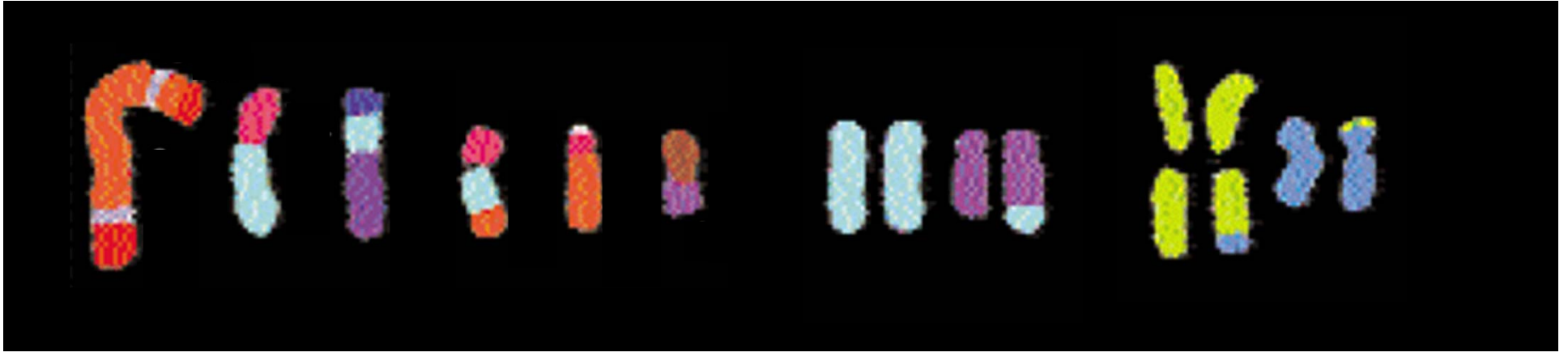




The Biogenesis of Chromosome Translocations

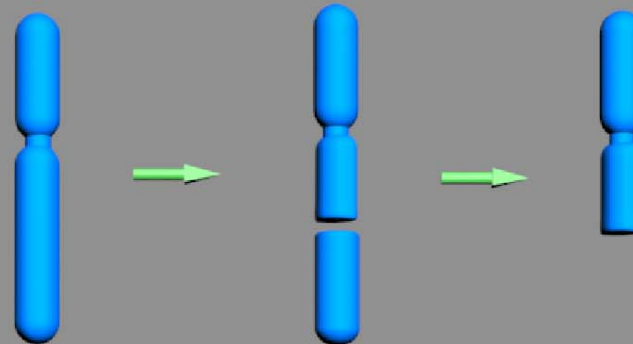
Vassilis Roukos

Tom Misteli Lab
National Institutes of Health

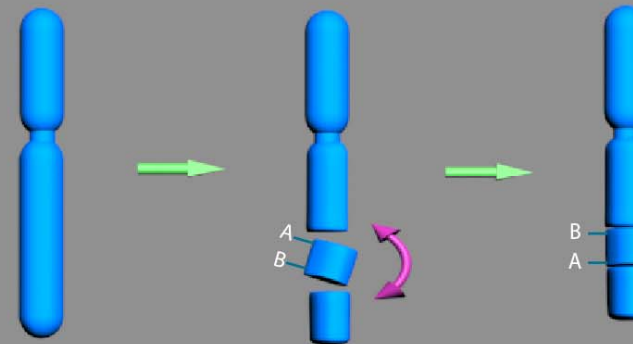


Genome Rearrangements

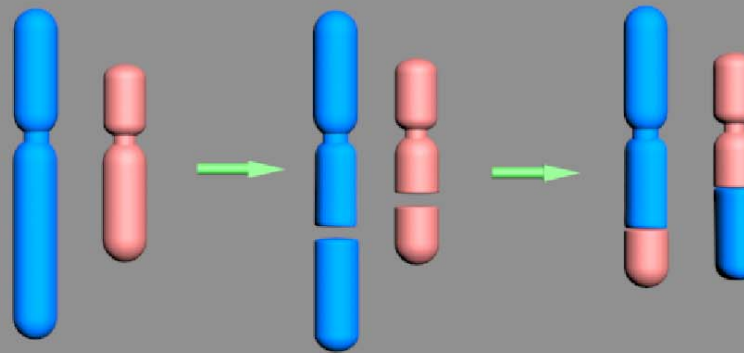
Deletion



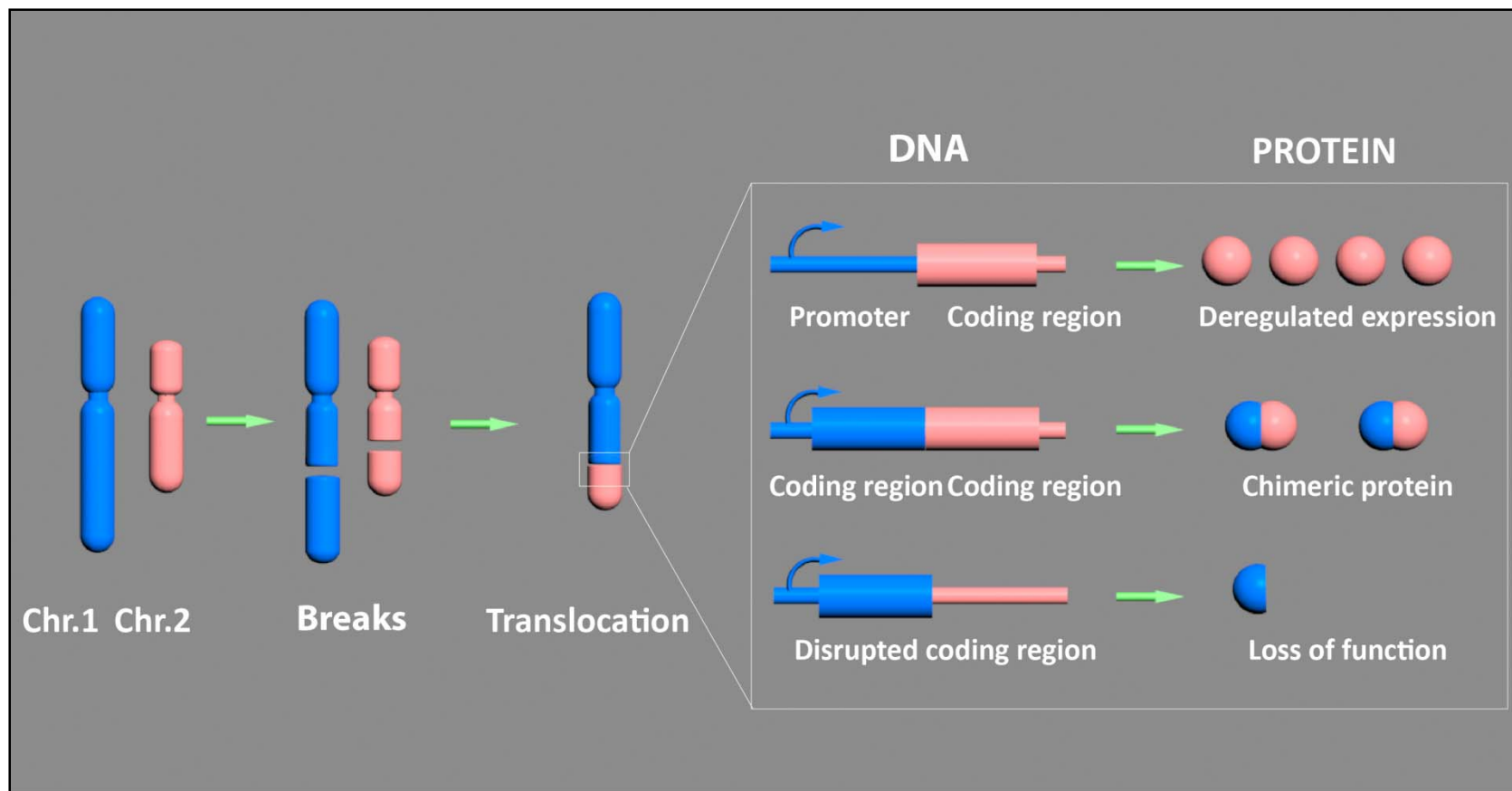
Inversion



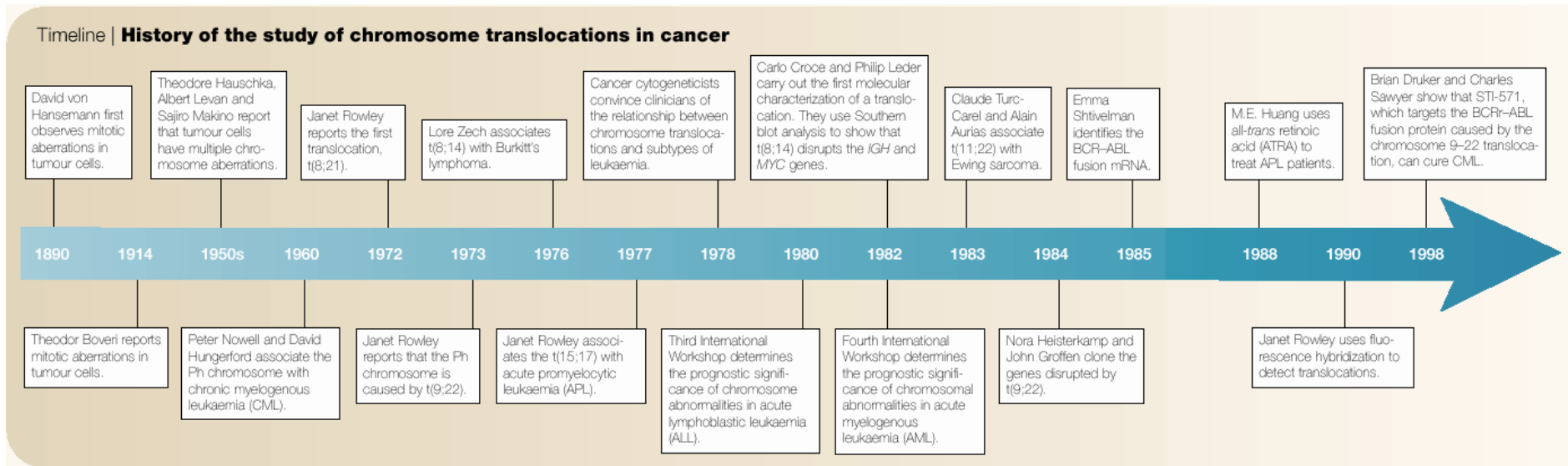
Translocation



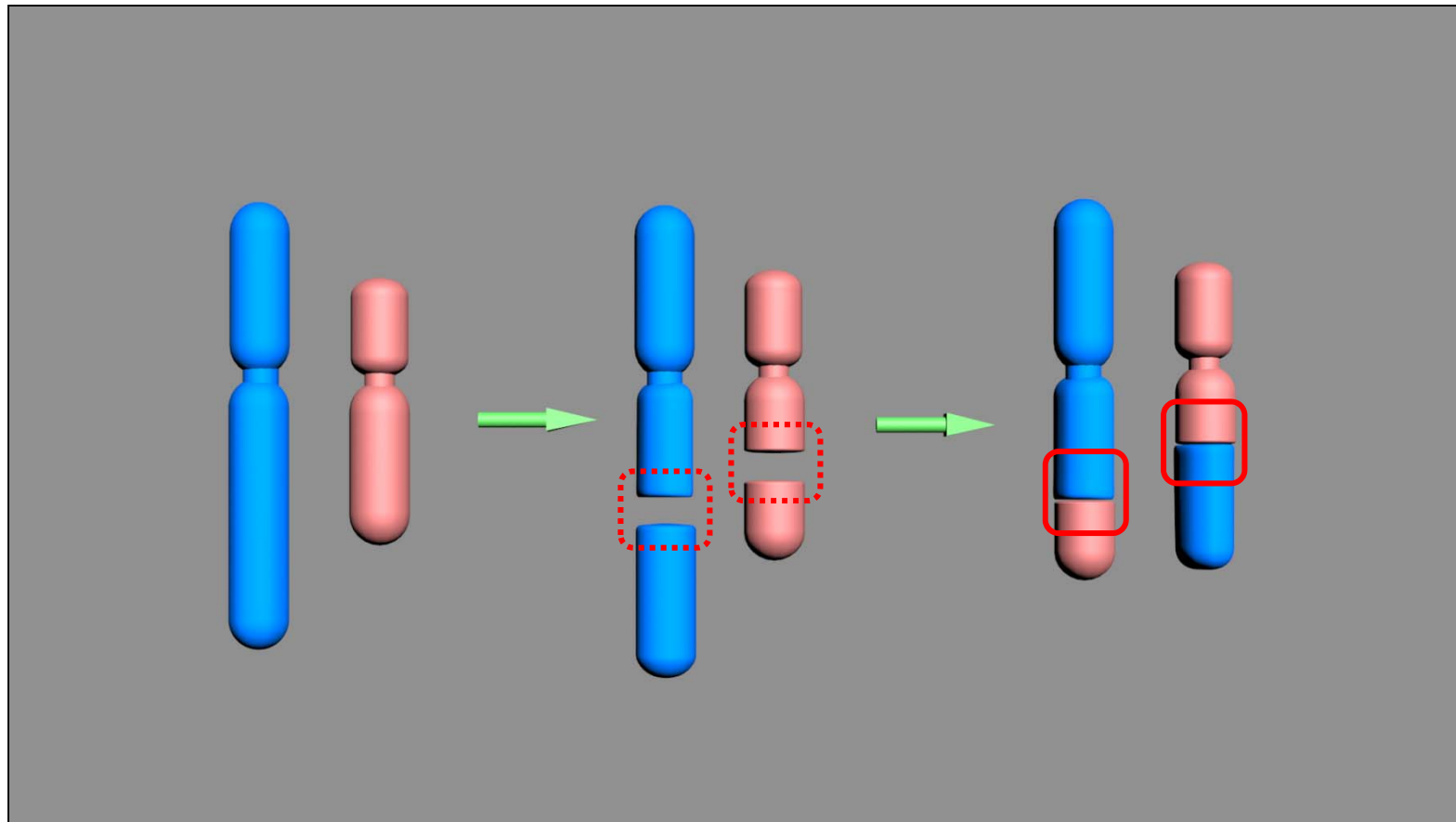
Chromosome Translocations



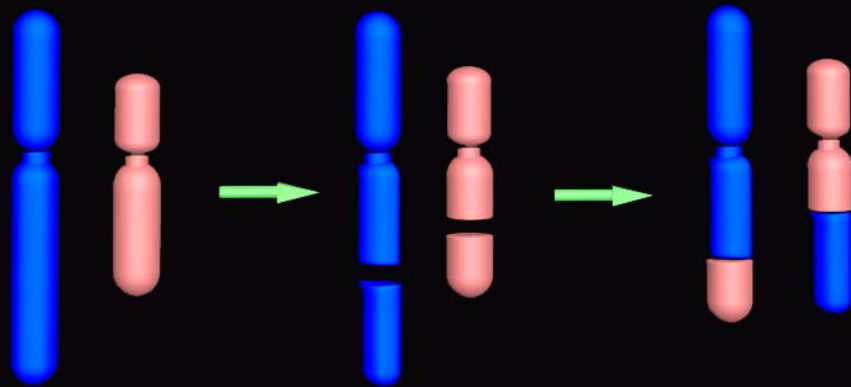
History...



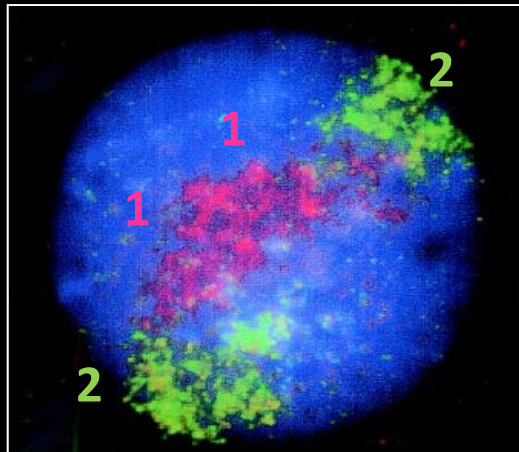
Chromosome Translocations



Genome Organization in the Nucleus: Non-random



Genome Organization in the Nucleus: Non-random

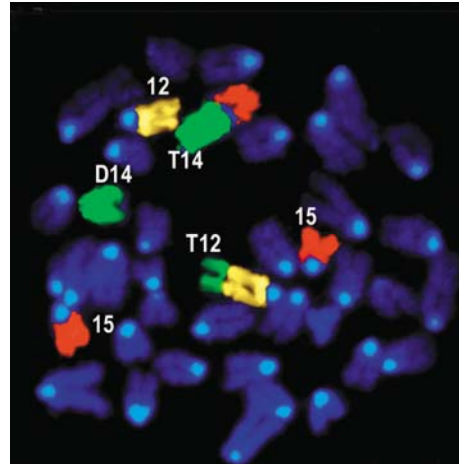


Boyle et al., *Hum Mol Genet*, 2001

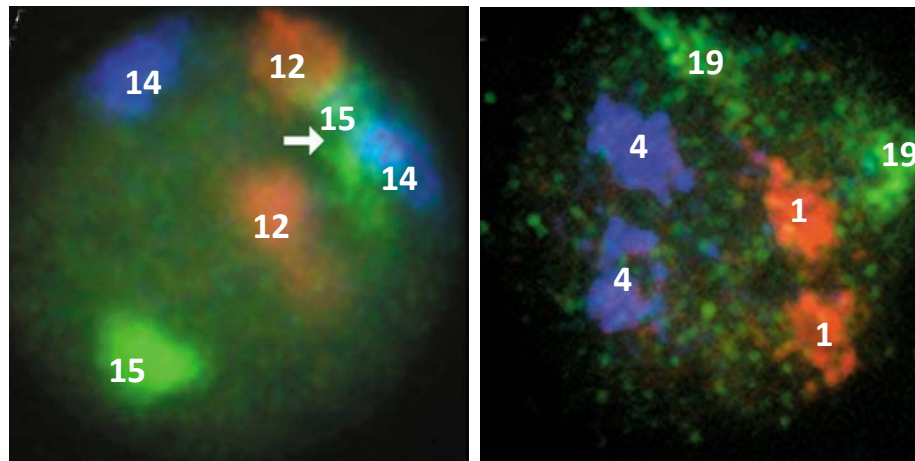


Where in the nucleus are translocating chromosomes?

