DDRNAs

DNA DAMAGE RESPONSE RNAs

LETTER

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

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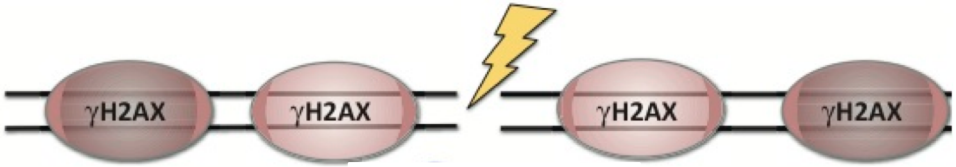
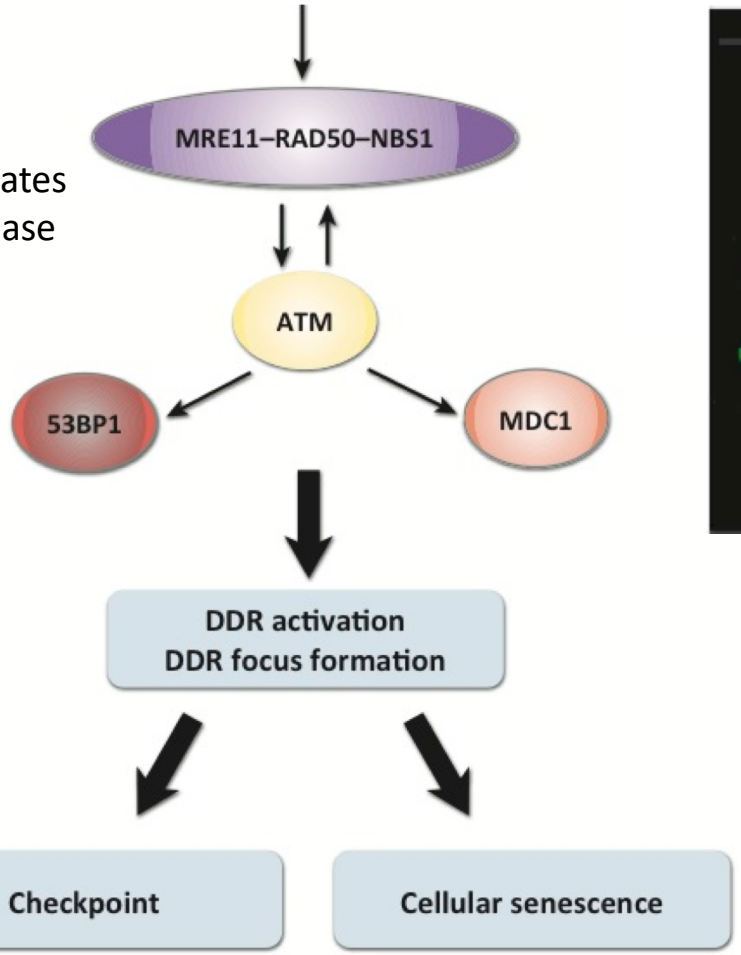
The DNA damage response revisited

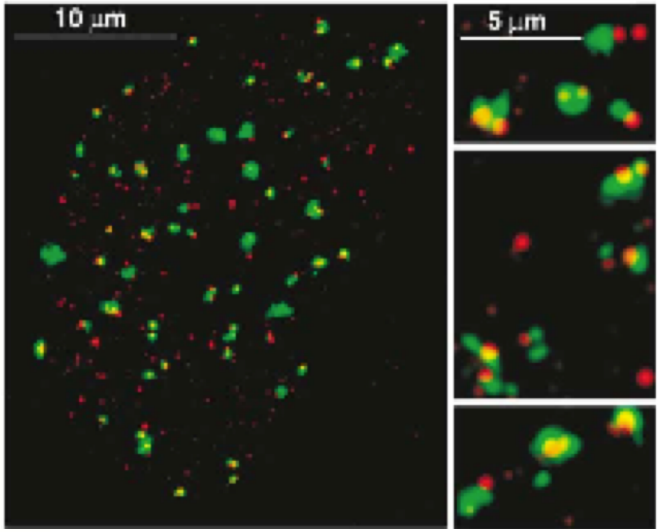


The MRN complex

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



DNA Damage Foci



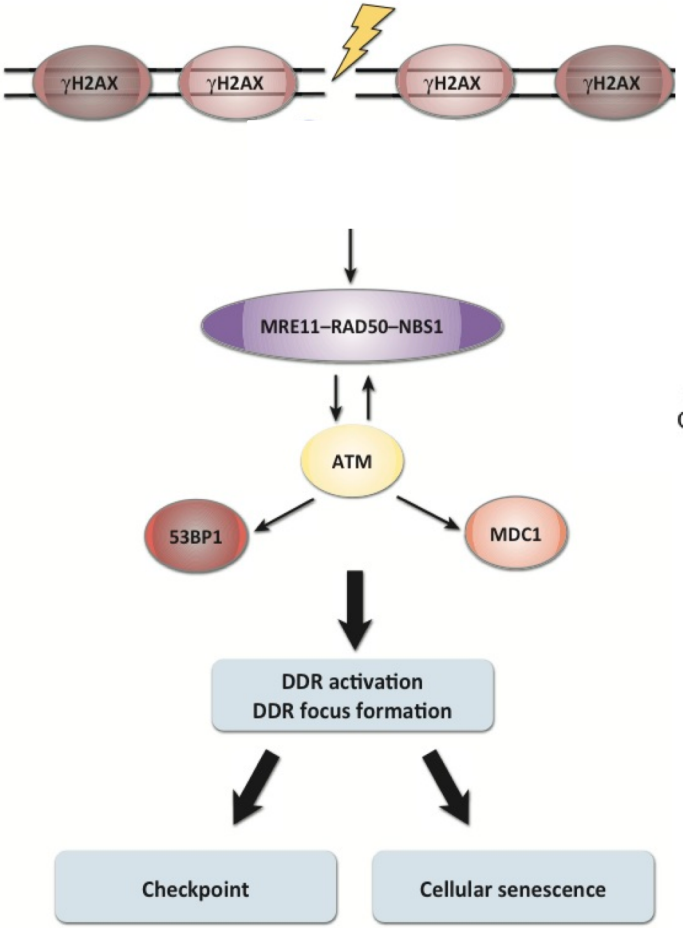
γH2AX

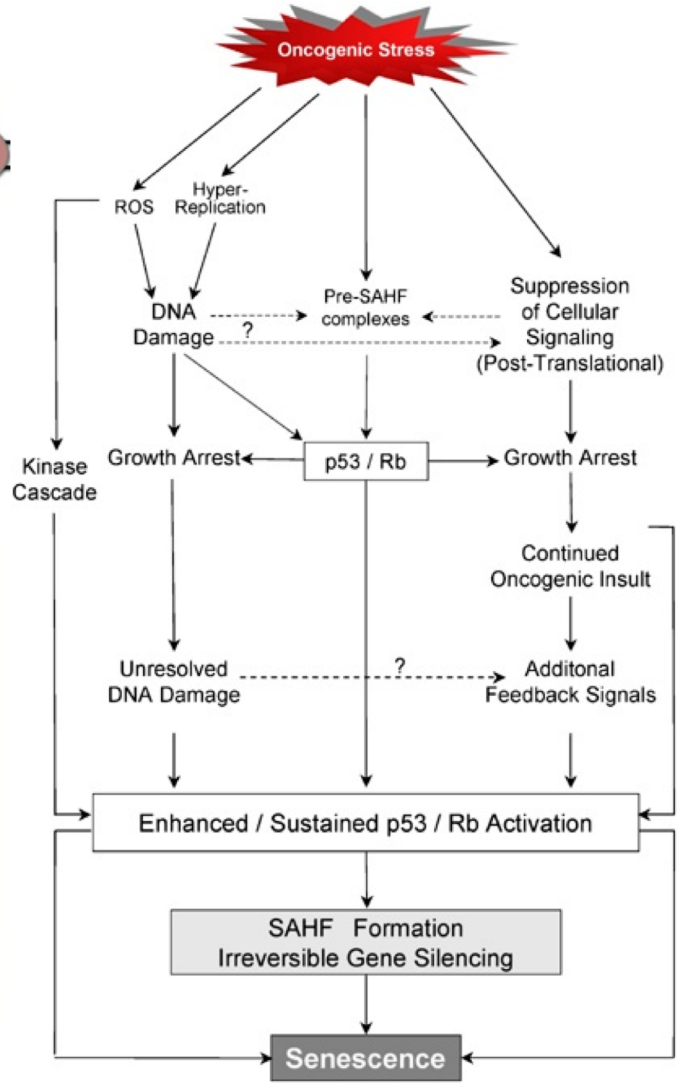
53BP1

MRE11

P-ATM

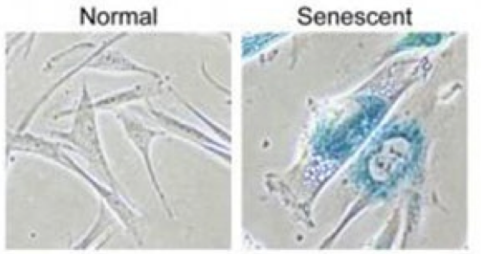
Model system for persistent DNA damage: ONCOGENE INDUCED SENESCENCE

