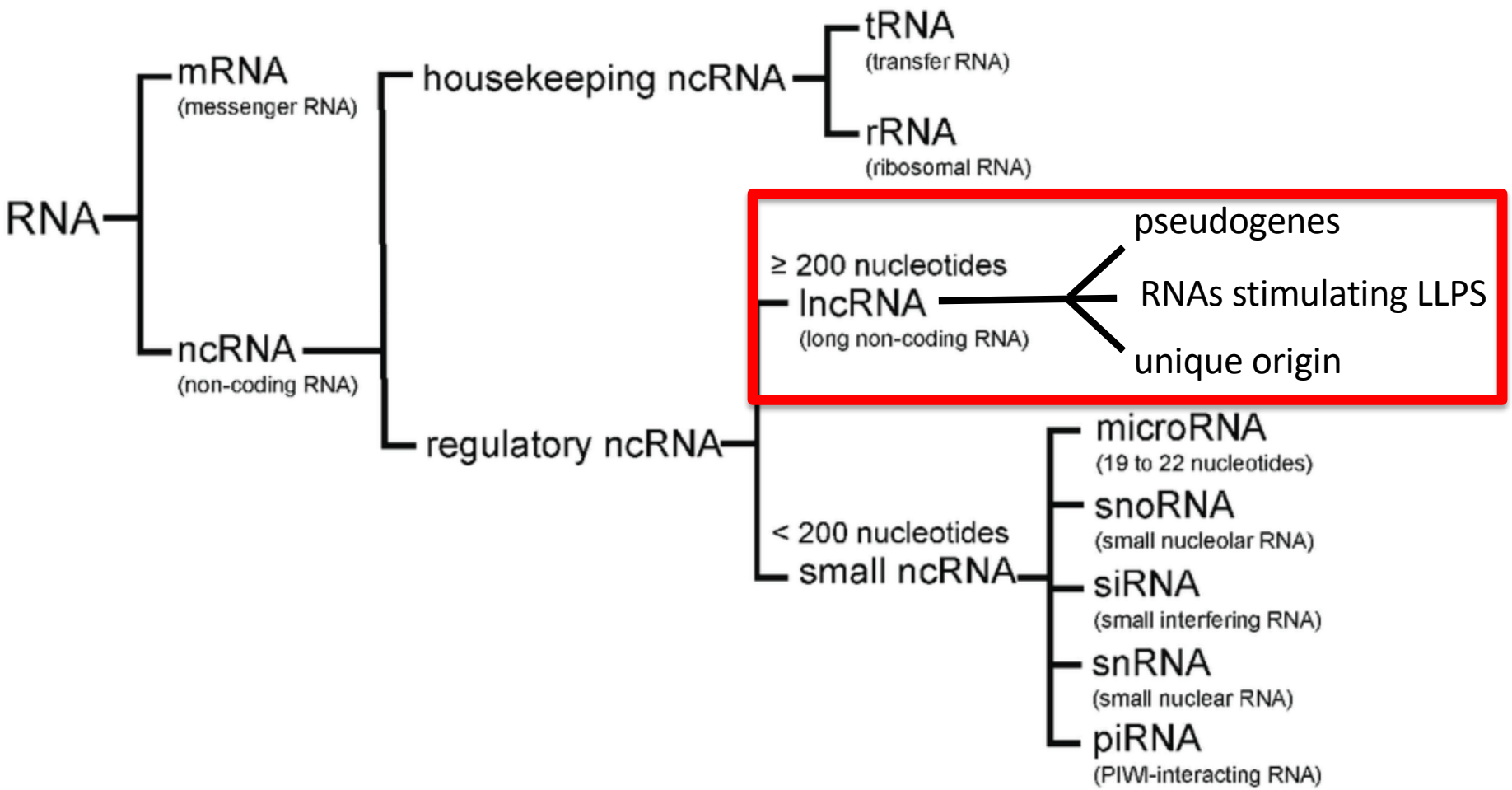
DDRNAs

DNA DAMAGE RESPONSE RNAs

RNA Seq identified new type of RNA elements – long non-coding RNAs



Most frequent, easy to annotate ncRNAs
(defined processing and classic gene regulation

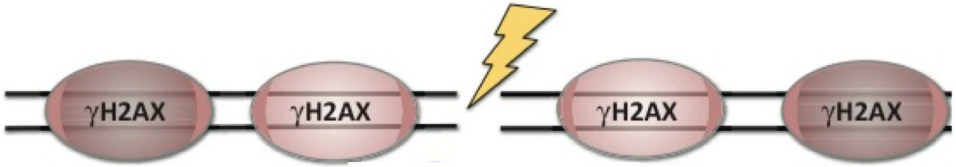
LETTER

doi:10.1038/nature11179

Site-specific DICER and DROSHA RNA products control the DNA-damage response

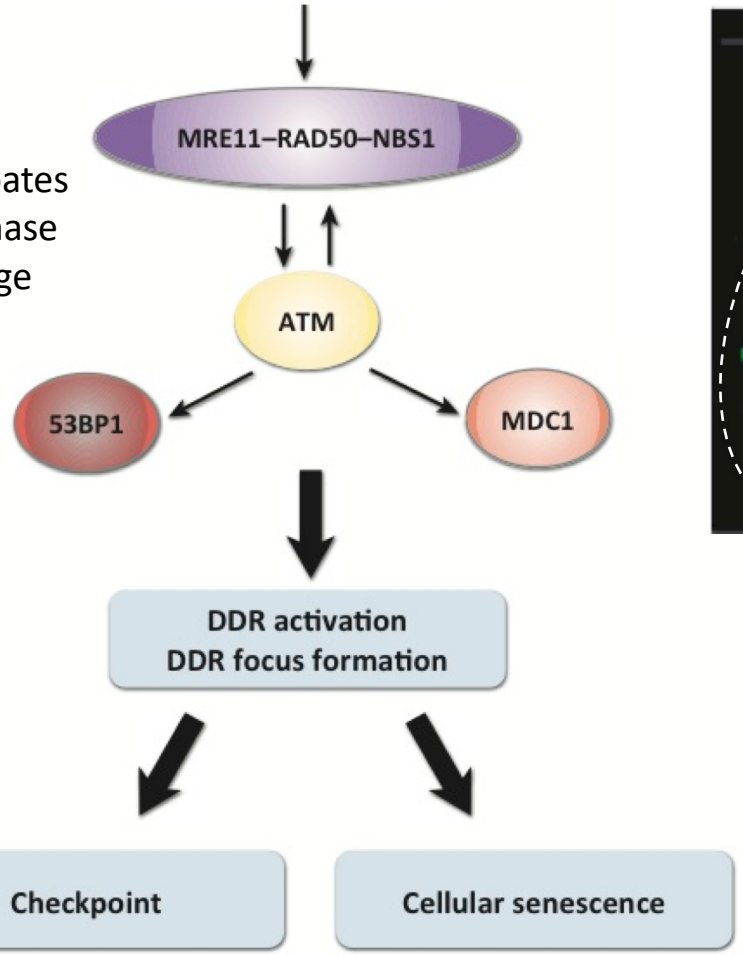
Sofia Francia[1,2], Flavia Michelini[1], Alka Saxena[3], Dave Tang[3], Michiel de Hoon[3], Viviana Anelli[1]†, Marina Mione[1]†, Piero Carninci[3] & Fabrizio d'Adda di Fagagna[1,4]

The DNA damage response revisited

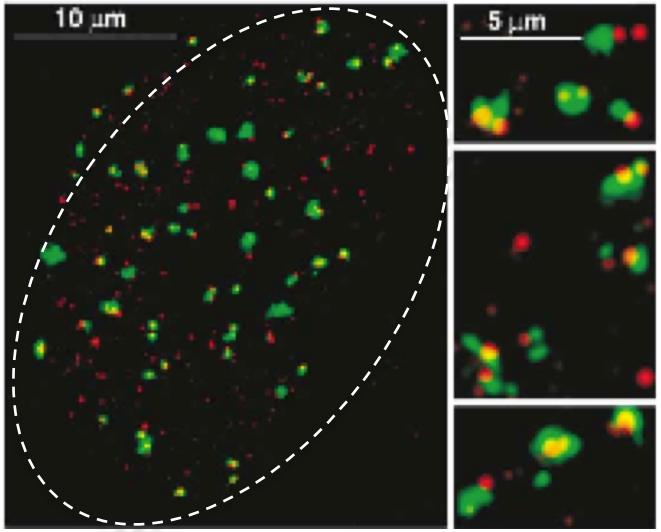


The MRN complex

- binds avidly to double-strand breaks
- The MRN complex also participates in activating the checkpoint kinase ATM in response to DNA damage



DNA Damage Foci



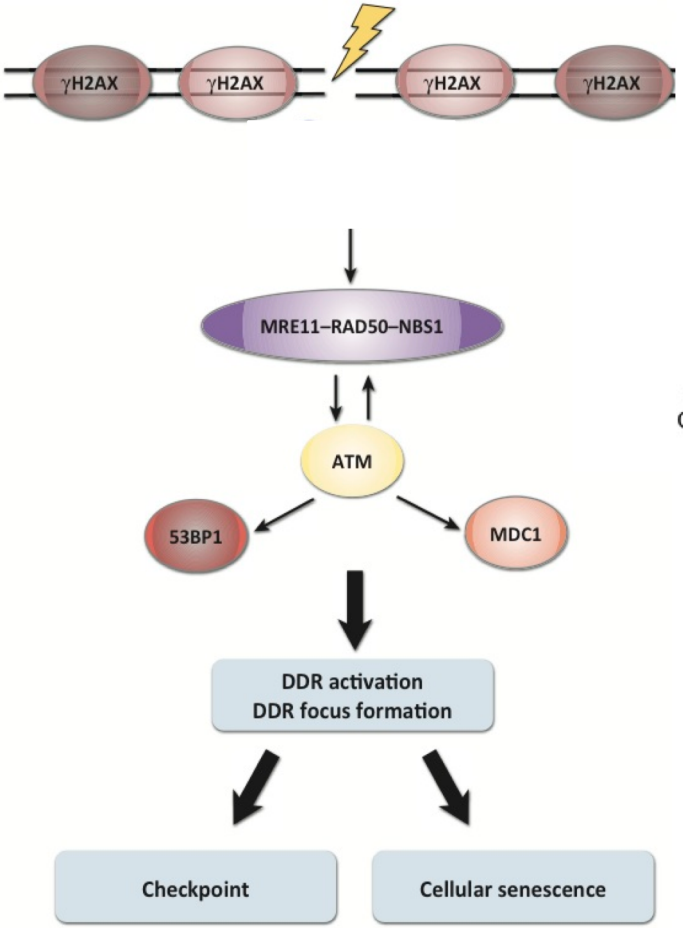
γH2AX

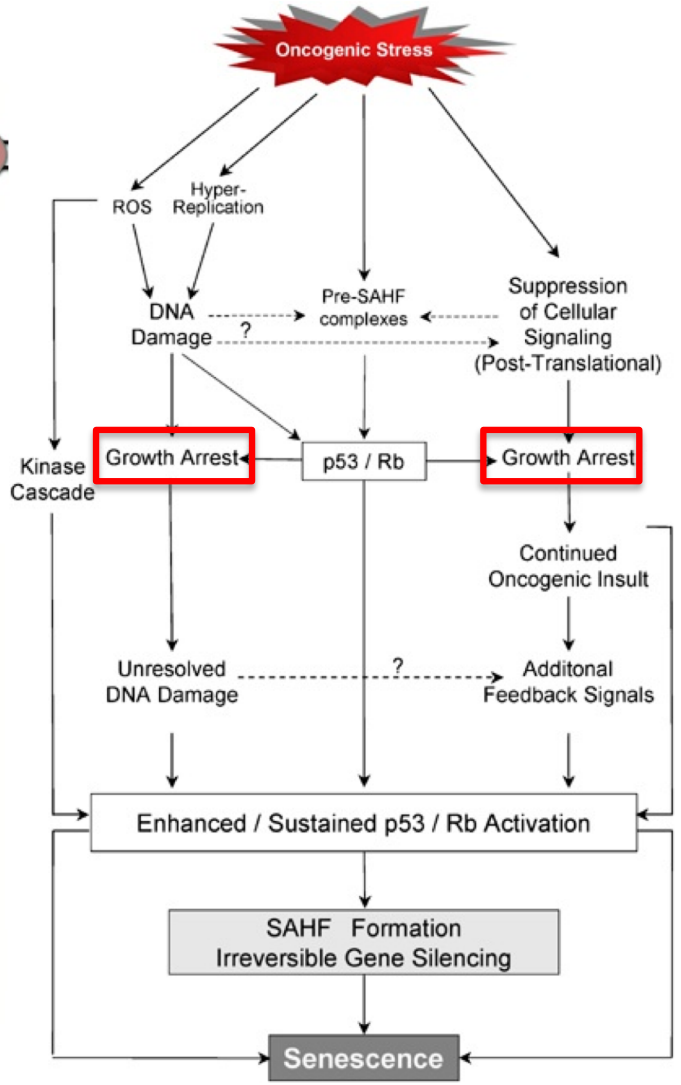
53BP1

MRE11

P-ATM

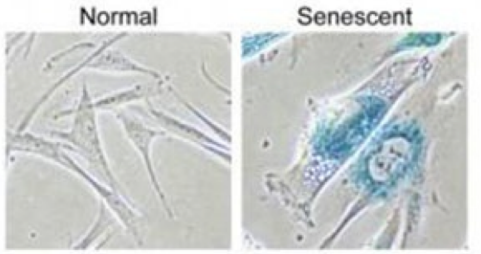
Model system for persistent DNA damage:
ONCOGENE INDUCED SENESCENCE