80% of lymphomas in *ATM*^{-/-} mice contain translocations involving combinations of 12/14/15 chromosomes



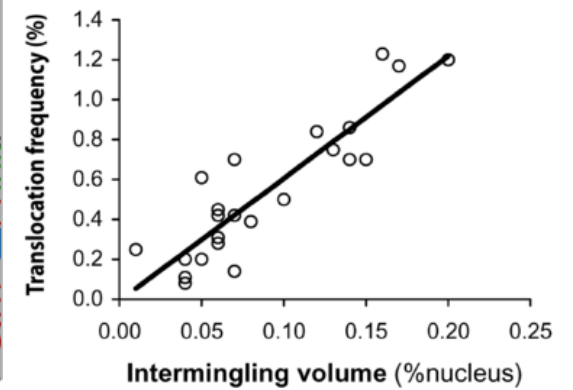
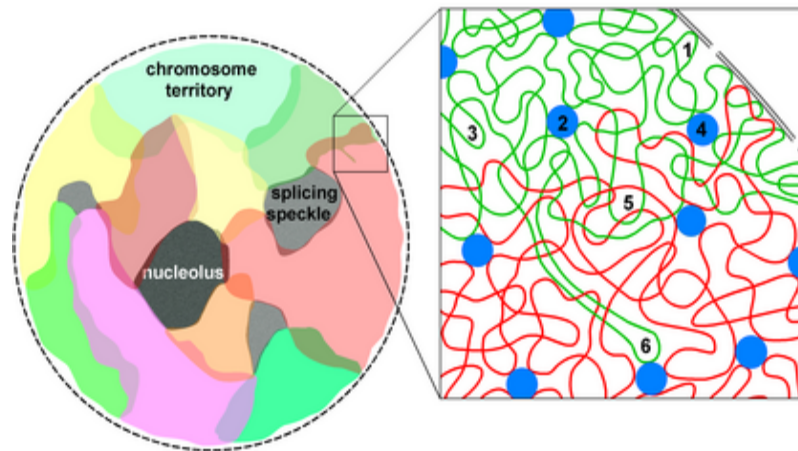
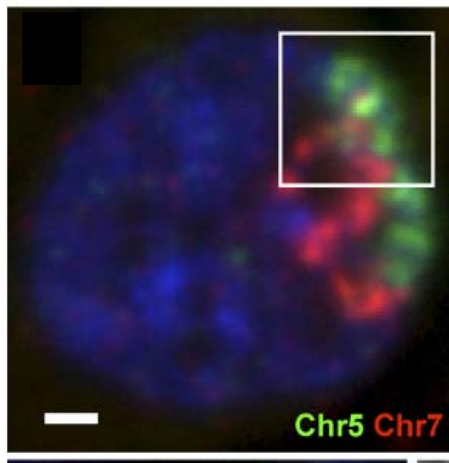
Liyanage M. et al., *Blood*, 2000



Parada L et al., *Cur Biol*, 2002

Spatial intermingling and translocation frequency

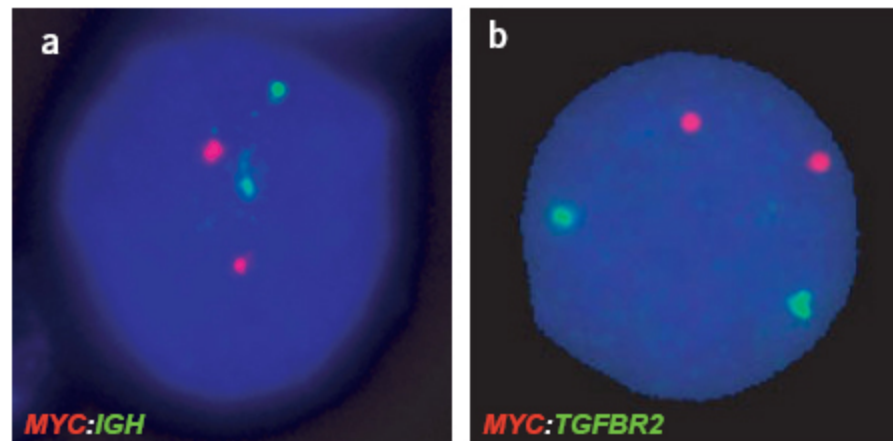
The degree of intermingling of chromosome territories in lymphocytes correlates with their translocation frequencies



Branco MR, Pombo A., *PLoS Biol*, 2006

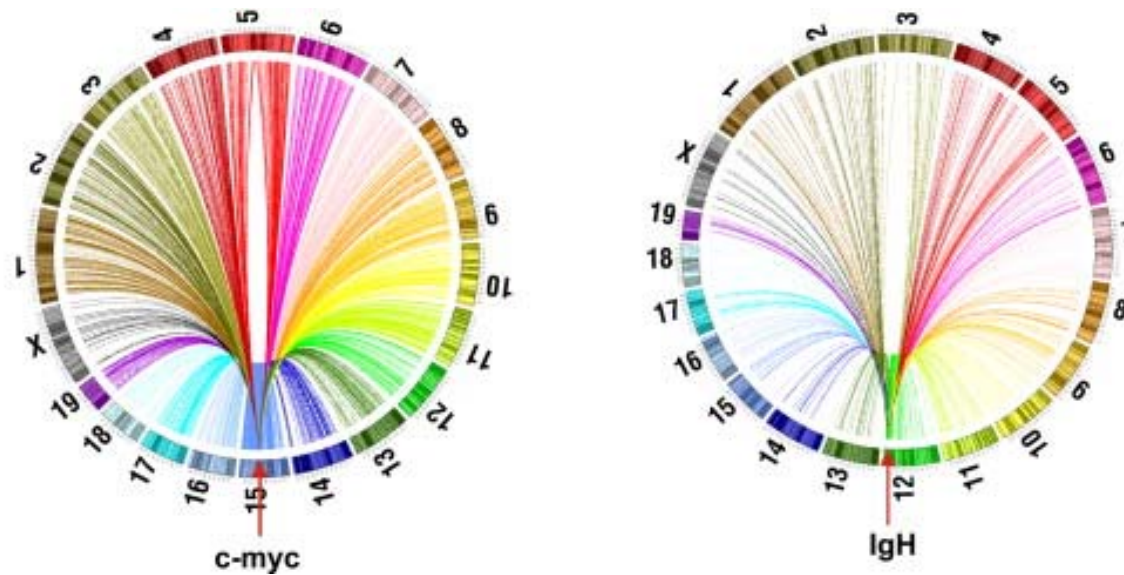
Proximity of Translocation Partners in Burkitt's Lymphoma

Genes that are involved in translocations are in closer proximity than other non translocating pairs



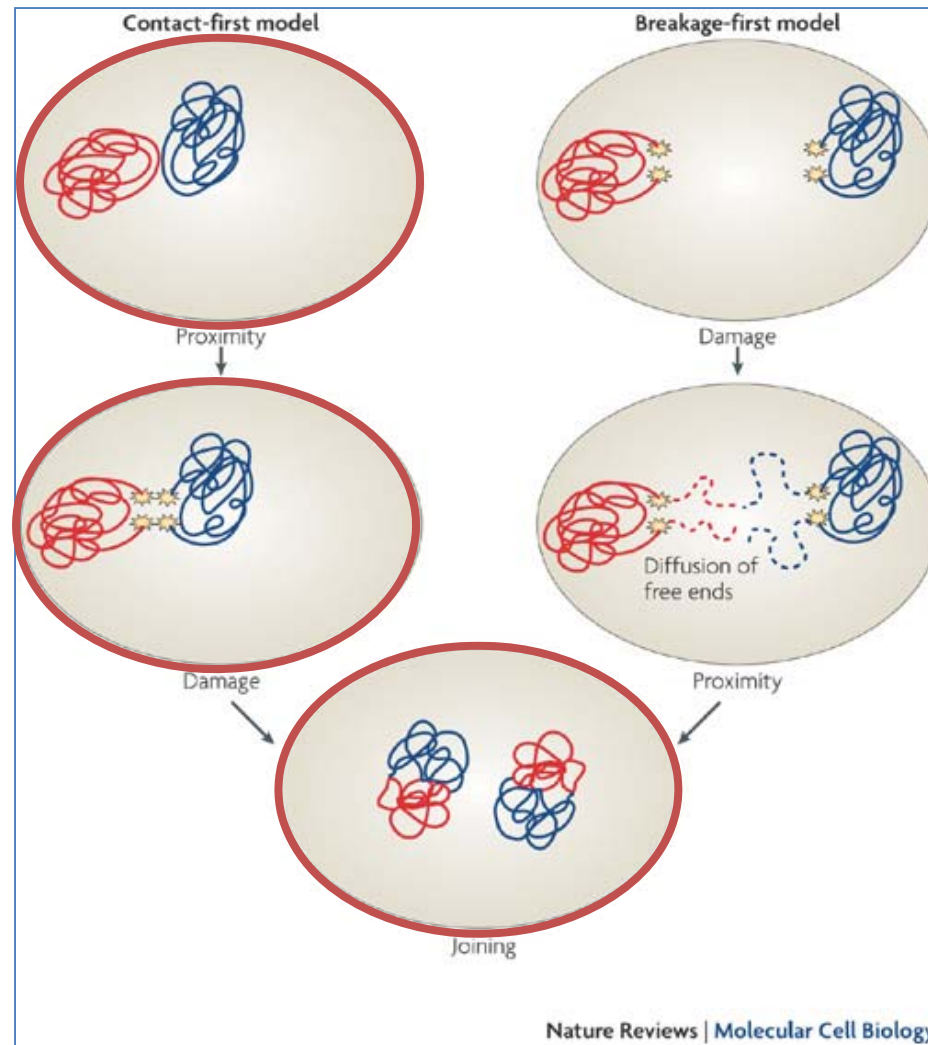
Roix J. et al., *Nature Genetics*, 2003

Proximity predisposes to translocation



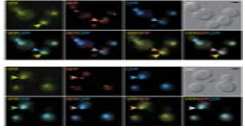
Chiarle et al., *Cell*, 2011
Klein et al., *Cell*, 2011
Hakim et al., *Nature*, 2012
Zhang et al., *Cell*, 2012
Rocha et al., *Mol Cell*, 2012

Contact-first vs. breakage-first



Soutoglou & Misteli, *Nature Reviews Mol. Cell Biol.*, 2009

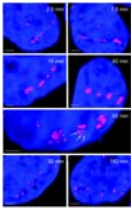
Mobility of Double Strand Breaks



Lisby et al., Nat Cell Biol 2003

Colocalization of multiple DNA double-strand breaks at a single Rad52 repair centre

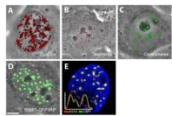
ISceI-HO, endonucleases (yeast)



Aten et al., Science. 2004

Dynamics of DNA double-strand breaks revealed by clustering of damaged chromosome domains

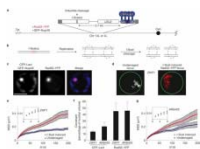
alpha-particles (mammals)



Krawczyk et al., J Cell Science. 2012

Chromatin mobility is increased at sites of DNA double-strand breaks

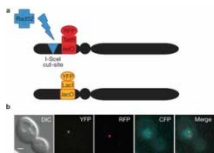
γ-irradiated (mammals)



Dion V, et al., Nat Cell Biol. 2012

Increased mobility of double-strand breaks requires Mec1, Rad9 and the homologous recombination machinery

ISceI endonucleases (yeast)

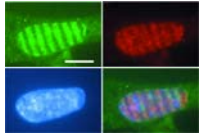


Miné-Hattab J, Rothstein R. Nat Cell Biol. 2012

Increased chromosome mobility facilitates homology search during recombination.

ISceI endonucleases (yeast)

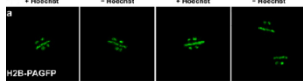
Immobi**l**ity of Double Strand Breaks



Nelms et al., Science. 1998

In situ visualization of DNA double-strand break repair in human fibroblasts.

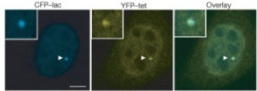
Ultrasoft X-rays (mammals)



Kruhlak M et al., J Cell Biol. 2006

Changes in chromatin structure and mobility in living cells at sites of DNA double-strand breaks

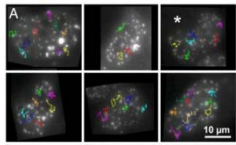
364 nm argon ion UV laser
(mammals)



Soutoglou et al., Nature Cell Biology 2007

Positional stability of single double-strand breaks in mammalian cells

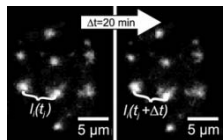
ISceI endonuclease
(mammals)



Jakob B et al., Proc Natl Acad Sci. 2009

Microscopy analysis of radiation-induced DNA double-strand break motion

Heavy ion irradiation



Girst et al Scientific Reports. 2013

Subdiffusion Supports Joining Of Correct Ends During Repair Of DNA Double-Strand Breaks

Carbon ion
micro-irradiation.

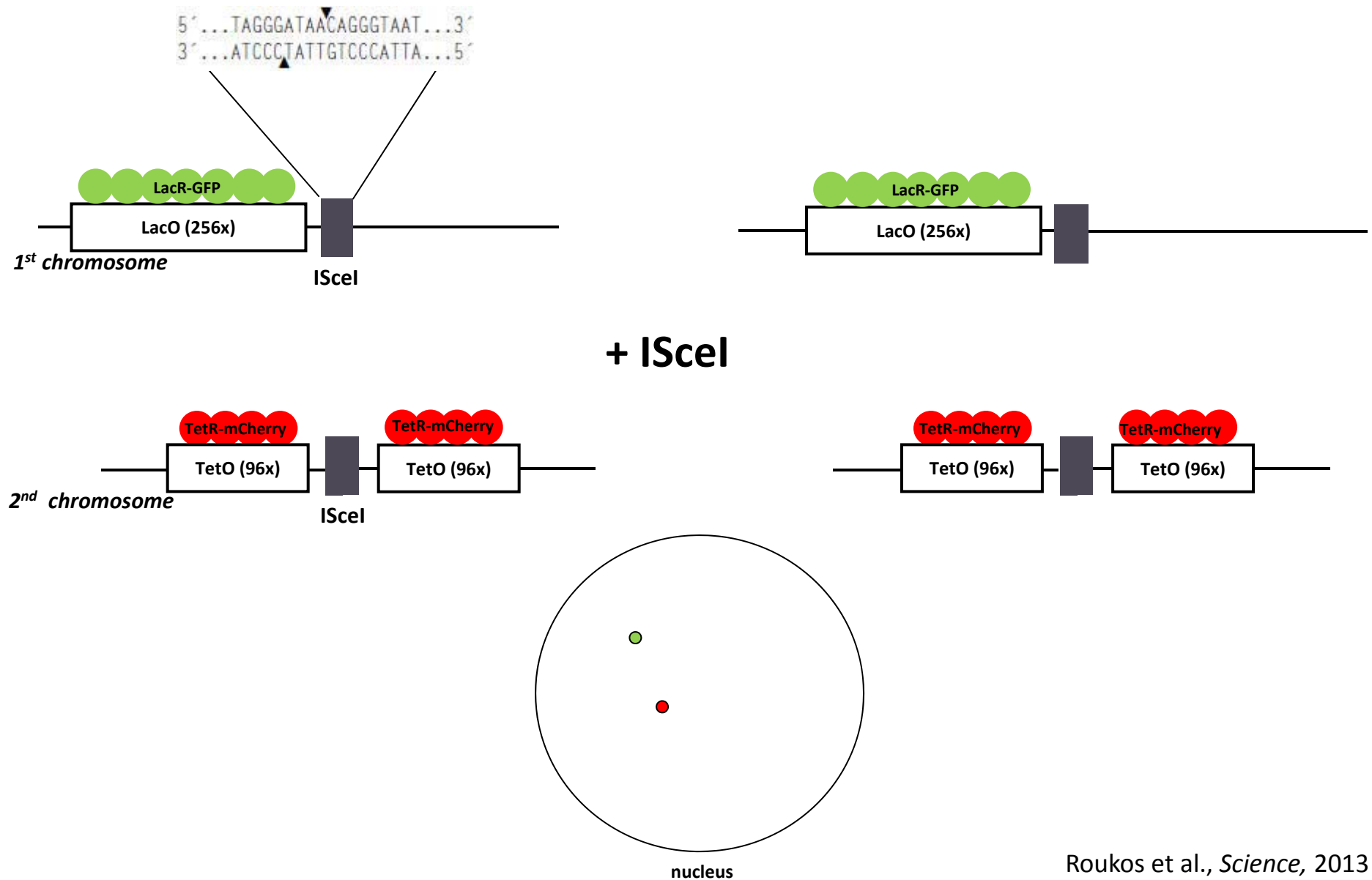
How do broken chromosomes meet in the nucleus?

How long does the translocation process take?