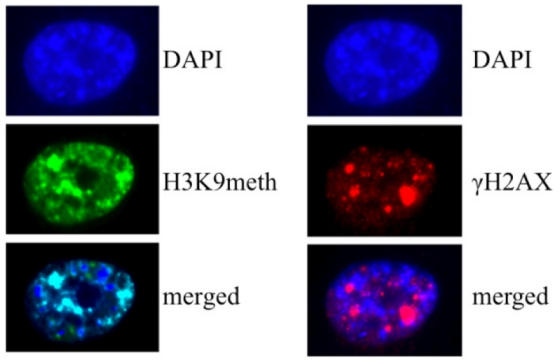




Senescence markers

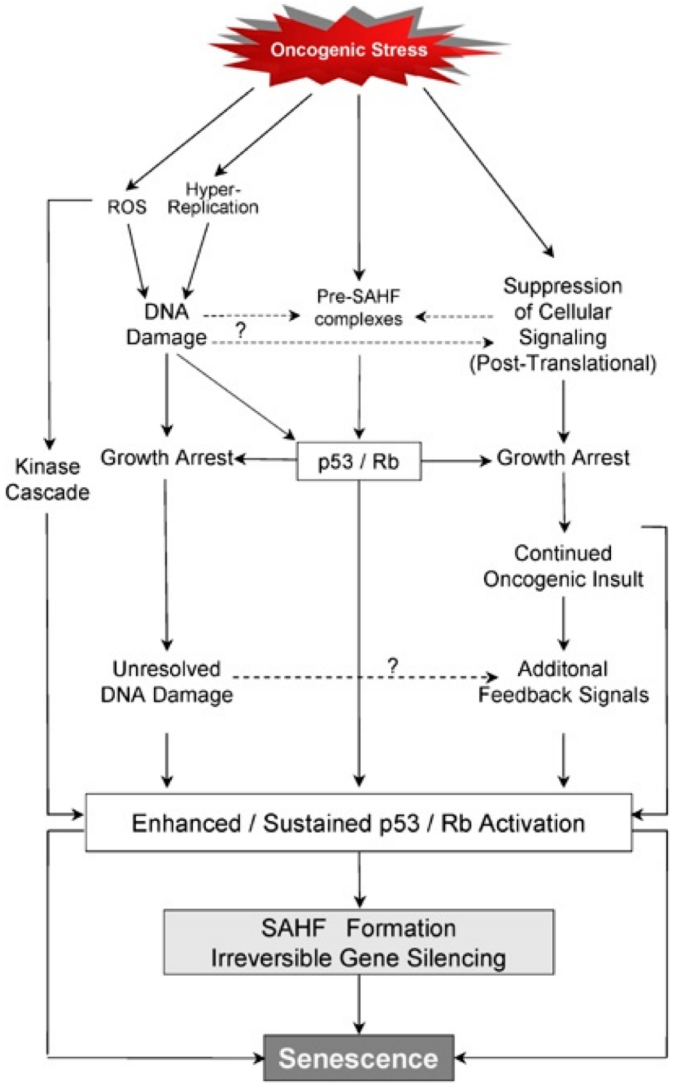
Beta galactosidase positve cells
Big foci of heterochromatin
That colocalize with gH2AX





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

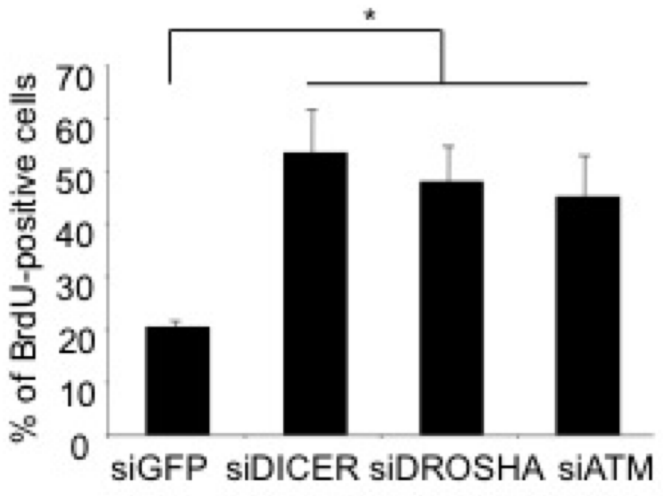
LOSS OF DICER/DROSHA REDUCES ONCOGENE INDUCES SENESCENCE



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")

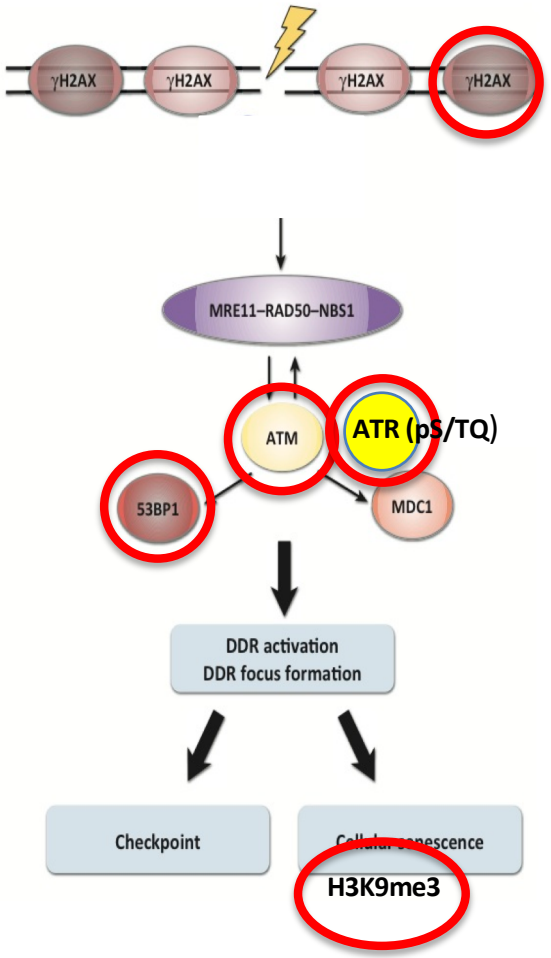
H-RasV12 drives excessive proliferation → Accumulation of DNA damage → Senescence → SAHF

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

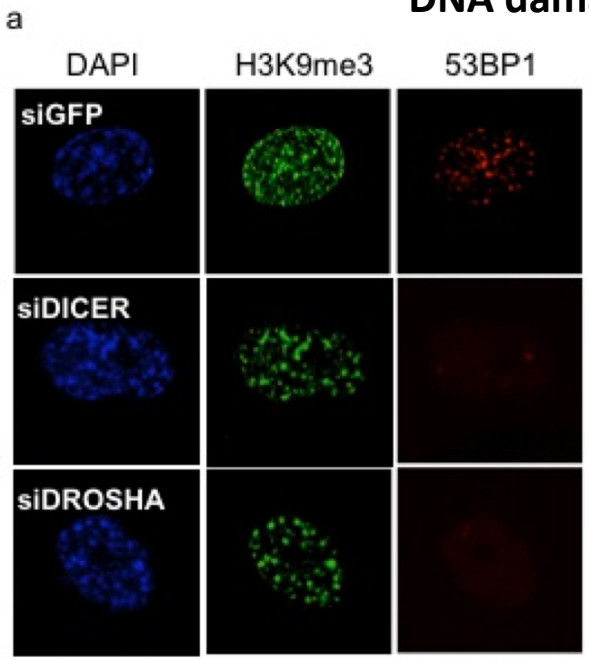


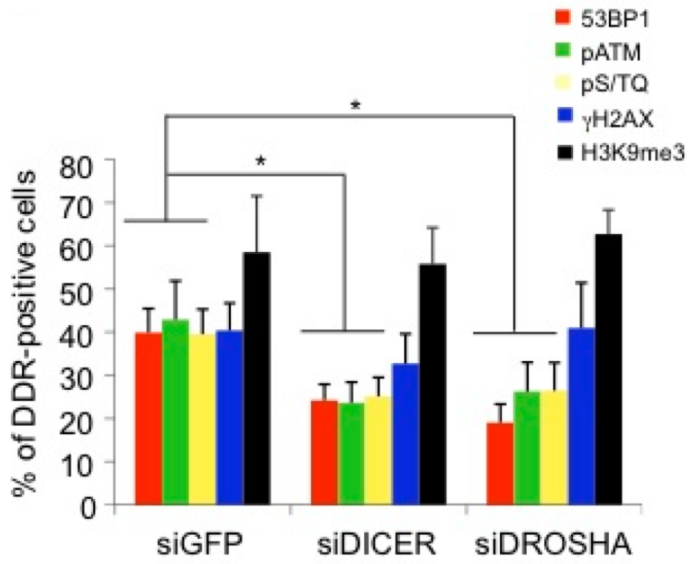
BrdU is incorporated in S-Phase and can be detected
Using an antibody (IF); more BrdU+ cells = more proliferation