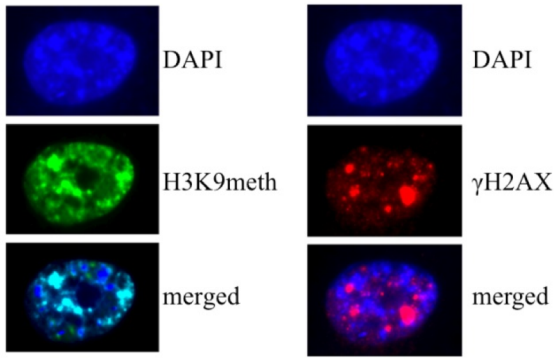




Senescence markers

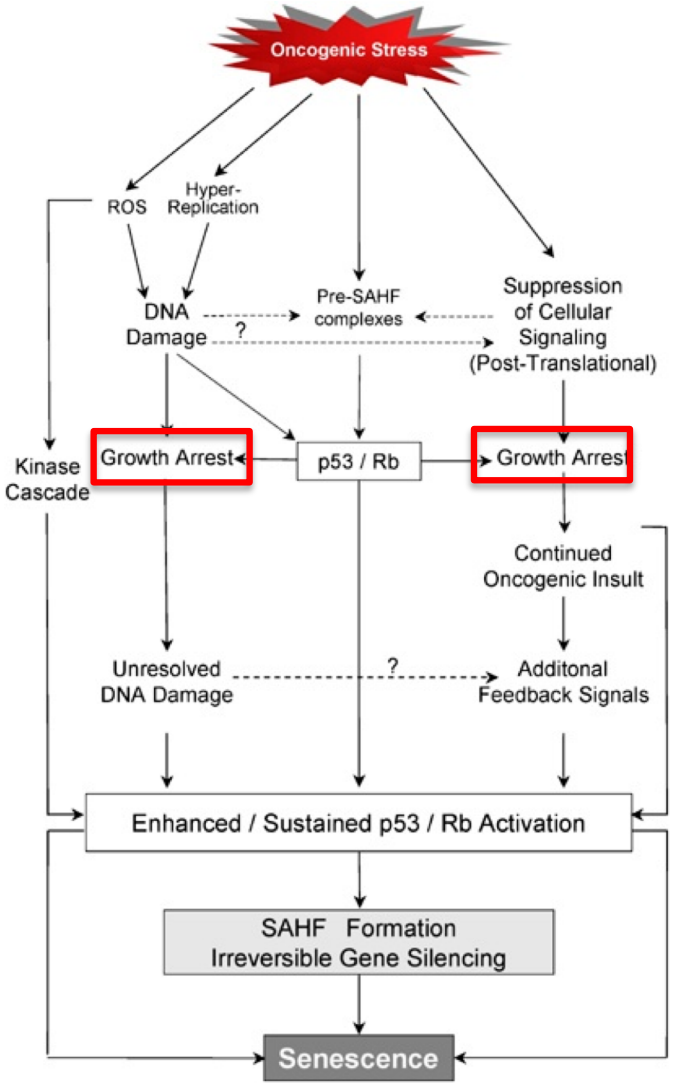
Beta galactosidase positve cells
Big foci of heterochromatin
That colocalize with gH2AX





Expression of oncogenes mediates increased DNA damage load
= tumorsuppressor mechanism
→Additional mutations required to escape from tumorsuppression
→Cancer formation

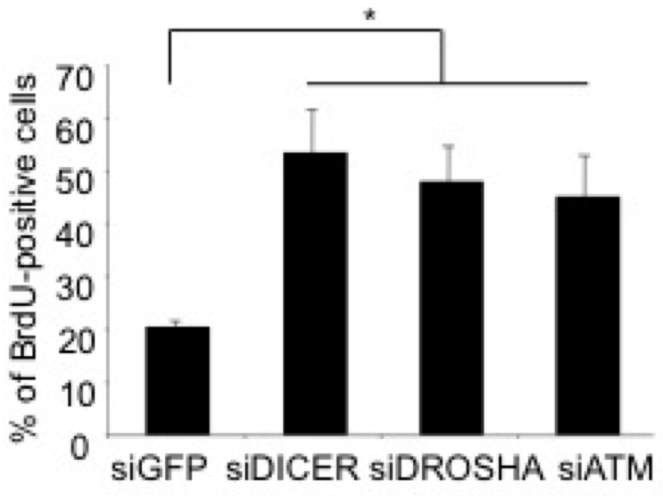
LOSS OF DICER/DROSHA REDUCES ONCOGENE INDUCES SENESCENCE



Primary, human foreskin fibroblasts (BJ cells) retroviraly transduced with a vector
encoding a Ras cDNA containing an ocogenic mutation = **H-RasV12**
=Oncogene induced senescent cells ("OIS cells")

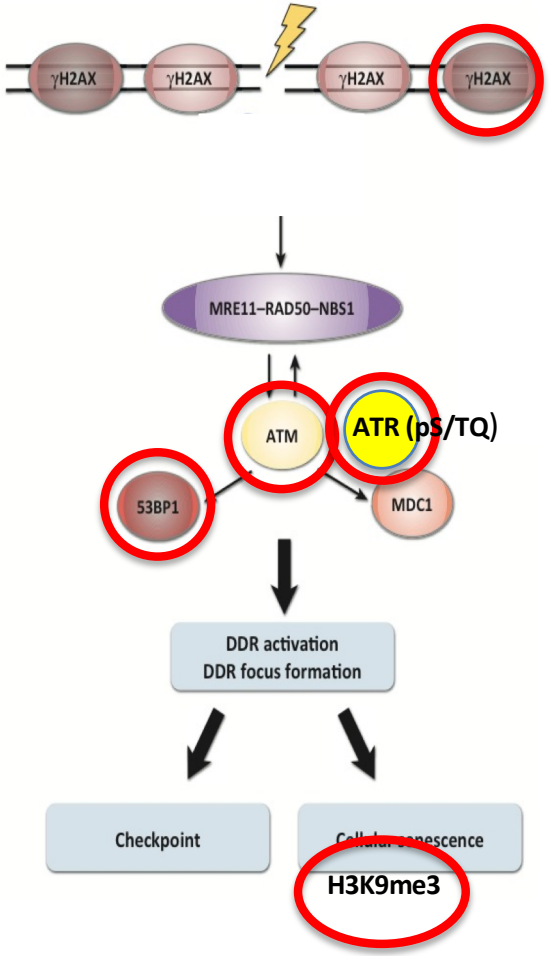
H-RasV12 drives excessive proliferation → Accumulation of DNA damage → Senescence → SAHF

OBSERVATION IN THE LABORATORY:
Knock-down of DICER and DROSHA or ATM increases cell proliferation markers

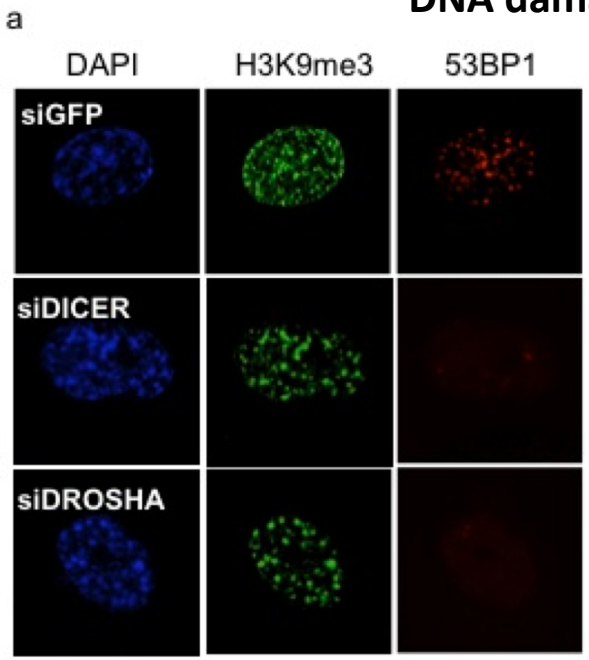


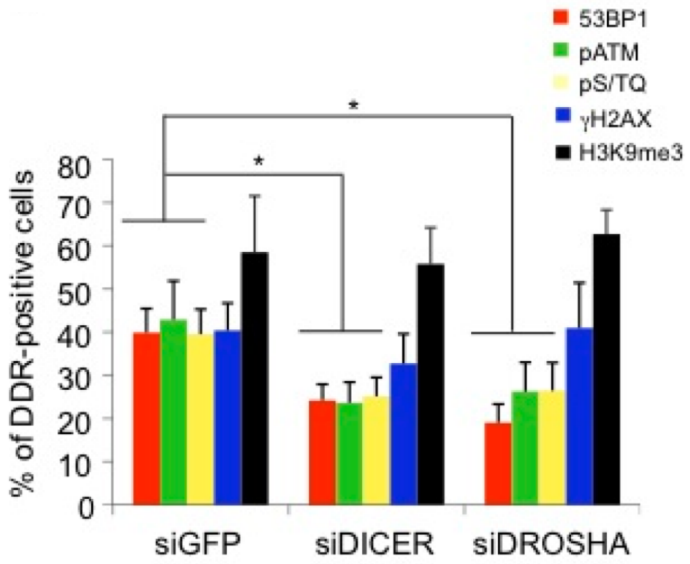
BrdU is incorporated in S-Phase and can be detected
Using an antibody (IF); more BrdU+ cells = more proliferation

LOSS OF DICER/DROSHA REDUCES DNA DAMAGE SIGNALLING IN OIS CELLS (MODEL 1)



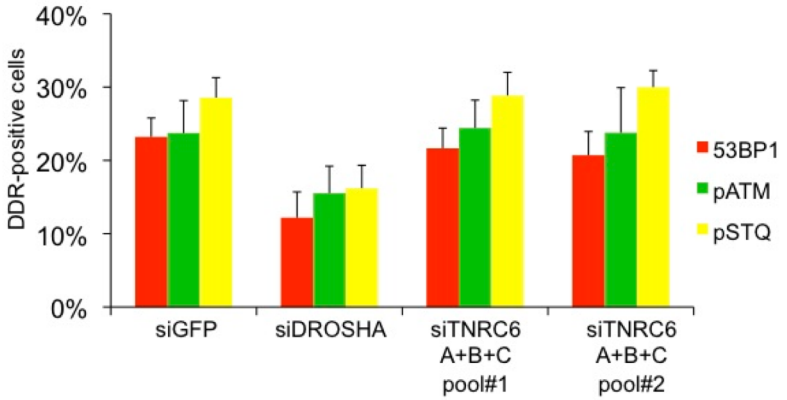
siRNA/miRNA pathways are important for DNA damage response





KNOCK DOWN OF GENES IMPORTANT FOR miRNA FUNCTION (TNRC6A,B,C; translational repression)