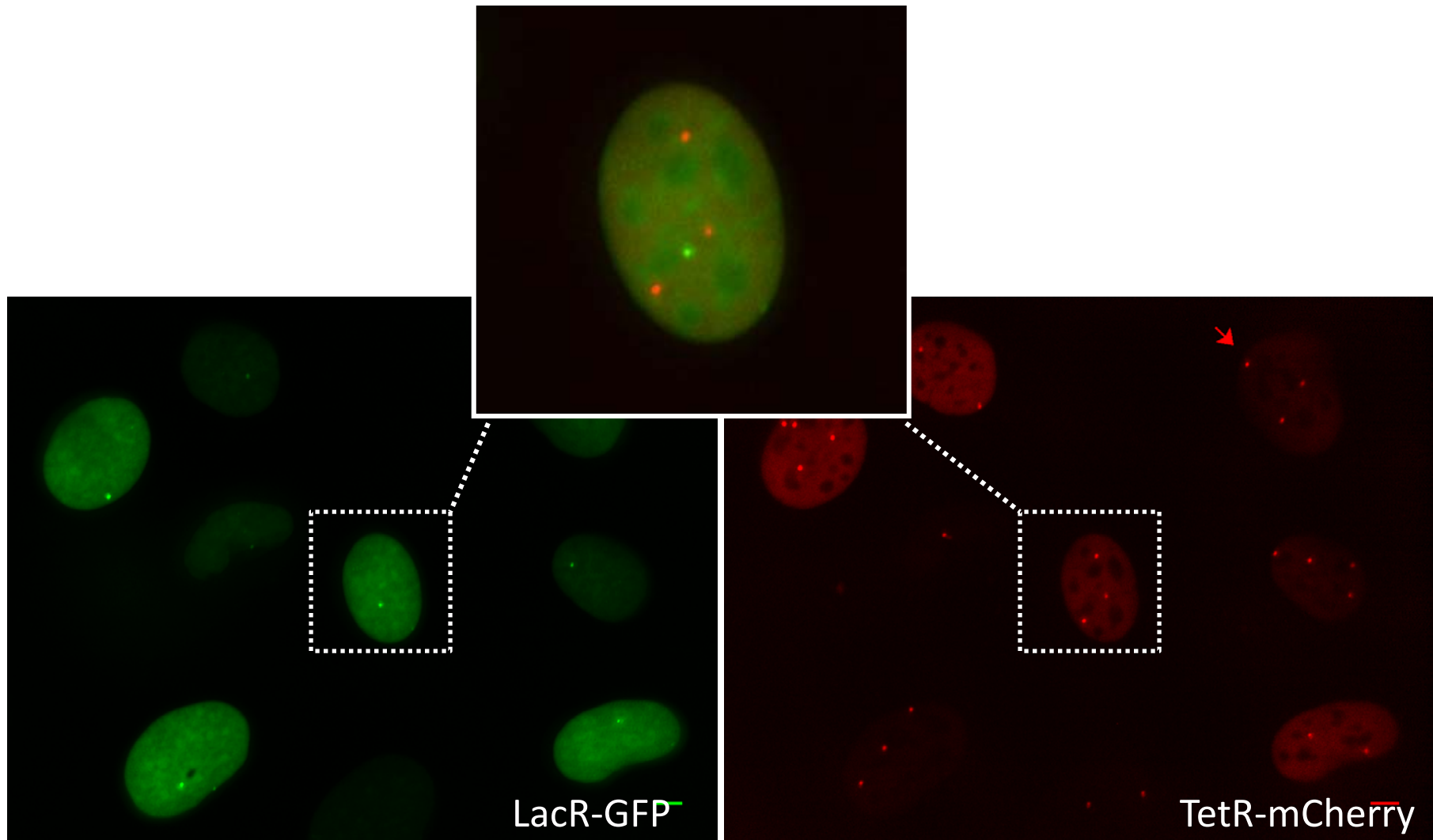
How does the cell cycle affect translocation formation?

Which are the molecular players?

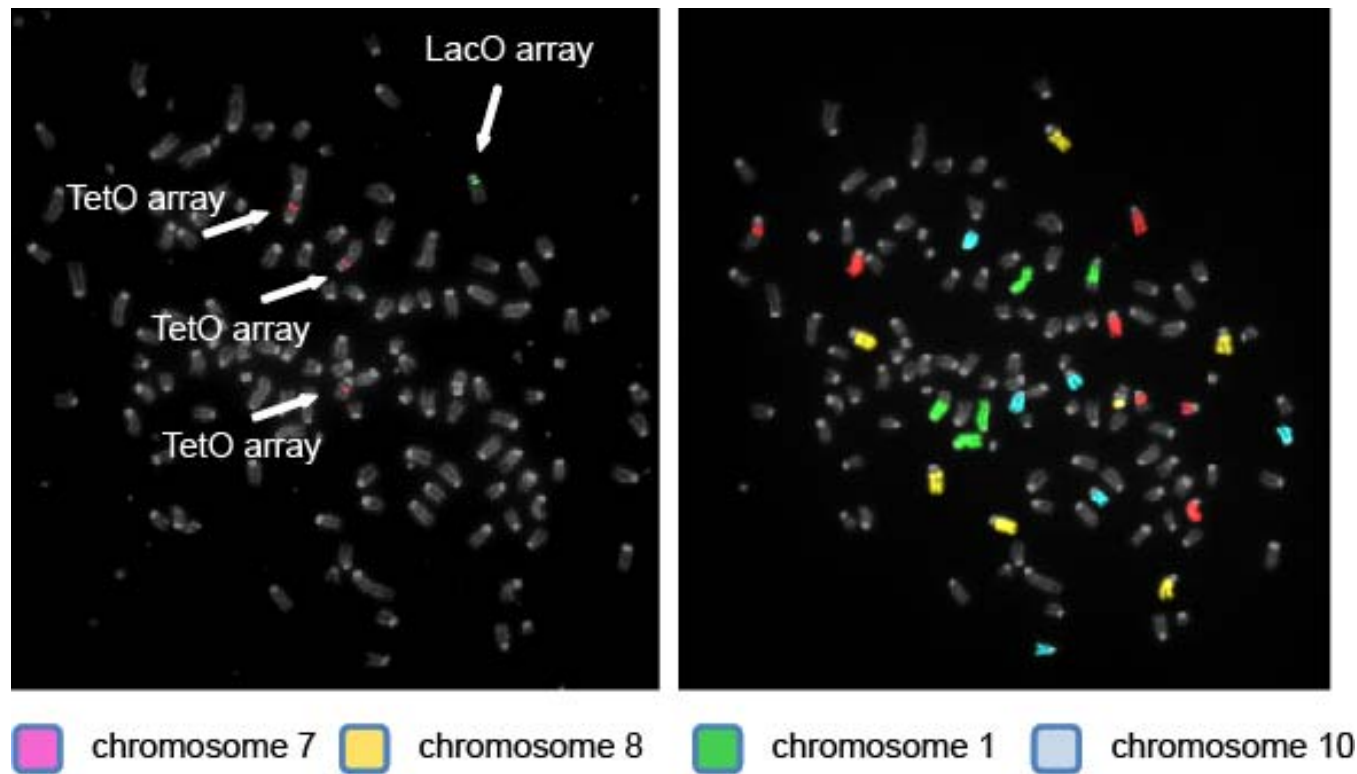
Experimental System to Visualize Translocations in vivo



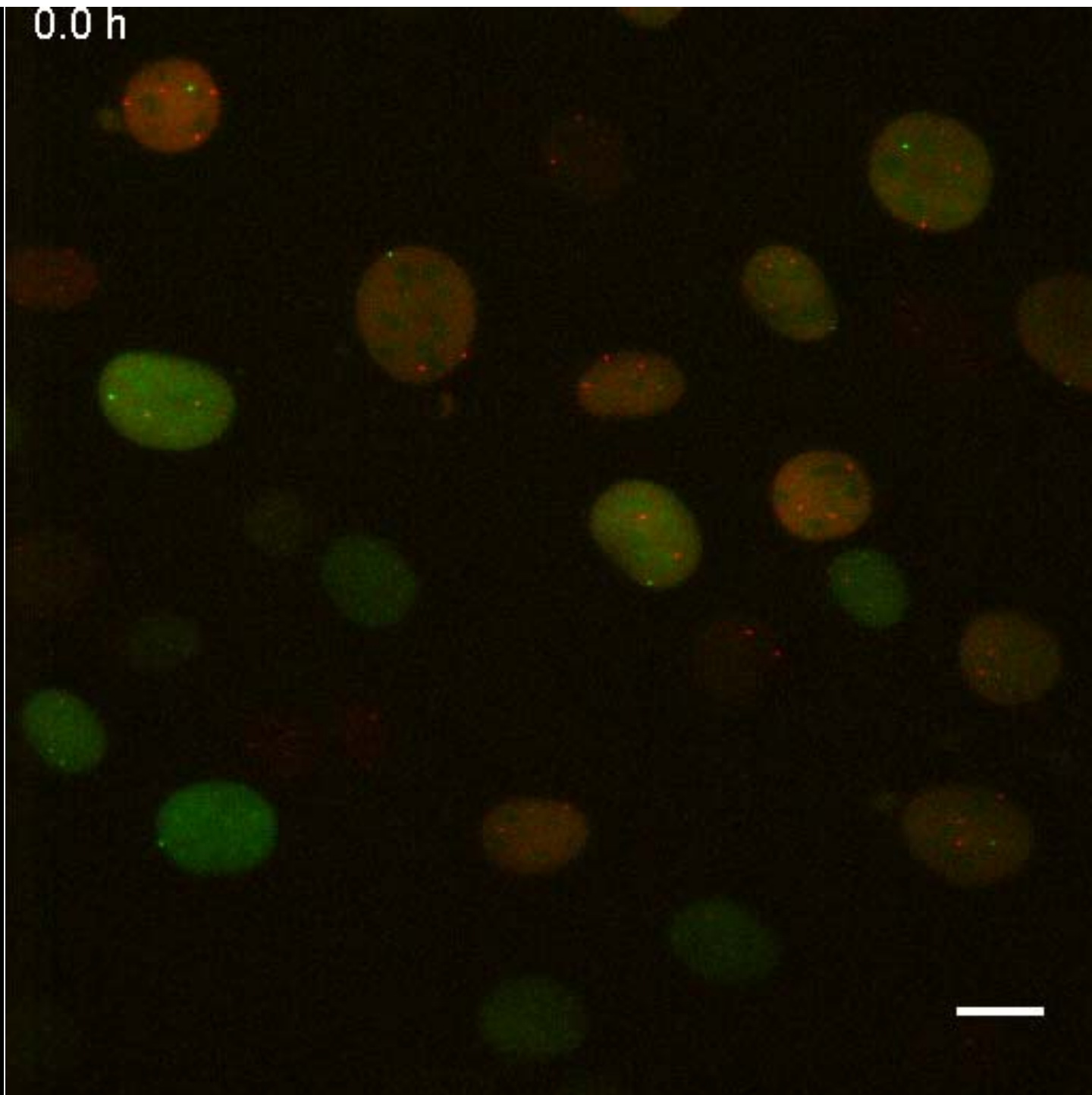
Experimental System to Visualize Translocations *in vivo*



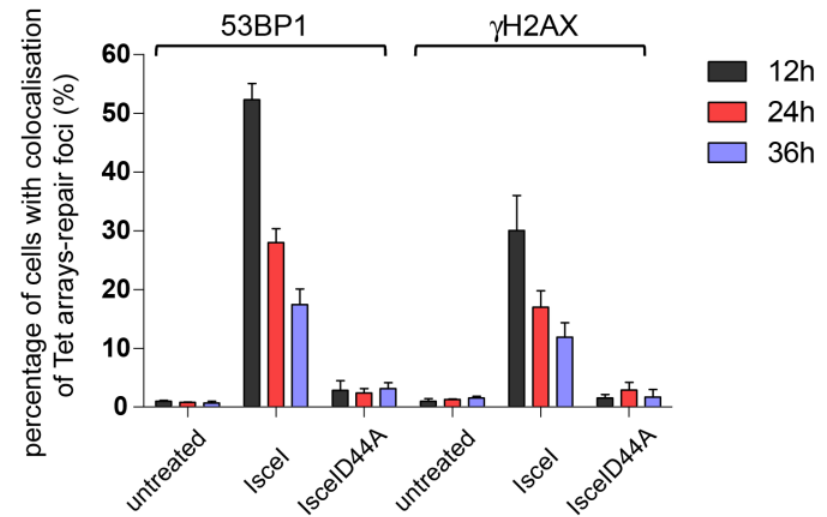
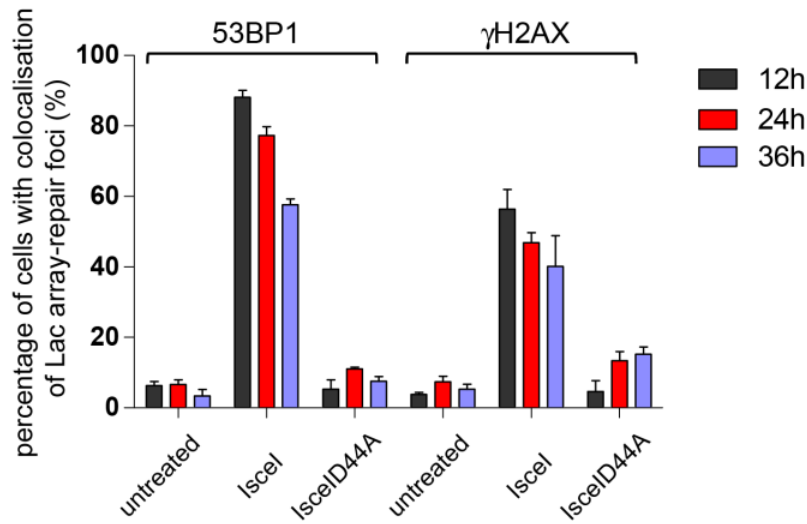
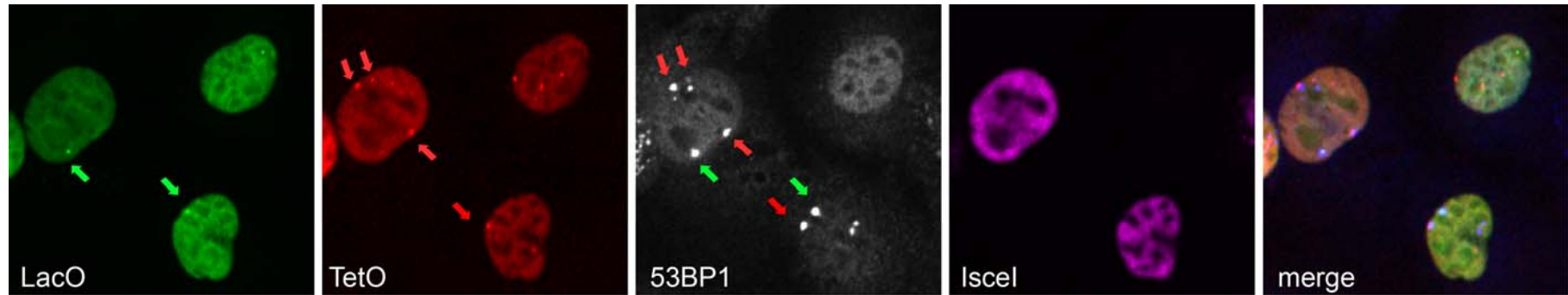
1 LacO and 3 TetO integrations in distinct chromosomes



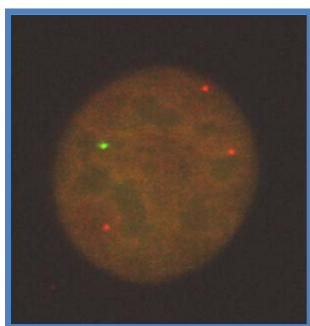
0.0 h



Double Strand Break formation and repair



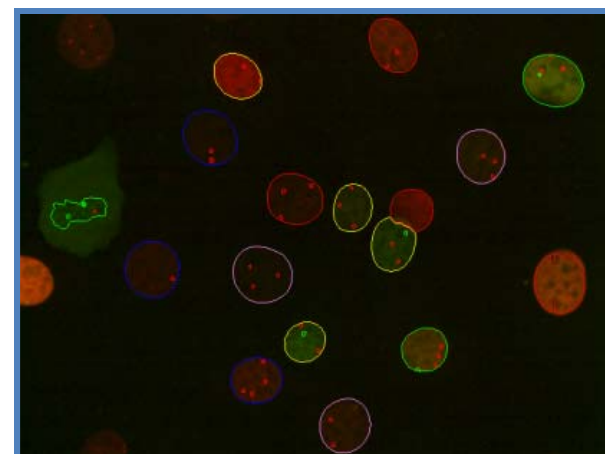
Capturing rare events: High-Throughput Imaging