LOSS OF DICER/DROSHA REDUCES DNA DAMAGE SIGNALLING IN OIS CELLS

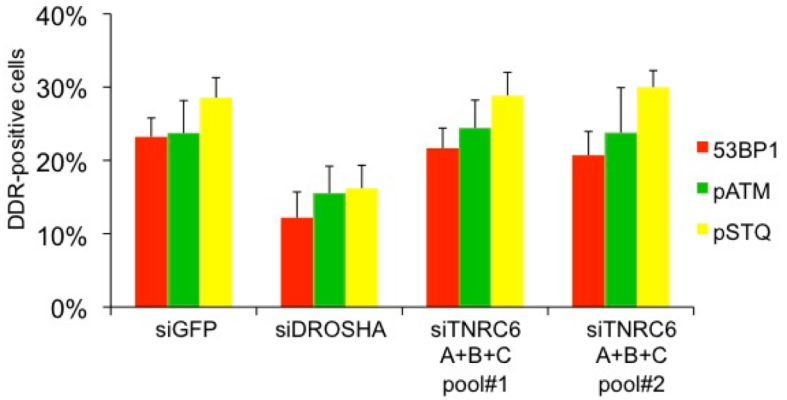


siRNA/miRNA pathways are important for DNA damage response





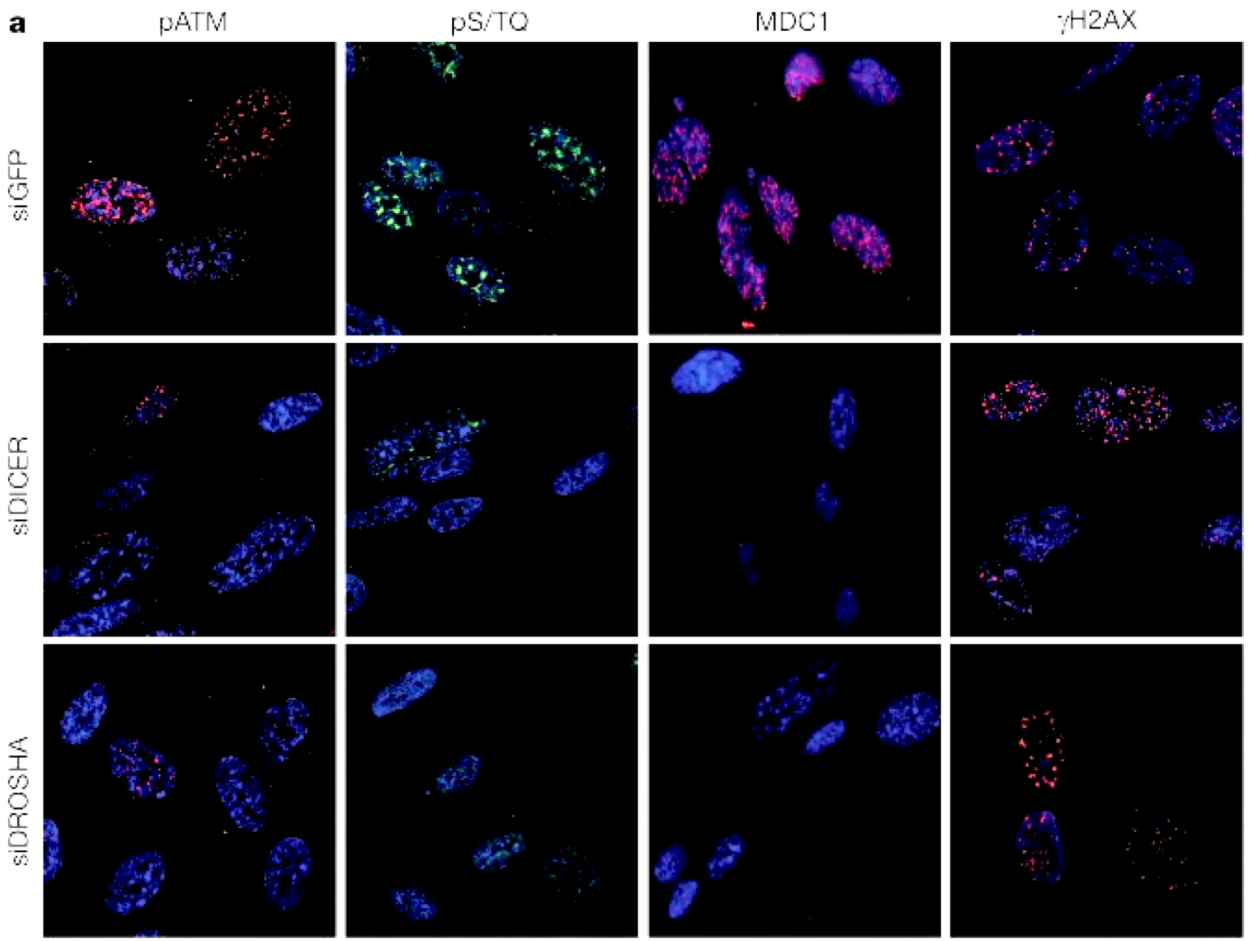
KNOCK DOWN OF GENES IMPORTANT FOR miRNA FUNCTION (TNRC6A,B,C; translational repression) **do not impact on DNA damage response**



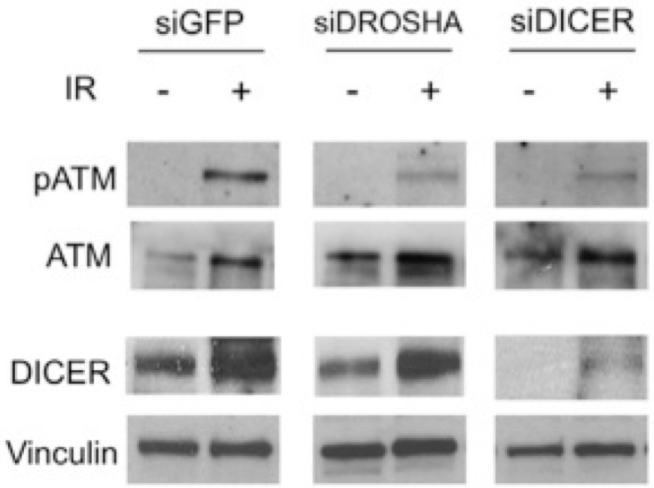
Note: multisite phosphorylation of SQ/TQ motifs is required for normal DNA-damage responses

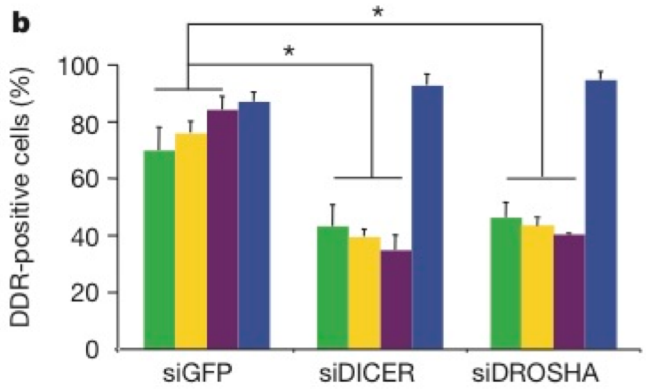
siRNA PATHWAYS ARE INVOLVED IN THE CONTROL OF DNA DAMAGE RESPONSE

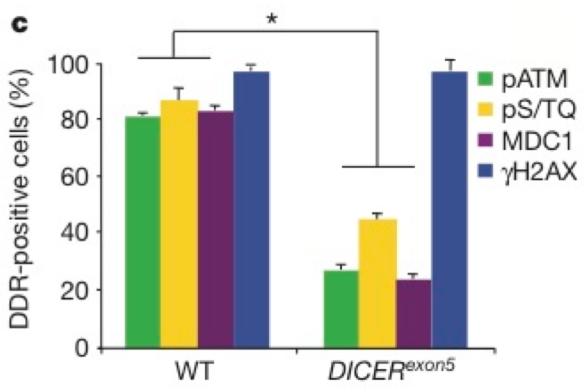
ANOTHER MODEL: GAMMA IRRADIATION OF NORMAL FIBROBLASTS



Knock-down of DICER and DROSHA Impairs the activation of a DNA damage response In gamma irradiated cells







IS RNA REQUIRED TO TRIGGER AN EFFICIENT DNA DAMAGE RESPONSE?