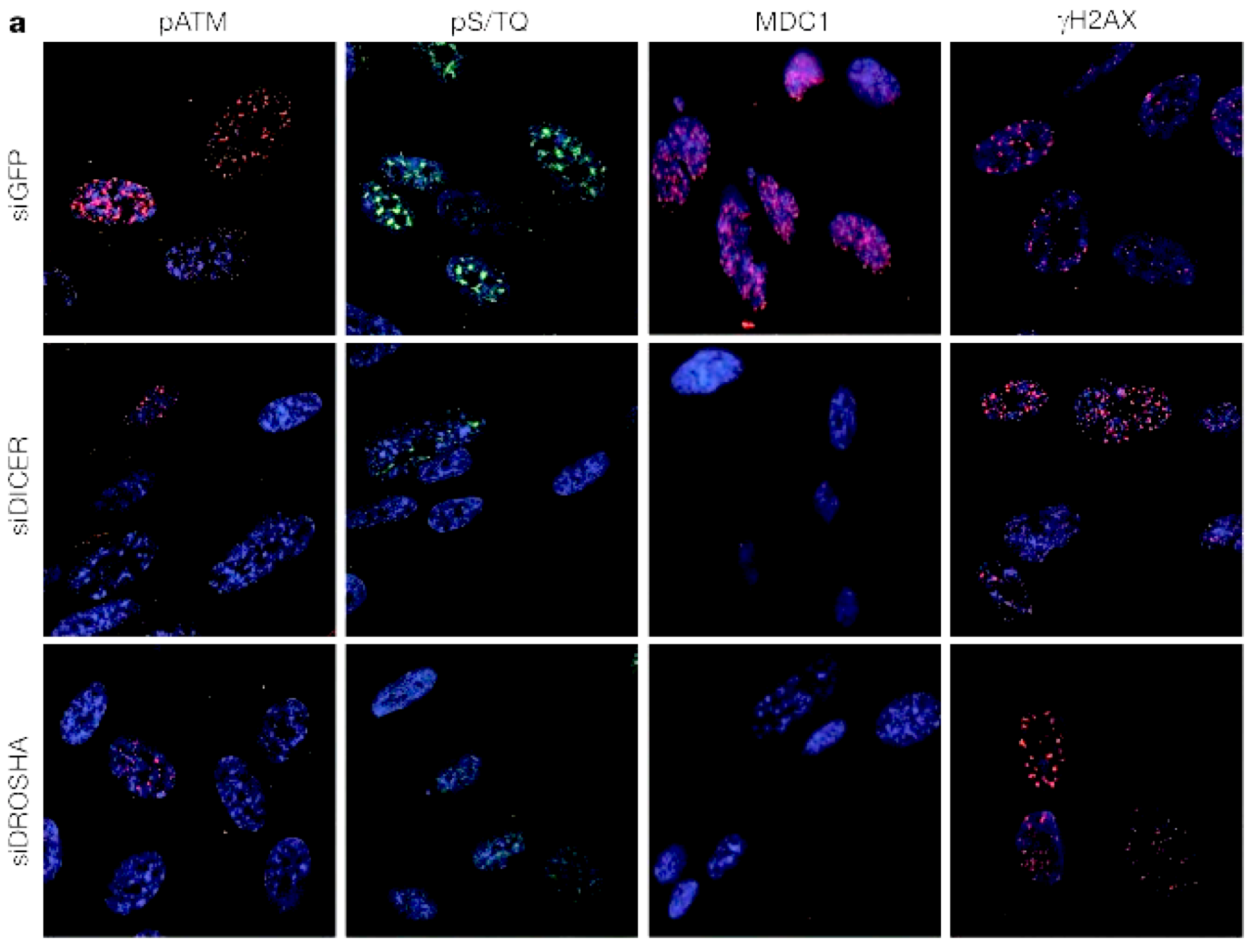
do not impact on DNA damage response



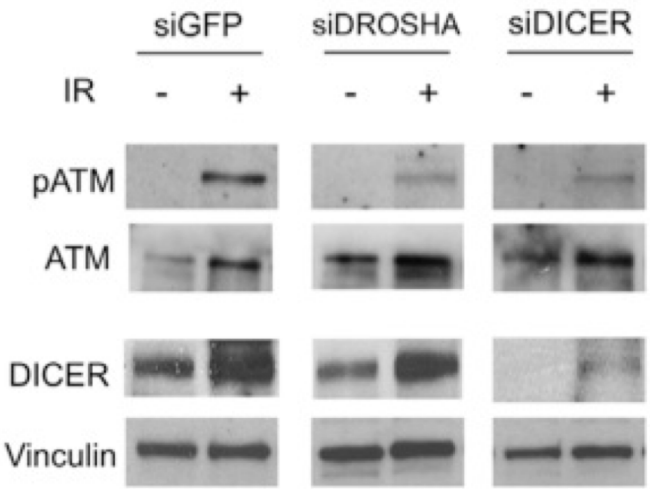
Note: multisite phosphorylation of SQ/TQ motifs is required for normal DNA-damage responses

siRNA PATHWAYS ARE INVOLVED IN THE CONTROL OF DNA DAMAGE RESPONSE

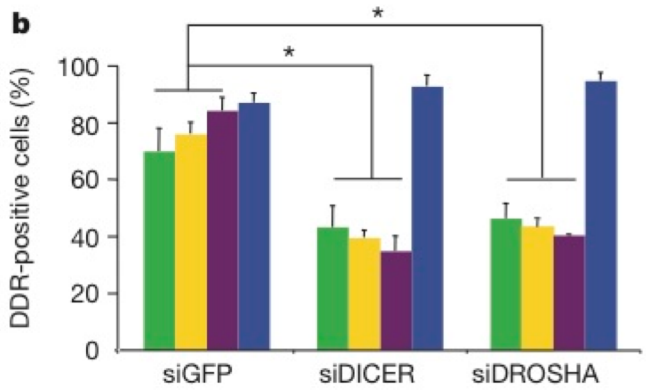
ANOTHER MODEL: GAMMA IRRADIATION OF NORMAL FIBROBLASTS (MOPDEL 2)

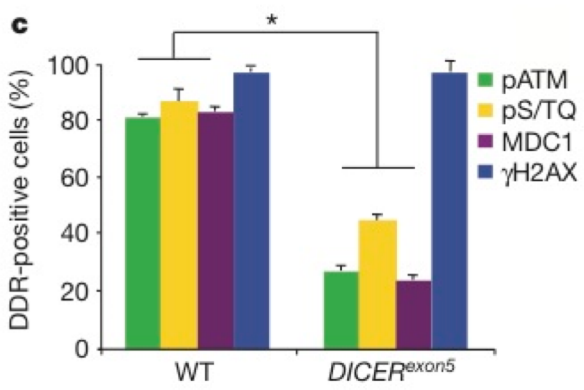


Knock-down of DICER and DROSHA Impairs the formation of DNA damage response foci in gamma irradiated cells



ATR expression is still maintained in Dicer/ Drosha LOF cells





pS/TQ antibodies are used to identify phosphorylated signaling proteins that are involved in the DNA damage response pathway,

IS RNA REQUIRED TO TRIGGER AN EFFICIENT DNA DAMAGE RESPONSE?

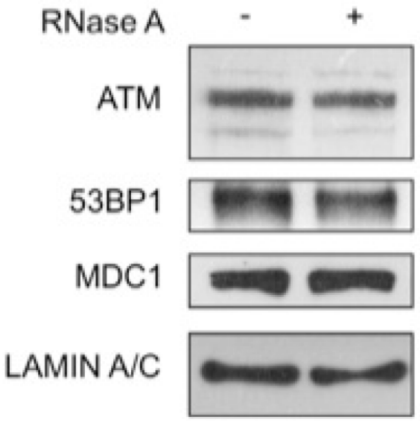
CREATE ARTIFICIAL MODEL SYSTEM USING RNaseA…..

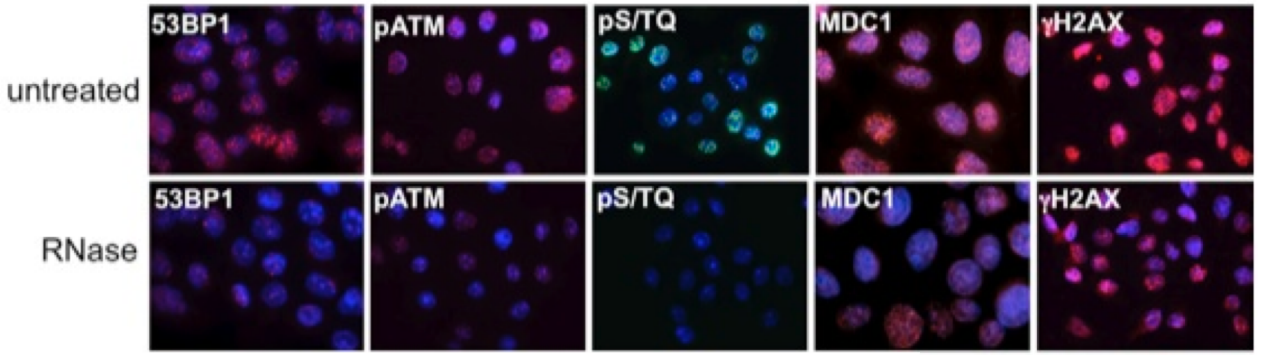
Model
Heterochromatin

Permeabilize Cells (detergent) → Treat with RNase → Heterochromatin Proteins are lost

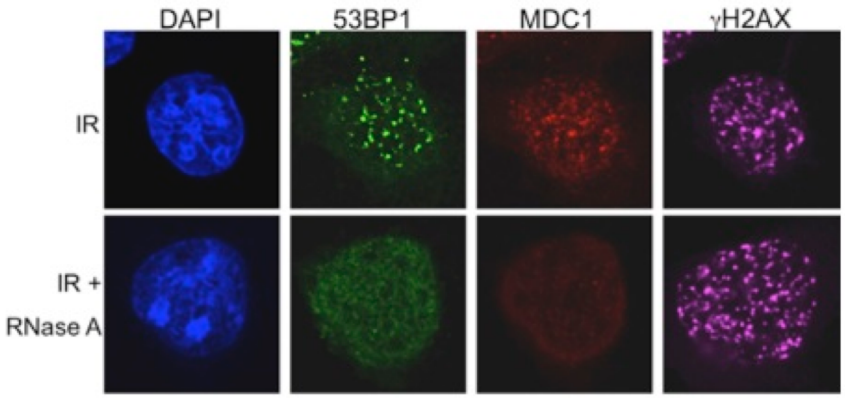
Model
DNA damage

HeLa cells + Gamma irradiation+ permeabilize → Treat with RNAse → Check protein Markers for DNA damage





IF: RNase treatments reduces the amount of DNA damage markers after gamma irradiation
WB: DNA damage proteins expressed in experimental cells

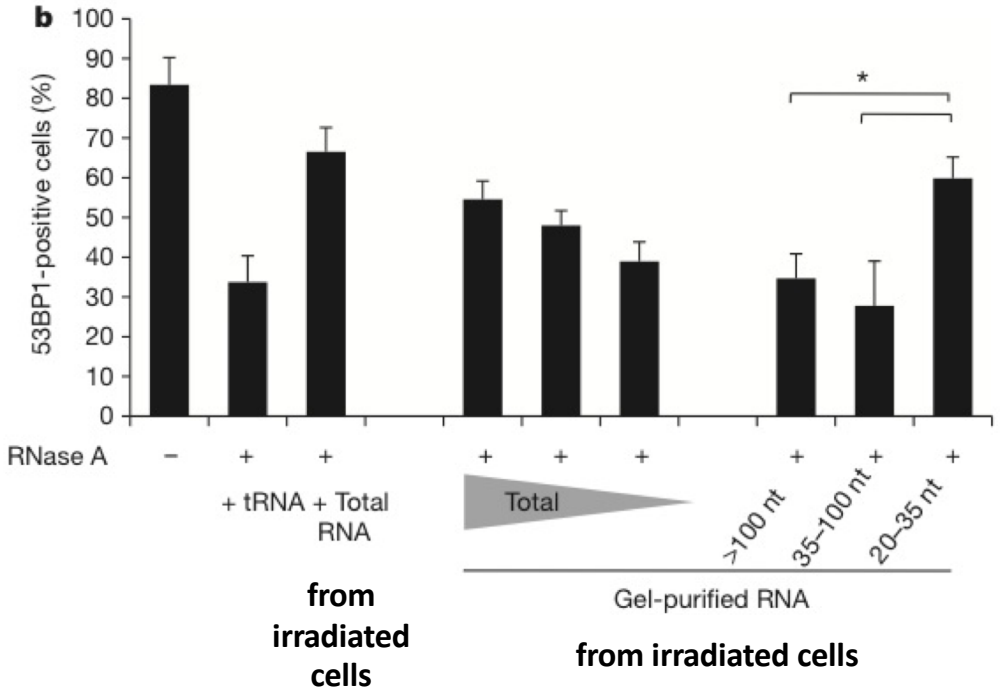


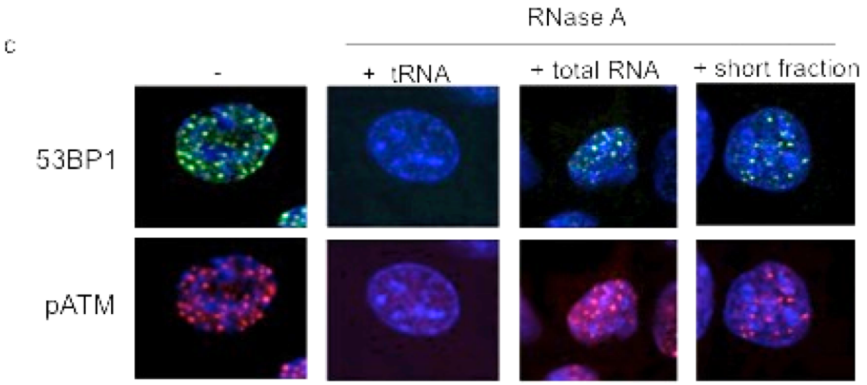
WHAT KIND OF RNA IS REQUIRED TO TRIGGER AN EFFICIENT DNA DAMAGE RESPONSE?

