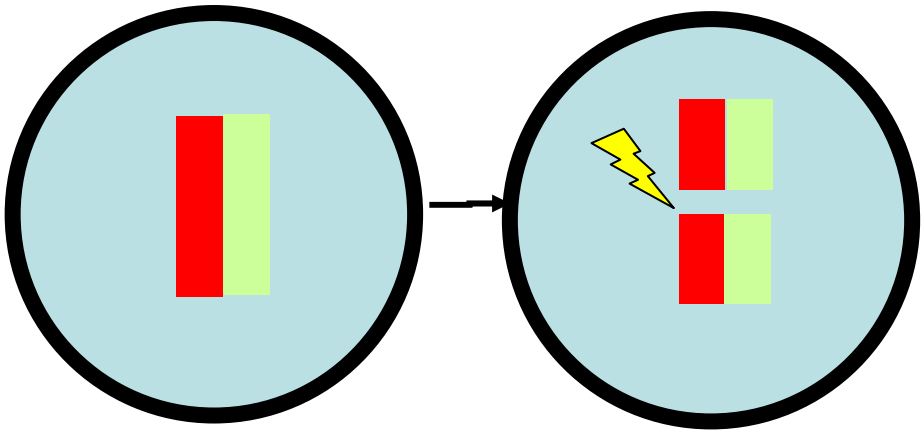
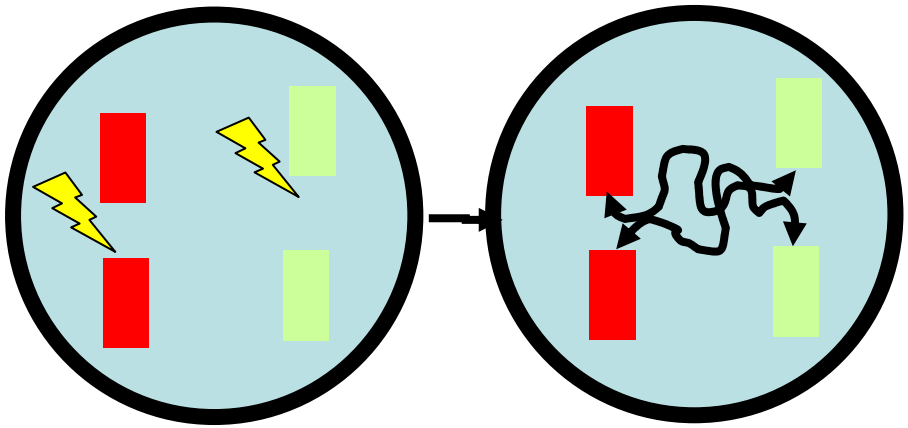


DSBs are immobile

Breakage first



DSBs must be mobile

Mobility of DSBs

Mobility

Lisby et al., NCB, 2003

Aten et al., Science, 2004

Immobility

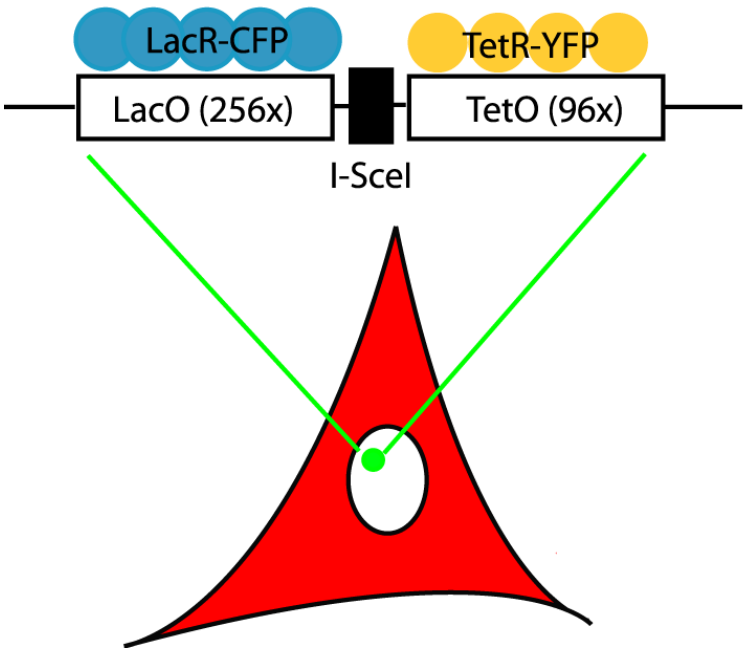
Nelms et al., Science, 1998

Kruhlak et al., JCB, 2005

An experimental system to study chromosome ends in vivo

yeast endonuclease I-SceI

TAGGGATAACAGGGTAAT
ATCCCTATTGTCCCATTA



Soutoglou et al., Nature Cell Bio., 2007

An experimental system to study chromosome ends in vivo

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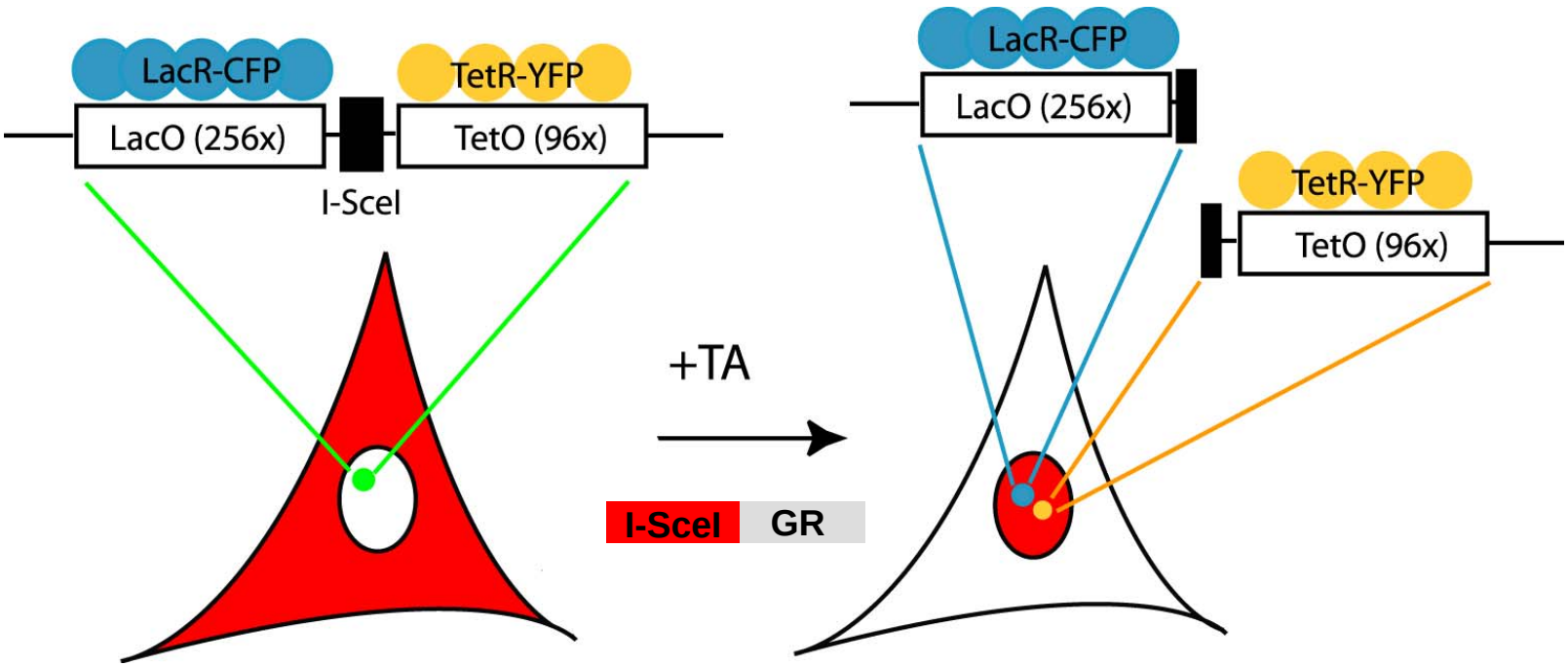
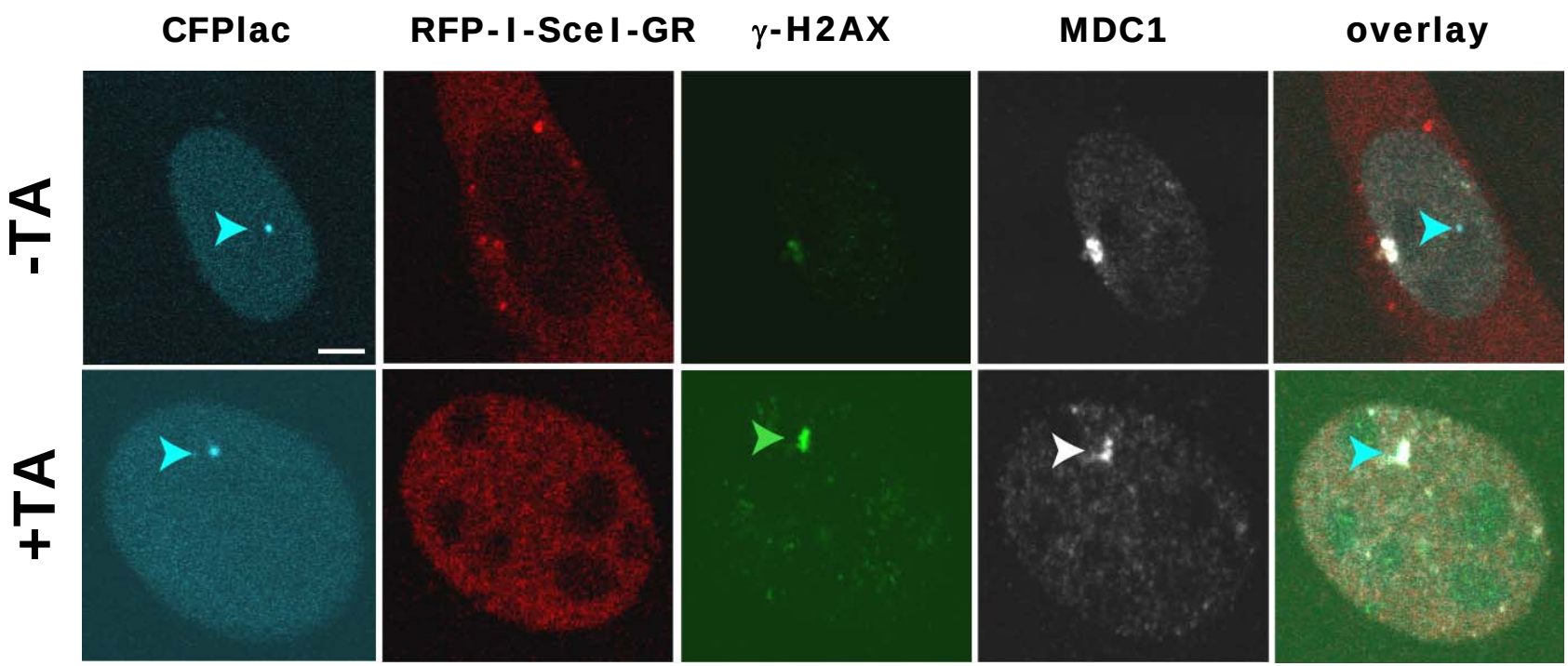


Soutoglou et al., Nature Cell Bio., 2007

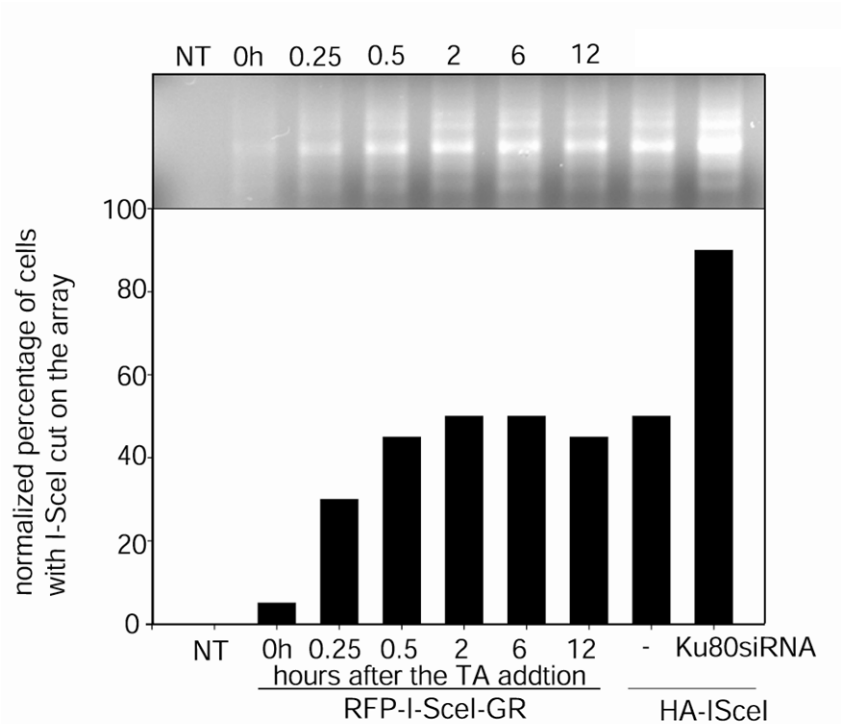
An experimental system to study chromosome ends in vivo