Model
Heterochromatin

Permeabilize
Cells (detergent)

→

Treat with
RNase

→

Heterochromatin
Proteins are lost

Model
DNA damage

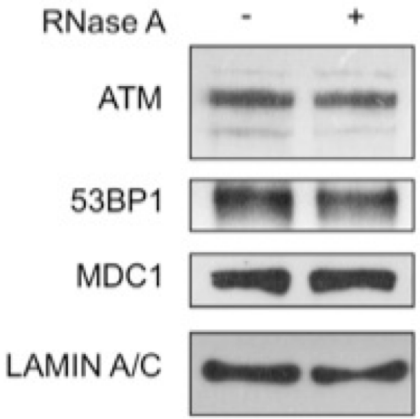
HeLa cells +
Gamma irradiation+
permeabilize

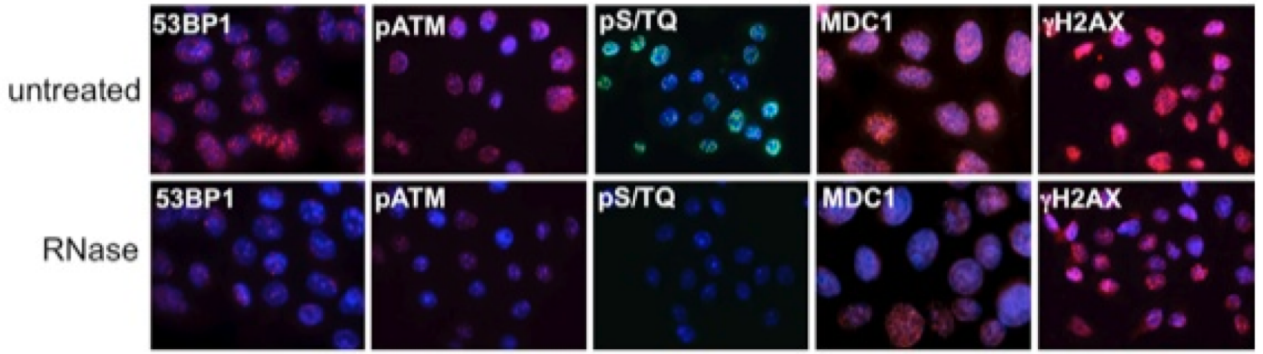
→

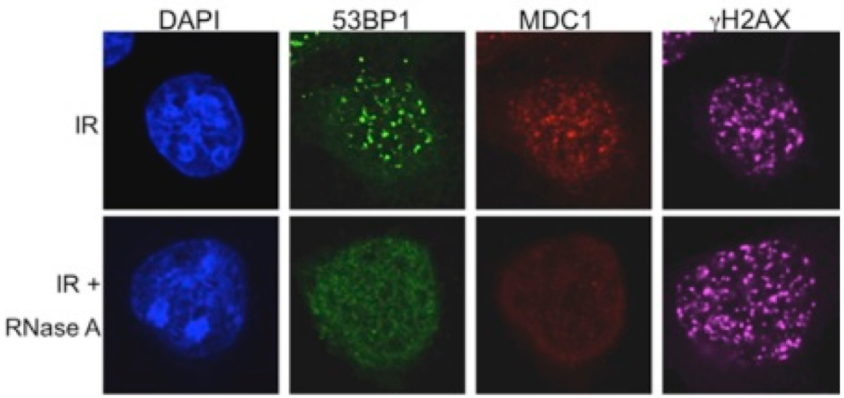
Treat with
RNAse

→

Check protein
Markers for DNA damage







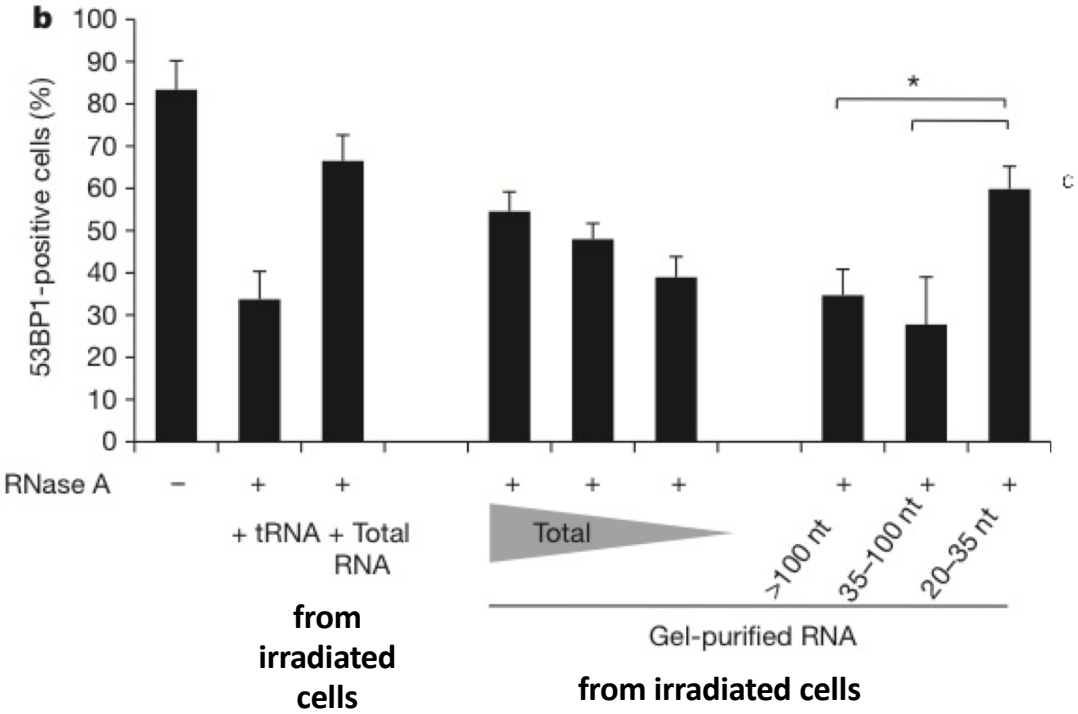
RNase treatments reduces the amount of DNA damage markers after gamma irradiation

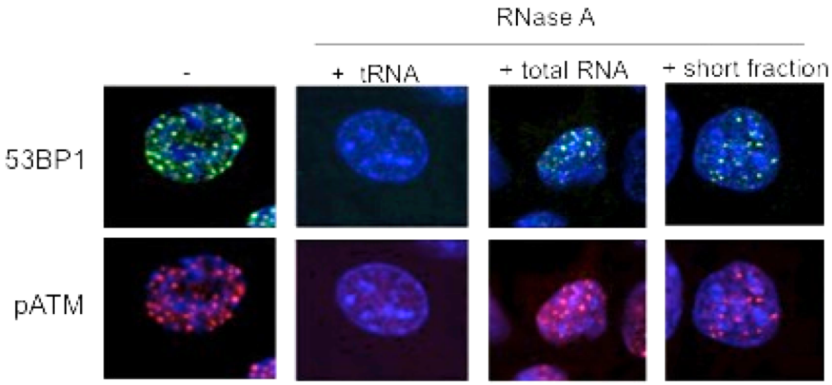
WHAT KIND OF RNA IS REQUIRED TO TRIGGER AN EFFICIENT DNA DAMAGE RESPONSE?

Model DNA damage

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

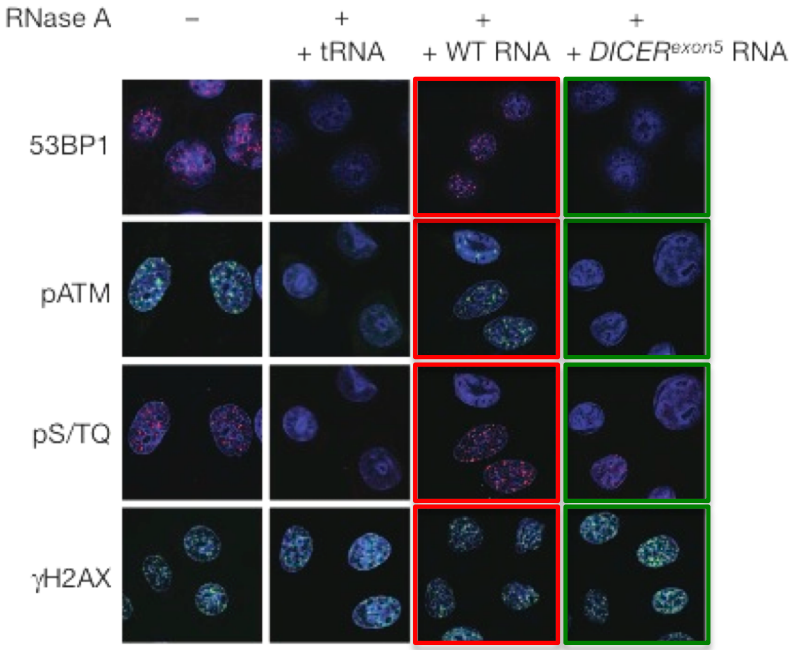
→ Add different classes of RNA → Check protein Markers for DNA damage