Model DNA damage

HeLa cells + Gamma irradiation+ permeabilize → Treat with RNAse → Check protein Markers for DNA damage

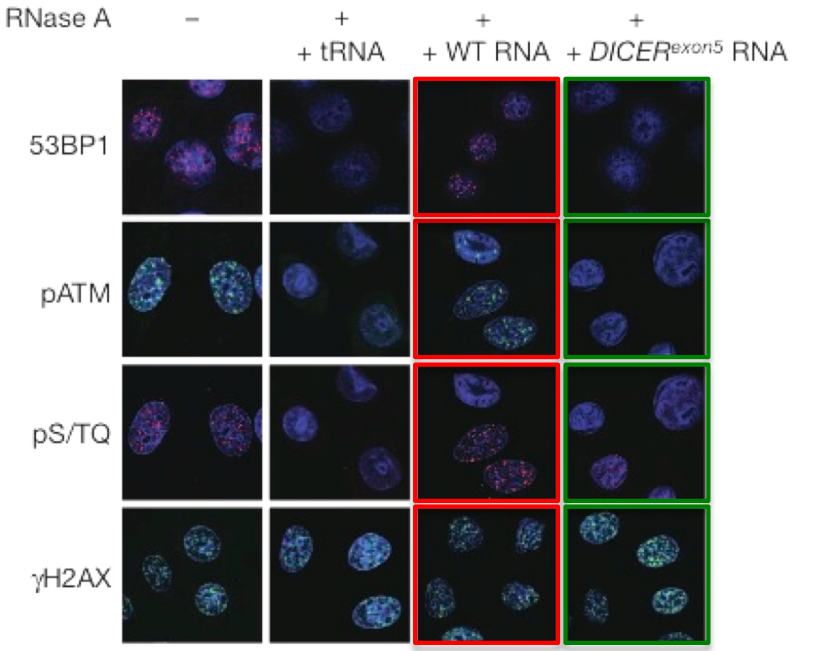
Treat with RNAse → Add different classes of RNA obtained from irradiated cells → Check protein Markers for DNA damage

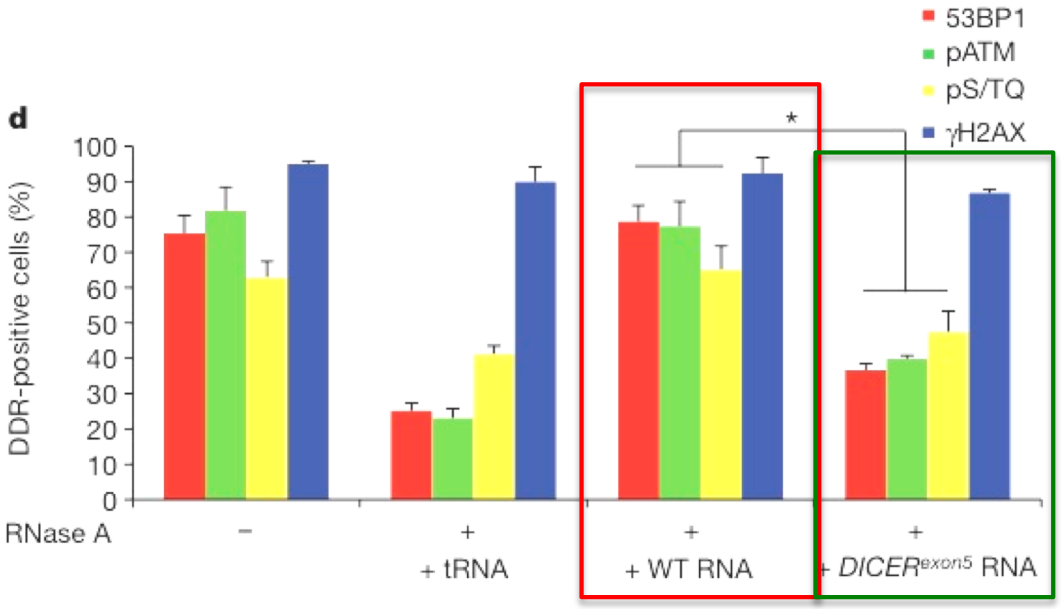




A short RNA fraction (20-35 nt) rescues DNA damage response after RNAse treatment
= POTENTIAL DICER/DROSHA PRODUCTS

WHAT KIND OF RNA IS REQUIRED TO TRIGGER AN EFFICIENT DNA DAMAGE RESPONSE?





Addition of RNA From Irradiated Dicer wt Cells = Small, DICER processed RNAs present

Addition of RNA From Irradiated Dicer KO Cells = NO small, DICER processed RNAs

Total RNA from Dicer null cells cannot rescue defects of DNA damage foci formation after RNAse treatment

→Dicer has a critical role in DNA damage response

CAN SMALL RNAs = DDRNAs (DNA DAMAGE RESPONSE RNAs) ACT DURING DNA DAMAGE RESPONSE AT A DEFINED SITE IN THE GENOME??

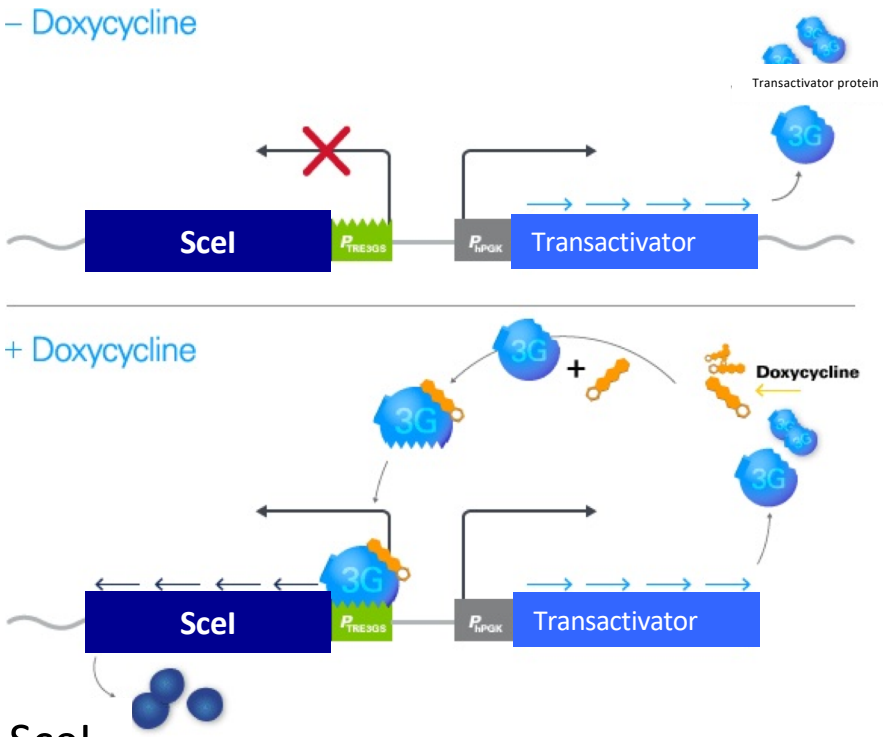
A CELL MODEL SYSTEM TO STUDY THE KINETICS OF DNA DAMAGE
→ Controlled and site-specific introduction of DNA Damage

Model cell line:
Contains:
1. an inducible transactivator

2. the restriction enzyme SceI under the control of a inducible promoter

3. A SceI site between Lac Repressor DNA sequences inserted into the genome of model cell line

4. The Lac Repressor that binds DNA seqeuences around the SceI sites



SceI

SceI

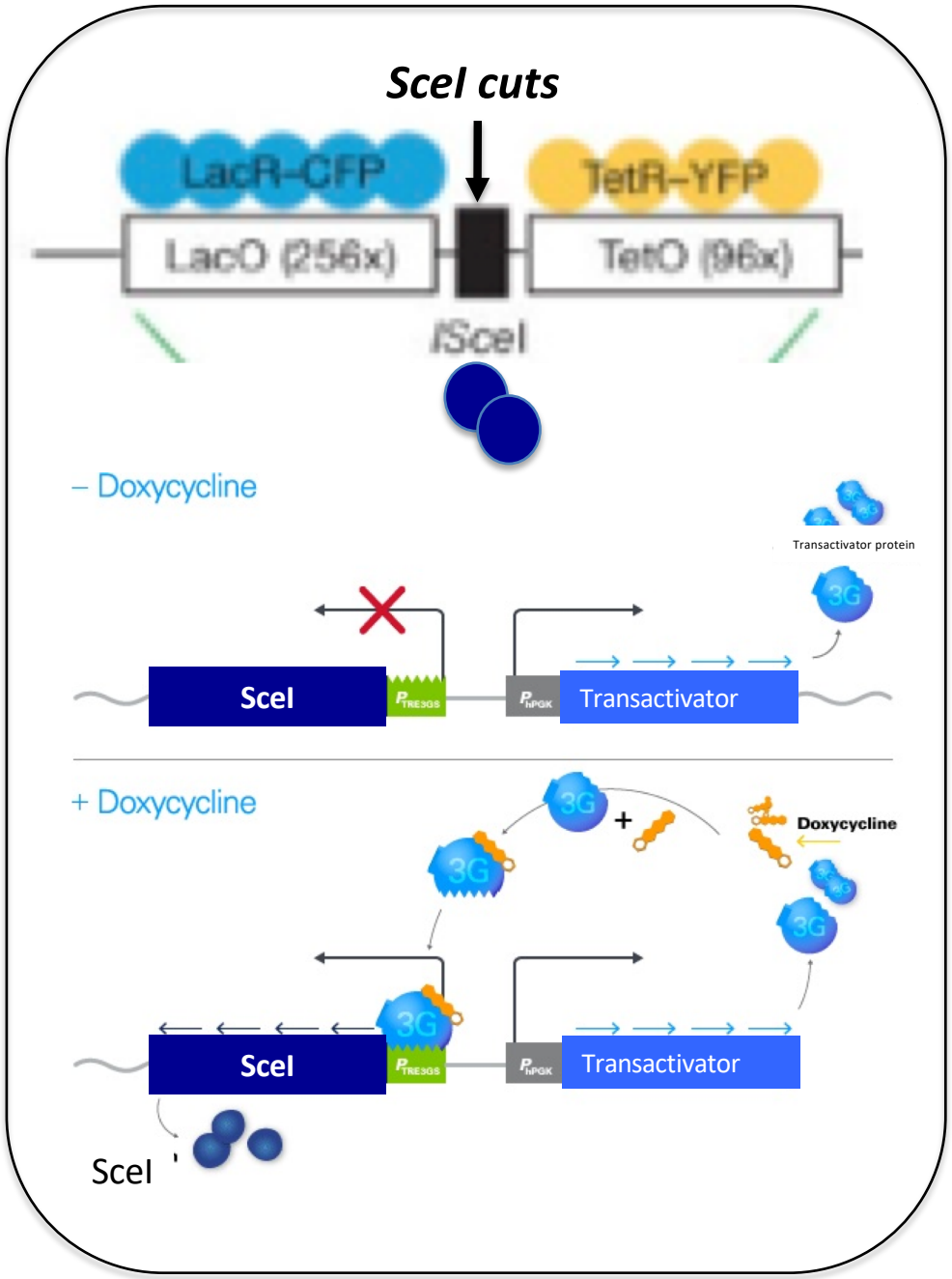
5′...TAGGGATAACAGGGTAAT...3′
3′...ATCCCTATTGTCCCATTA...5′

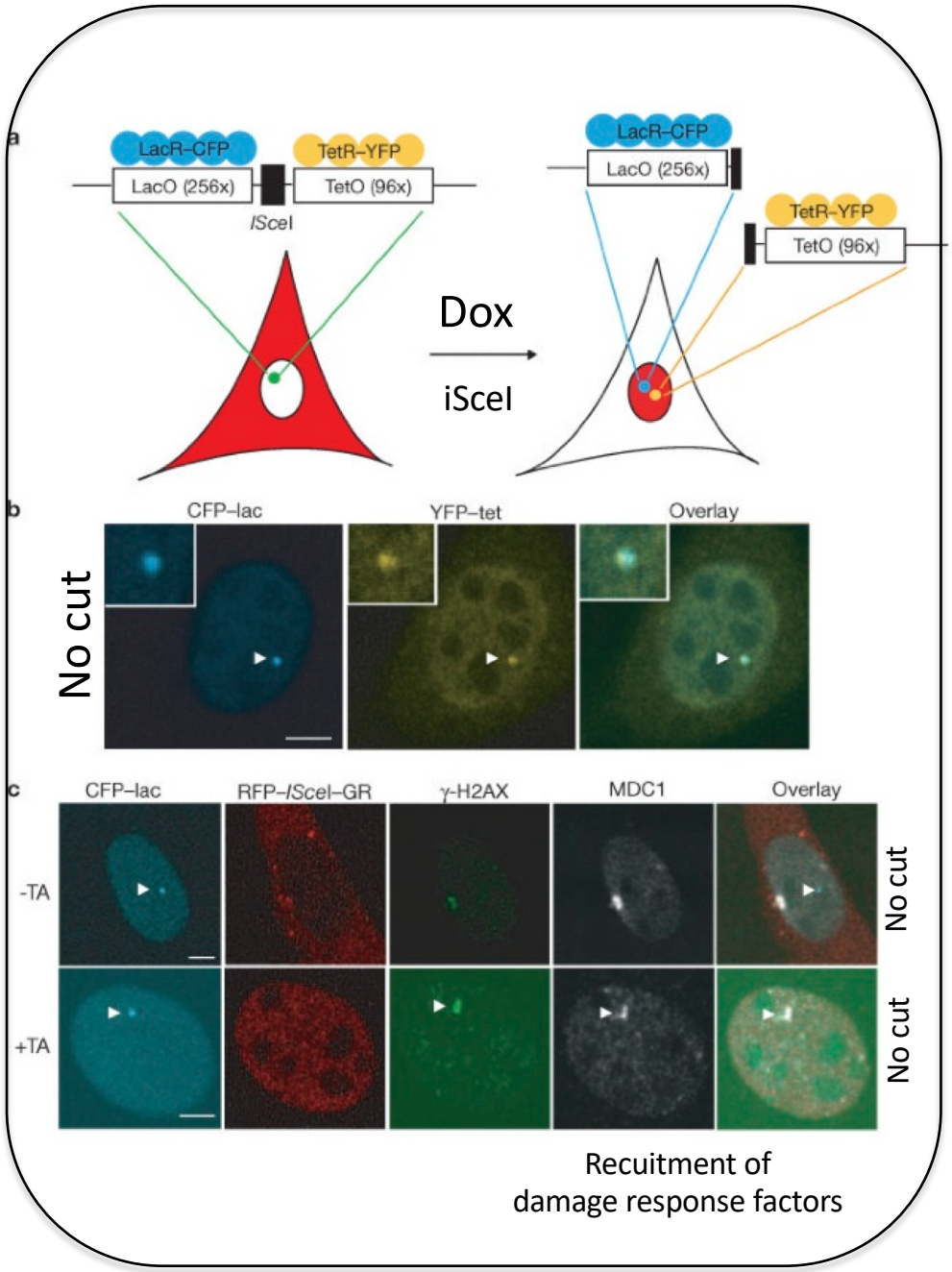
SceI is a restiction enzyme That does not cut in the human genome

Inducing SceI expression does not cut genomic DNA in human cells!