NIH3T3 cells

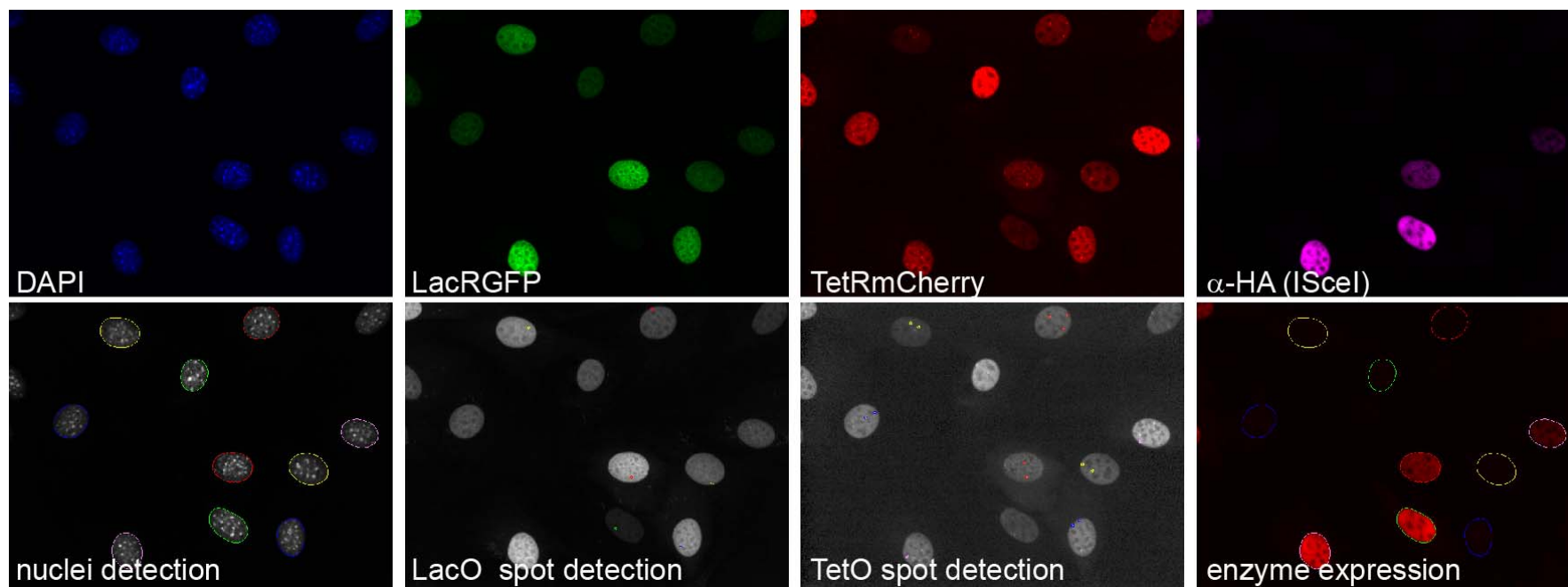


High-Throughput Microscopy

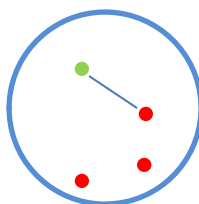


Automated Image Analysis

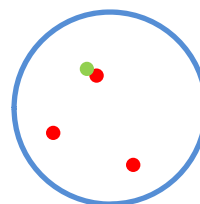
Image analysis



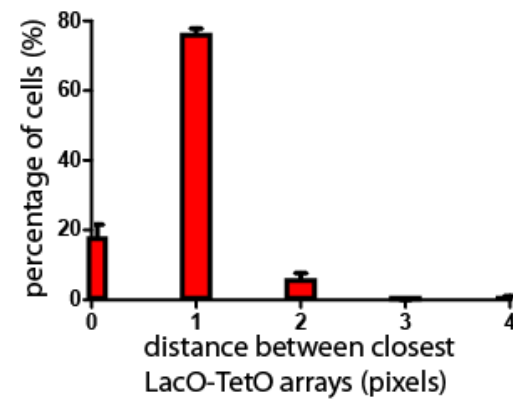
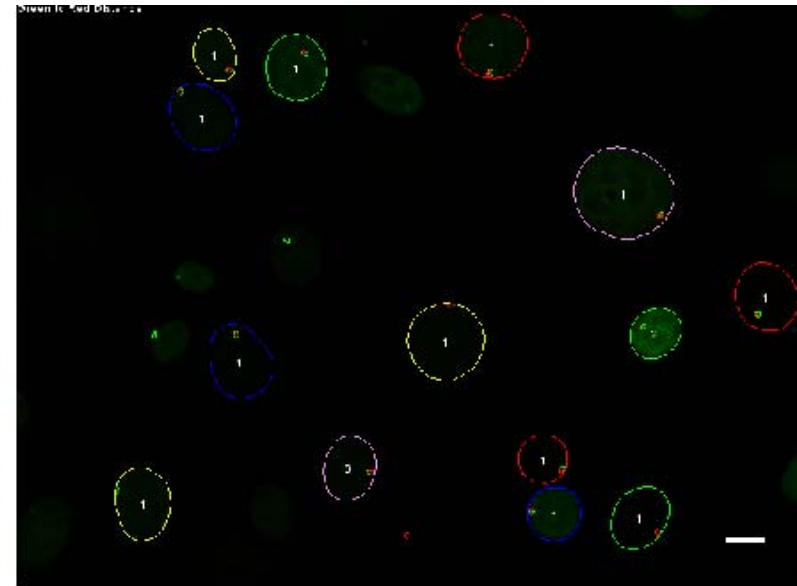
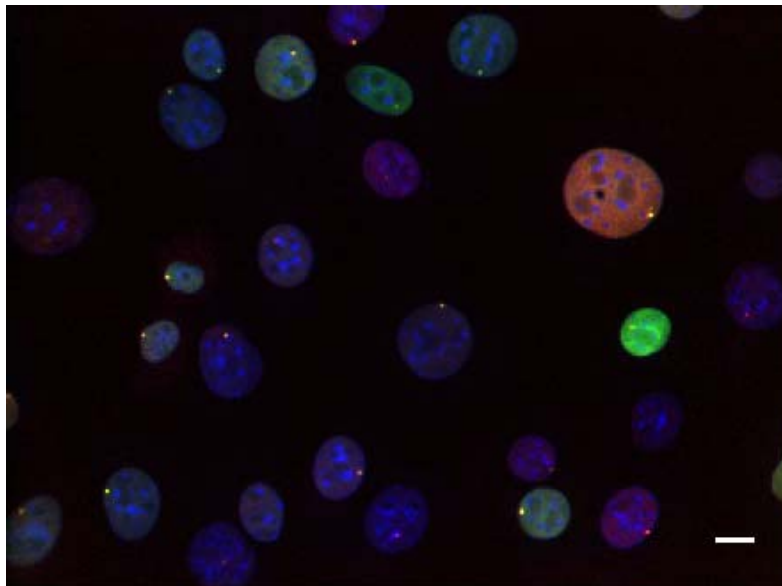
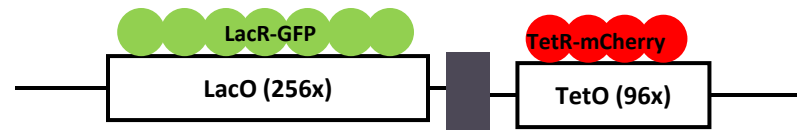
20 pixels



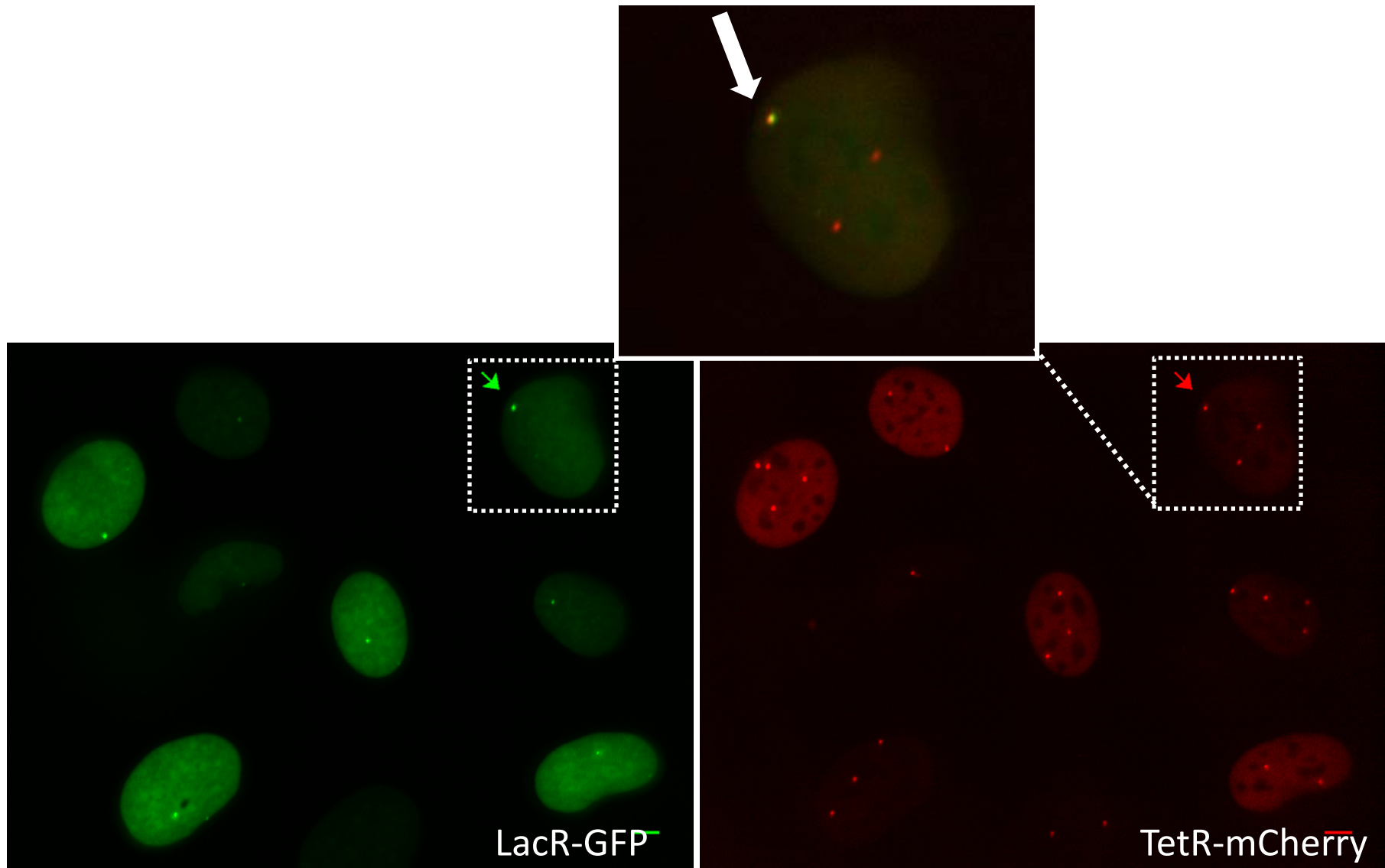
2 pixels



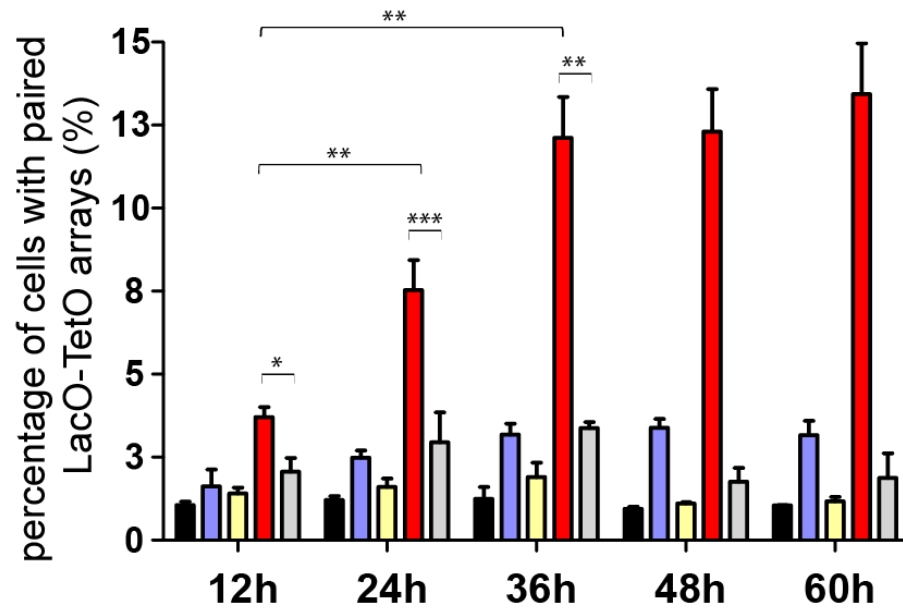
How does a translocation look like?



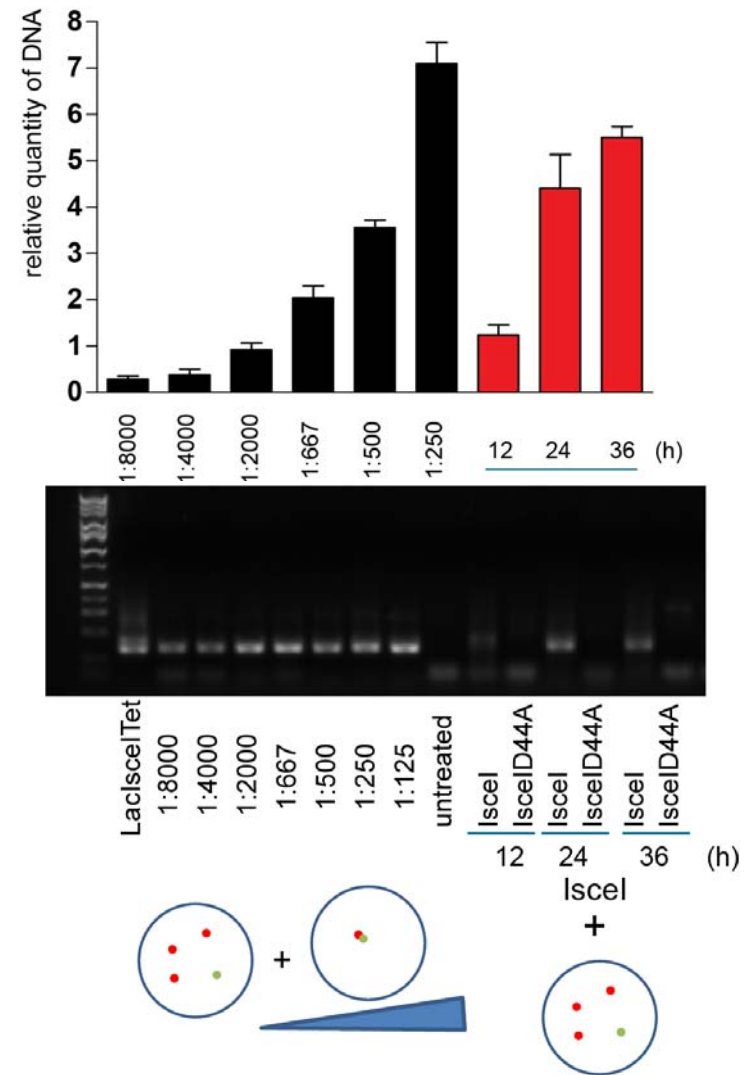
How many cells with translocations?



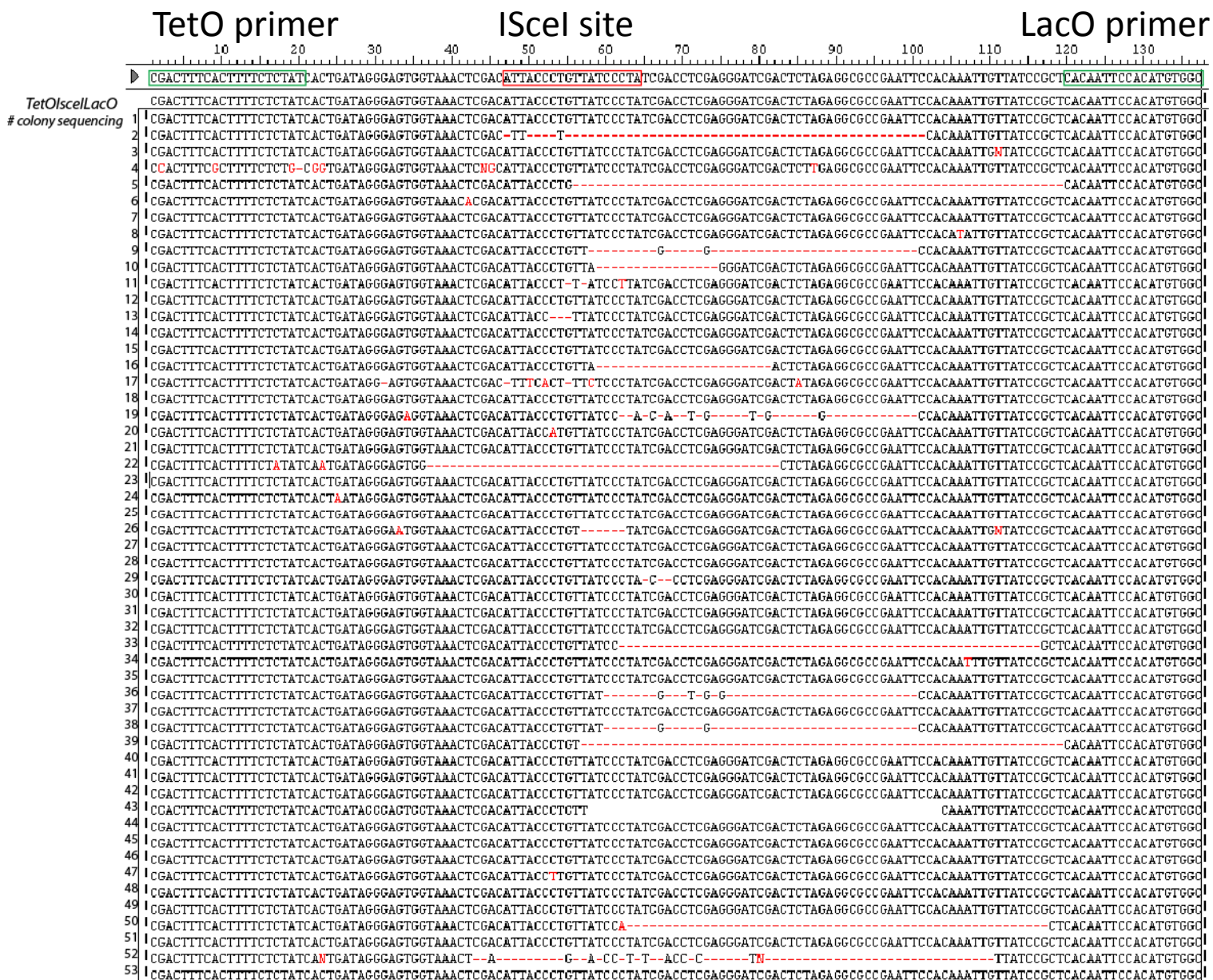
Colocalization of arrays and translocation formation upon DSB-induction



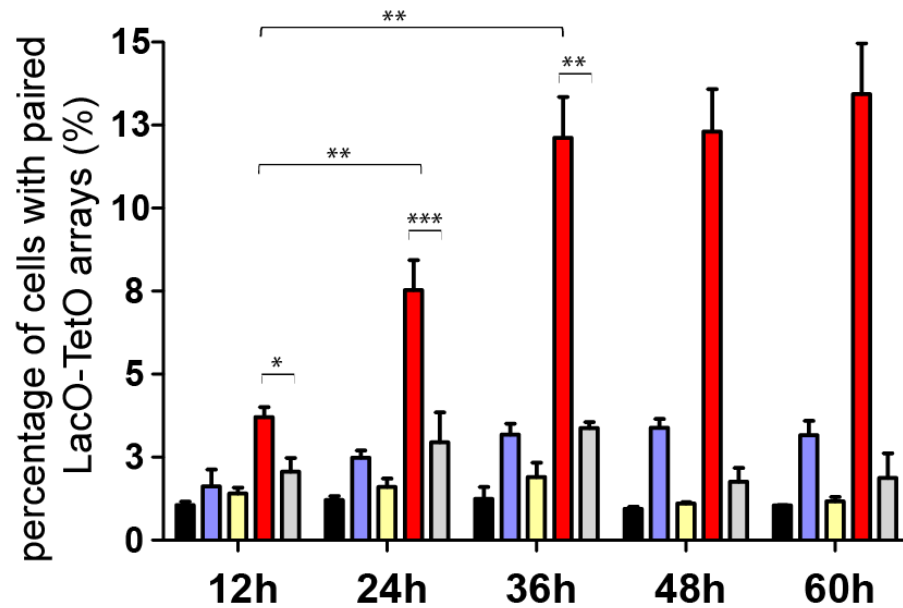
- untreated
- Iscl-no expression
- IsclD44A-no expression
- Iscl-expression
- IsclD44A-expression