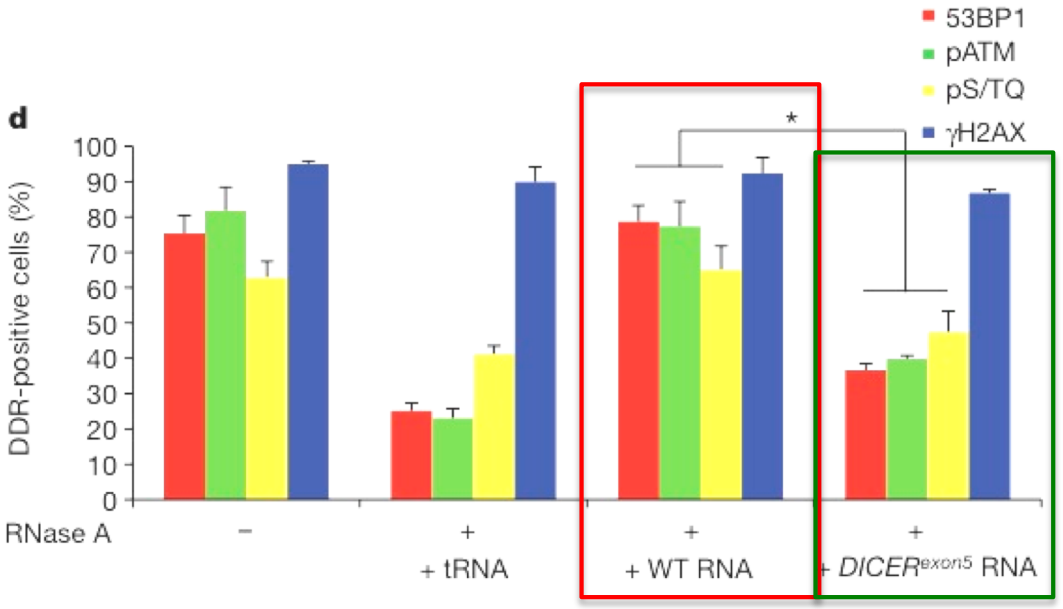
A short RNA fraction (20-35 nt) rescues DNA damage response after RNAse treatment
= POTENTIAL DICER/DROSHA PRODUCTS

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



RNA From Irradiated Dicer wt Cells = Small RNAs present

RNA From Irradiated Dicer KO Cells = NO small RNAs



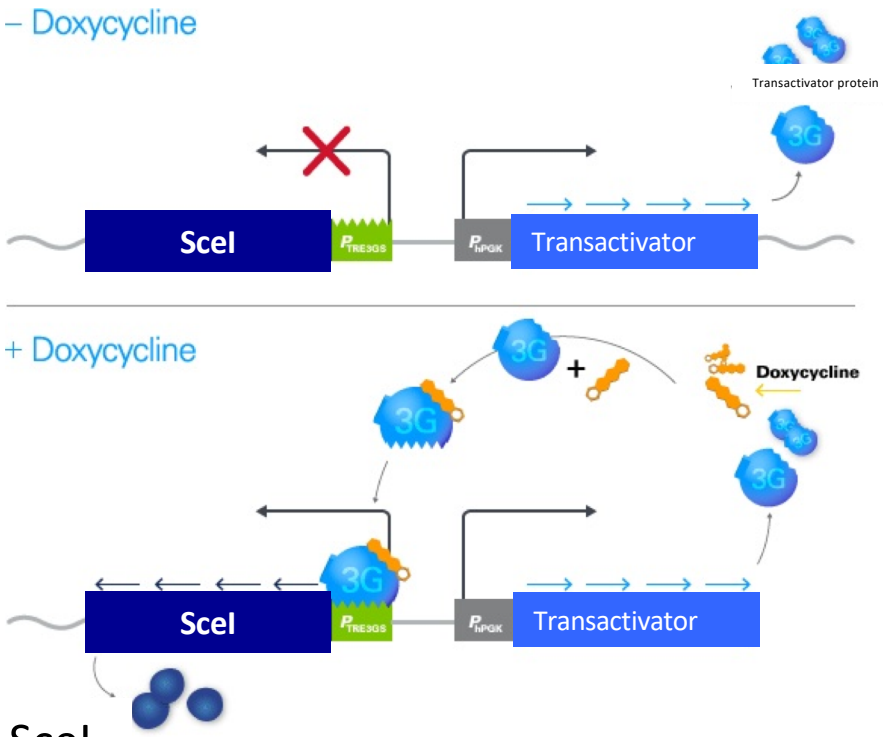
Total RNA from Dicer null cells cannot rescue defects of DNA damage foci formation after RNAse treatment