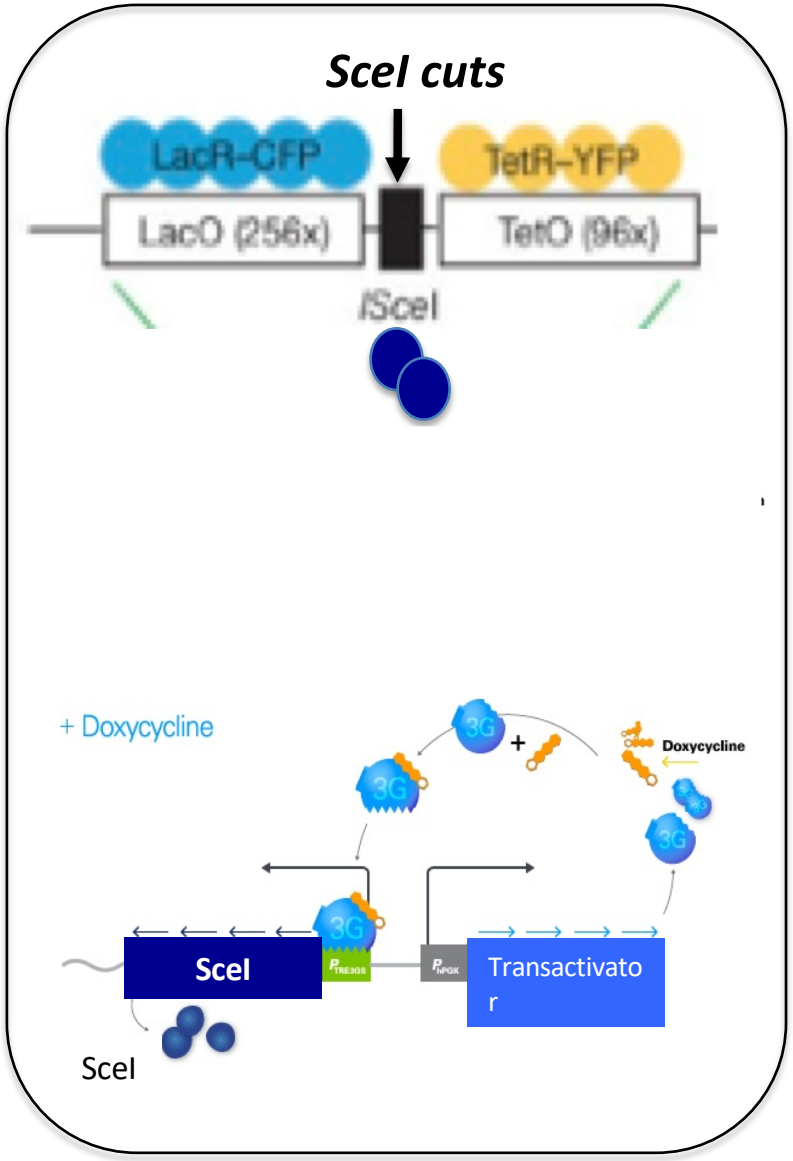
LETS INTRODUCE A SceI SITE AND MARK THE SceI SITE USING SEQEUNCES BOUND BY THE Lac REPRESSOR

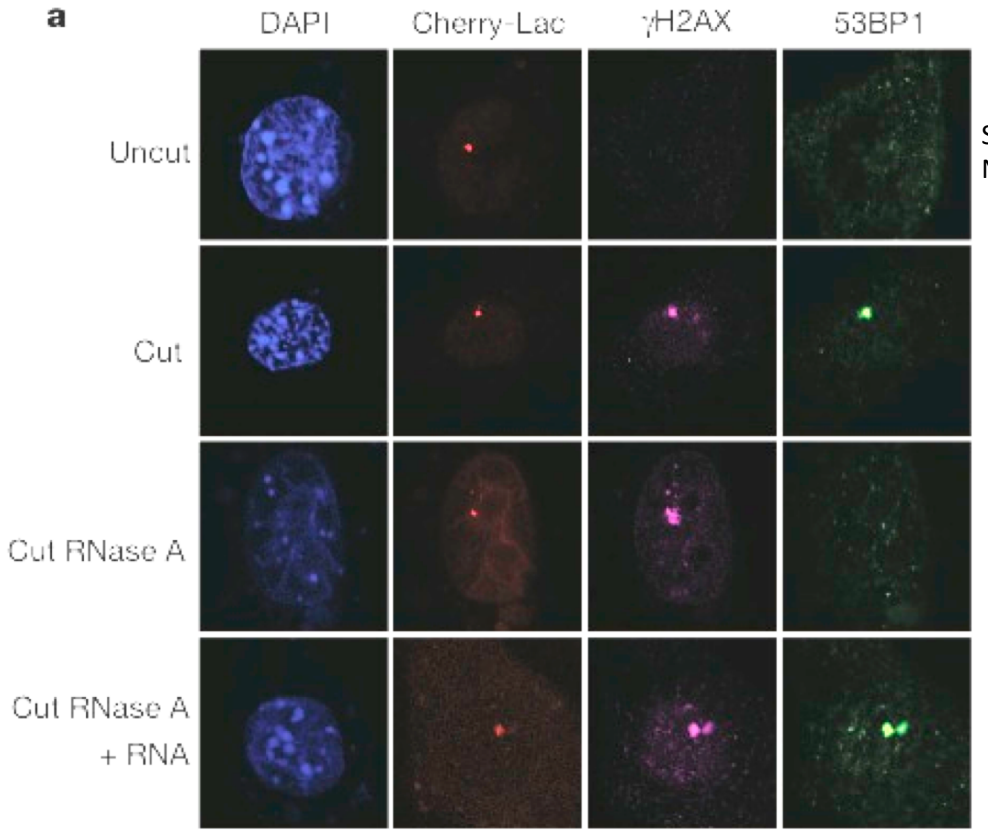
A MODEL SYSTEM TO STUDY THE KINETICS OF DNA DAMAGE





DEFINED RNAs FROM DNA DAMGE SITES ARE IMPORTANT FOR DNA DAMAGE RESPONSE





SceI site visible
No damage

SceI site visible:
damage
signaling
(DNA damage
focus)

+ RNase
SceI site visible:
no damage
signalling

+ RNase+RNA
prepared from
cells with cut
in SceI site.
Now SceI site
again visible:
damage
signalling

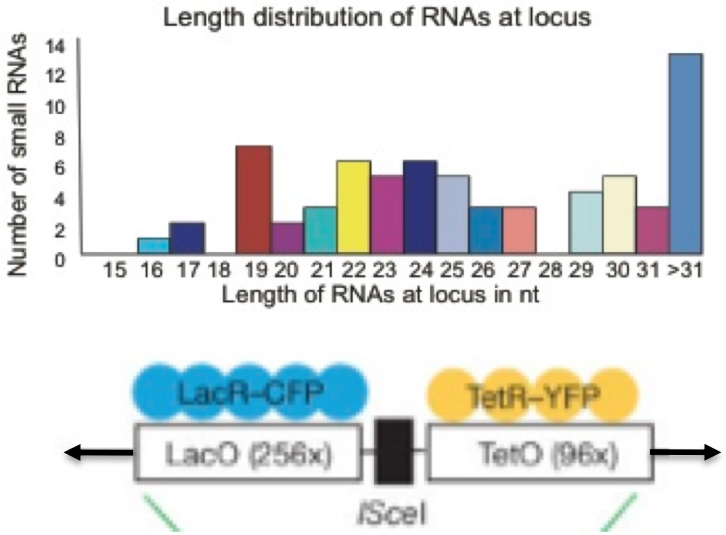
→ RNA was prepared from cells that have cut the SceI site

→ RNA from cells that do not have the SceI site but express SceI cannot rescue!!!!!

DEFINED RNAs FROM DNA DAMGE SITES ARE IMPORTANT FOR DNA DAMAGE RESPONSE - EVIDENCE 1

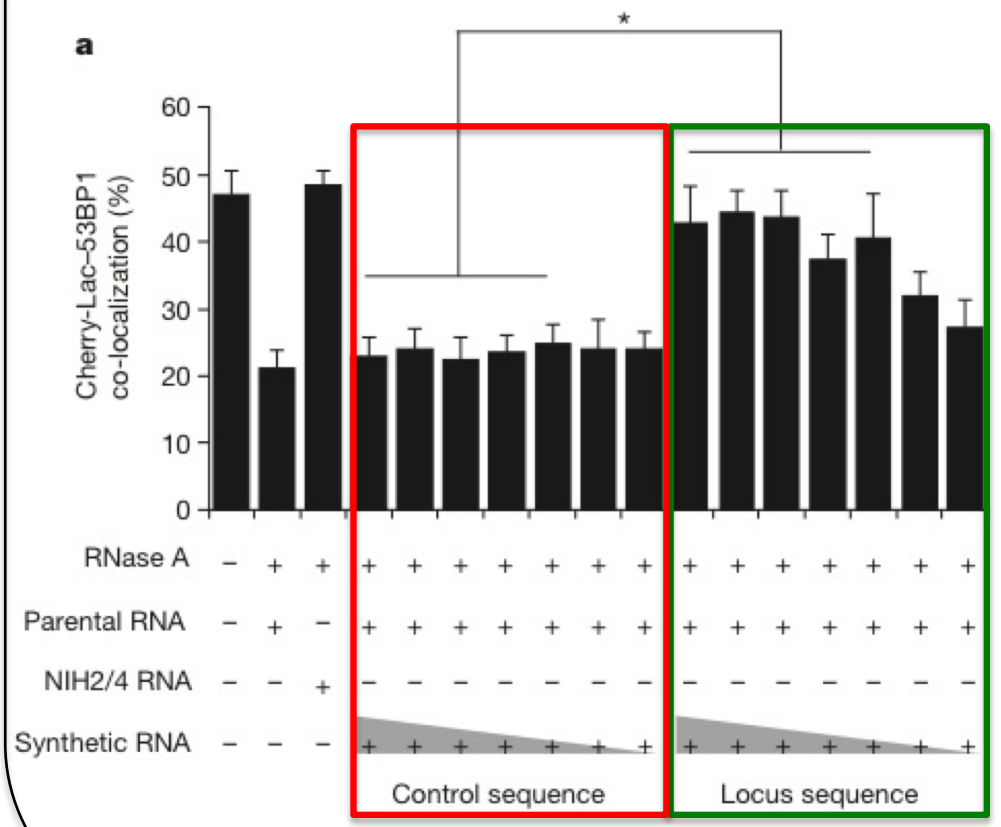
Take SceI cells:

-induce cutting
- prepare small RNA fraction
-make small RNASeq
detect small RNAs from locations around the SceI sites