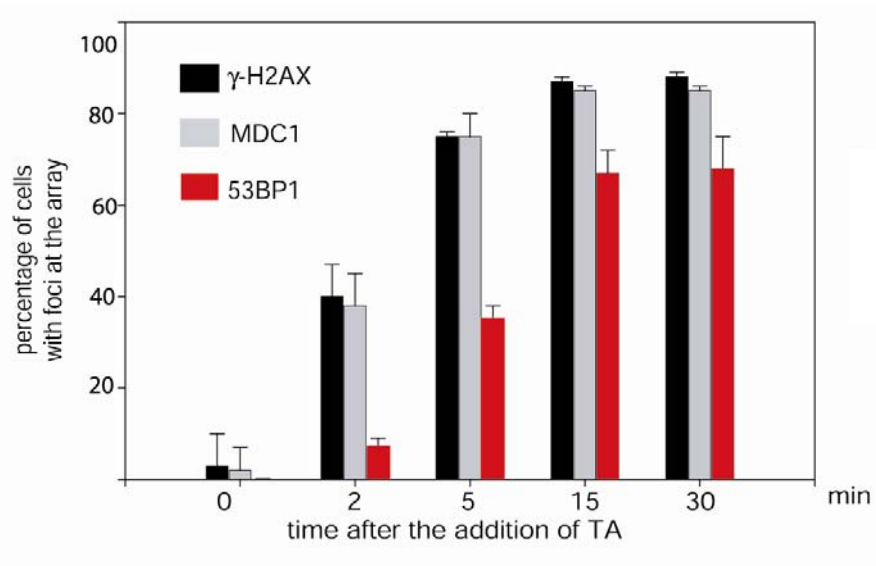
Soutoglou et al., Nature Cell Bio., 2007

Rapid repair kinetics

Cutting (Ligation-mediated PCR)

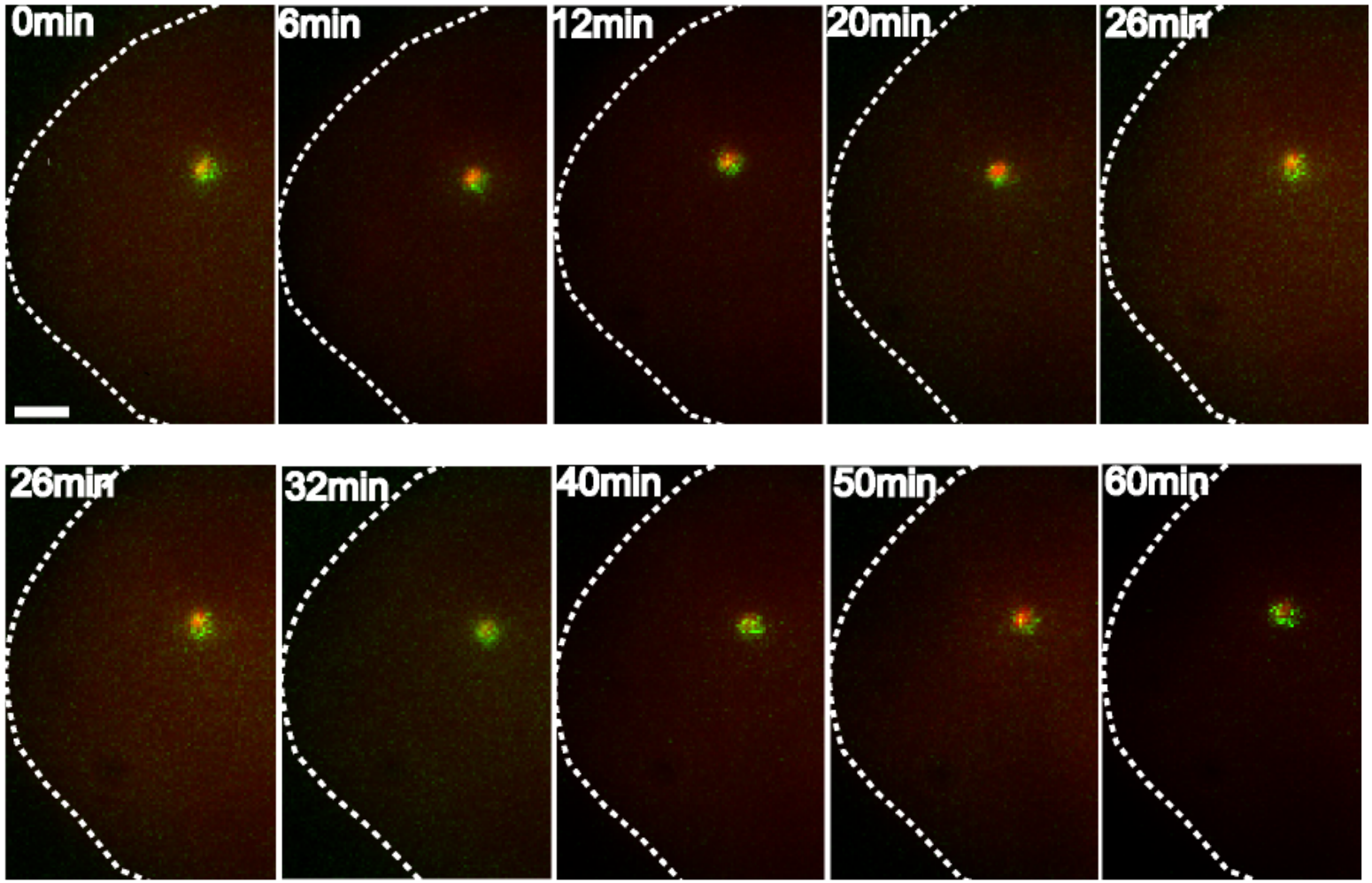


Recruitment of repair factors

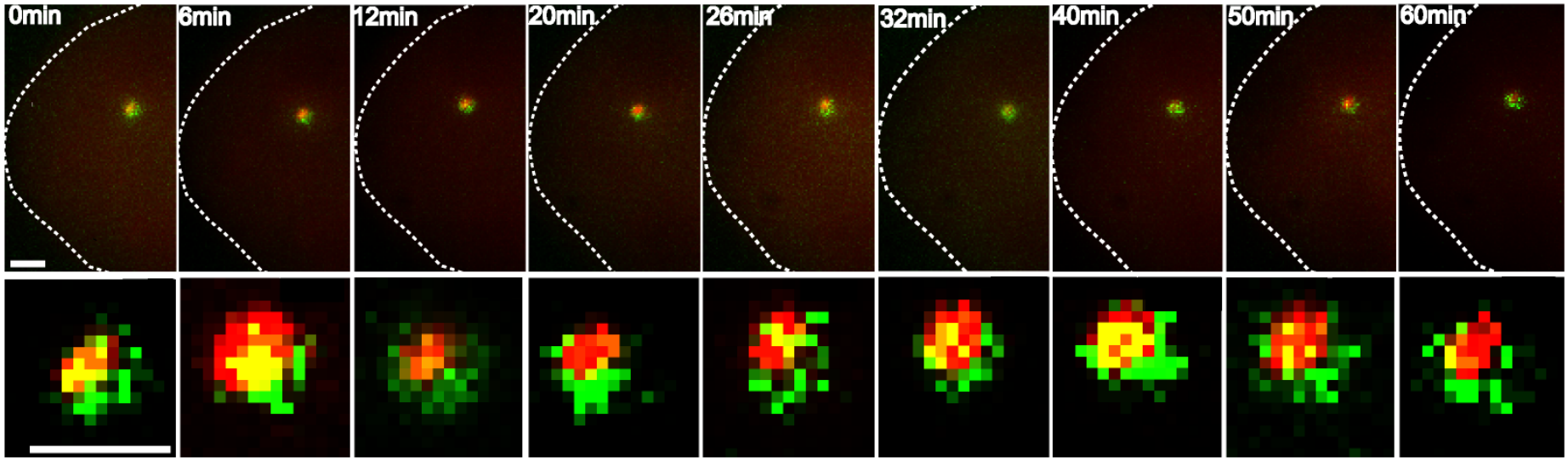


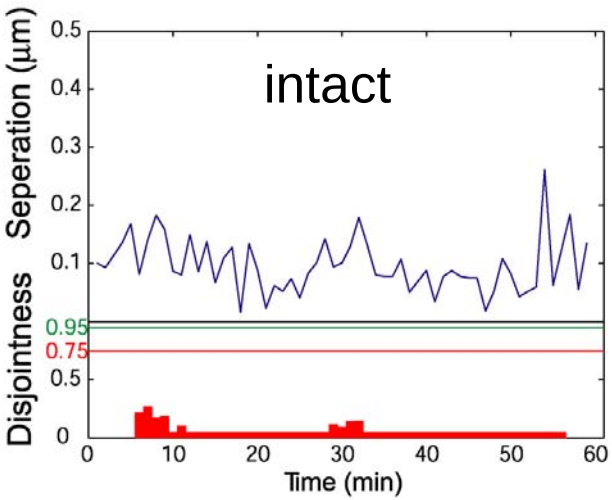
Soutoglou et al., Nature Cell Bio., 2007