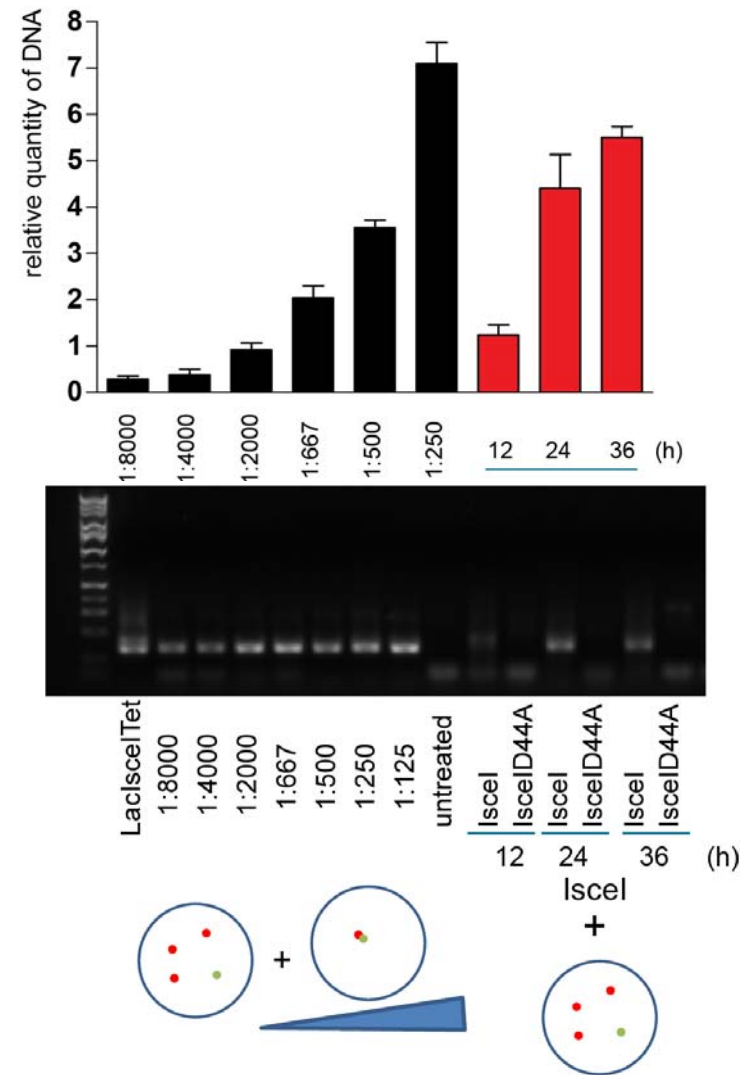
Sequencing the translocated junctions



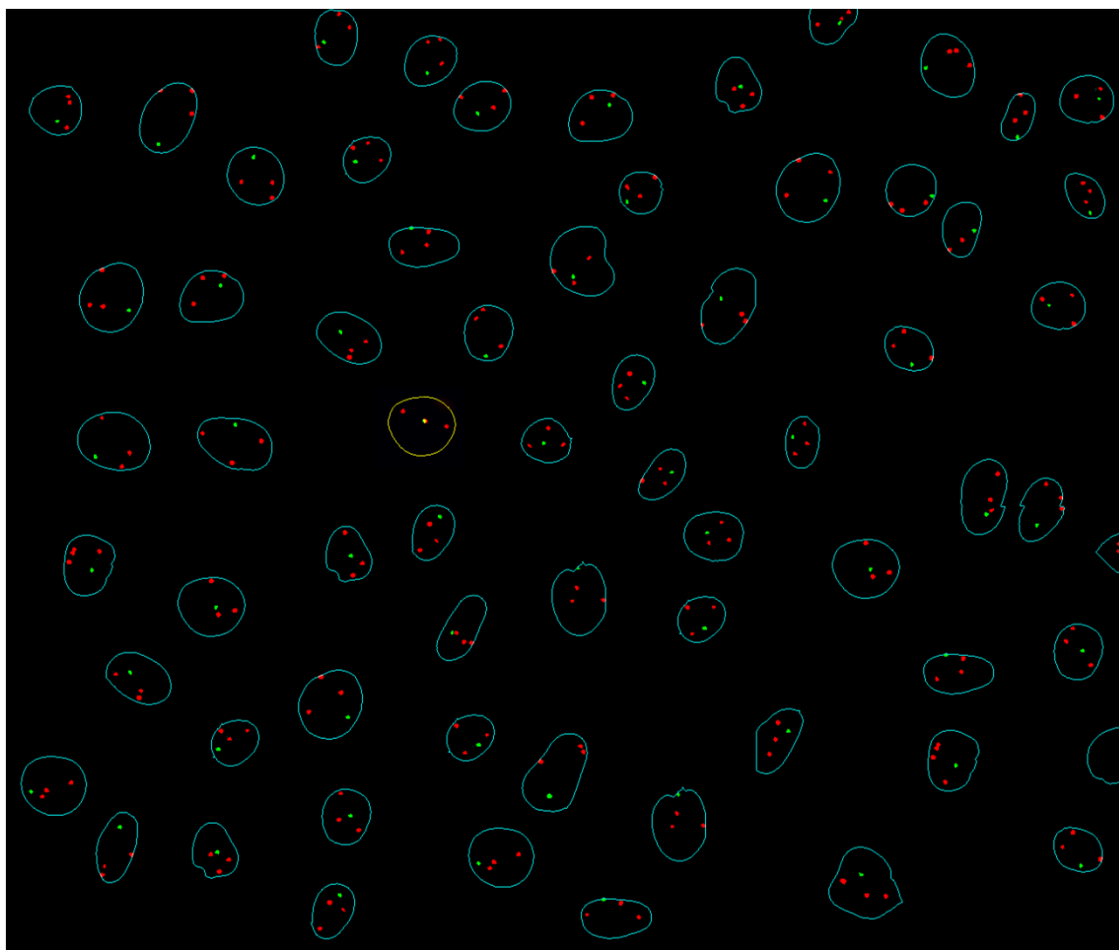
Colocalization of arrays and translocation formation upon DSB-induction



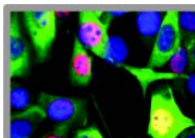
- untreated
- Iscel-no expression
- IscelD44A-no expression
- Iscel-expression
- IscelD44A-expression



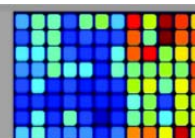
High-throughput timelapse microscopy to capture translocations



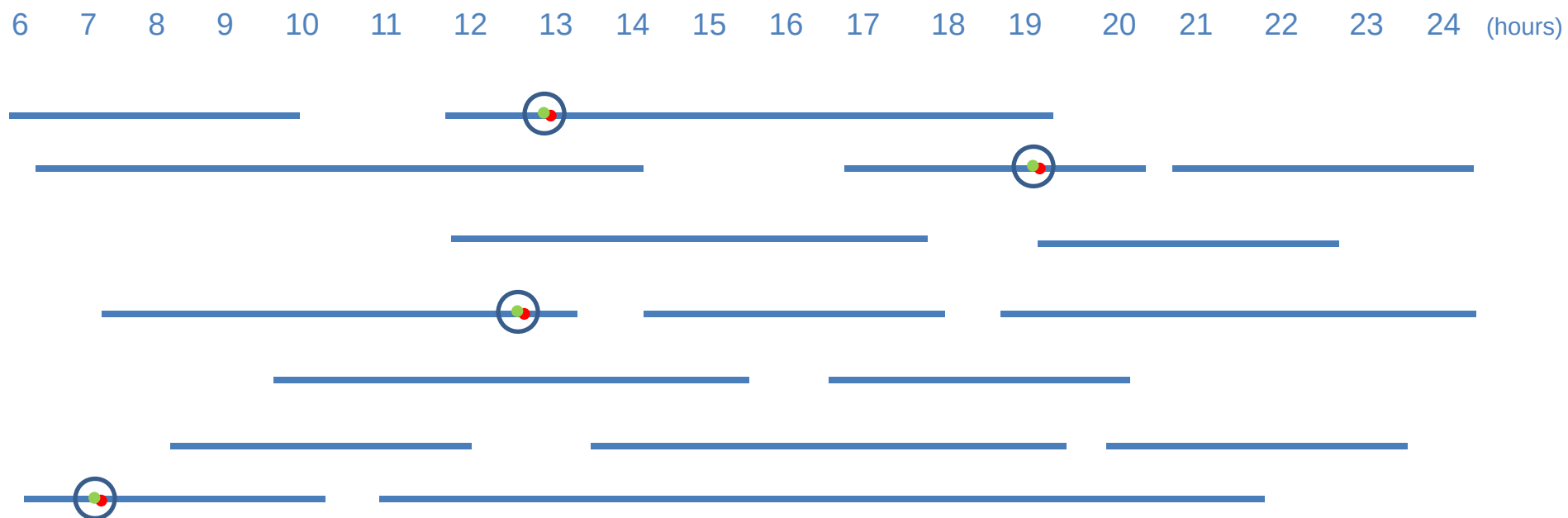
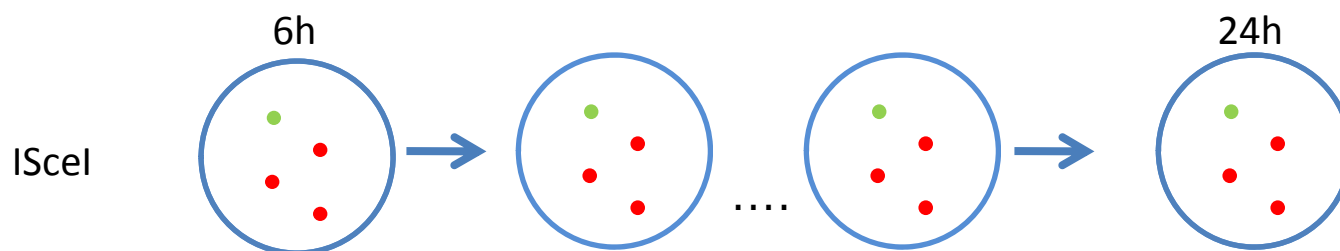
3 z stacks/field, 14 fields/well, 60 wells/experiment, imaging for 18 hours, 1h interval
840 movies for 18h = 90720 images ~50GB of data



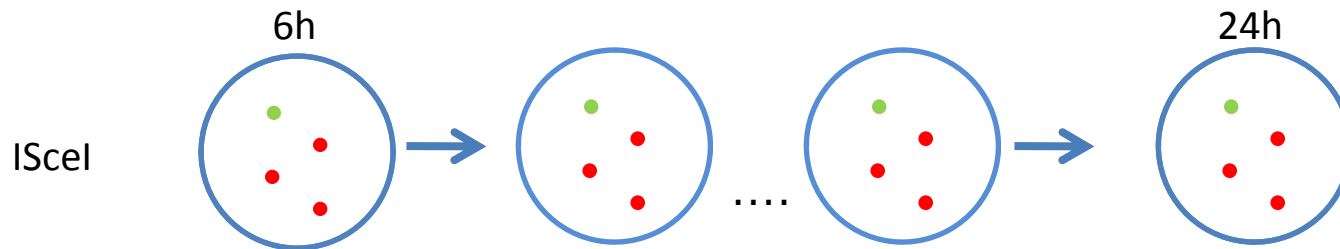
HiTIF
High-Throughput
Imaging Facility



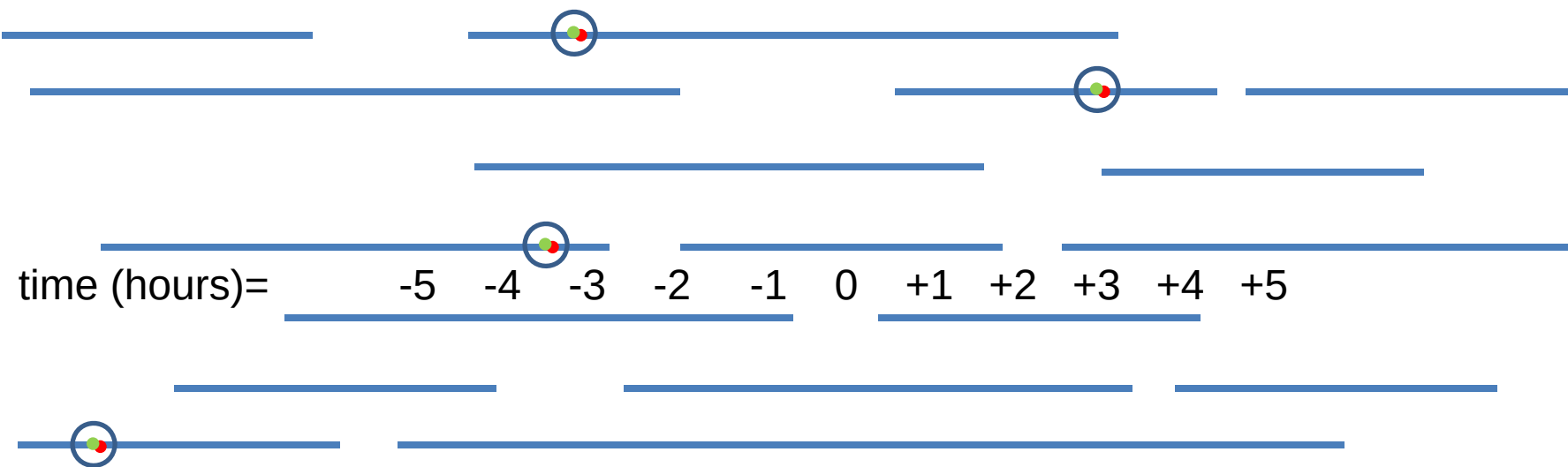
Unbiased, automated image analysis



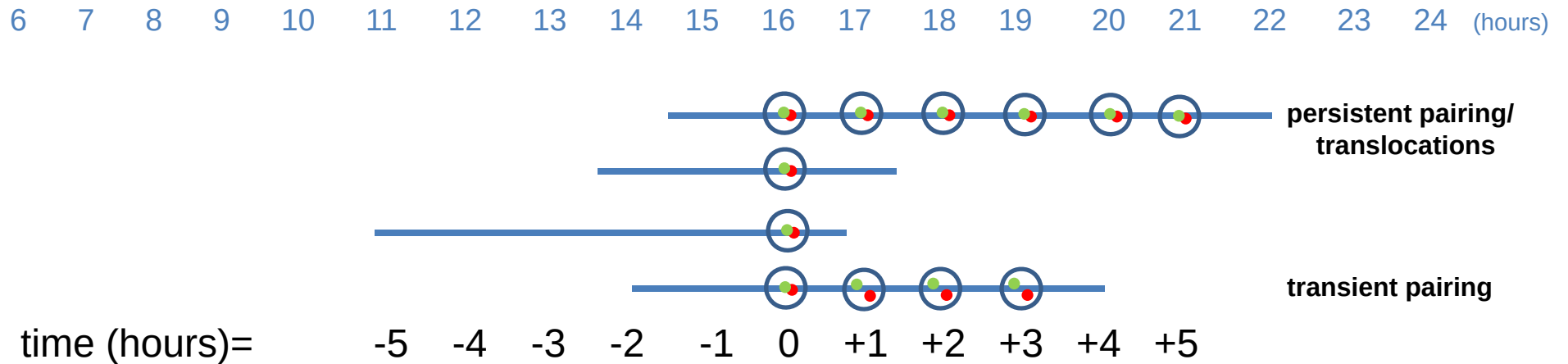
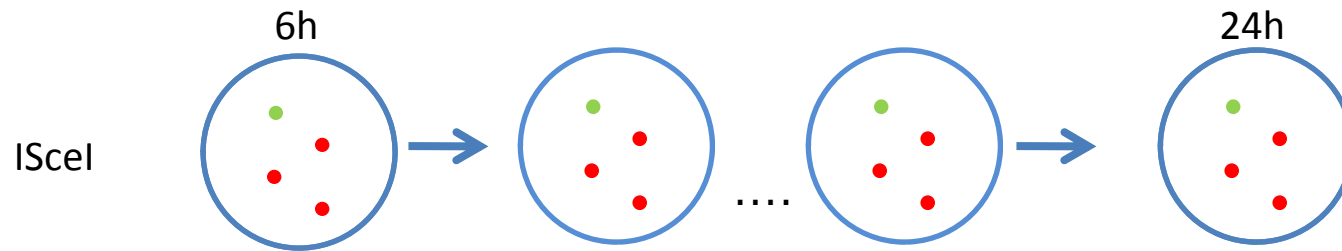
Align tracks with paired DSBs



6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 (hours)

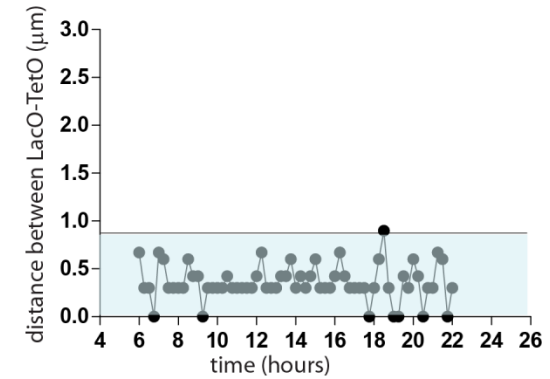
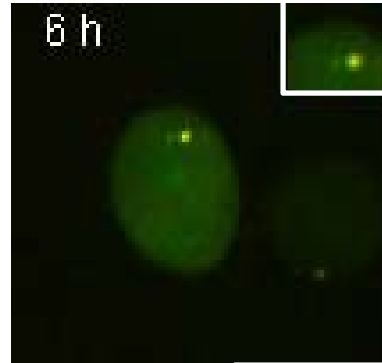
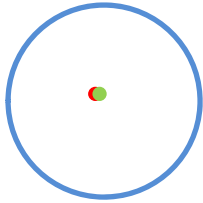