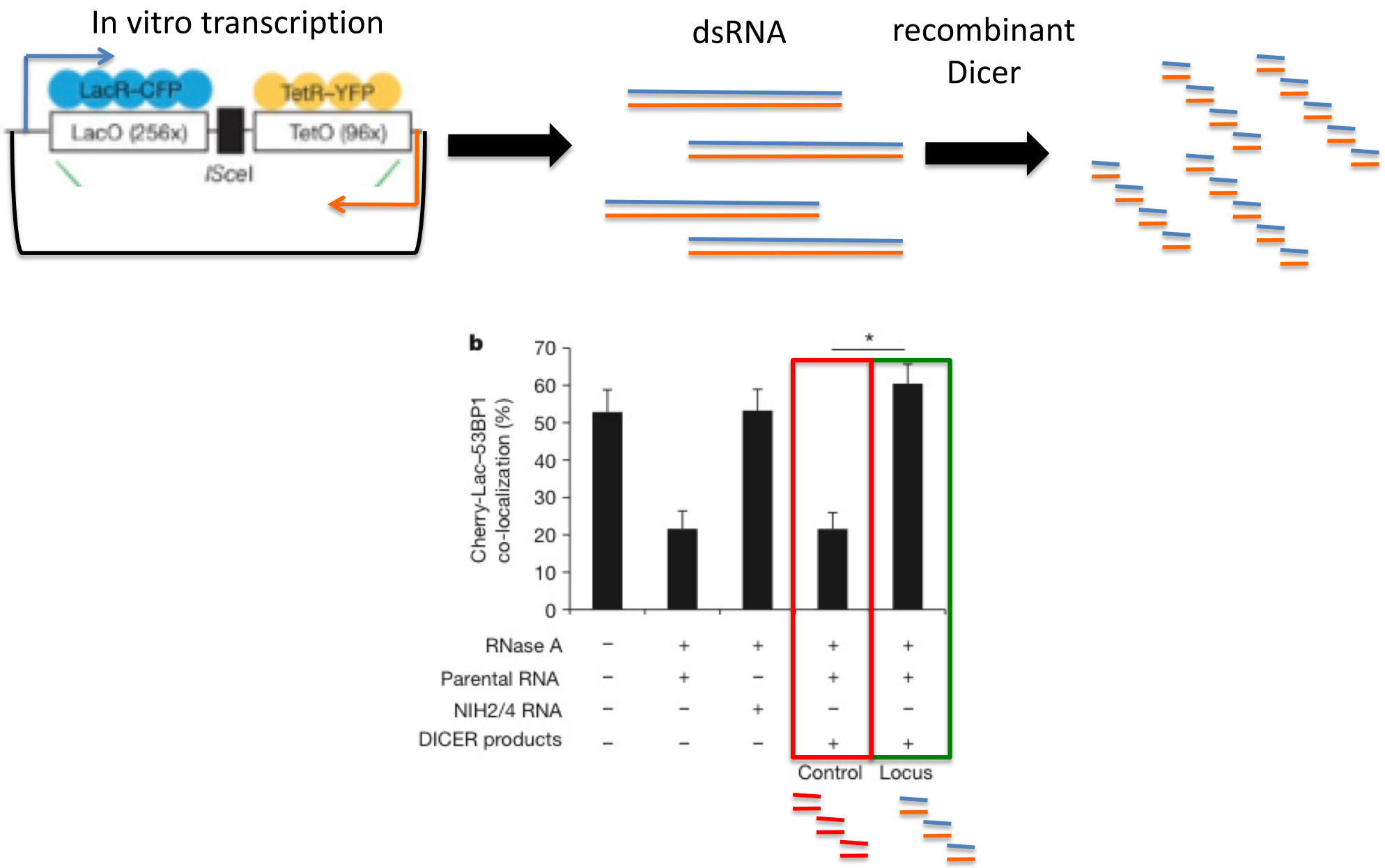
!!!!!Small RNAs (16-31) arising from regions around the SceI site can be identified !!!!!

SYNTHETIC, small RNAs with sequences that resemble the region around the SceI site can rescue the formation of DNA damage foci at the SceI site



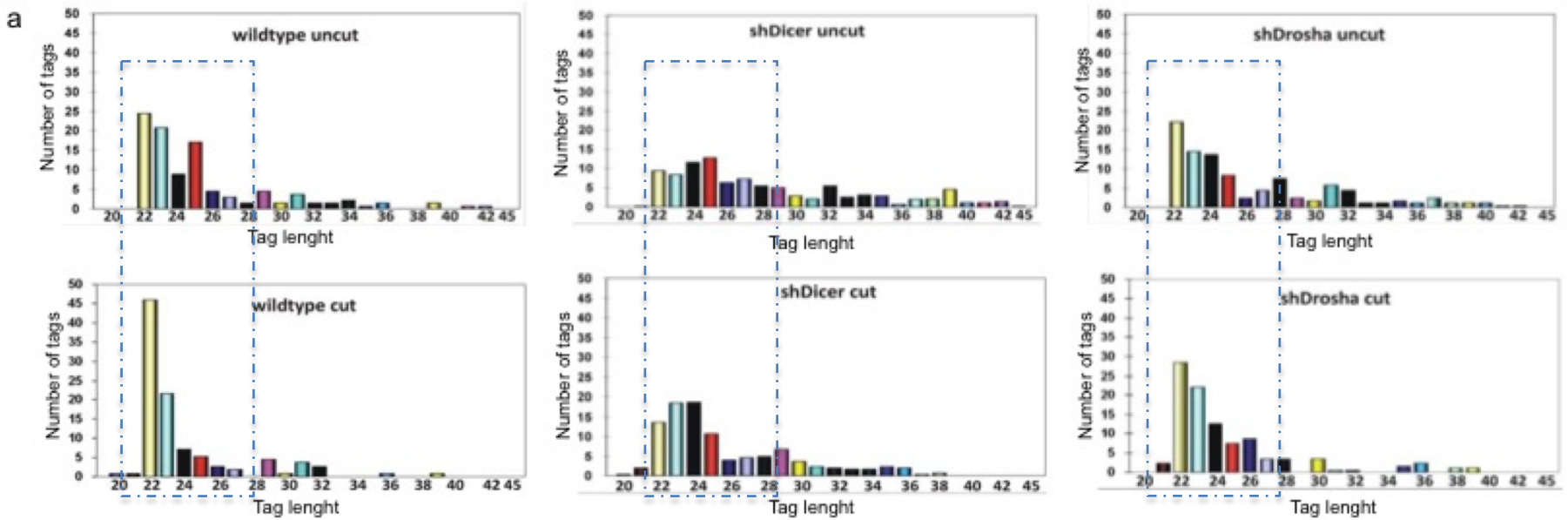
SceI region

DEFINED RNAs FROM DNA DAMGE SITES ARE IMPORTANT FOR DNA DAMAGE RESPONSE - EVIDENCE 2



DEFINED RNAs FROM DNA DAMGE SITES ARE IMPORTANT FOR DNA DAMAGE RESPONSE - EVIDENCE 2

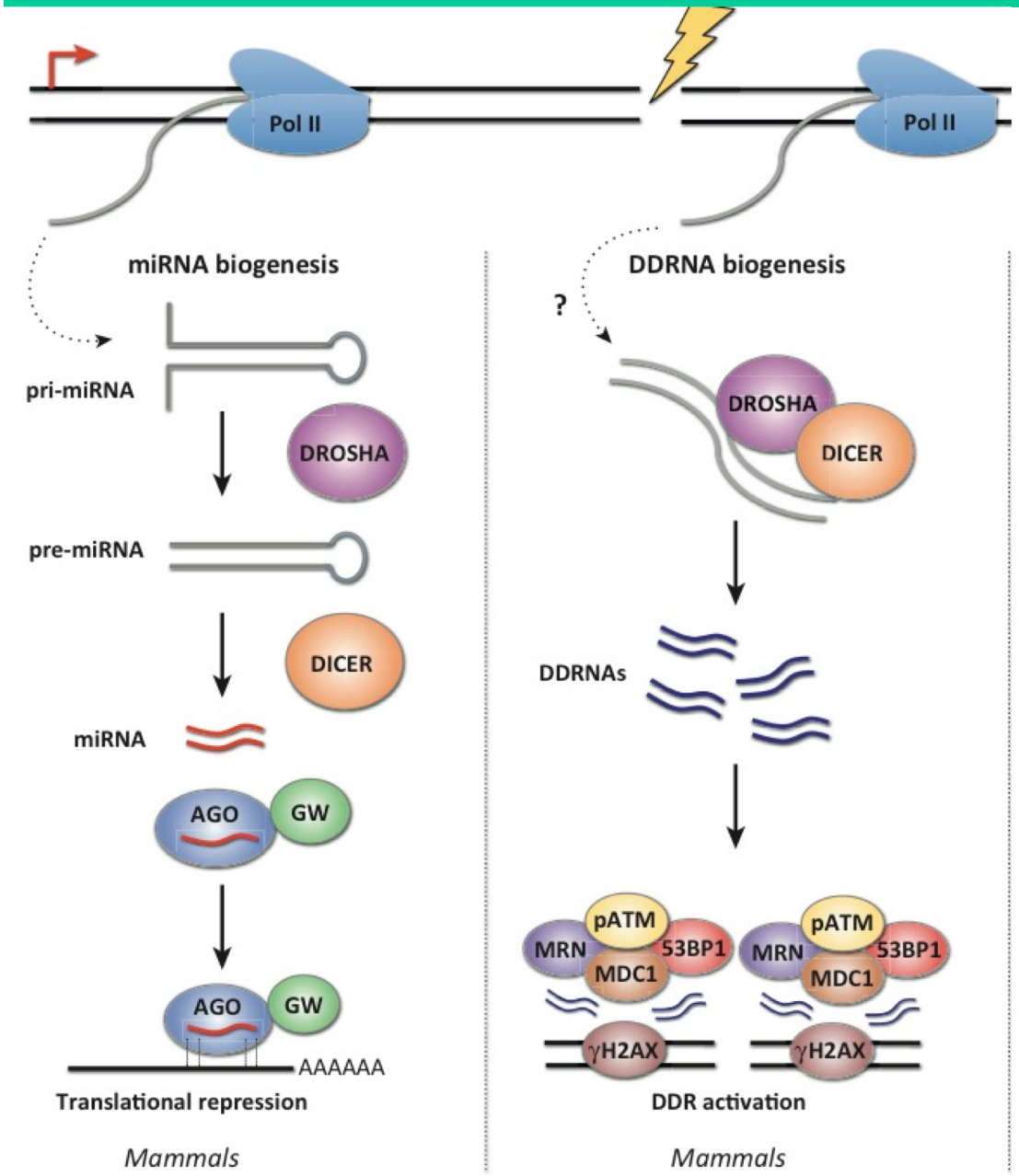
Take SceI cells
- Knock down DICER or DROSHA:
-induce cutting by Sce-I
- prepare RNA
-make RNA-Seq
detect small RNAs from locations around the SceI sites

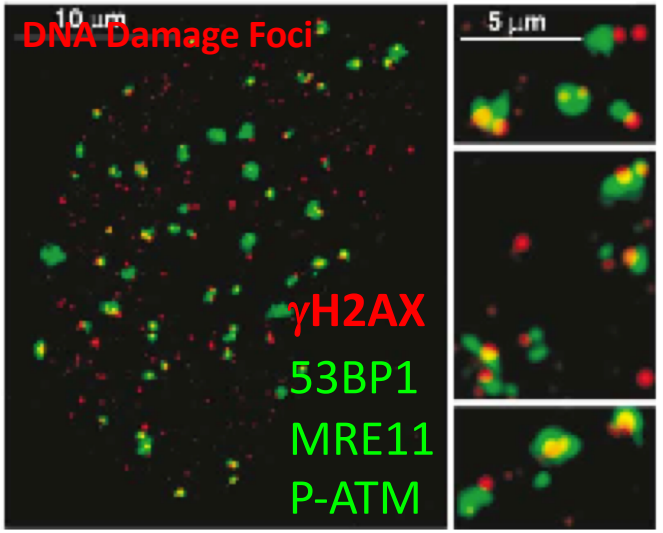


DICER RNAi *DROSHA RNAi*

Small RNAs (16-31) arising from
regions around the SceI site is reduced in DICER/DROSHA knock-down cells

DNA DAMAGE RESPONSE RNAs (DDRNA) CONTROL THE DNA DAMAGE RESPONSE





In summary, we demonstrate that different sources of DNA damage, including oncogenic stress, ionizing radiation and site-specific endonucleases, activate the DDR in a manner dependent on DDRNAs, which are DICER- and DROSHA-dependent RNA products with the sequence of the damaged site. DDRNAs control DDR foci formation and maintenance, checkpoint enforcement and cellular senescence in cultured human and mouse cells and in different cell types in living zebrafish larvae. They act differently from canonical miRNAs, as inferred by their demonstrated biological activity independent of other RNAs and of GW182-like proteins.

How is RNA Polymerase recruited to DNA damage?

ARTICLES
https://doi.org/10.1038/s41556-019-0392-4
nature cell biology

Functional transcription promoters at DNA double-strand breaks mediate RNA-driven phase separation of damage-response factors

Fabio Pessina[1], Fabio Giavazzi[2], Yandong Yin[3], Ubaldo Gioia[1], Valerio Vitelli[1], Alessandro Galbiati[1], Sara Barozzi[1], Massimiliano Garre[1], Amanda Oldani[1], Andrew Flaus[4], Roberto Cerbino[2], Dario Parazzoli[1], Eli Rothenberg[3] and Fabrizio d'Adda di Fagagna[1,5]*

Damage-induced long non-coding RNAs (dilncRNA) synthesized at DNA double-strand breaks (DSBs) by RNA polymerase II are necessary for DNA-damage-response (DDR) focus formation. We demonstrate that induction of DSBs results in the assembly of functional promoters that include a complete RNA polymerase II preinitiation complex, MED1 and CDK9. Absence or inactivation of these factors causes a reduction in DDR foci both in vivo and in an in vitro system that reconstitutes DDR events on nucleosomes. We also show that dilncRNAs drive molecular crowding of DDR proteins, such as 53BP1, into foci that exhibit liquid–liquid phase-separation condensate properties. We propose that the assembly of DSB-induced transcriptional promoters drives RNA synthesis, which stimulates phase separation of DDR factors in the shape of foci.