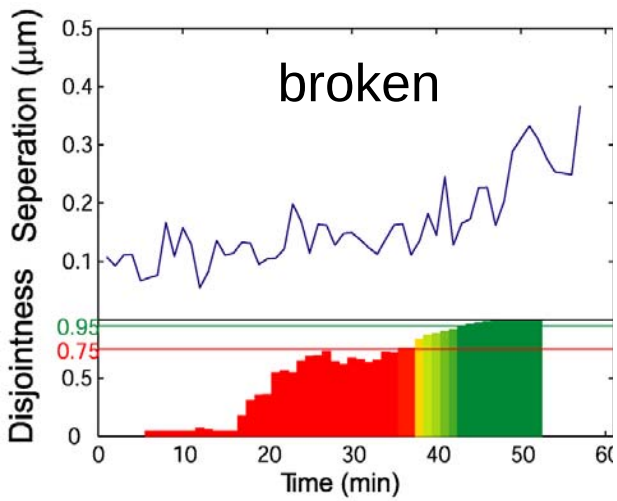
DSB are positionally stable

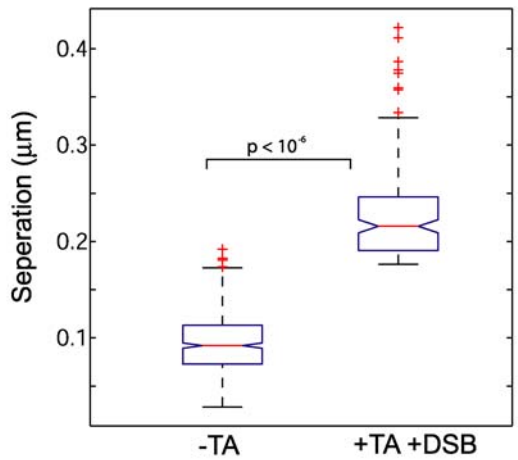


Local separation of chromosome ends

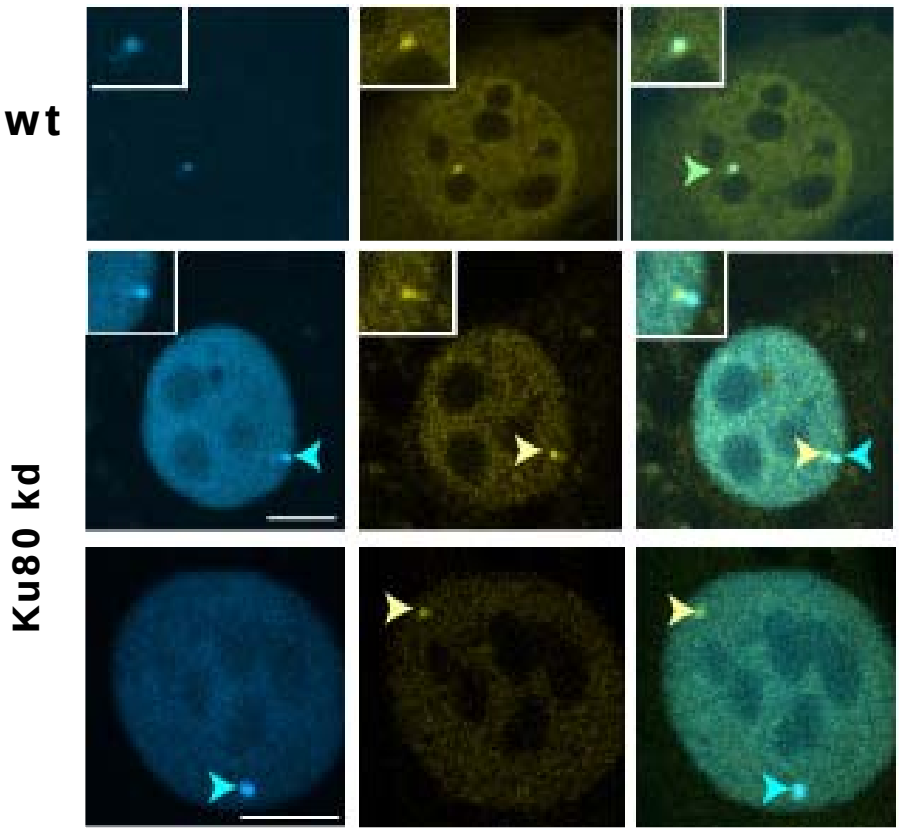


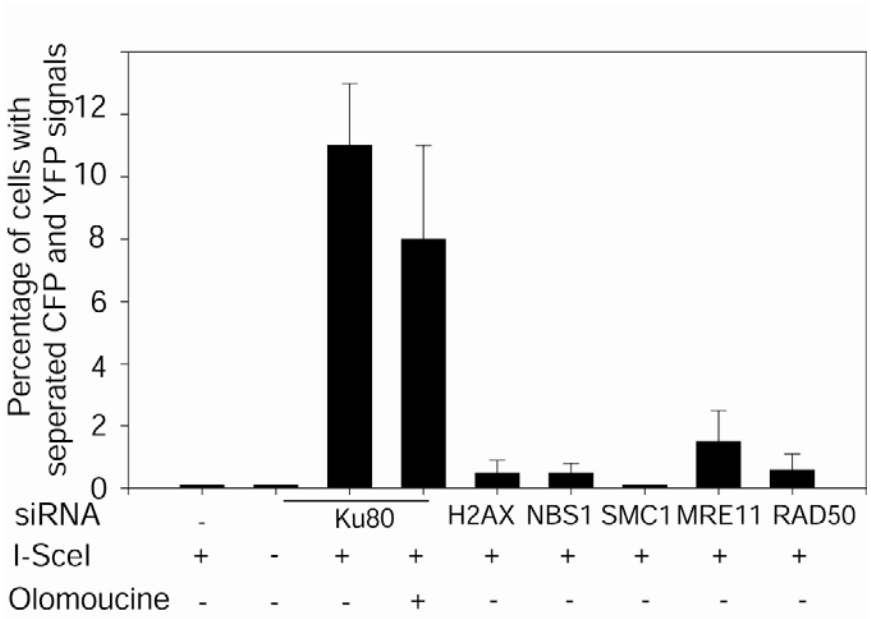






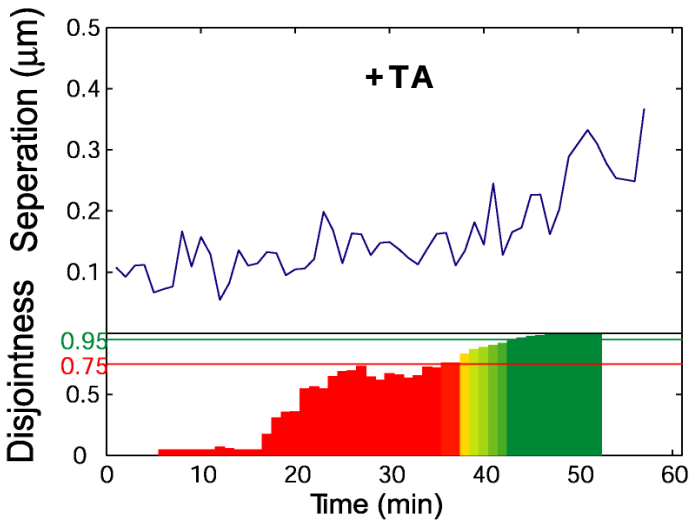
Ku80 mediates chromosome end stability

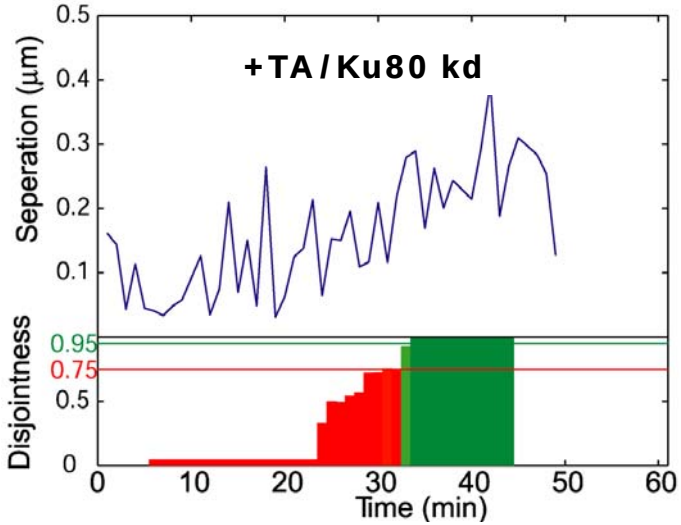


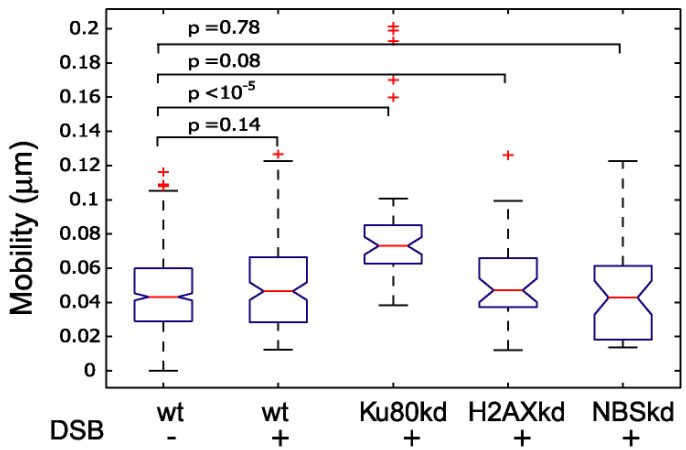


Soutoglou et al., Nature Cell Bio., 2007

Increased mobility in the absence of Ku80



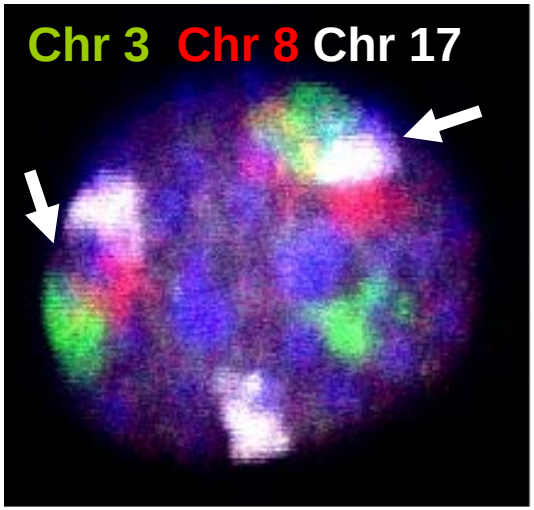


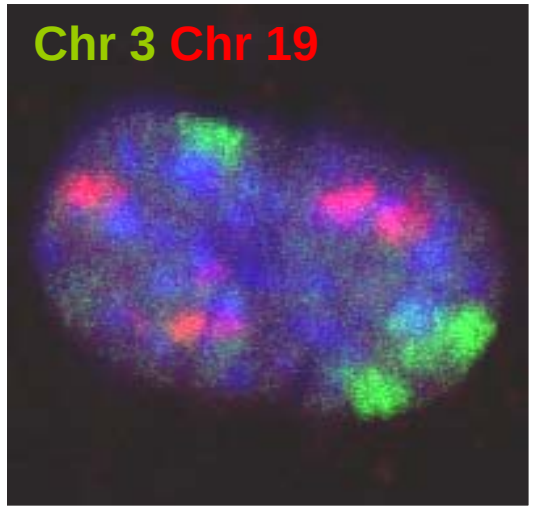


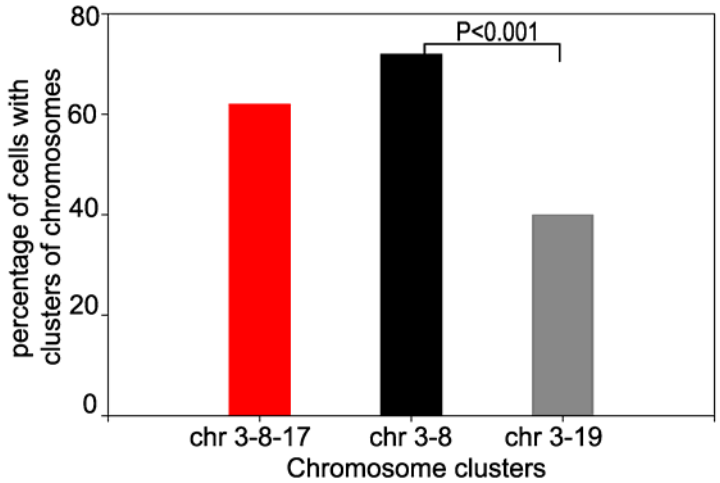
Identification of a recurrent array translocation

Soutoglou et al., Nature Cell Bio., 2007

Proximity of array translocation partners

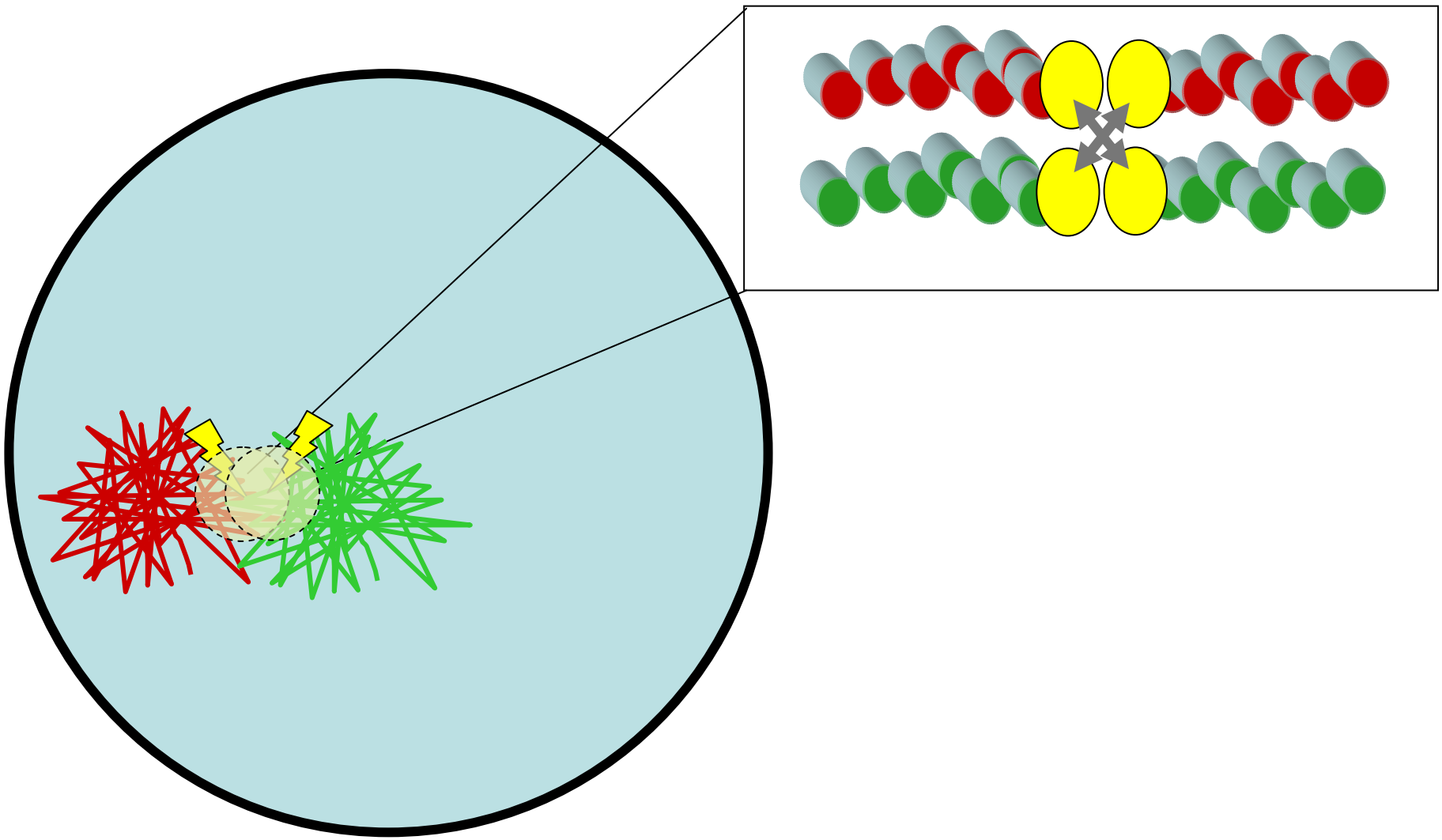






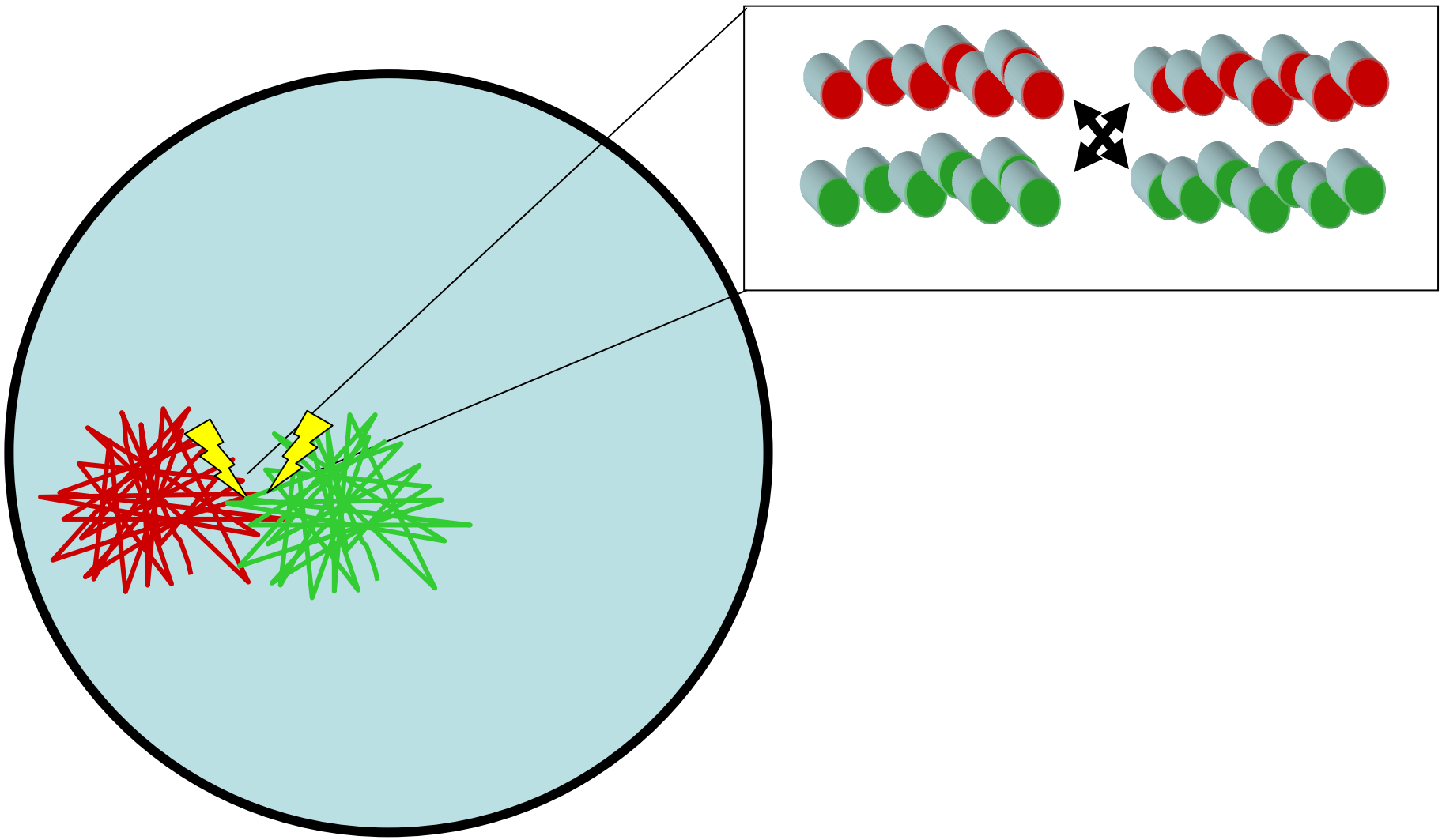
Soutoglou et al., Nature Cell Bio., 2007