→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 MODEL SYSTEM TO STUDY THE KINETICS OF DNA DAMAGE

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



SceI

SceI

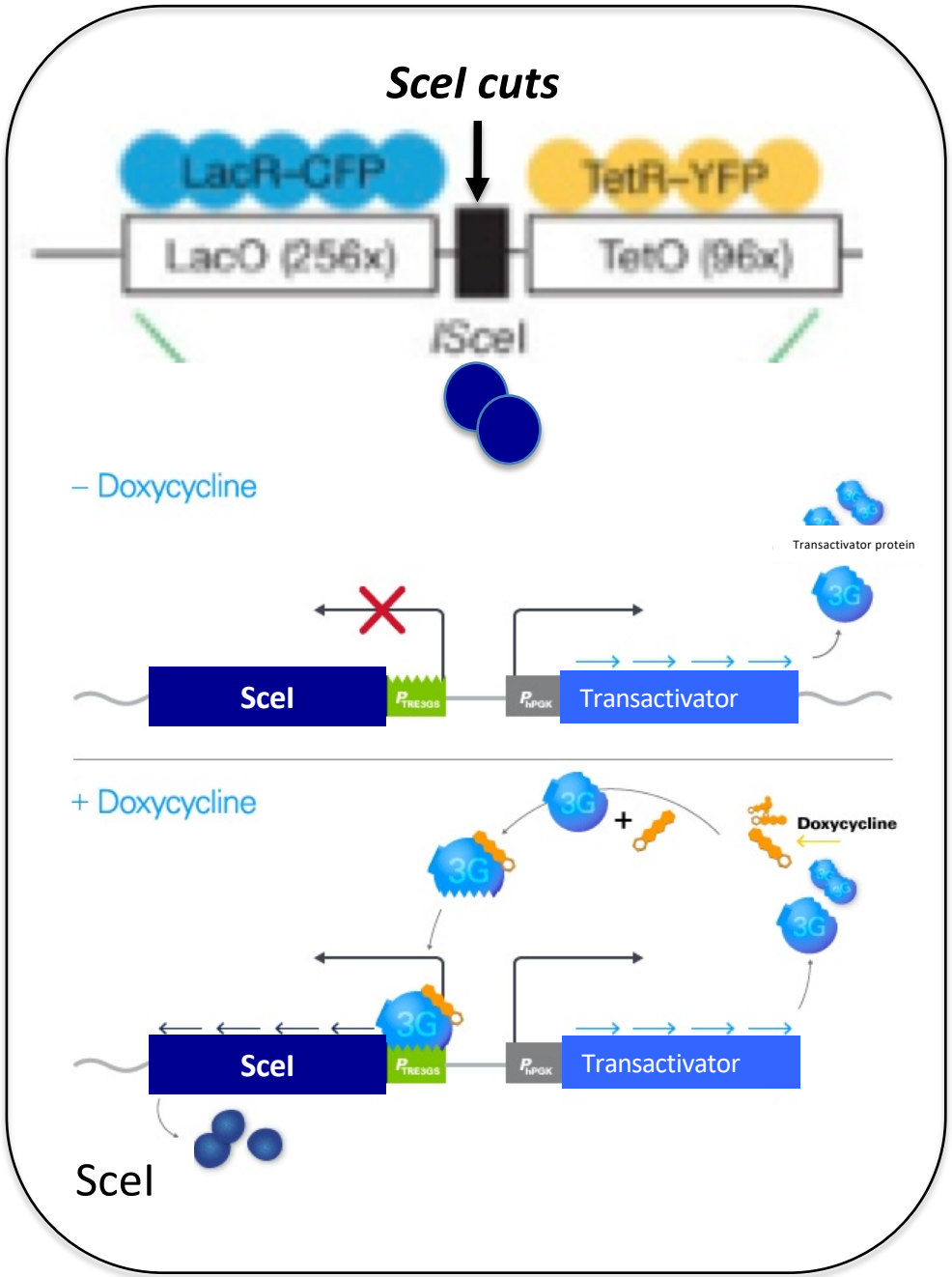
5′...TAGGGATAACAGGGTAAT...3′
3′...ATCCCTATTGTCCCATTA...5′

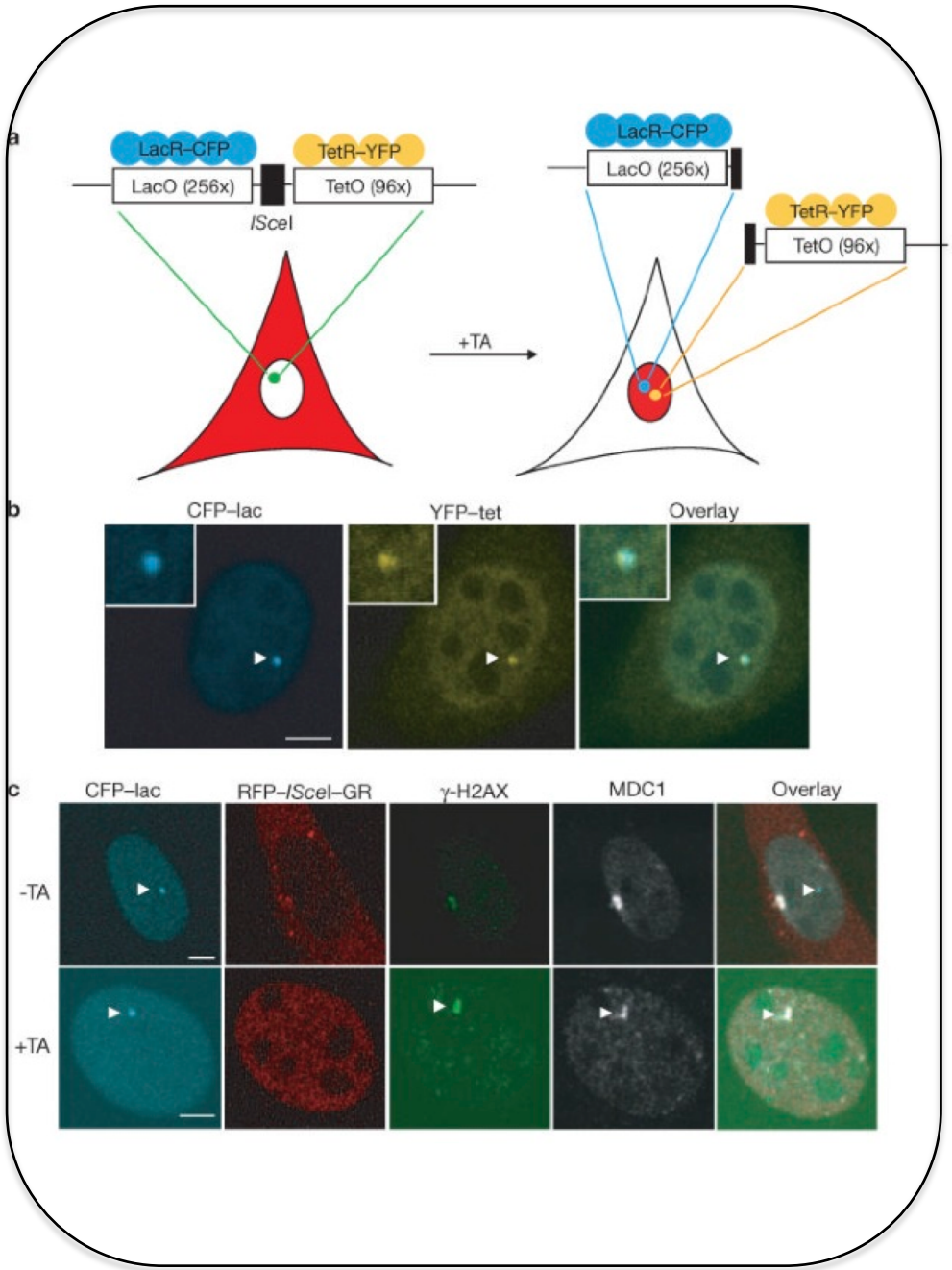
**SceI is a restiction enzyme
That does not cut in the human
genome**

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

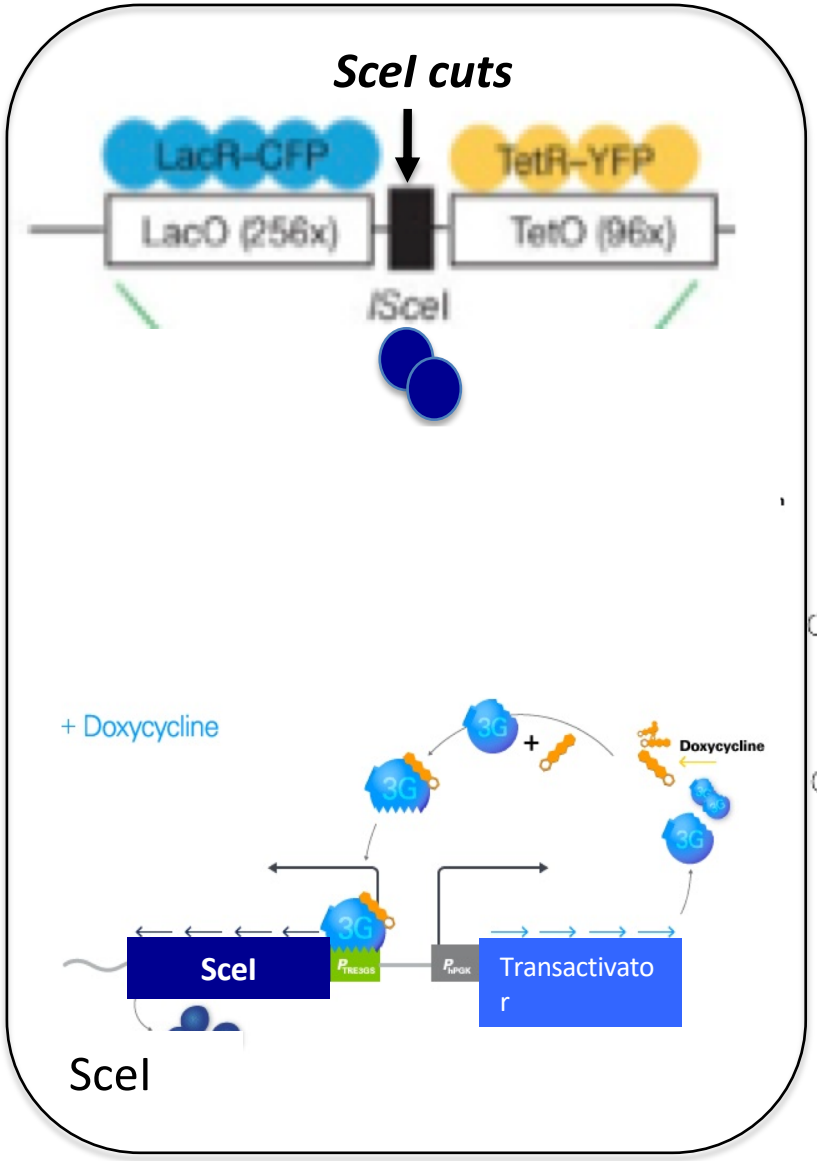
LETS INTRODUCE A SceI SITE AND MARK THE SceI SITE USING SEQEUNCES BOUND BY THE Lac REPRESSOR