Persistent vs transient pairing

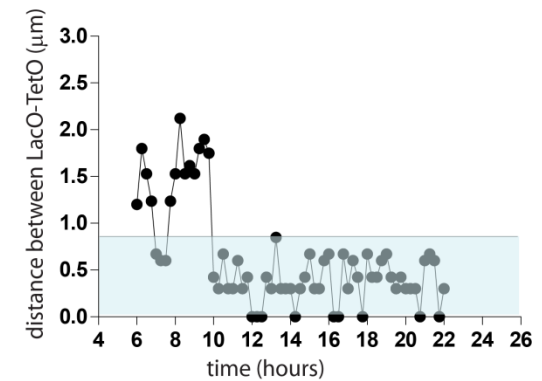
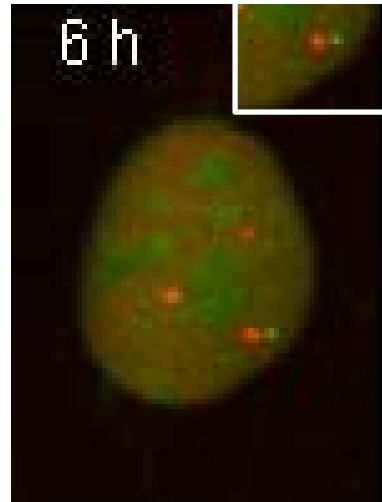
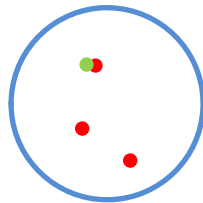


Synchronized motion of translocated DSBs

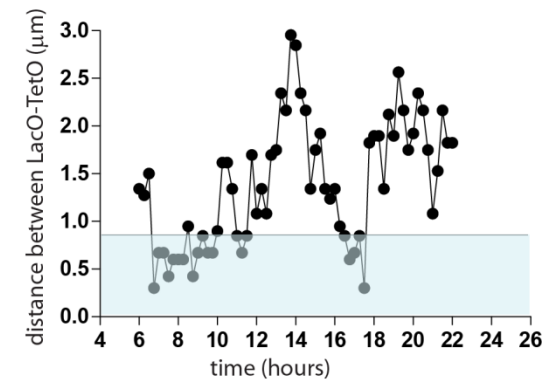
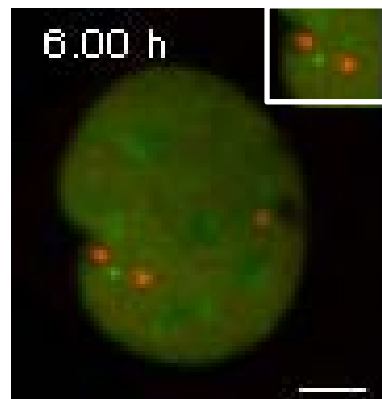
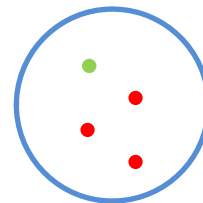
Calibration cells



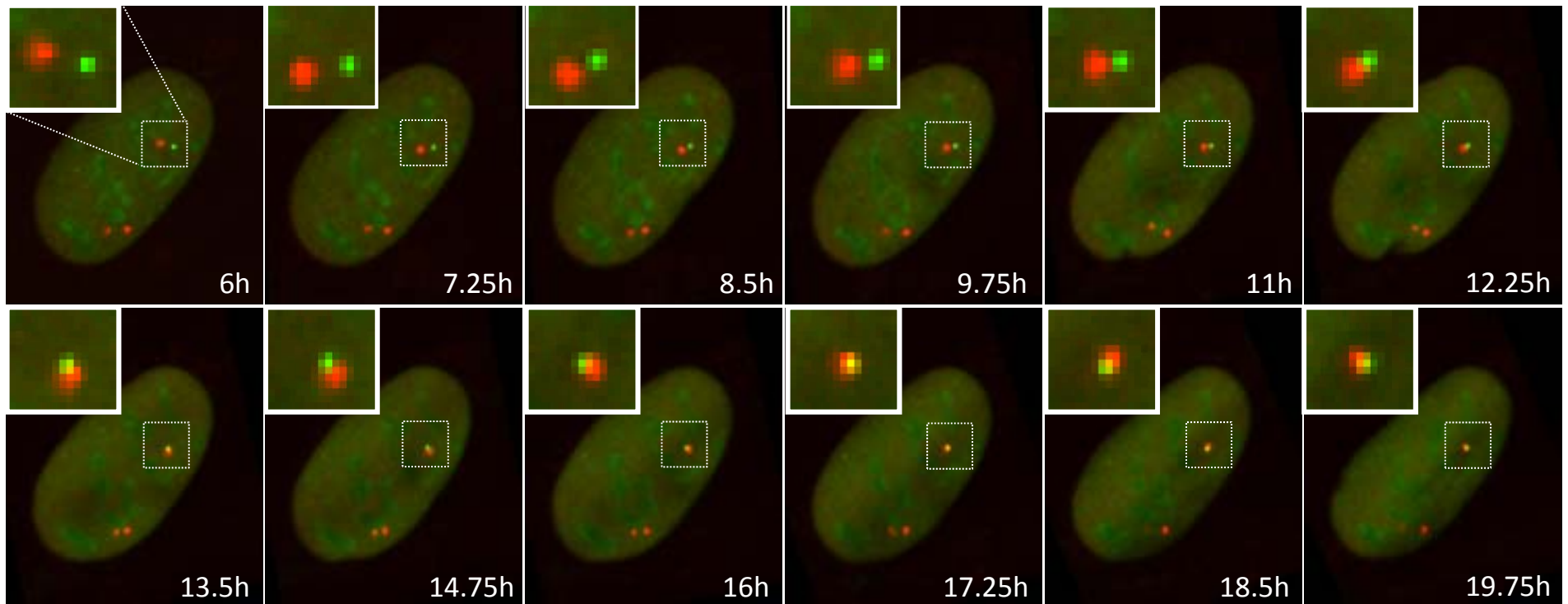
Persistent pairing, translocations



Transient pairing

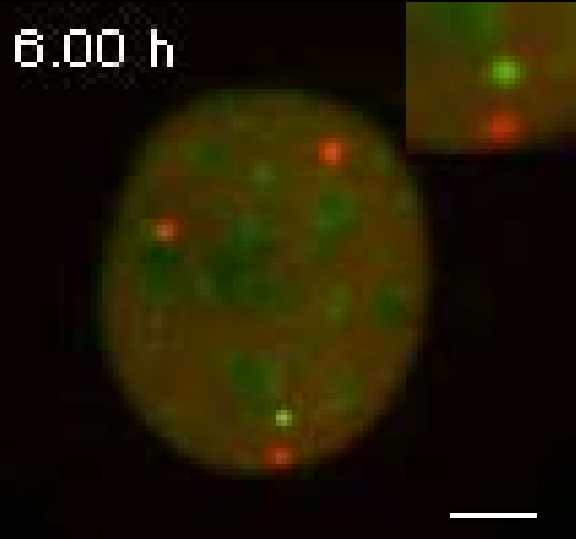


Translocations in real life

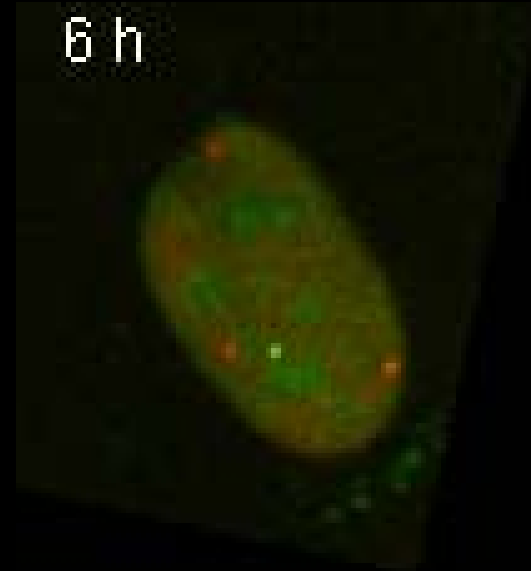


Translocations in real life

6.00 h

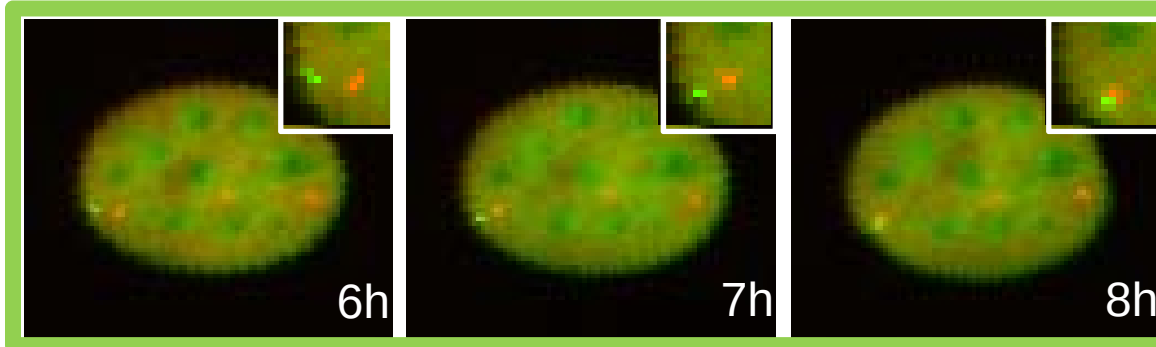


6 h

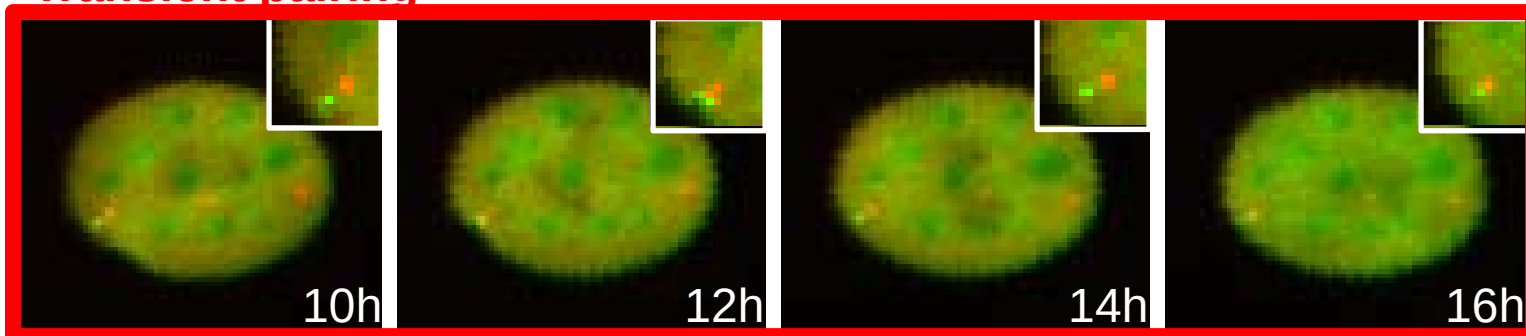


Distinct phases of translocation formation

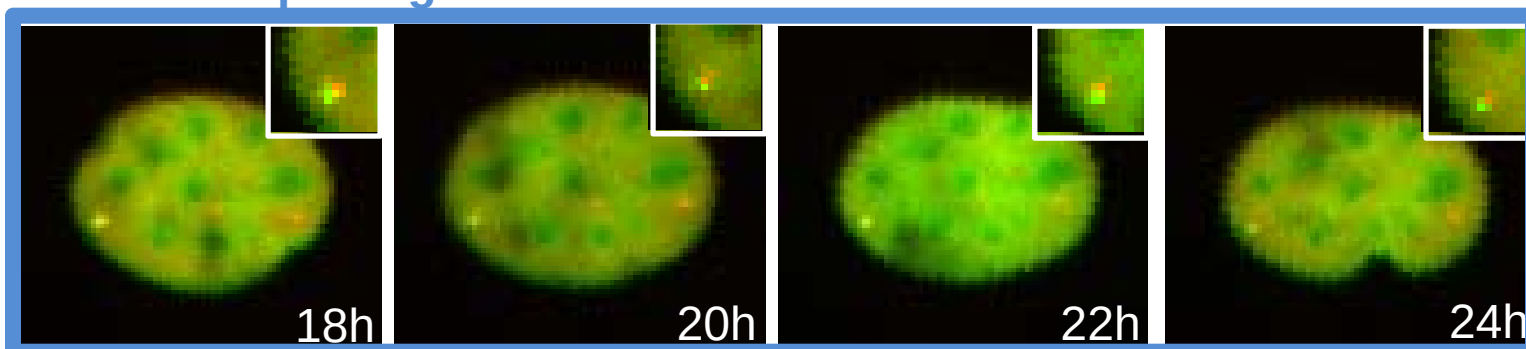
Partner search



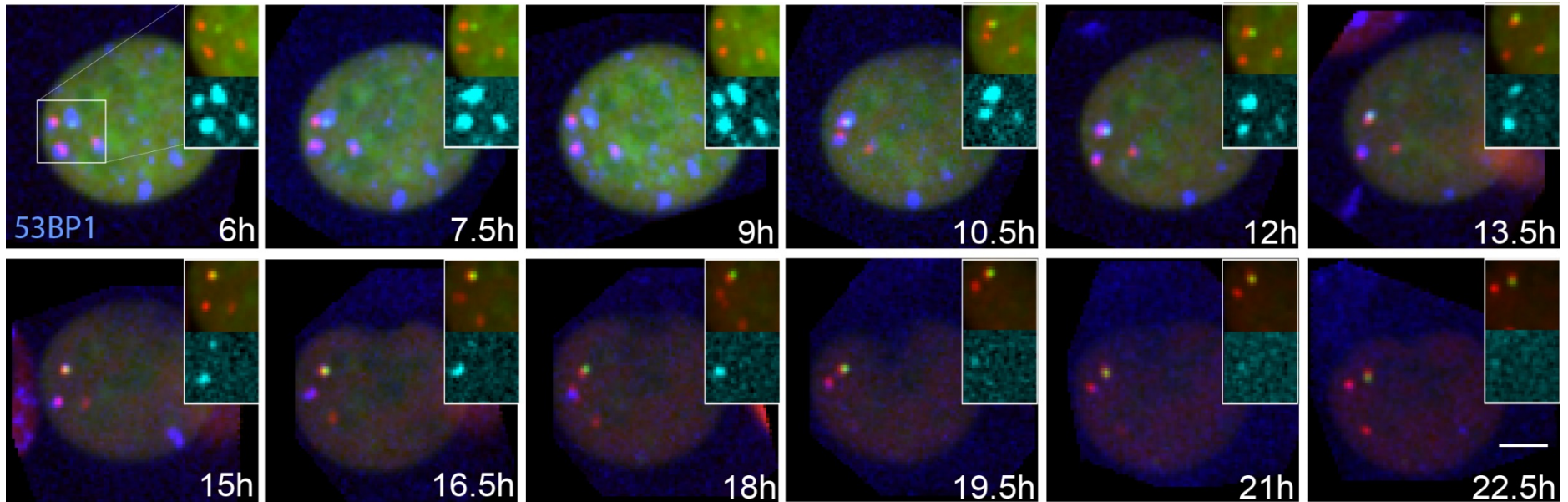
Transient pairing



Persistent pairing

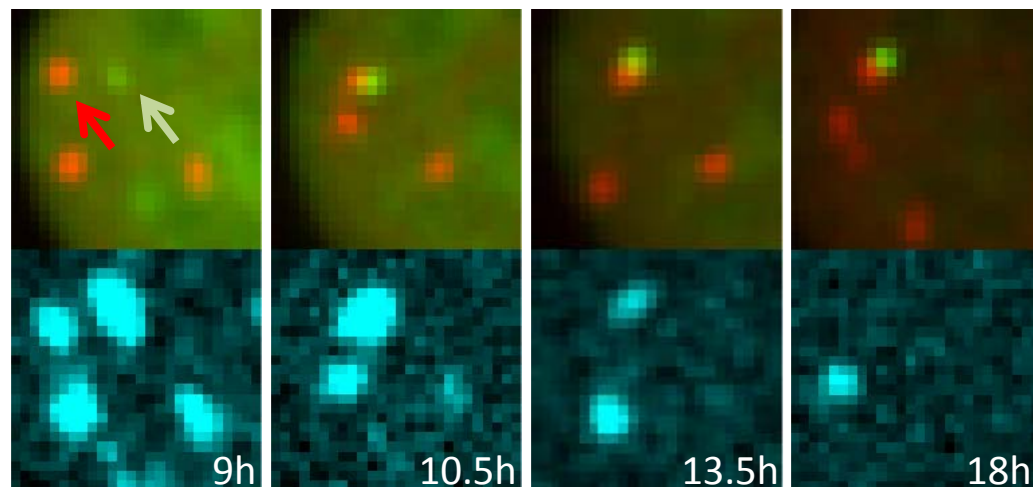


Translocations in real life - monitoring DNA repair



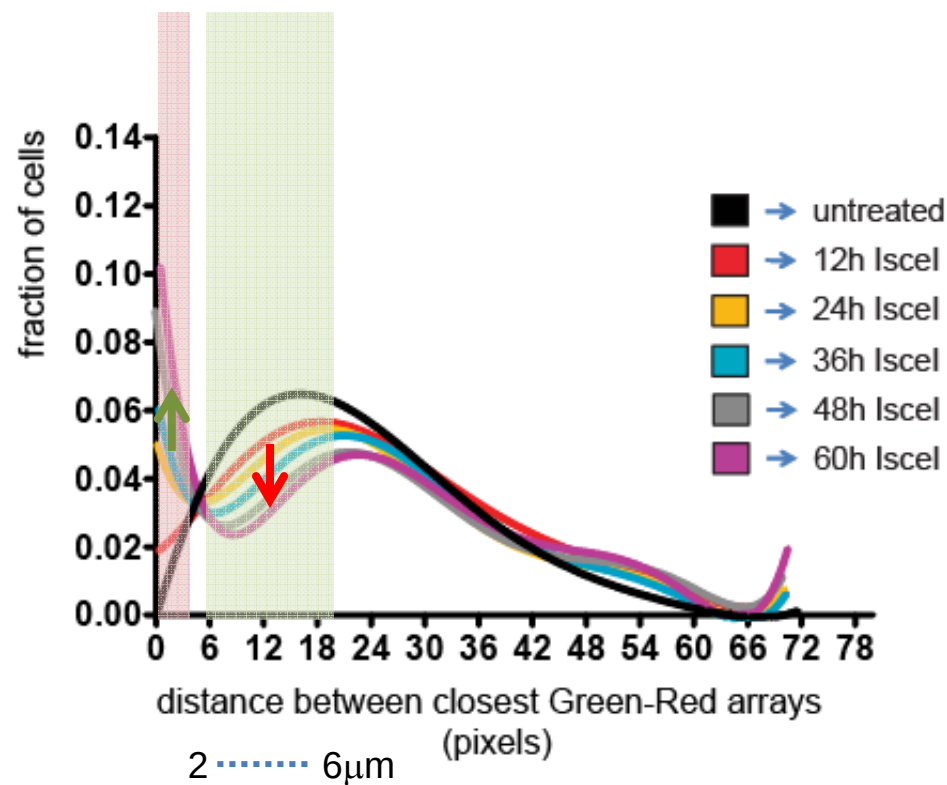
LacO-TetO

53BP1

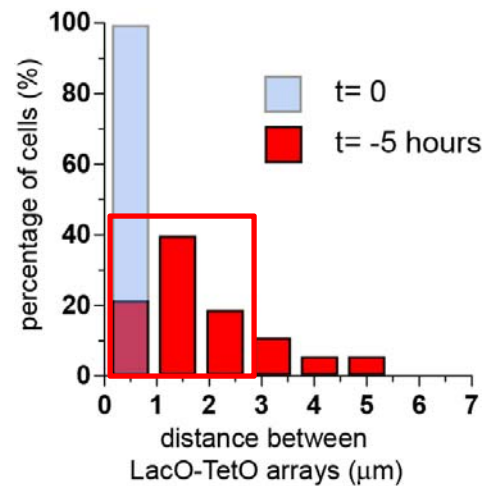


Where do translocation partners come from?