Formation of chromosome translocations



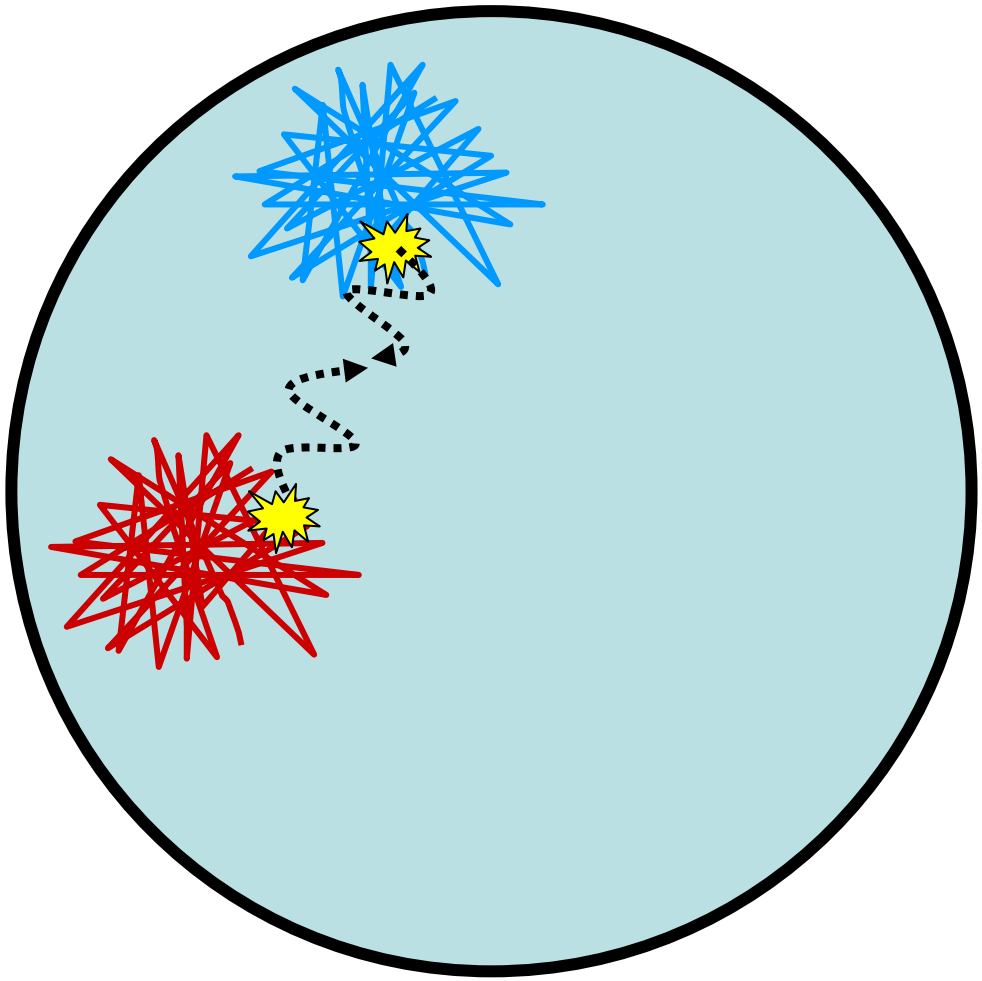
Soutoglou and Misteli, Current Opinion in Cell Biology, 2007
Misteli, Cell, 2007

Formation of chromosome translocations



Soutoglou and Misteli, Current Opinion in Cell Biology, 2007
Misteli, Cell, 2007

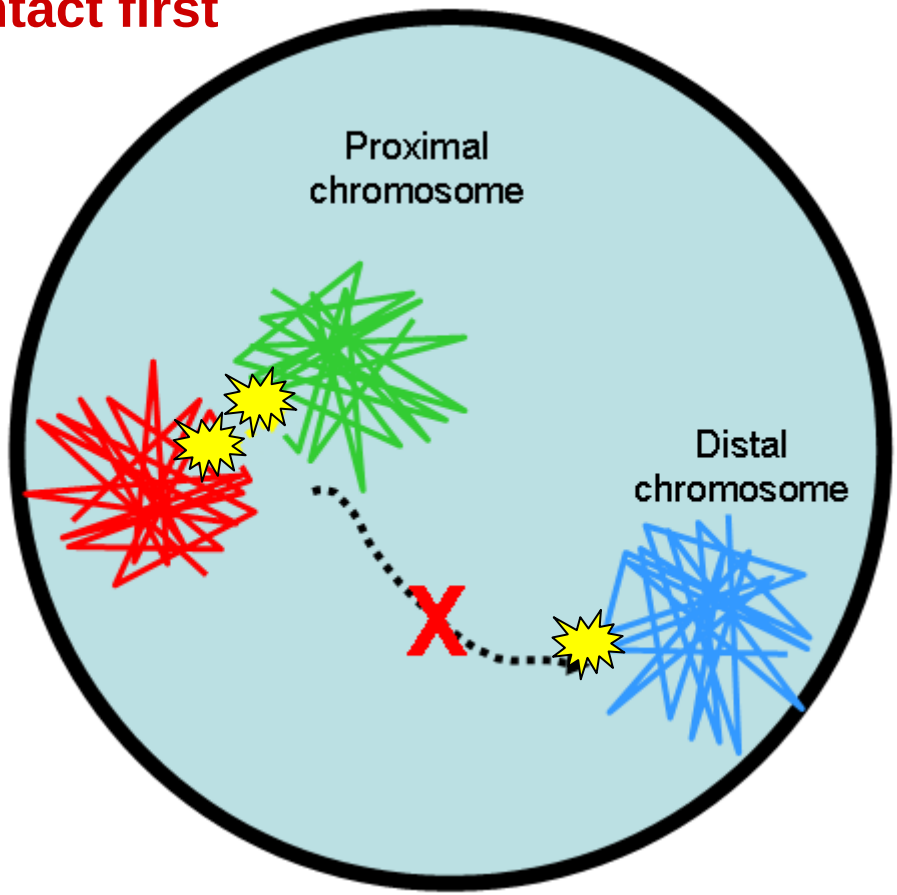
Formation of chromosome translocations



Soutoglou and Misteli, Current Opinion in Cell Biology, 2007
Misteli, Cell, 2007

Formation of chromosome translocations

Contact first



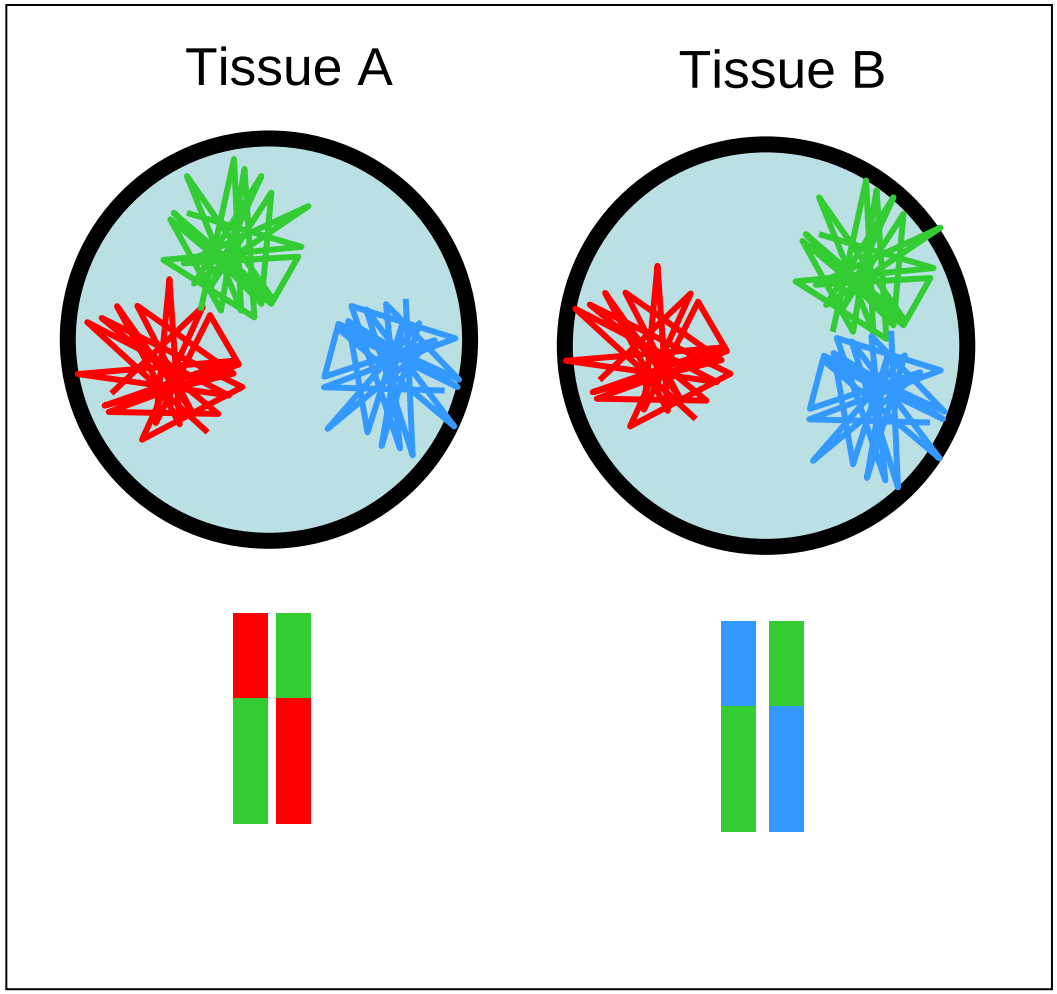
DSBs are immobile

Correlation between translocation frequency and spatial proximity

Non-random spatial arrangement of the genome is a significant determinant of translocations

Soutoglou and Misteli, Current Opinion in Cell Biology, 2007
Misteli, Cell, 2007

Determinants of translocations: tissue-specific genomes

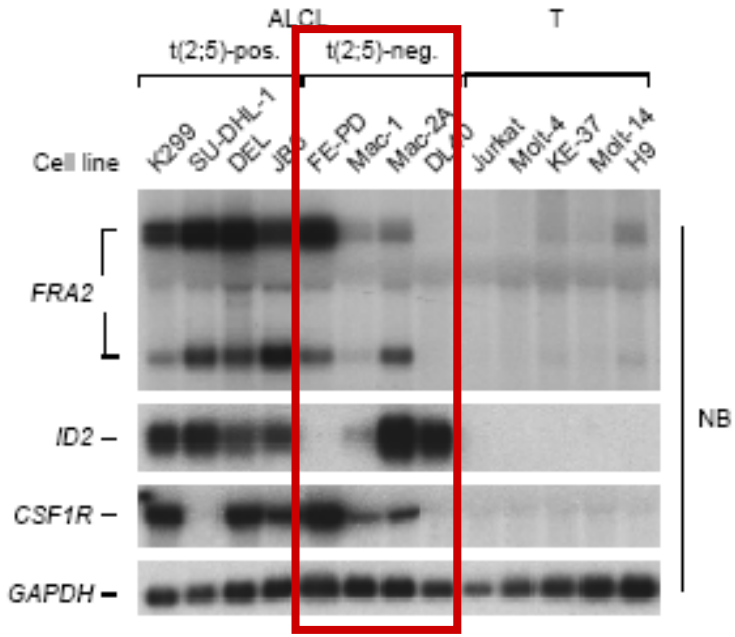


Determinants of translocations: gene expression

Anaplastic large cell lymphoma

patients:

- patients with no translocations

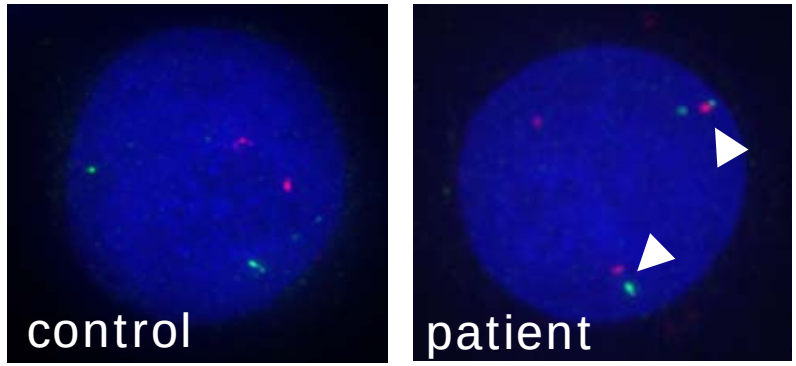


Mathas et al., PNAS, in press

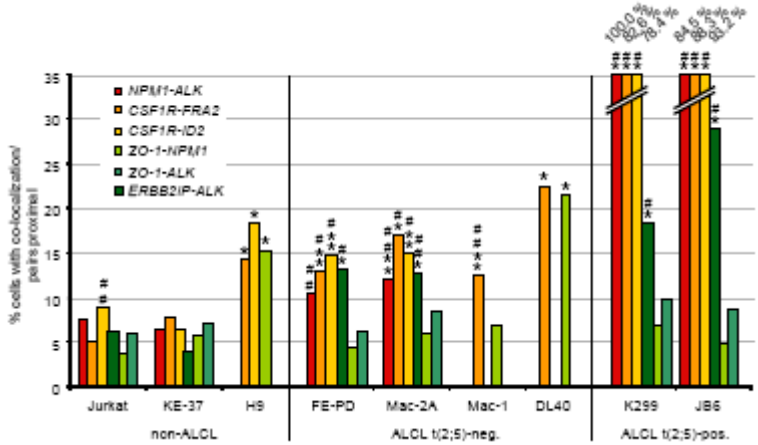
Determinants of translocations: gene expression

Anaplastic large cell lymphoma

patients:



- patients with no translocations

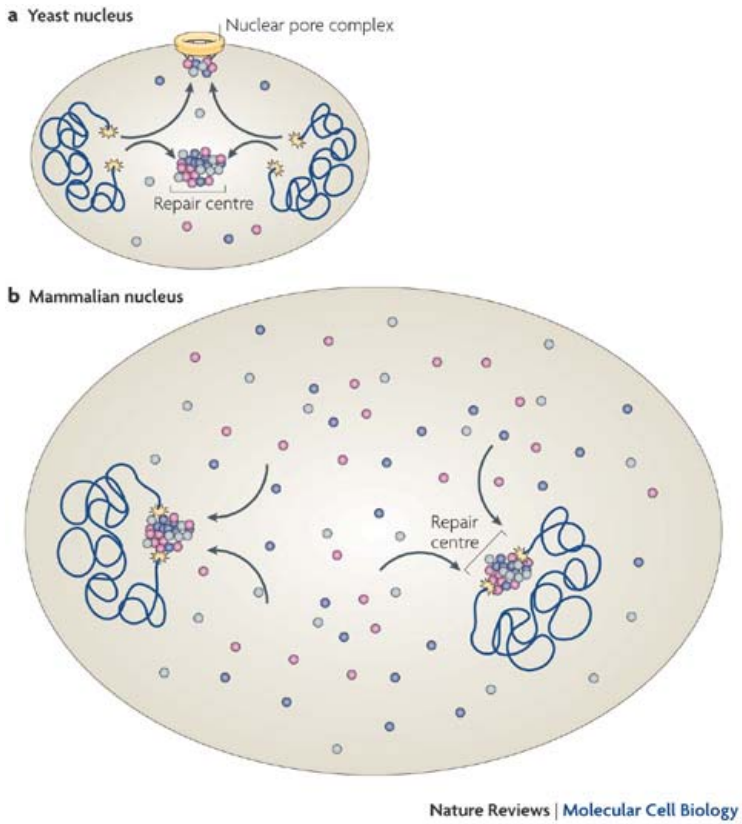


Mathas et al., PNAS, in press

Yeast vs. mammalian

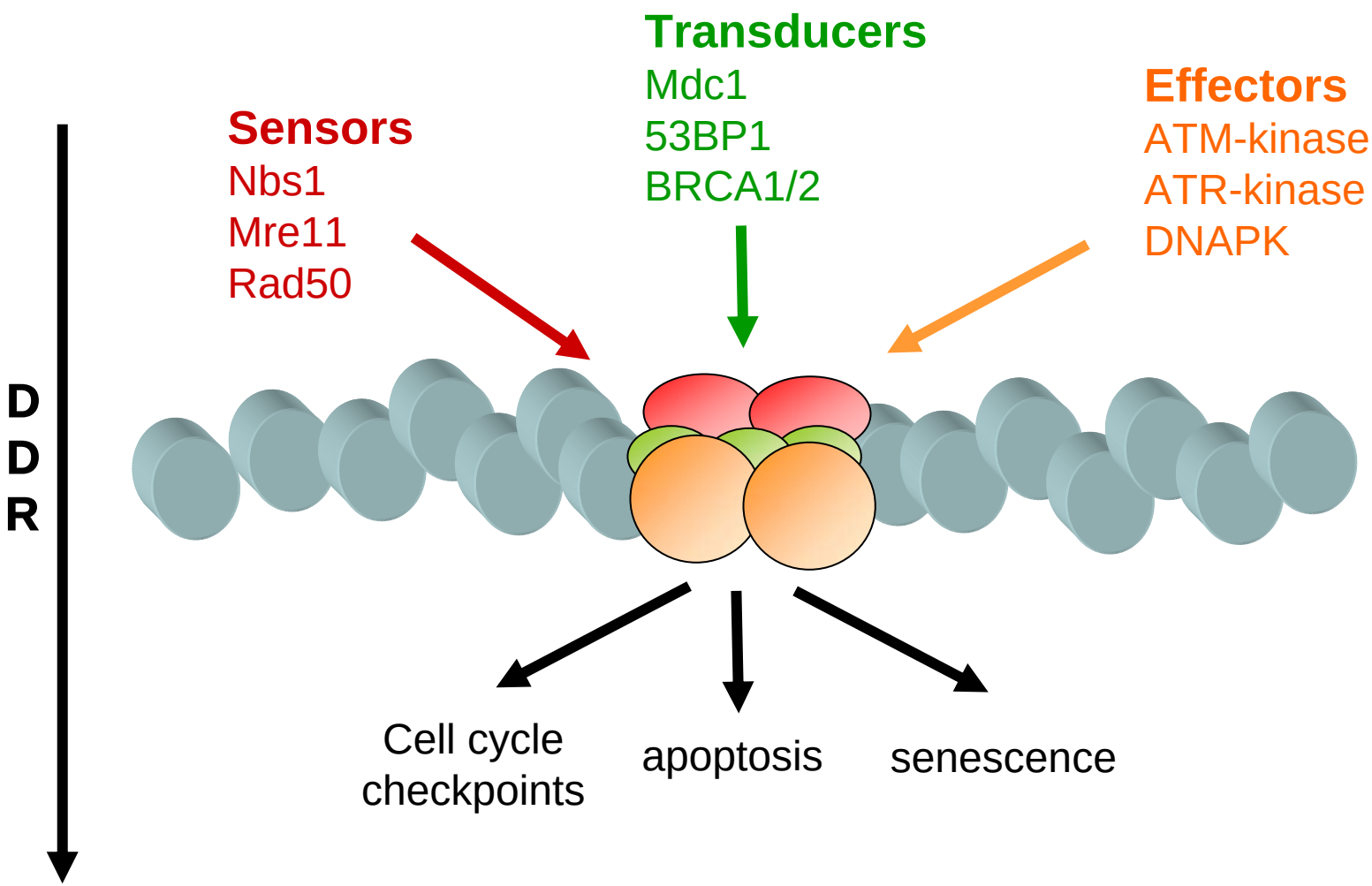
Yeast - mobility
Lisby et al., NCB, 2003
Nagai et al., Science, 2008
Kalocsay, Mol. Cell, 2009

Mammalian – immobility
Nelms et al., Science, 1998
Kruhlak et al., JCB, 2005
Soutoglou et al., NCB, 2007
but
Aten et al., Science, 2004
Dimitrova et al., Nature, 2009

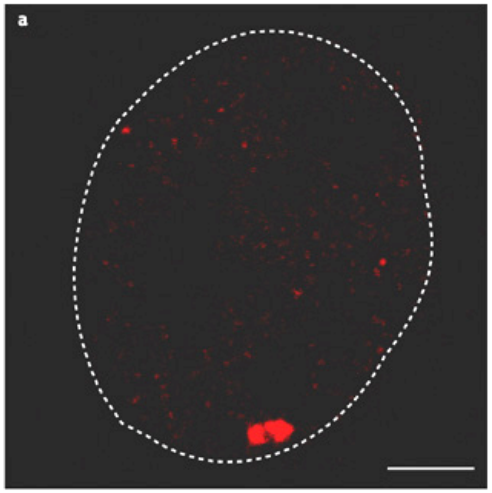


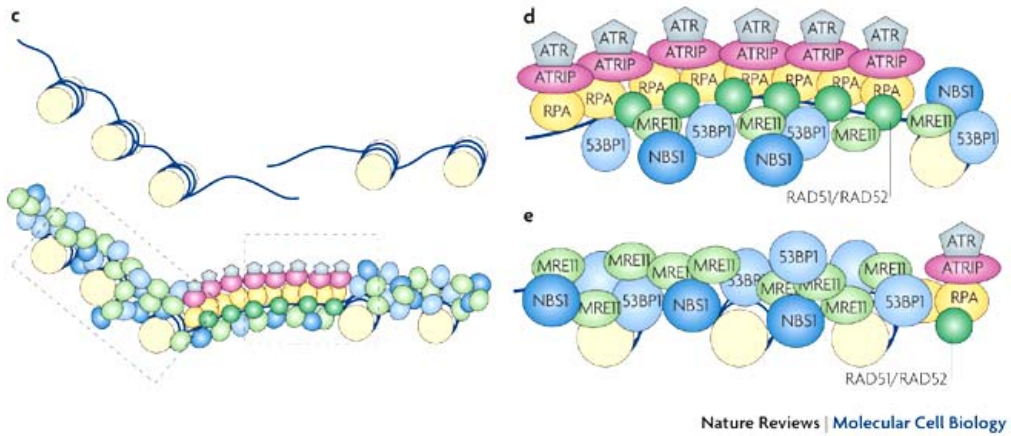
Soutoglou and Misteli, 2009
Nature Reviews Mol Cell Bio

DSB repair


Transducers
Mdc1
53BP1
BRCA1/2
Sensors
Nbs1
Mre11
Rad50
Effectors
ATM-kinase
ATR-kinase
DNAPK
D
D
R
Cell cycle checkpoints
apoptosis
senescence

Repair foci: cytological manifestations of DNA repair

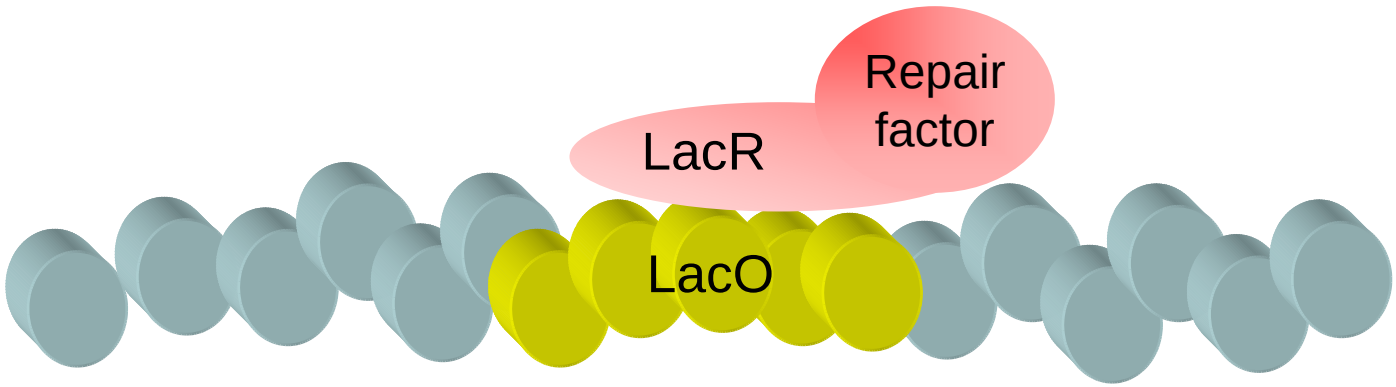




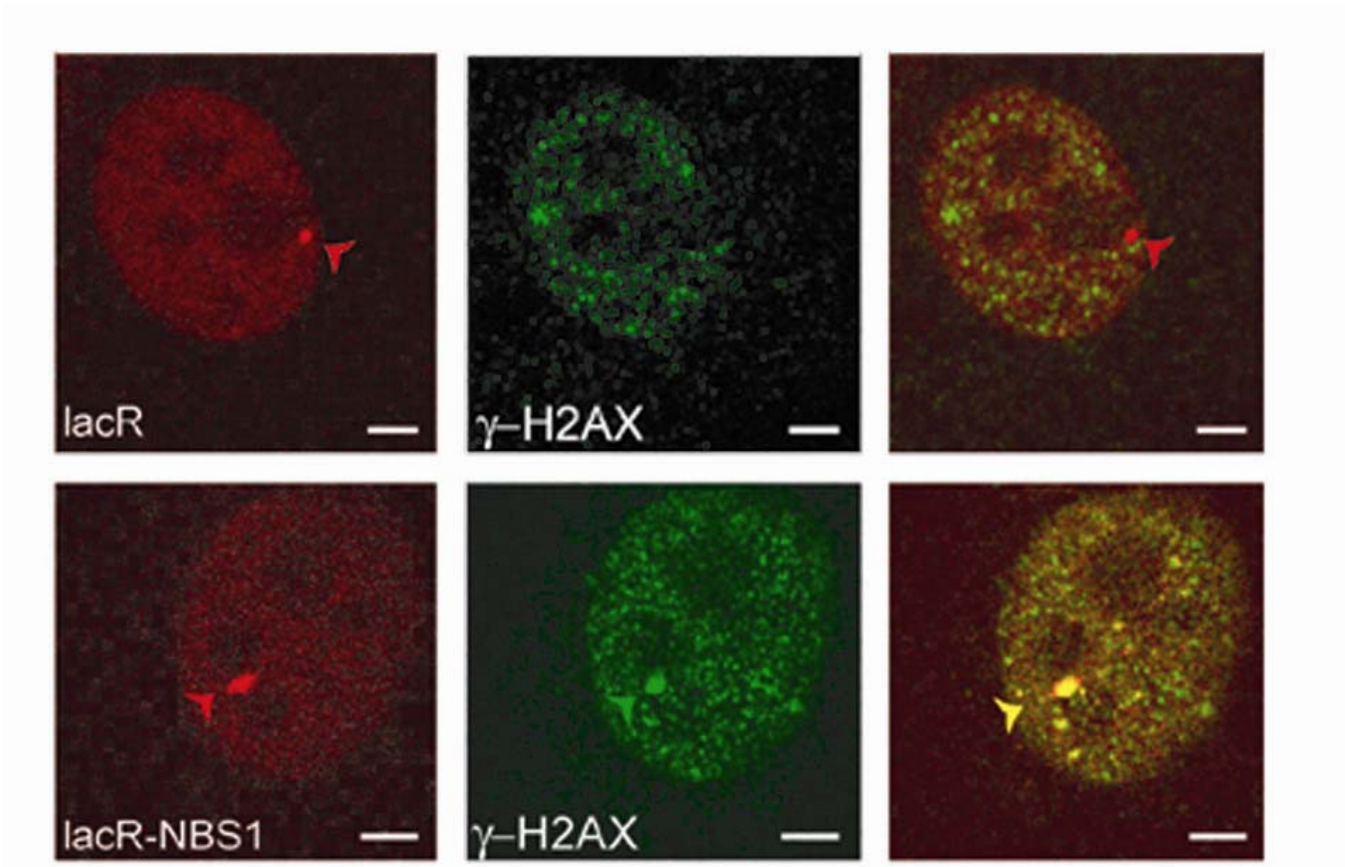
Soutoglou and Misteli, 2009
Nature Reviews Mol Cell Bio

What is the functional relevance of repair foci?
How do they assemble? What is their structure?