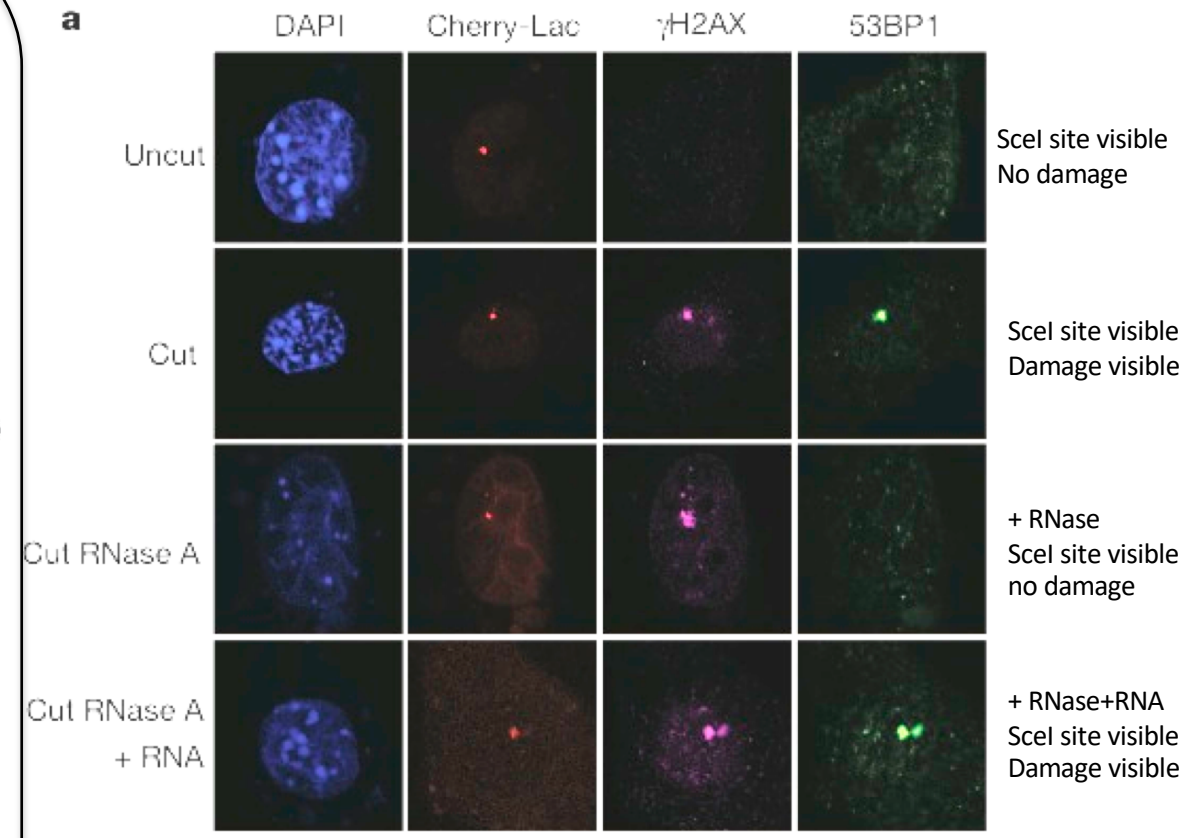
A MODEL SYSTEM TO STUDY THE KINETICS OF DNA DAMAGE





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



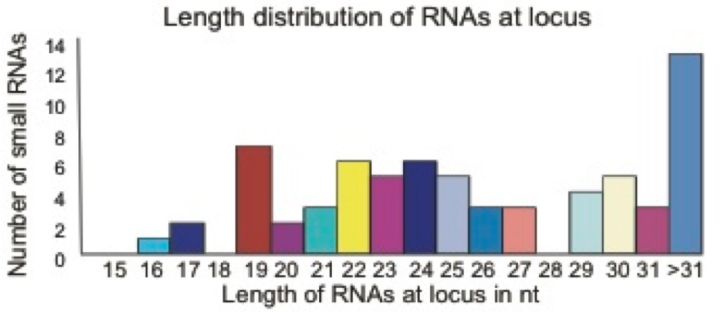


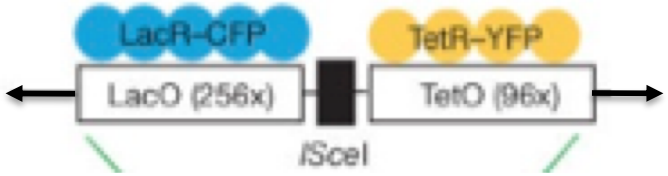
→ **RNA was prepared from cells that have cut the SceI site**

→ **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

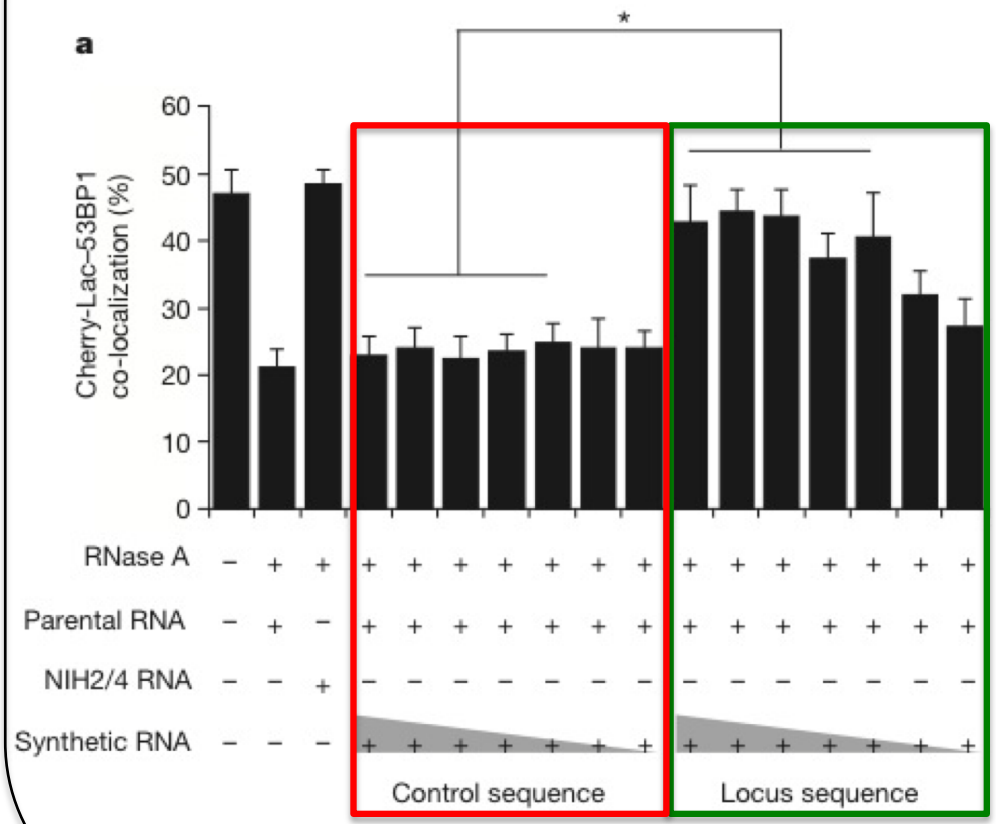
Take SceI cells:
-induce cutting
- prepare smallRNA fraction
-make RNASeq
detect small RNAs from locations around the SceI sites





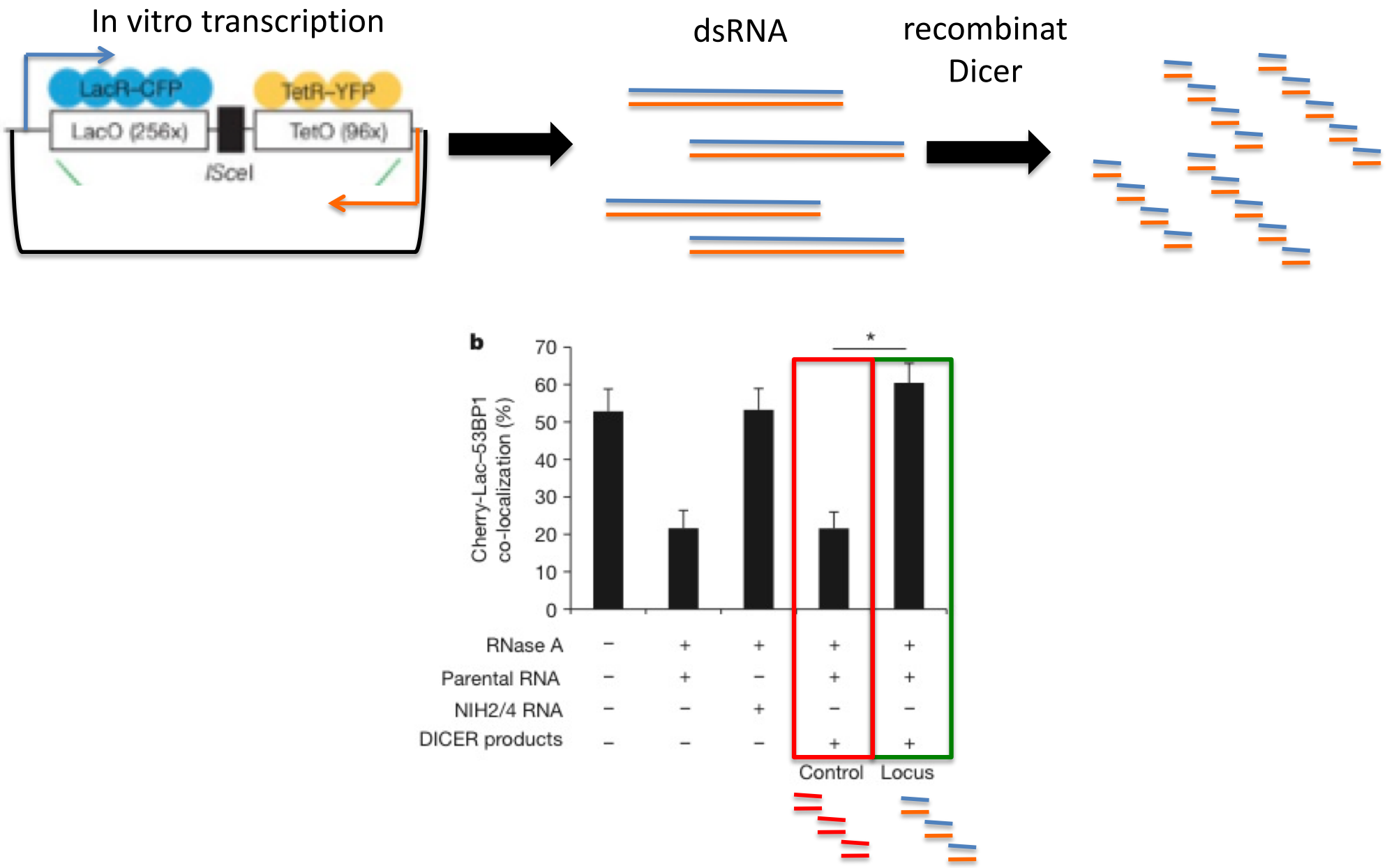
!!!!!Small RNAs (16-31) arising from regions around the SceI site can be identified !!!!!