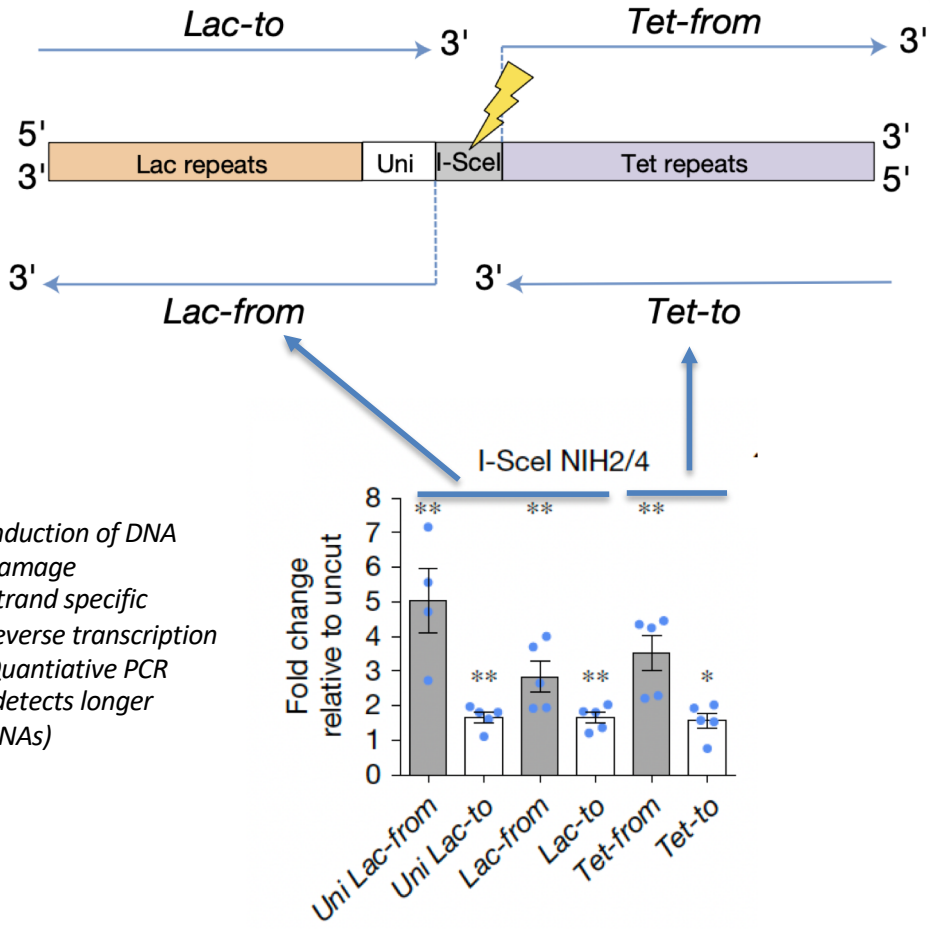
ARTICLES
nature cell biology

Damage-induced lncRNAs control the DNA damage response through interaction with DDRNAs at individual double-strand breaks

Flavia Michelini[1], Sethuramasundaram Pitchiaya[2,5], Valerio Vitelli[1], Sheetal Sharma[1], Ubaldo Gioia[1], Fabio Pessina[1], Matteo Cabrini[3], Yejun Wang[4], Ilaria Capozzo[3], Fabio Iannelli[1], Valentina Matti[1], Sofia Francia[1,3], G. V. Shivashankar[1,4], Nils G. Walter[2] and Fabrizio d'Adda di Fagagna[1,3,6]

HOW ARE TRANSCRIPTS GENERATED AT DNA DAMAGE SITES

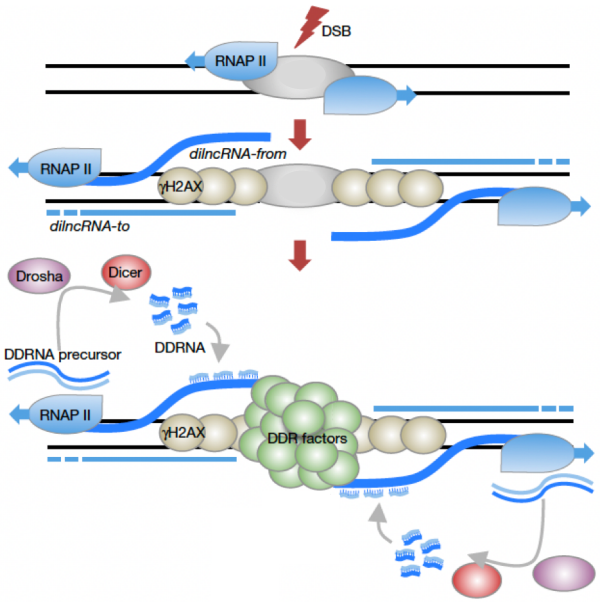
dlincRNAs: DNA damage induced long, non-coding RNAs



- *Induction of DNA damage*
- *Strand specific reverse transcription*
- *Quantiative PCR (detects longer RNAs)*

- **Major fraction of dlincRNAs are transcribed from damage - outwards**
- **Minor fraction of dlincRNAs are transcribed towards site of damage**

MODEL



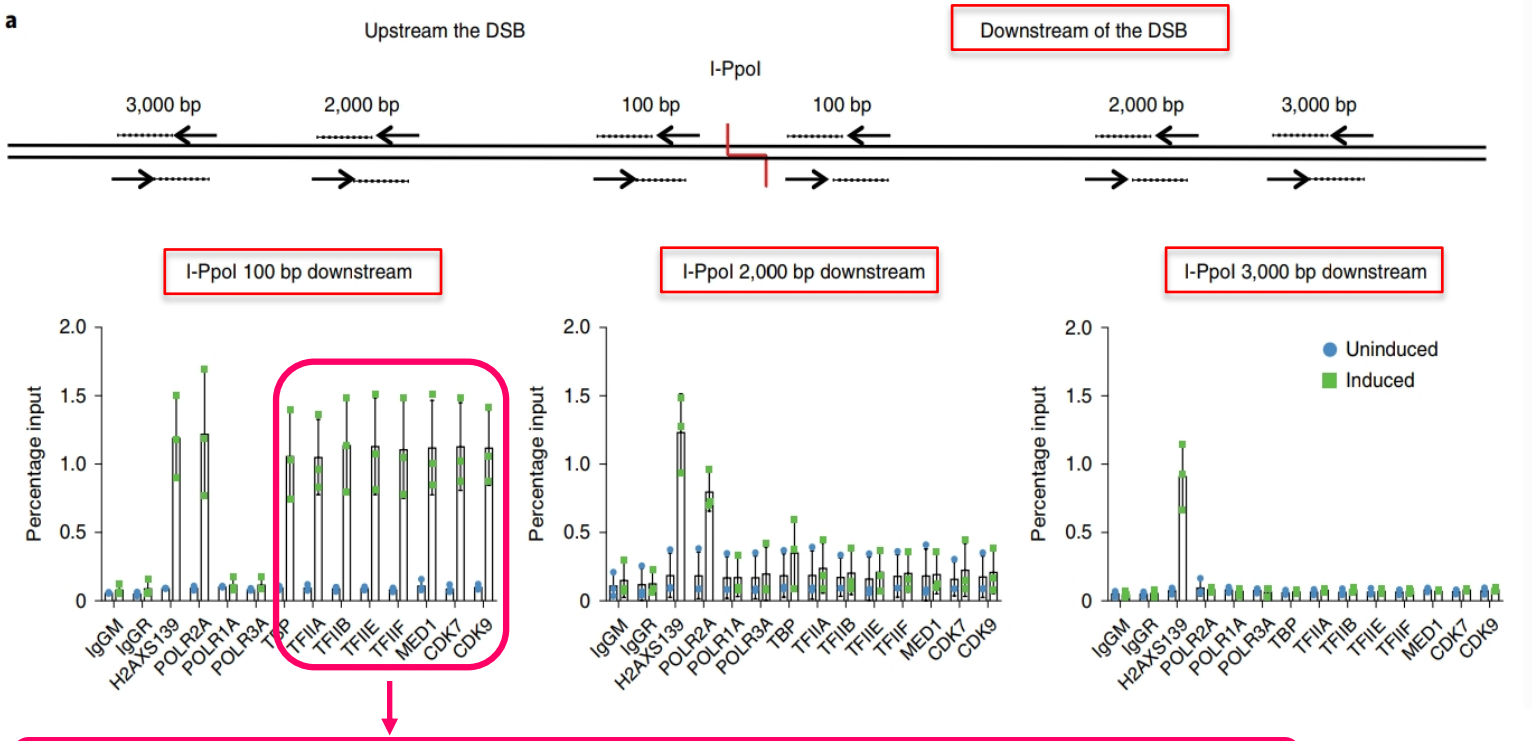
MODEL: RNAPII at the DSB triggeres the bidirectional synthesis of dilncRNA-from (blue) and, in general less abundantly, of dilncRNA-to (light blue). DROSHA and DICER process the long double-stranded RNA, probably the outcome of paired or folded dilncRNAs, generating DDRNAs that pair with nascent unprocessed single-stranded dilncRNAs; together they bind to 53BP1 and fuel DDR focus formation. Interfering with dilncRNA–DDRNA interactions through ASOs allows site-specific inhibition of DDR.

IS RNA POLYMERASE II RECRUITED TO SITES OF DNA DAMAGE?

PMC: Preinitiation Complex for transcription (PIC, TFIIA, B, E, F,) + MED1 + CDK9

HeLa cells whit a specific endogenous locus cleaved using and inducible I-Ppol endonuclease (analpgy to SceI) to induce DSB → ChIP analyses at different distances to the cut

ChIP: Primers located at damage site, upstream and downstream of DNA damage site (I-Ppol)

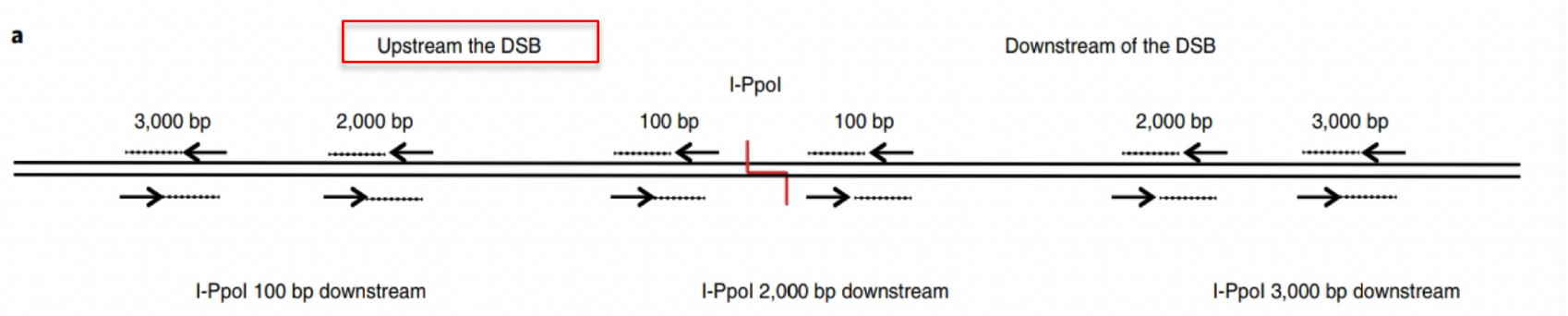


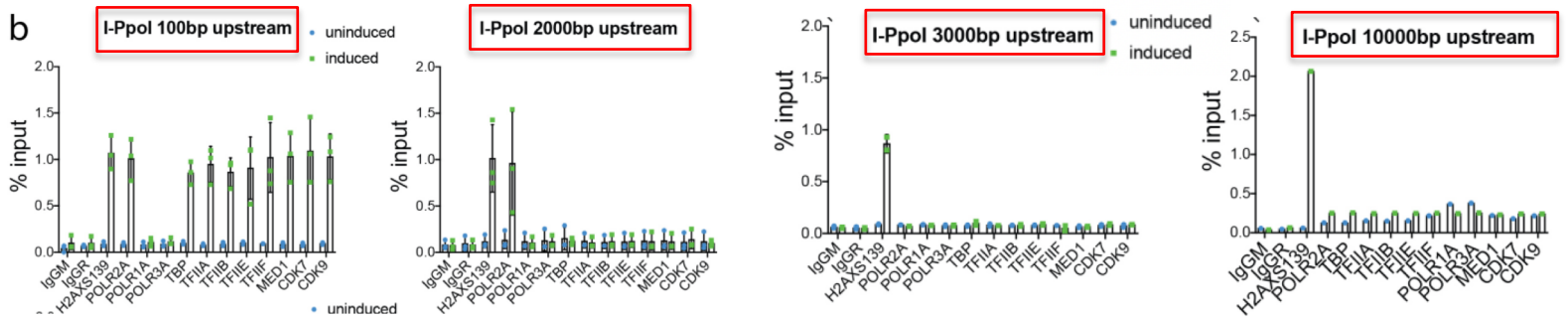
PMC components and RNAPII are strongly associated only in proximity of the DSB (100bp) → **Active transcription at DSB?**

IS RNA POLYMERASE II RECRUITED TO SITES OF DNA DAMAGE?