Bringing repair factors to chromatin

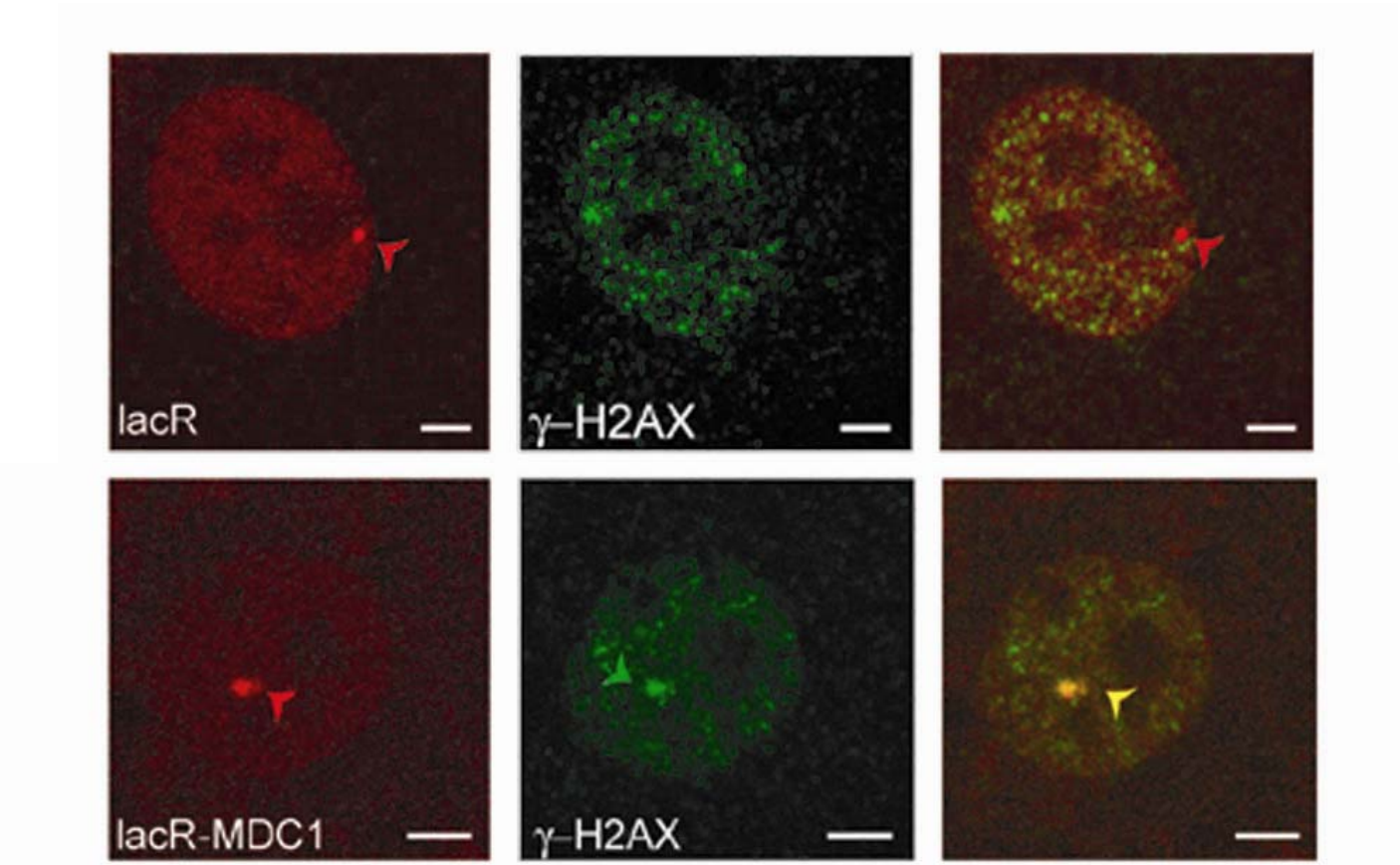


Activation of DDR by repair factor tethering



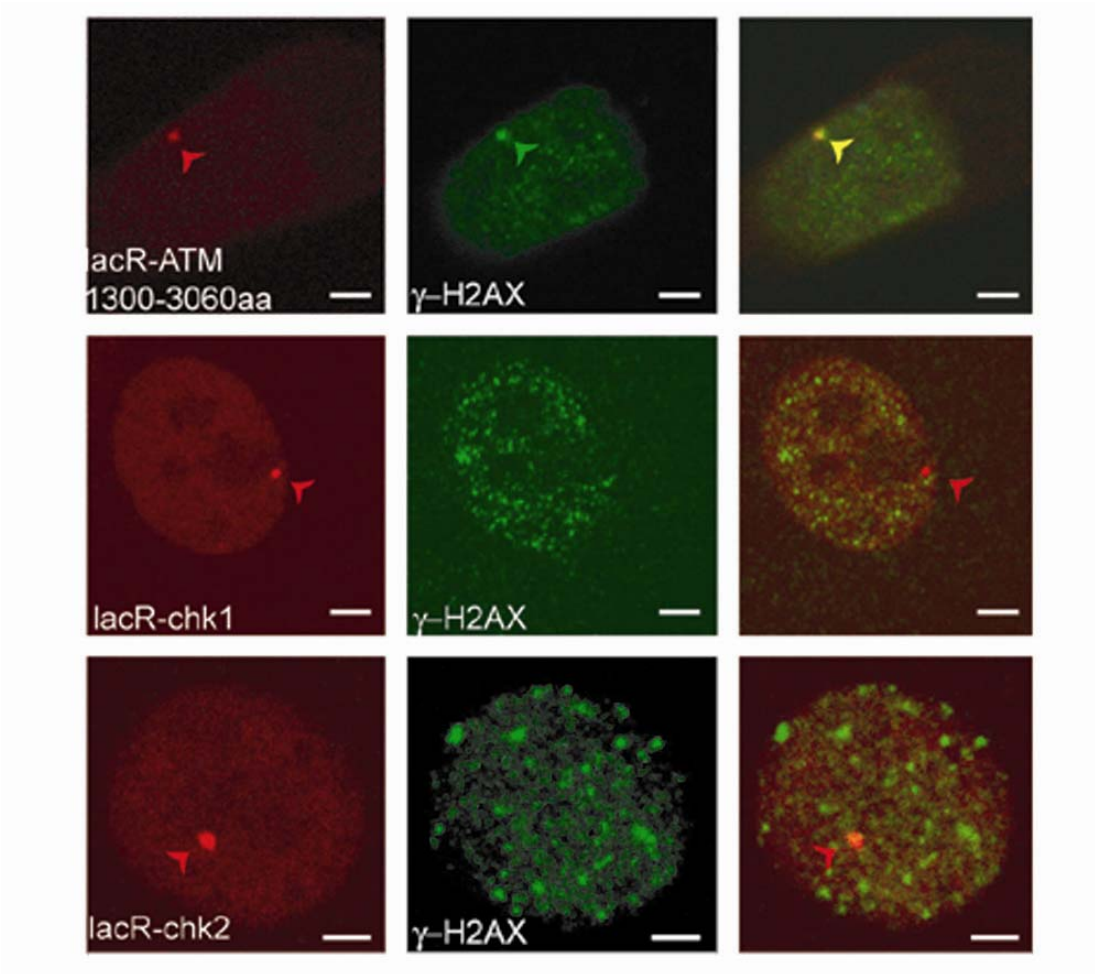
Soutoglou and Misteli, Science, 2008

Activation of DDR by repair factor tethering



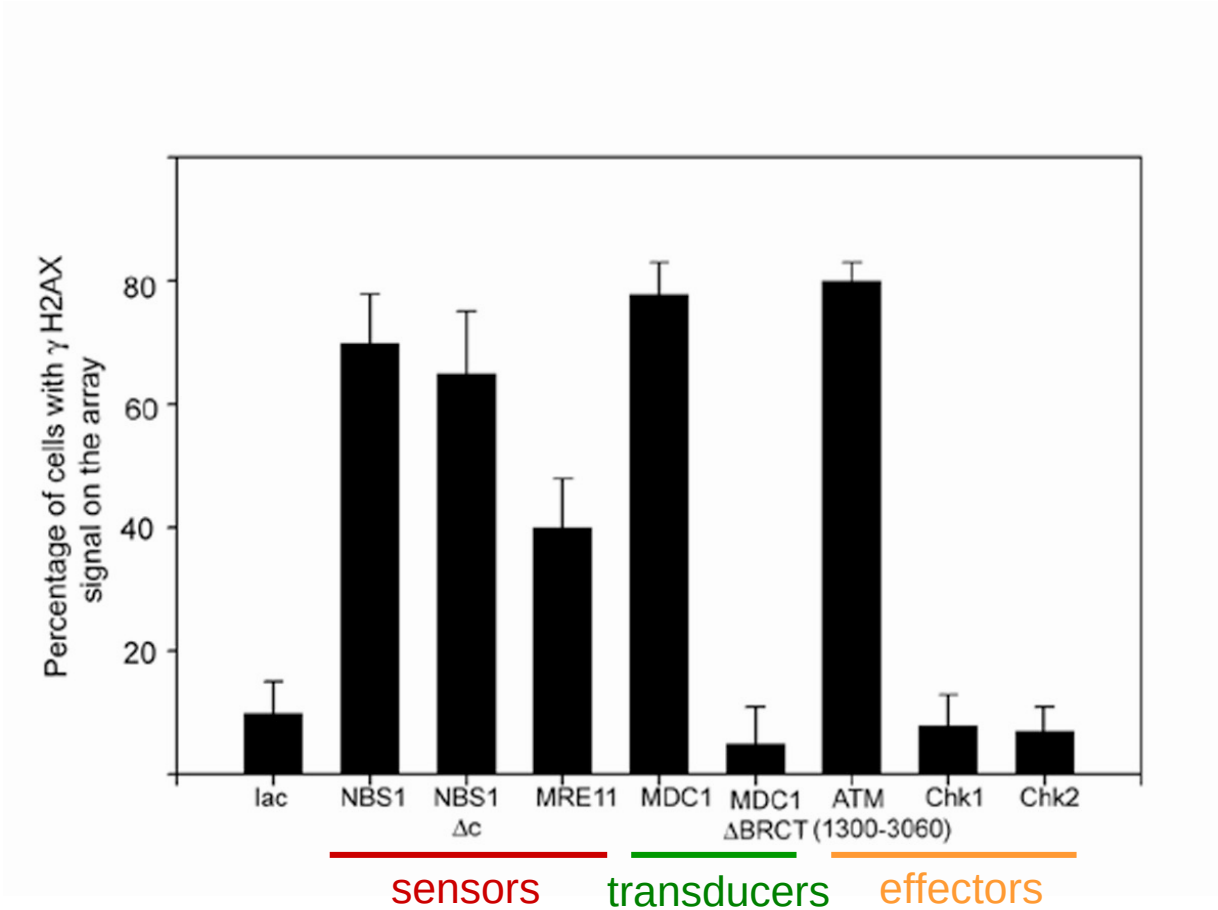
Soutoglou and Misteli, Science, 2008

Activation of DDR by repair factor tethering



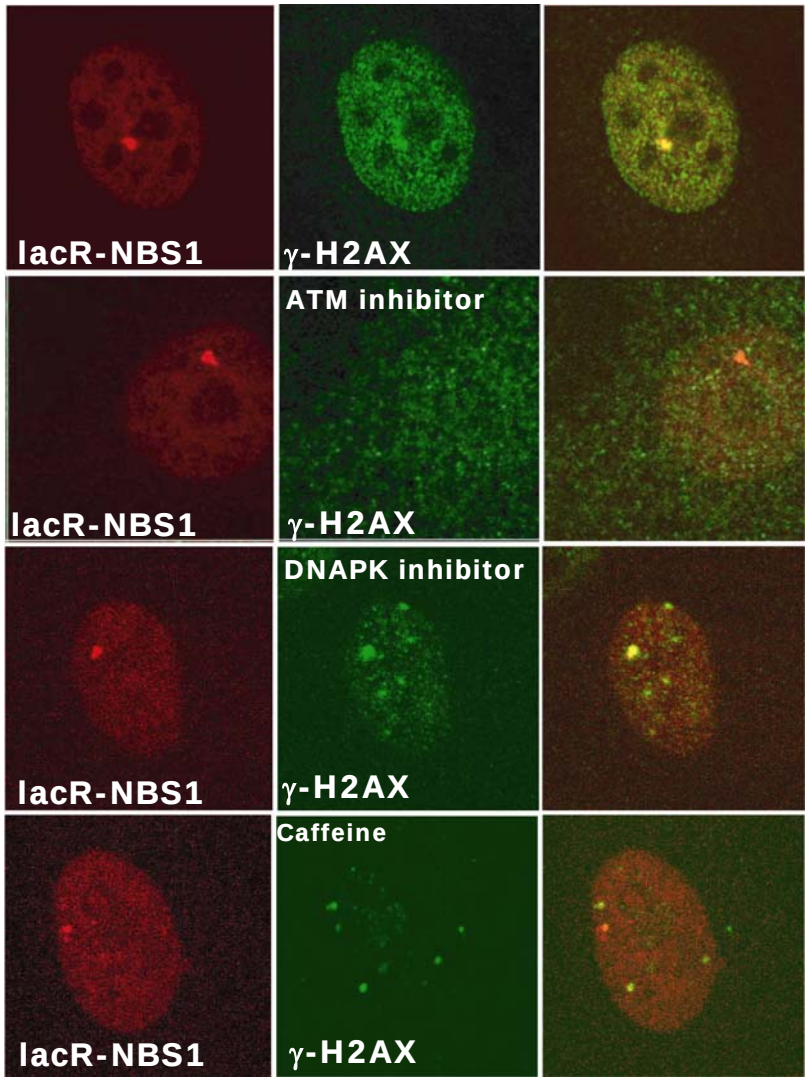