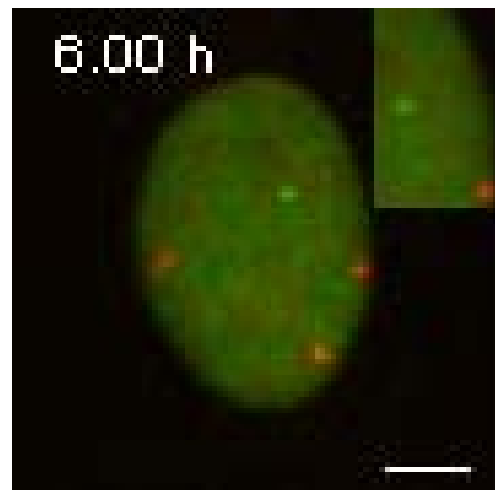
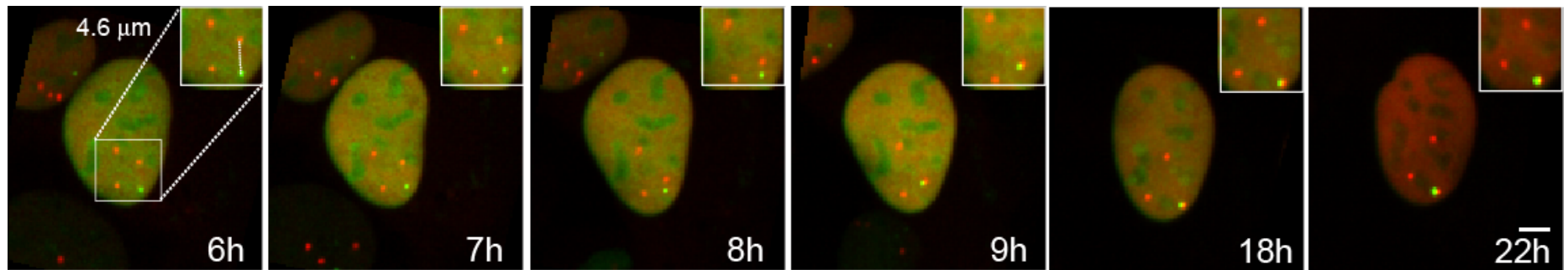
DSB-pairing occurs from proximal breaks



Translocations occur predominantly from proximal DSBs



Translocations between distal breaks

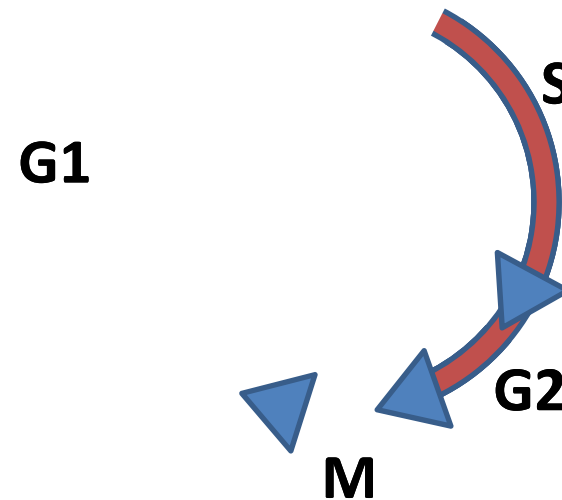


When in the cell cycle do translocations form?

When in the cell cycle do translocations form?

Non homologous end-joining (NHEJ)

Homologous recombination (HR)



- Chromosomal loci/breaks show different mobilities according to cell cycle stage
- Distinct pathways repair DSBs in a cell cycle-dependent manner

Heun et al., *Science*, 2001

Gerlich et al., *Cell*, 2003

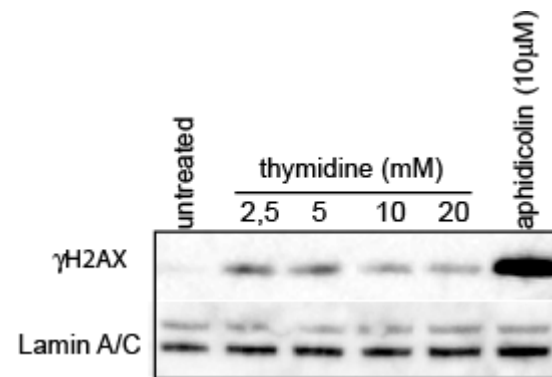
Walter et al., *J Cell Biol*, 2003

Thomson et al., *Cur Biol*, 2004

Krawczyk et al., *J Cell Sci*, 2012

Symington and Gautier, *Annu Rev Genet*, 2011

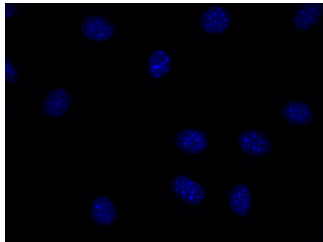
An imaging tool to determine cell cycle phase using DAPI staining



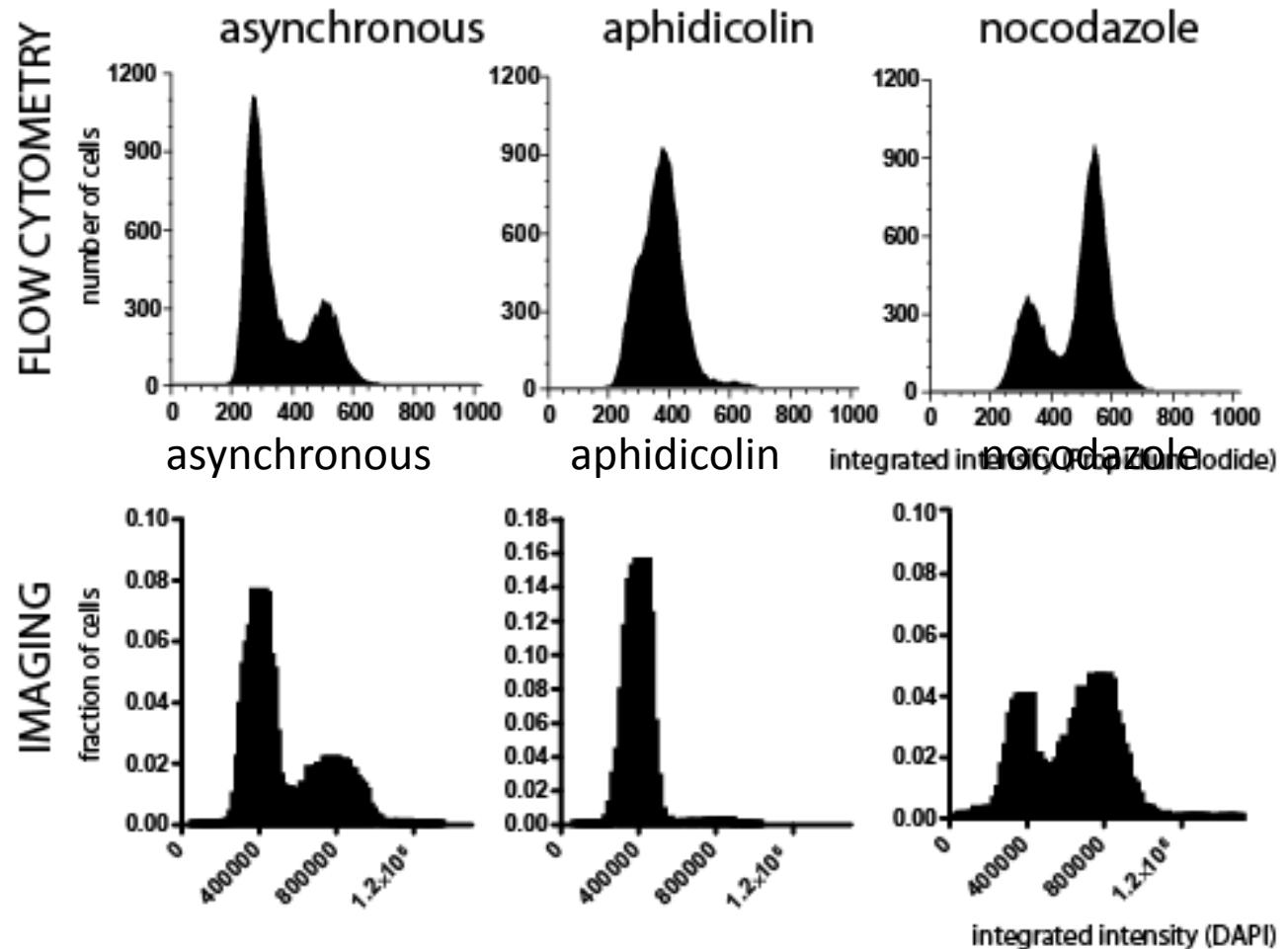
An imaging tool to determine cell cycle phase using DAPI staining



PI staining

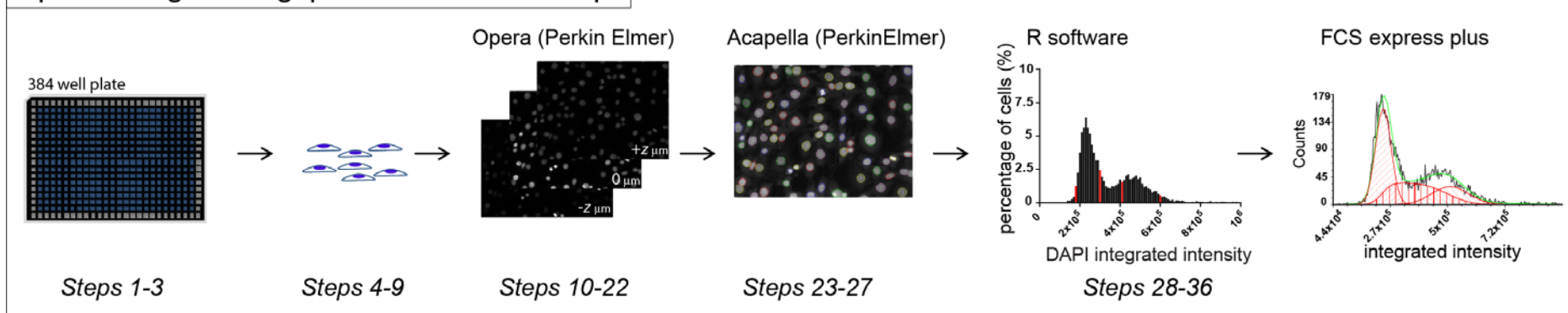


DAPI staining

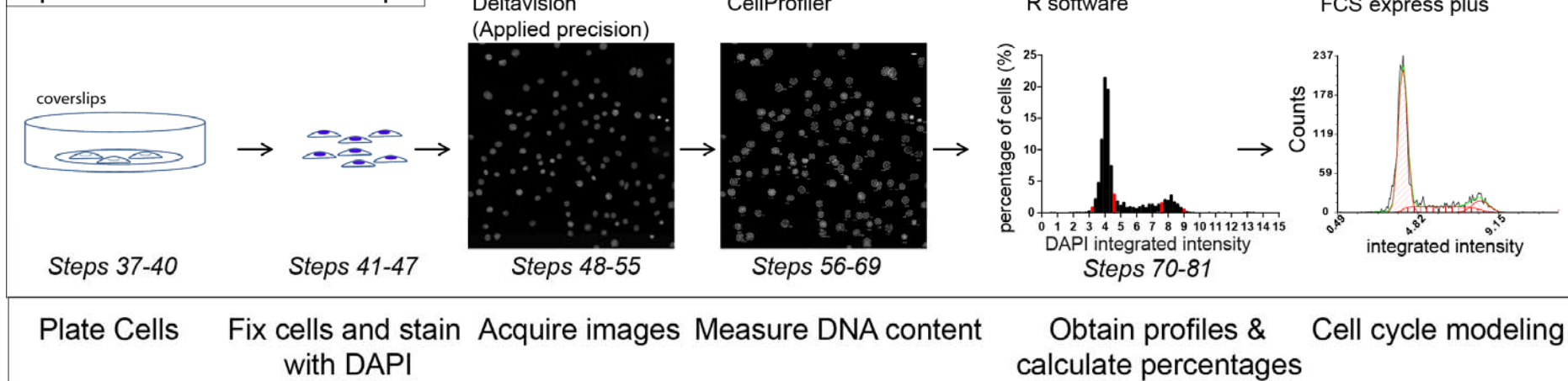


Cell cycle staging of individual cells by fluorescence microscopy

Option A: high-throughput confocal microscope

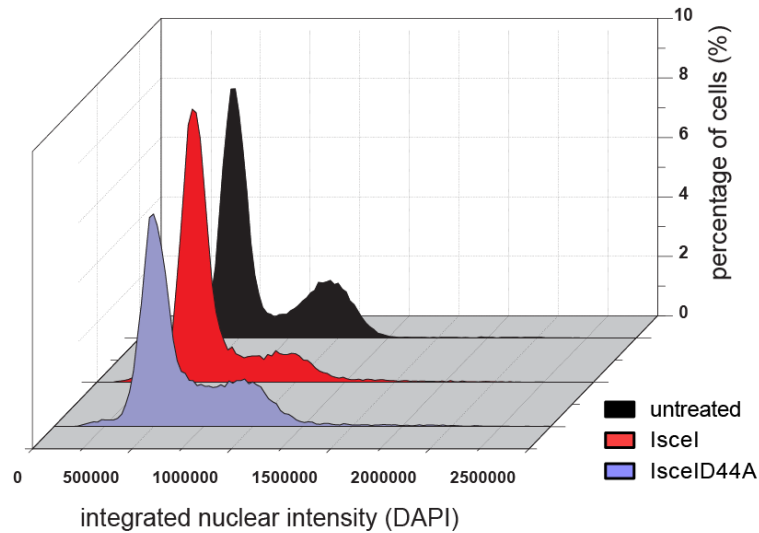


Option B: wide-field microscope

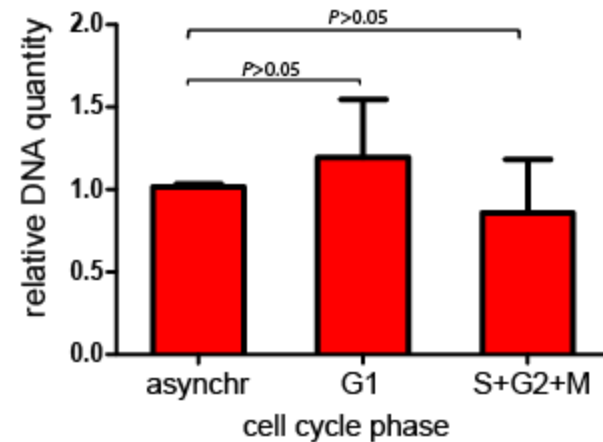
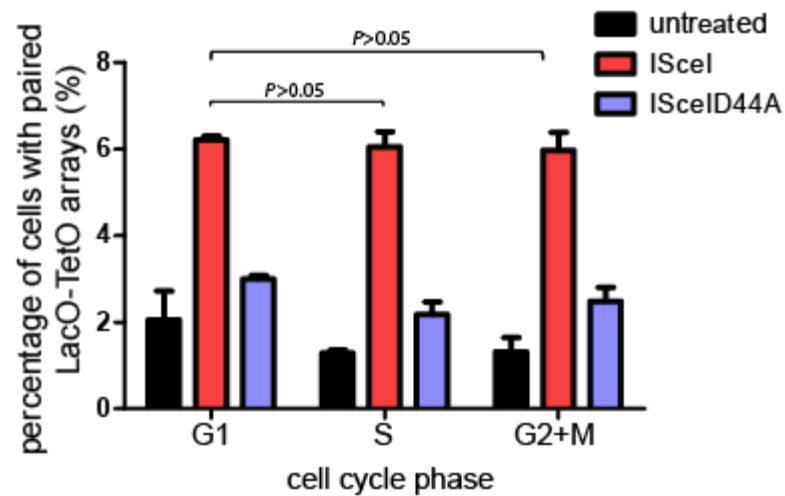
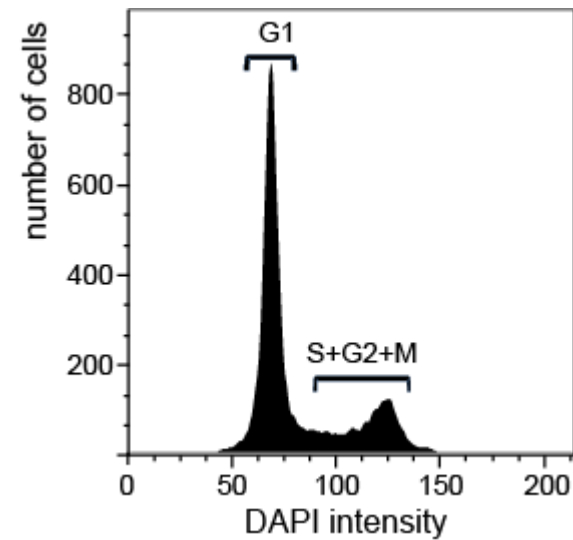