PMC: Preinitiation Complex (PIC, TFIIA, B, E, F,) + MED1 + CDK9
HeLa cells whit a specific endogenous locus cleaved using and inducible I-PpoI endonuclease (analpgy to SceI) to induce DSB
ChIP analyses at different distances to the cut

ChIP: Primers locatet at, upstream and downstream of DNA damage site (I-PpoI)



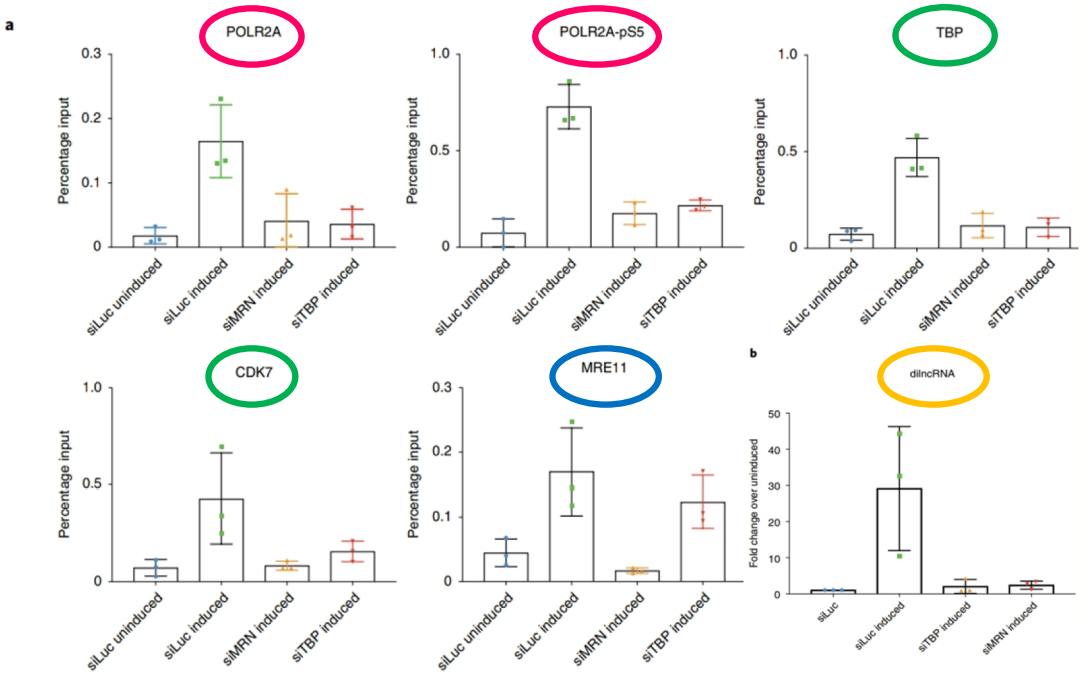


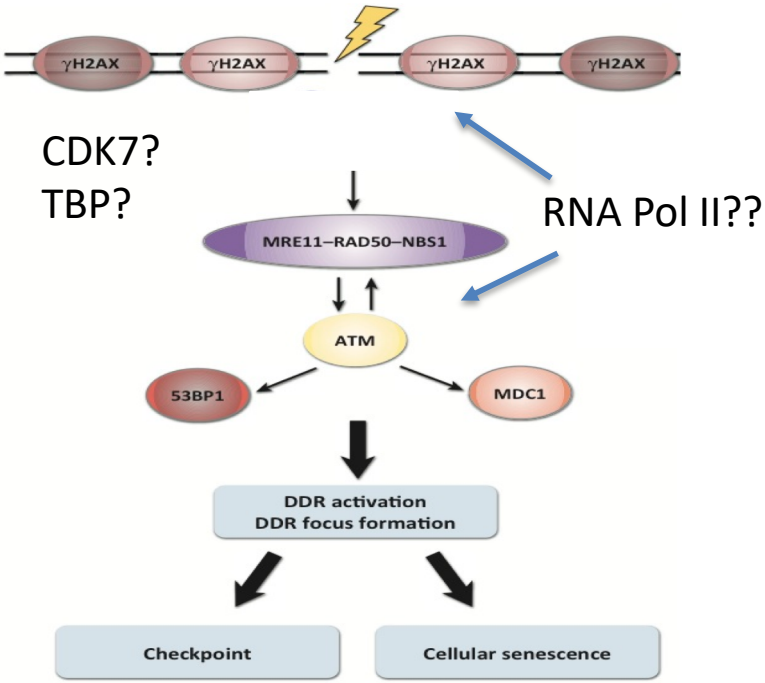
PMC components and RNAPII are strongly associated only in proximity of the DSB (100bp) → **Active transcription at DSB?**

DO DNA DAMAGE SIGNALLING FACTORS RECRUIT RNA Pol II TO SITES OF DNA DAMAGE

How is RNAPII recruited ad DSB site?

HeLa cells with locus cleaved by I-PoI endonuclease → RNA interference by transfection with siMRN (MRN complex) and siTBP (PIC complex)





siTBP and siMRN inhibited the accumulation of RNAPII

Similar to their assembly at promoters:

- CDK7 recruitment depends on TBP and MRN
- TBP recruitment depends on MRN

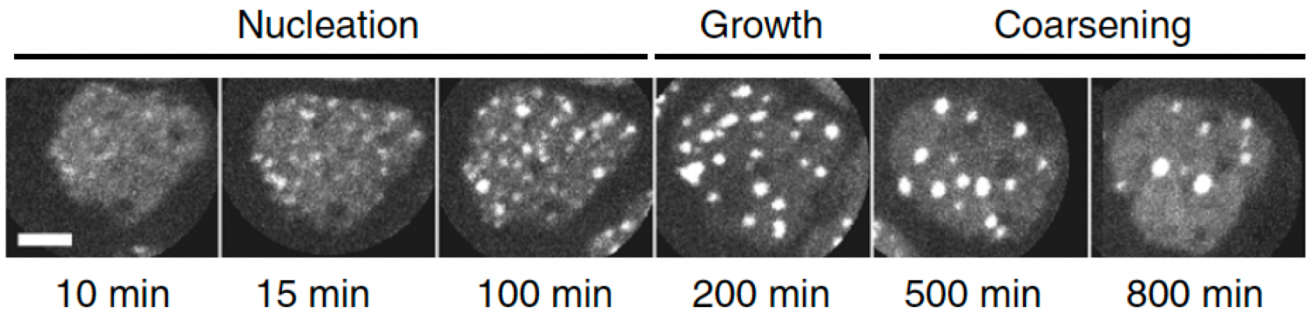
MRN recruitment does NOT depends on TBP

siTBP and siMRN inhibited DNA DamageRNA synthesis

MRN and PIC are important for recruitment and stabilitazion of RNAPII and also impact each other

Note: RNAPII pSer5 = evasion to transcriptional elongation

DNA damage drives LLPS at breaks

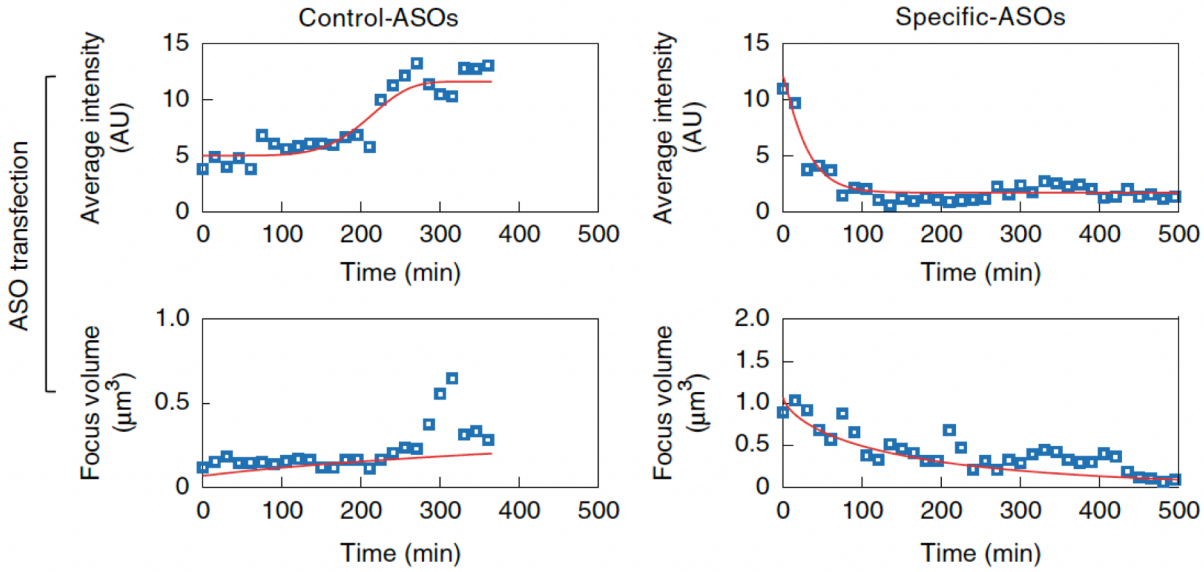


53BP1-GFP TIFF formation via LLPS

Cells expressing 53BP1-GFP with a specific endogenous locus cleaved using and inducible I-PpoI endonuclease (analogy to SceI) to induce DSB

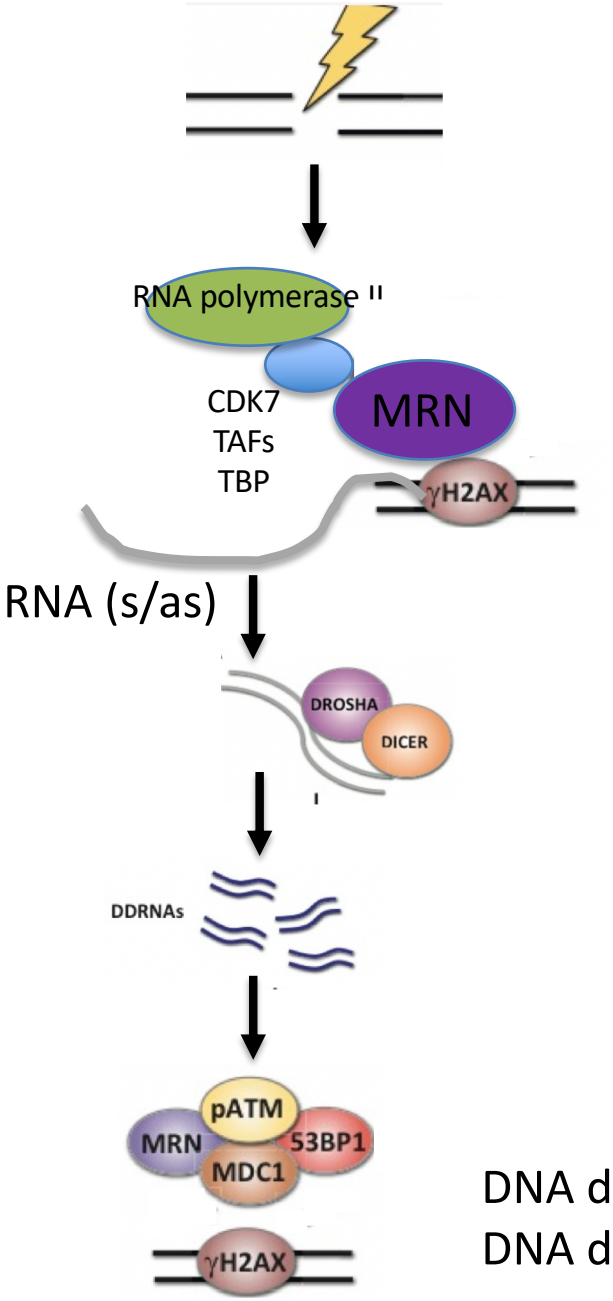
→ Induce cutting and transfect cells with anti-sense oligos (control and taregted toward DNA damage site derived RNAs)

→ Measure foci intensity and diameter



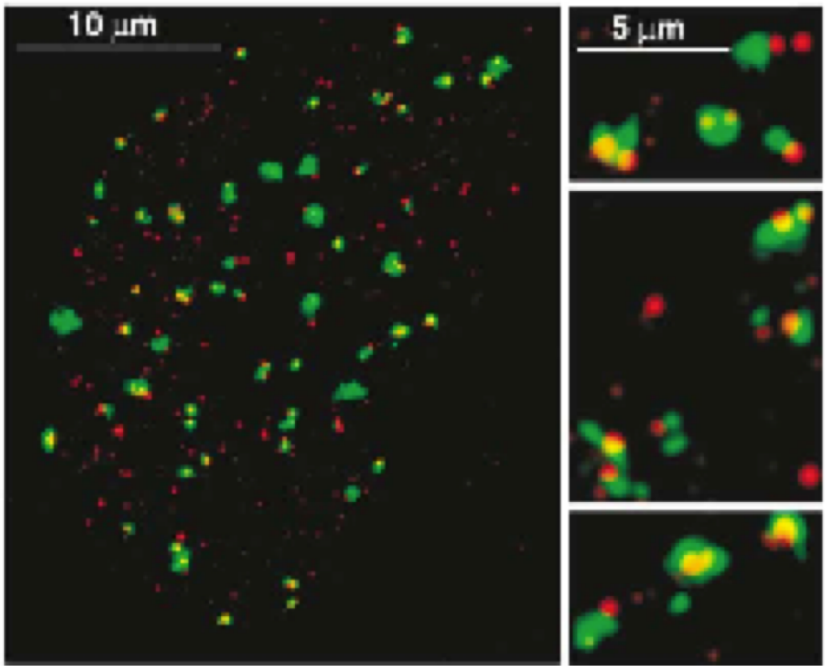
DNA damage RNAs are central for DNA damage foci formation in life cells

DNA DAMAGE RESPONSE RNAs (DDRNA) CONTROL THE DNA DAMAGE RESPONSE



DNA damage foci formation
DNA damage sigmalling

DNA Damage Foci



γH2AX

53BP1
MRE11
P-ATM