Soutoglou and Misteli, Science, 2008

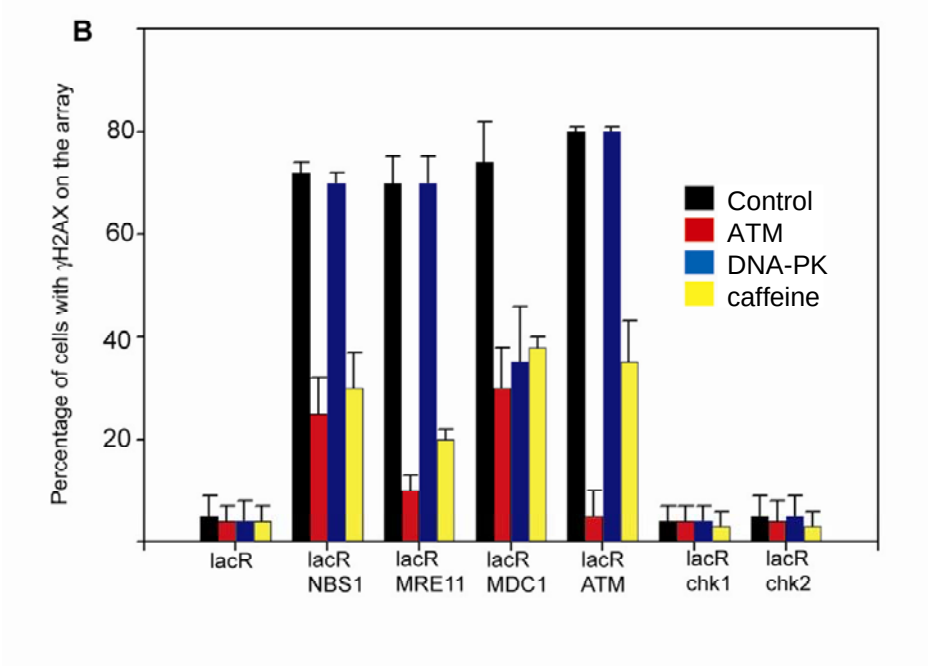
Activation of DDR by repair factor tethering



Soutoglou and Misteli, Science, 2008

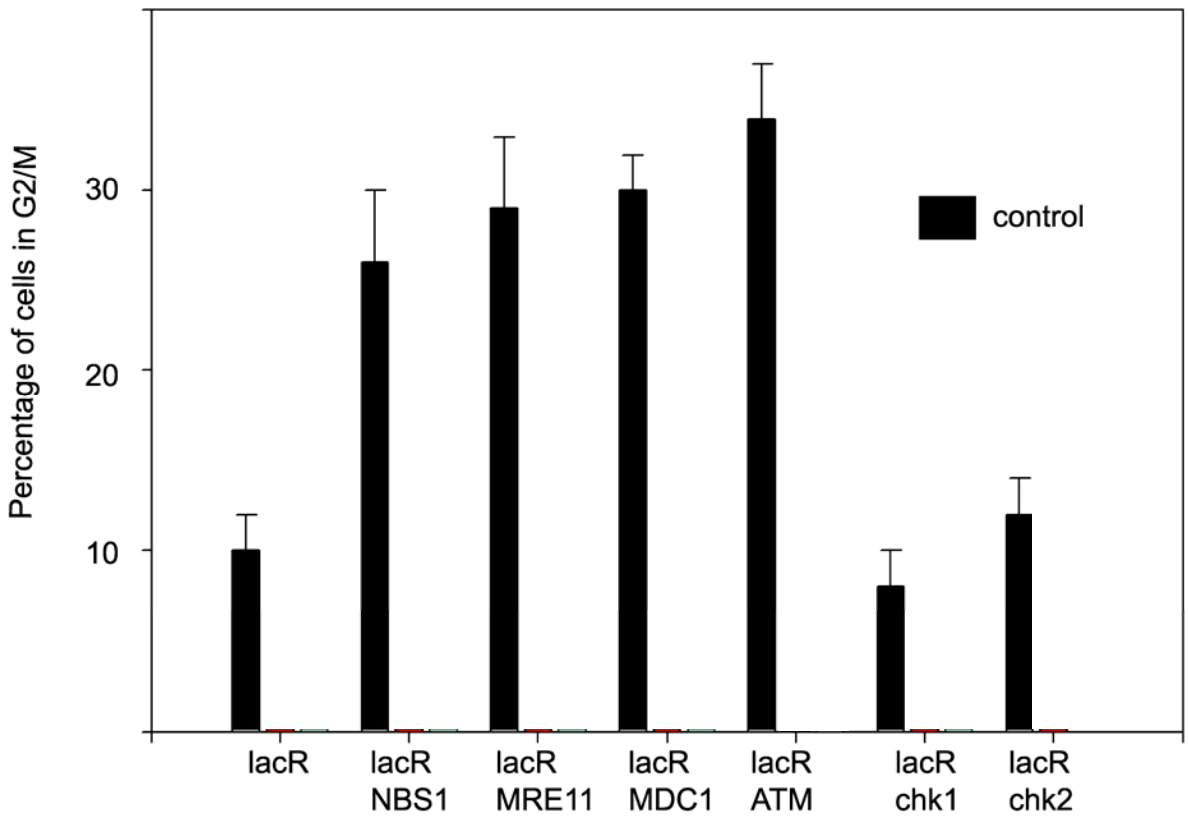
DDR activation by tethering is ATM-dependent





Soutoglou and Misteli, Science, 2008

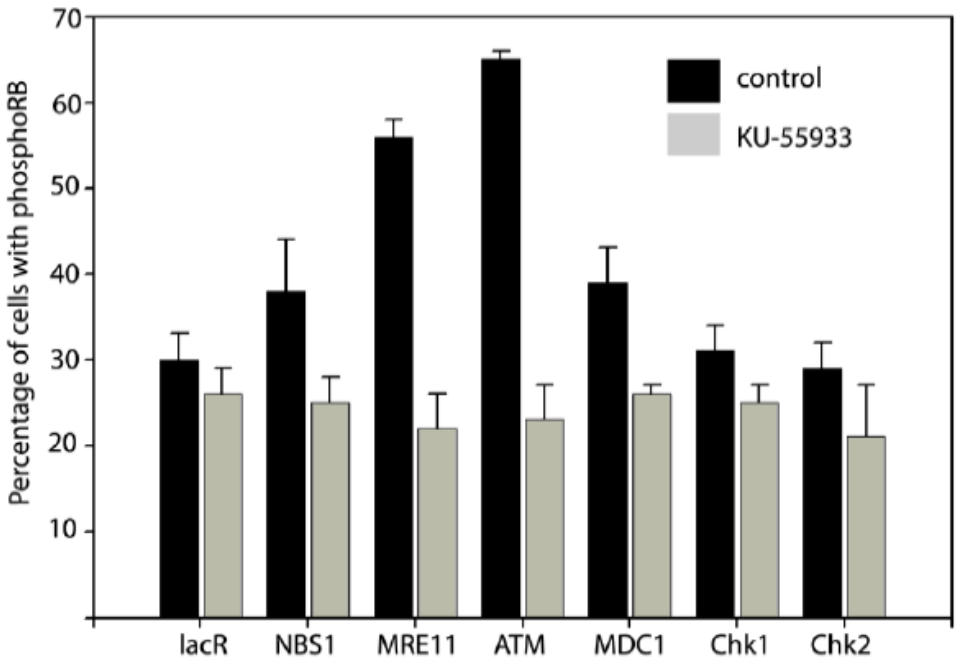
Repair factor tethering leads to cell-cycle delays