Translocations occur in all stages of the cell cycle

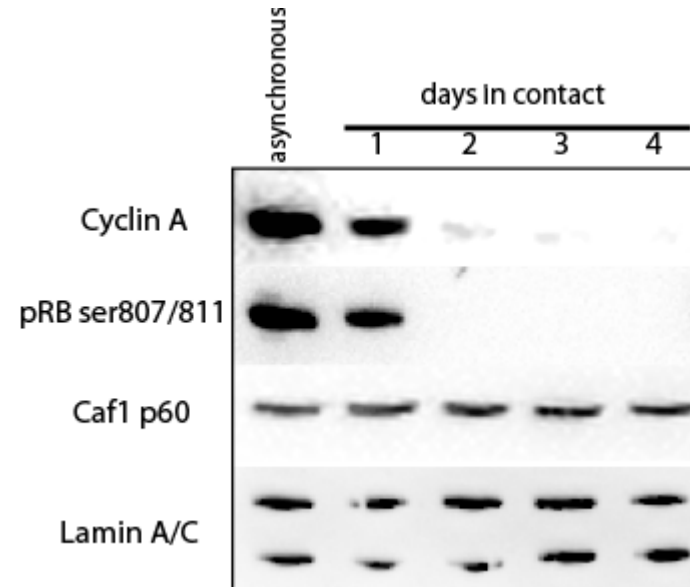
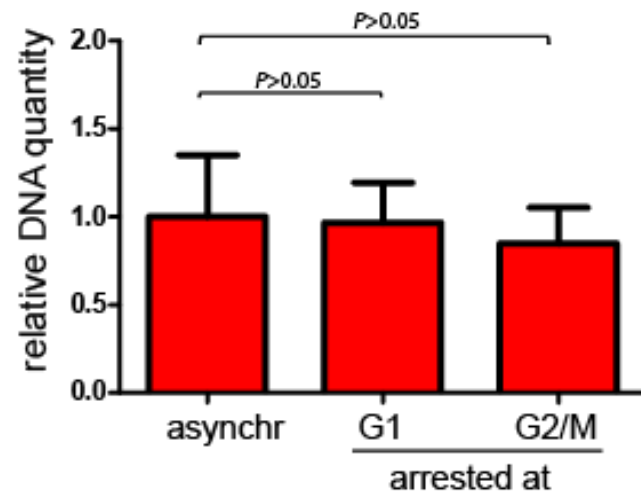
Pairing



Translocations



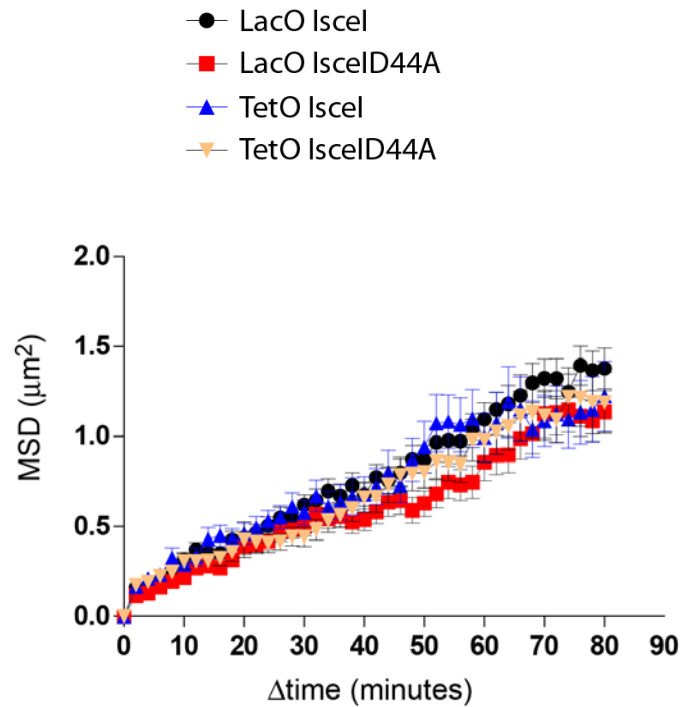
Translocations occur in all stages of the cell cycle



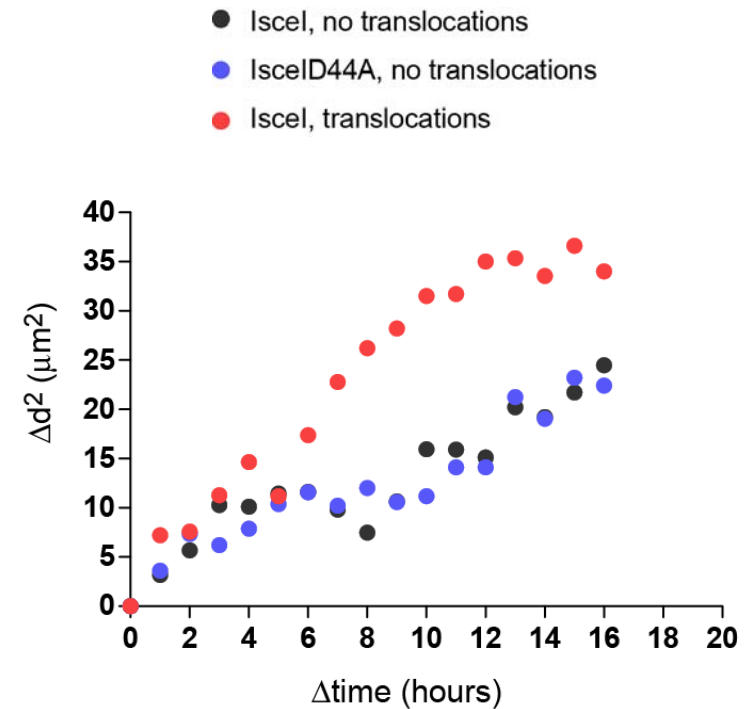
Is there anything special about translocating DSBs?

Increased mobility of translocating DSBs

All DSBs



Translocating vs. non-translocating



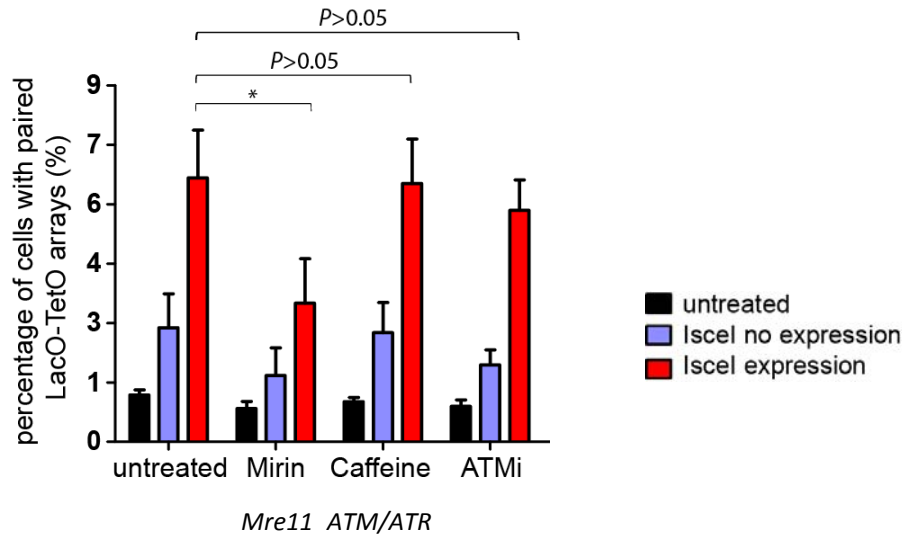
M. J. Kruhlak et al., *J. Cell Biol.*, 2006

B. Jakob et al., *PNAS*, 2009

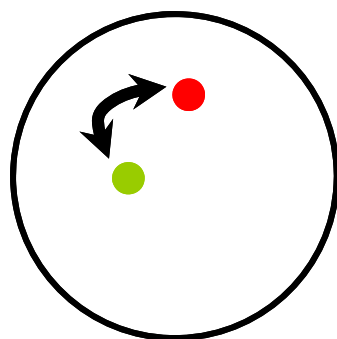
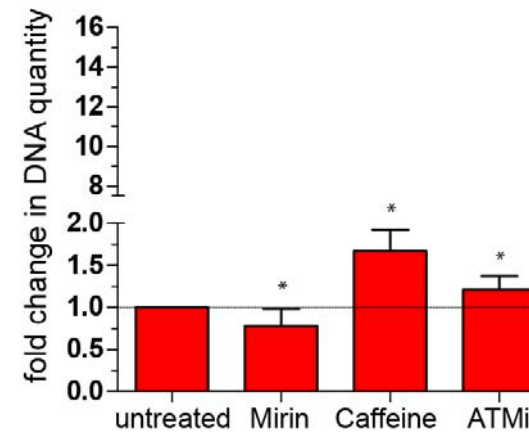
How do DNA repair processes affect translocation formation?

Inhibition of Mre11 reduces pairing and translocations

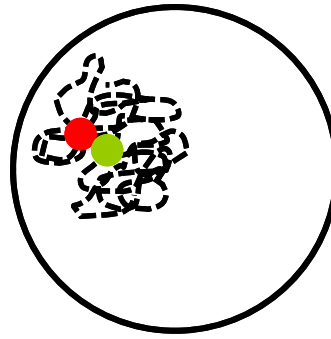
Pairing



Translocations

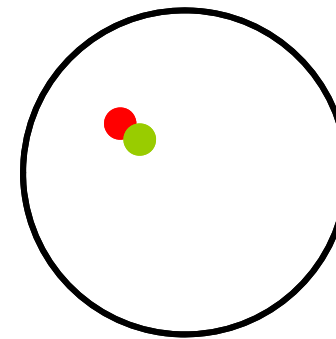


Partner search



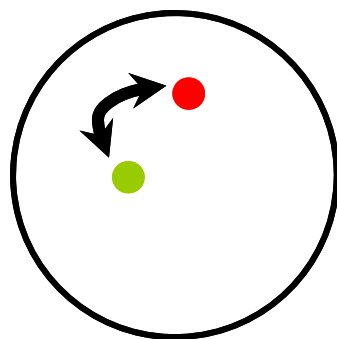
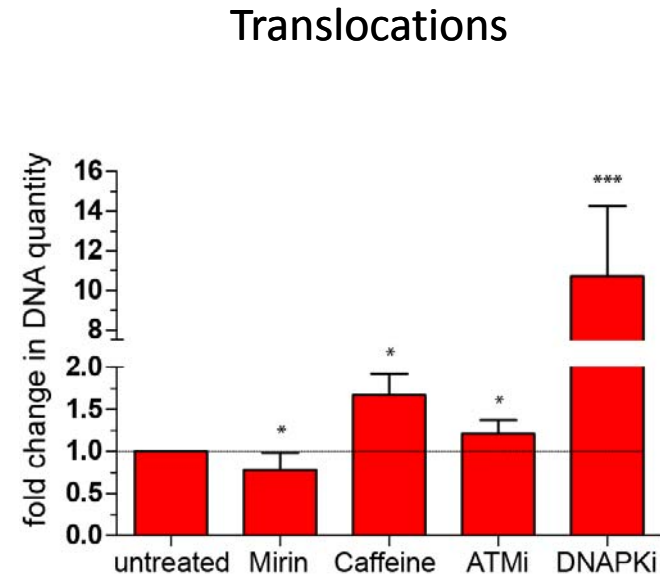
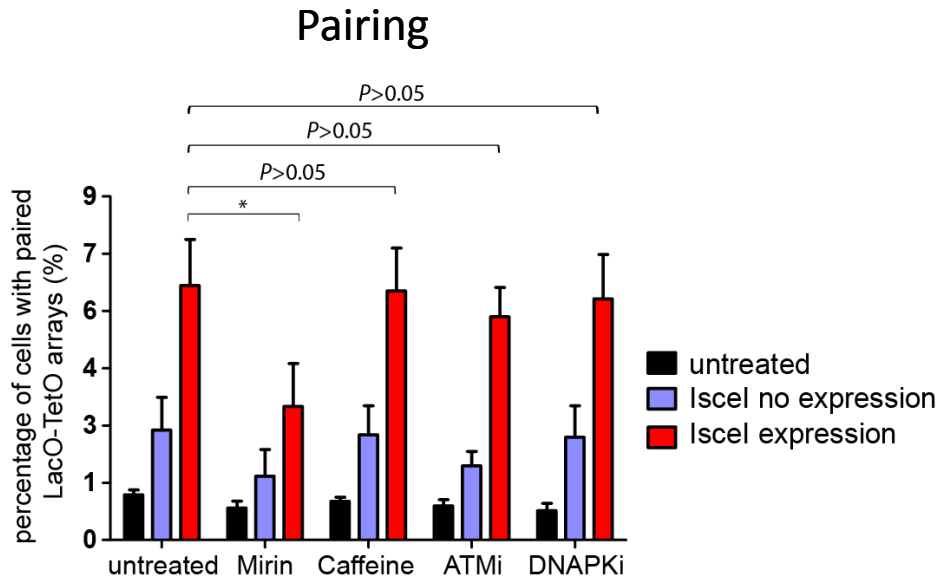
Transient pairing

Mre11

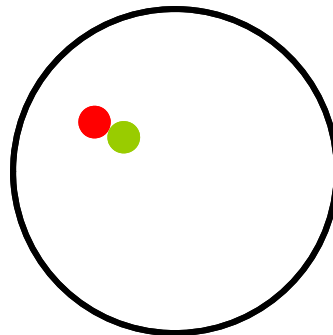


Joining
Translocation

Inhibition of DNAPKcs does not affect pairing but promotes translocations

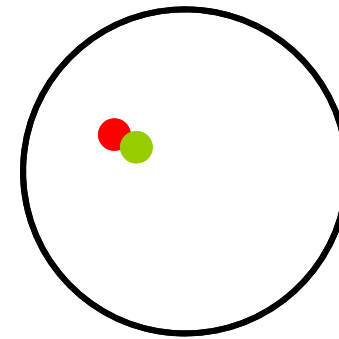


Partner search



Transient pairing

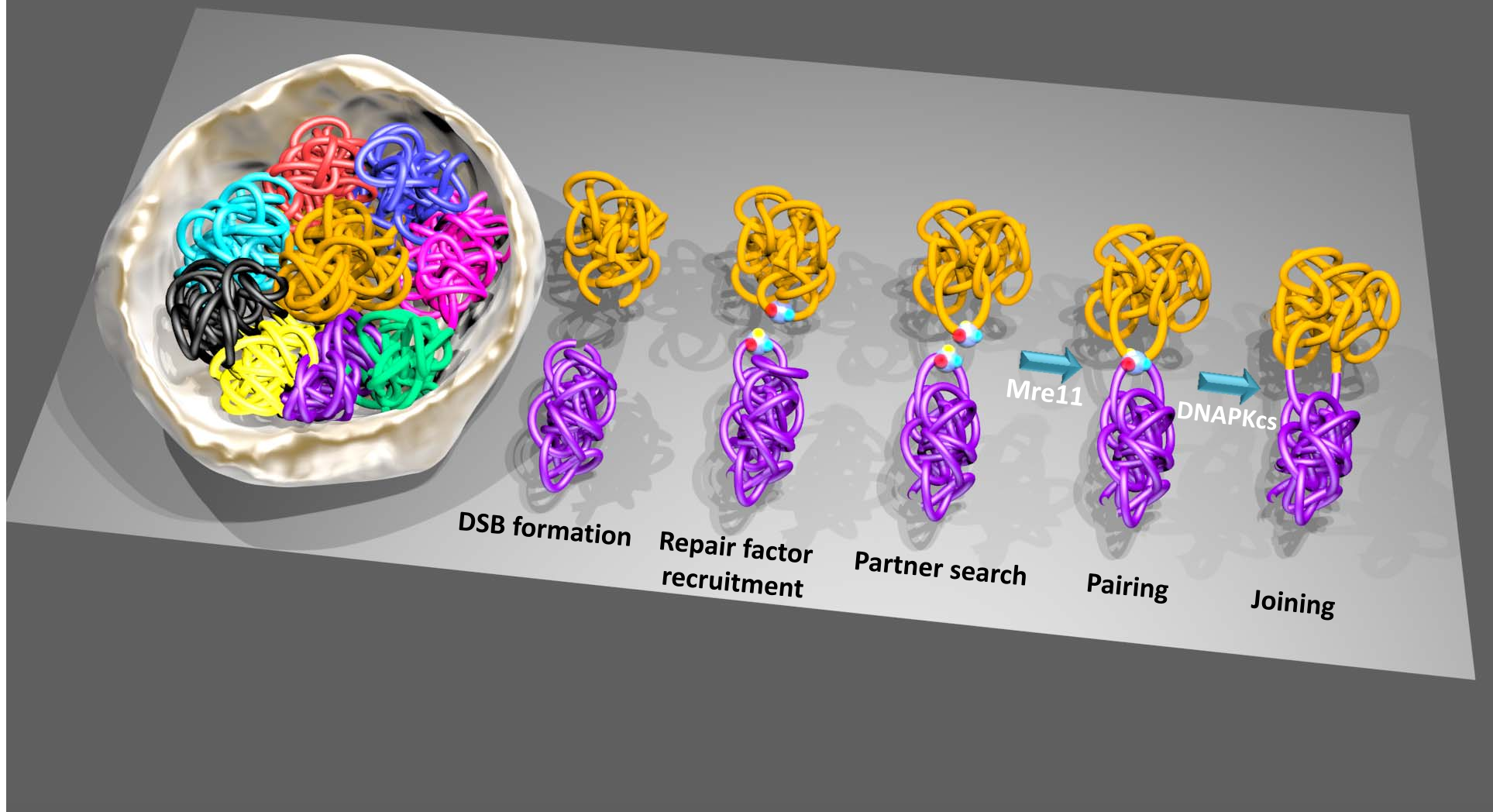
Mre11



Joining/ Translocation

DNAPKcs

A spatiotemporal framework for the formation of translocations



Roukos V. et al., *Science*, 2013
Roukos V. & Misteli T. *Nature Cell Biology*, 2014

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