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



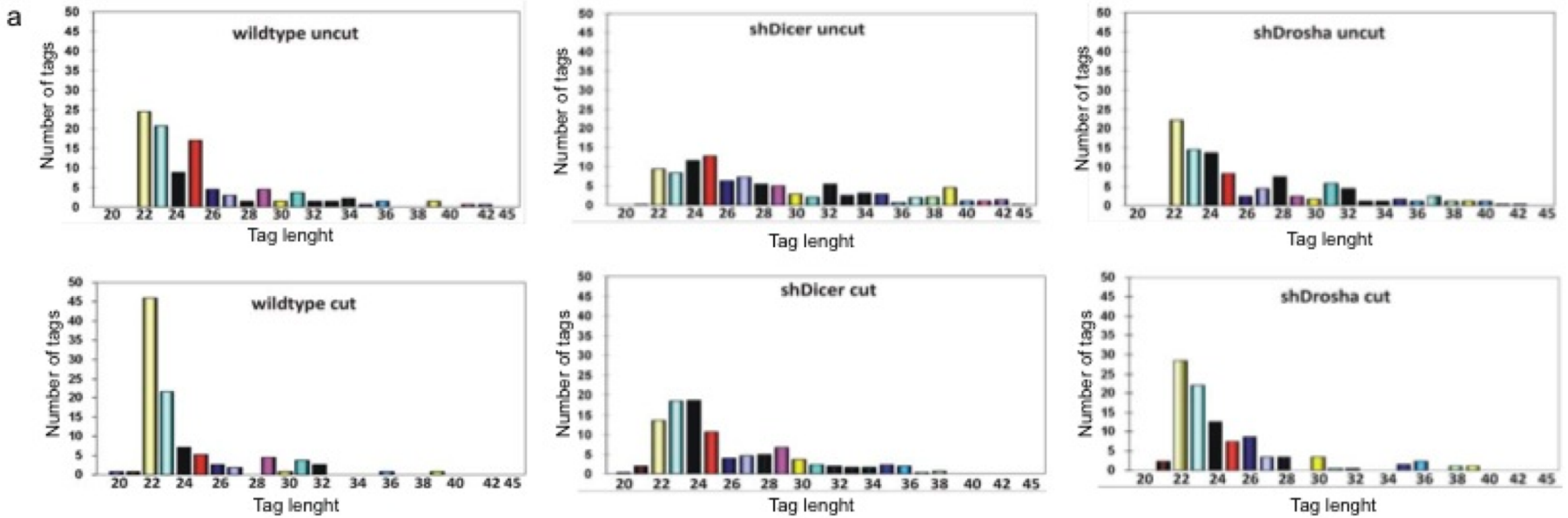
SceI region

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

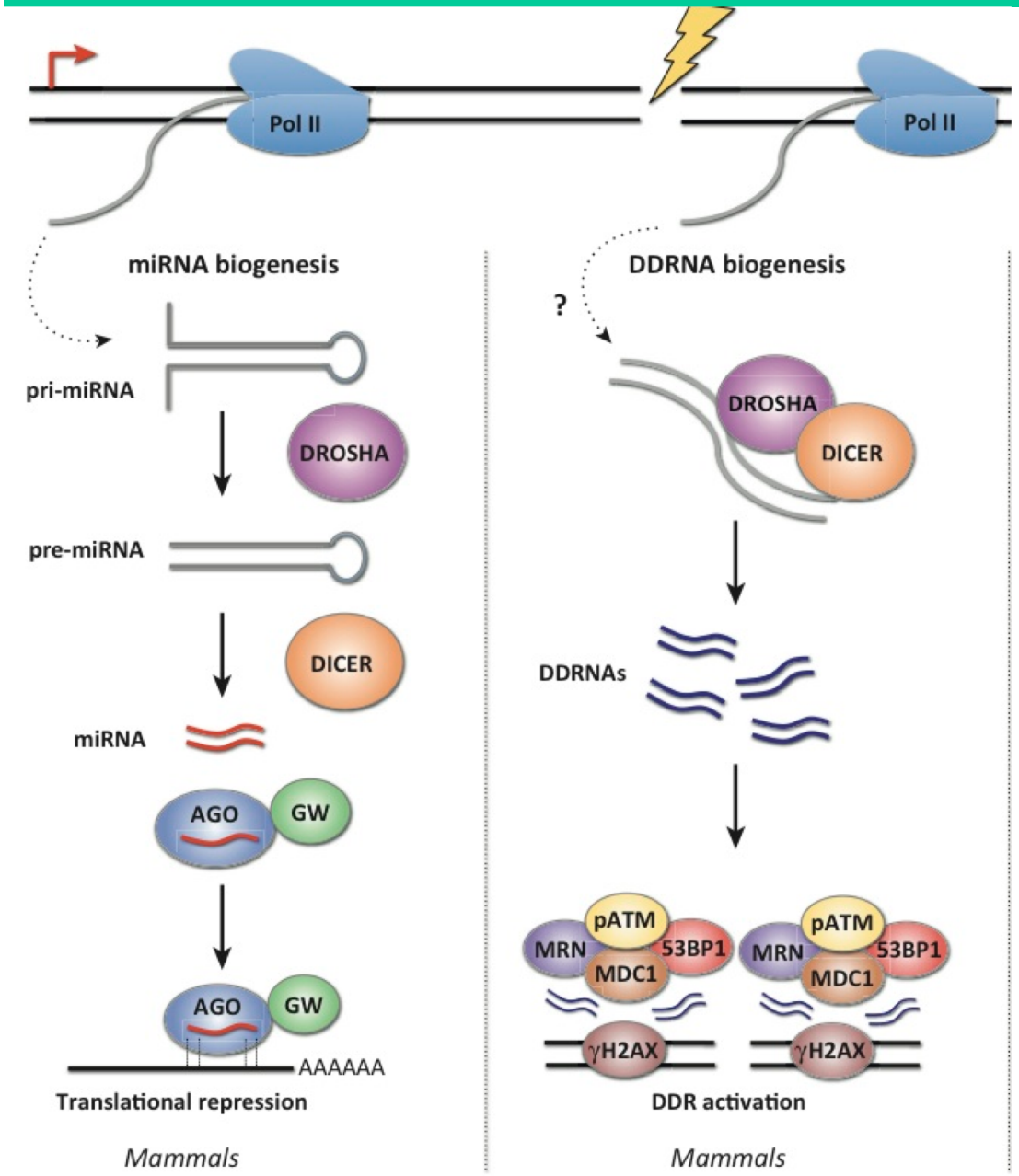
Take SceI cells
- Knock down DICER or DROSHA:
-induce cutting
- prepare RNA
-make RNASeq
detect small RNAs from locations around the SceI sites

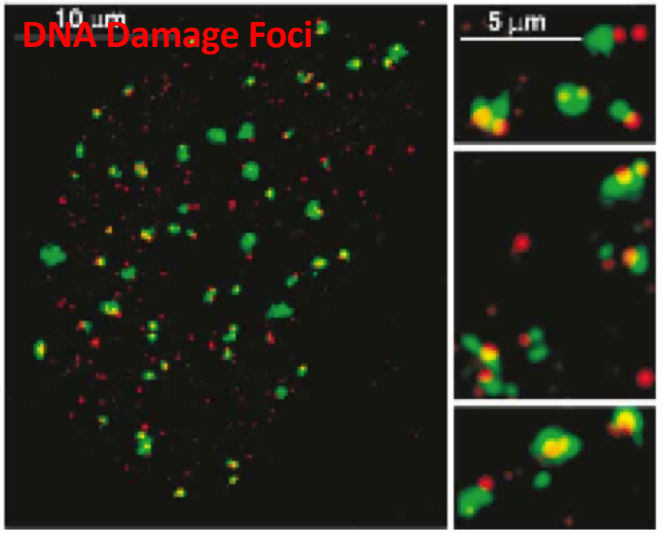


DICER RNAi *DROSHA RNAi*

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





γH2AX
53BP1
MRE11
P-ATM

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.