Soutoglou and Misteli, Science, 2008

Repair factor tethering leads to cell-cycle delays

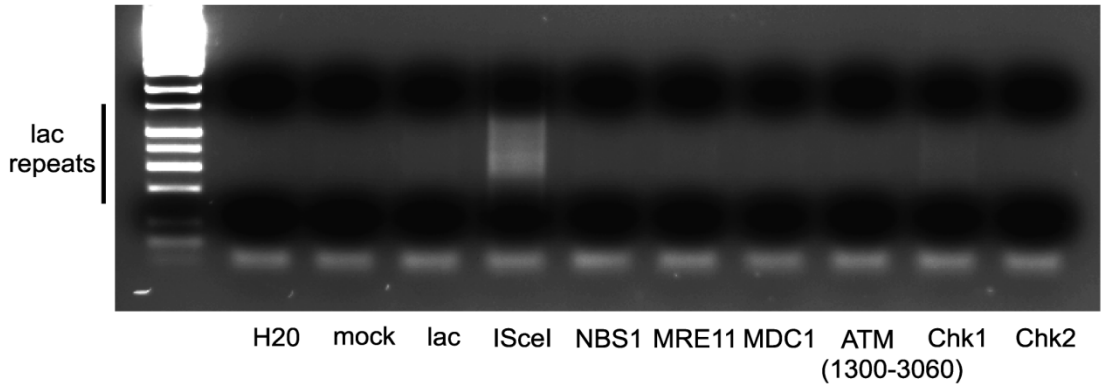
Phosphorylation of Rb



Soutoglou and Misteli, Science, 2008

Activation of DDR does not require DNA lesions

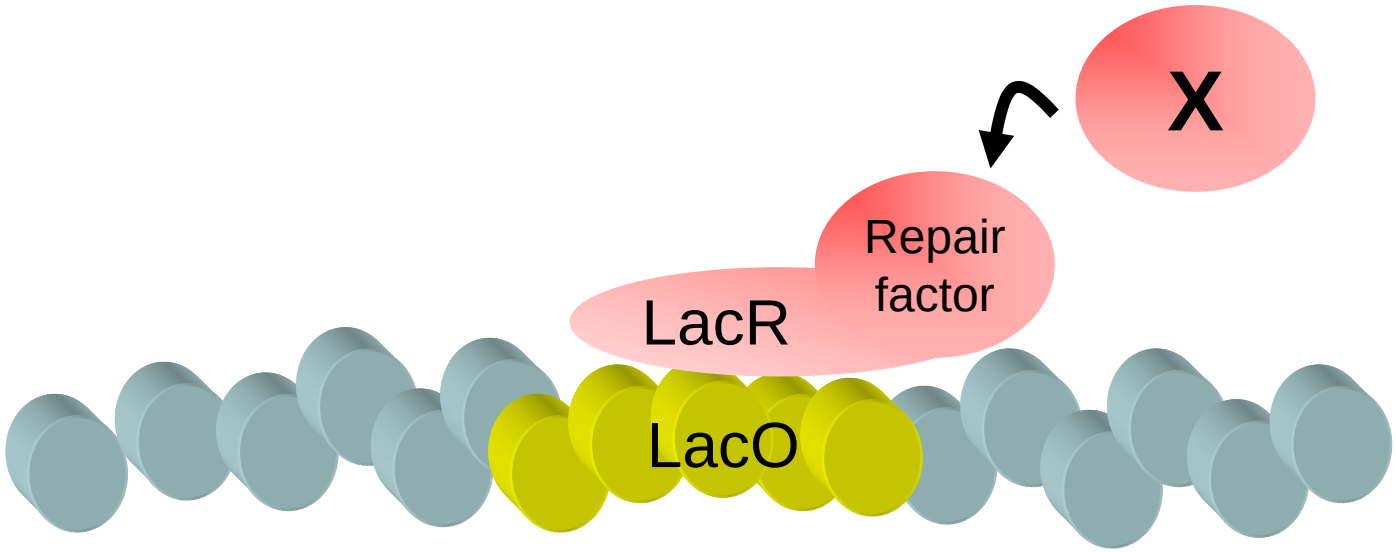
Ligation-mediated PCR



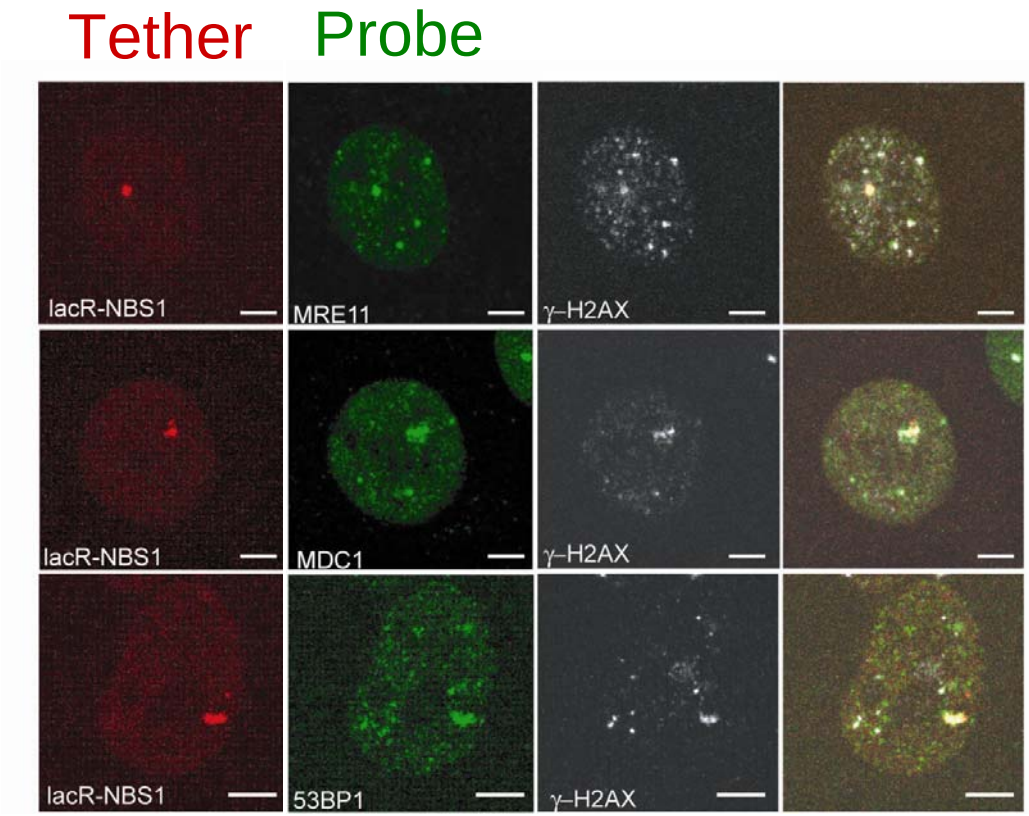
-DNA damage is not required to assemble the repair machinery
-DNA damage is not required to propogate/maintain DDR

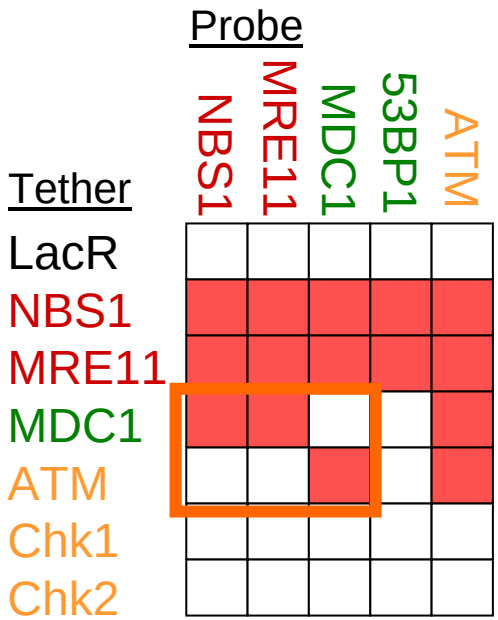
Soutoglou and Misteli, Science, 2008

Probing repair factor interplay



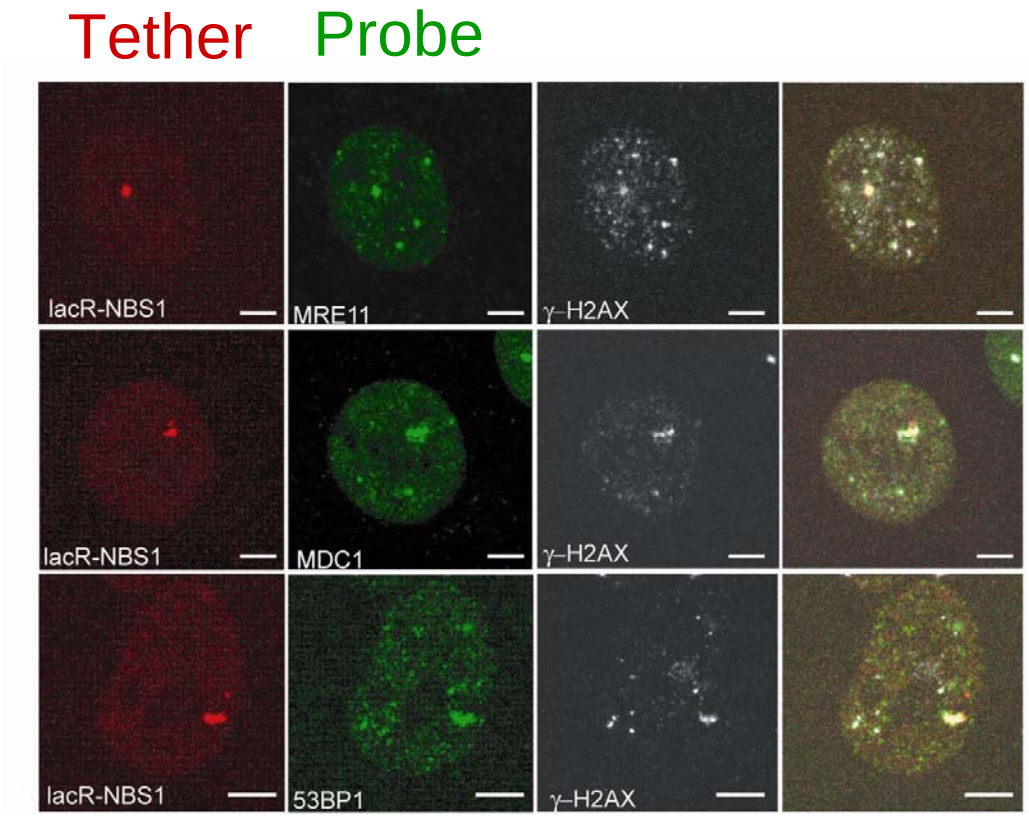
Interdependencies in repair factor recruitment

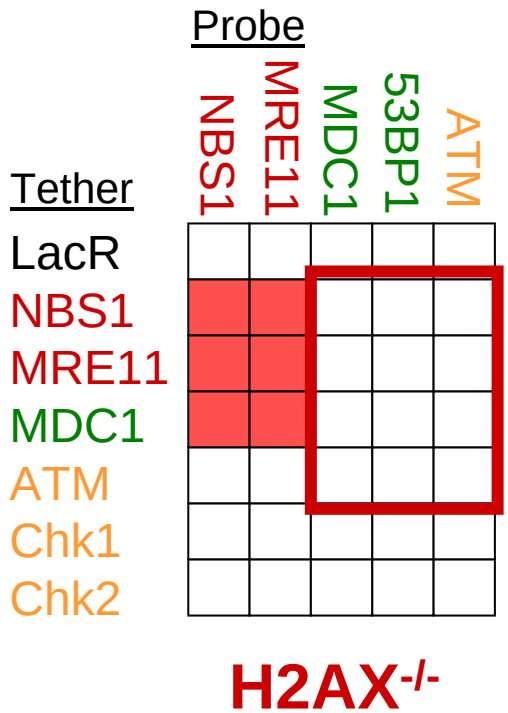




Downstream factors can recruit upstream components

Interdependencies in repair factor recruitment

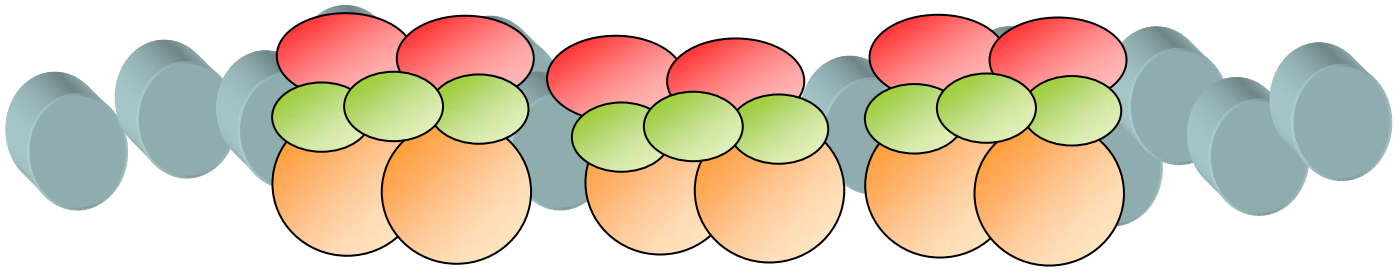


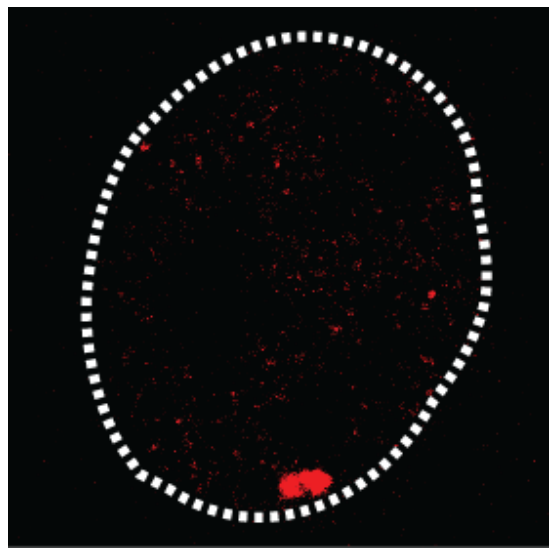


Downstream, but not upstream, recruitment events are dependent on H2AX

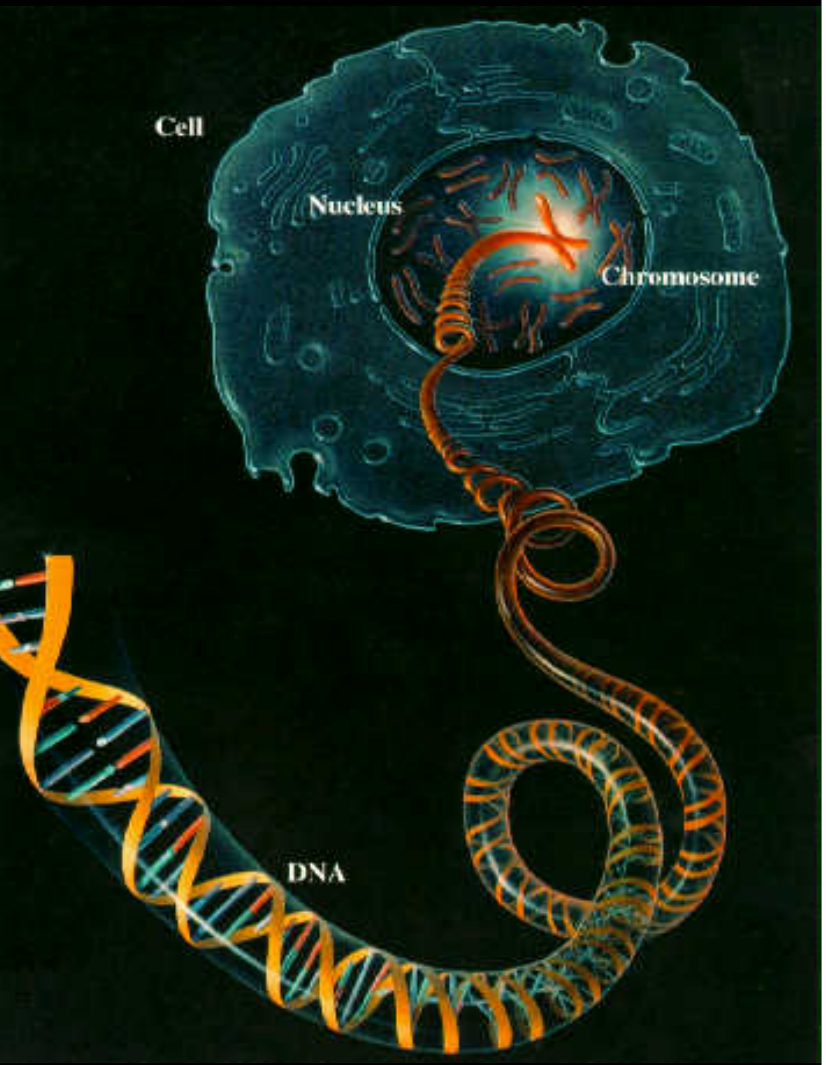
Amplification and spreading of DDR via cyclical recruitment

Amplification of DDR signal
Spreading of repair machinery





Spatial genome organization and genome stability



Global genome organization

- **Effect of non-random organization**
- **Mechanisms of translocations**

Local chromatin organization

- **Assembly of the repair machinery**
- **Role of chromatin in assembly**
- **Effect of chromatin on repair signaling**

Reini Luco
Steve Mabon
Karen Meaburn
Alexandre Mejat
Paola Scaffidi
Gianluca Pegoraro
Sara Snyder
Evi Soutoglou (Strasbourg)
Travis Dittmer
Vassillis Roukos
Nicolas Rascovan
Pilar Saladores
Nard Kubben

Luis Parada
Bilbao, Spain

Jeffrey Roix
Constellation Pharma

Phil McQueen
NIH

Maria Jasin
Sloan Kettering

Thomas Ried
Andre Nussenzweig
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