Microirradiation for single strand break repair

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Creating sites of DNA damage

Localized sites of DNA damage

Adapted from Polo and Jackson Genes Dev. 2011

El-Khamisy et al. Nucl. Acids Res. 2003

Agagoshi et al. DNA Repair 2010
Laser microirradiation

External laser source:
- UV (337 – 400 nm)
- Two-photon source (700-850 nm)

Diagram showing the setup with a sample, dichroic mirrors, confocal scanner, detector, and a graph of mean intensity over time.
What can a microirradiation experiment reveal?

1. Determine if a protein responds to induced damage site (screening for novel interactants or critical protein domains)
2. Peak recruitment time of proteins to site of induced damage
3. Residence time of the protein at the site of damage
4. Co-localization of proteins at site of DNA damage
5. Time courses for repair

Information you get out is only as good as the rigor used to characterize the system.

1. Types of damage induced
2. Cellular background used
3. Fluorescent proteins vs. endogenous proteins
Designing your microirradiation experiment

1. Induce a specific type of DNA damage
   A. Power
   B. Time

2. Monitor the response of repair proteins to that site of damage
   A. Live cell
   B. Immunofluorescence
UV and near UV Wavelengths

266 nm irradiation (Dinant J. Cell Sci. 2007)

337 nm irradiation (Kong NAR 2009)

405 nm irradiation (Hanssen-Bauer EMM 2011)

365 nm irradiation (Lan PNAS 2004)

Endogenous photo-sensitizers

Type I - reaction

Type II - reaction

O_2

Fe^{3+}

- OH

Superoxide

Hydroxyl radicals

Singlet oxygen

Pyrimidine dimers

Oxidative DNA modifications

XRCC1

53BP1

8-oxoG

GFP-XPA

CPD

Merge

\gamma H2AX

3-OHdG

2 min

10 min

30 min

60 min

120 min
Inducing a specific mixture of breaks

1. Laser power- ideally the amount of energy that passes through the objective into the sample

2. Time- the duration the laser spends on the defined ROI (pixel dwell time, frame rate, iterations)

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Power Reported</th>
<th>Damage Characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>337 nm</td>
<td>~0.08 μJ</td>
<td>8-oxodG, 6,4PPs, CPDs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>γH2AX positive</td>
</tr>
<tr>
<td>364 nm</td>
<td>~ 0.17 μJ</td>
<td>γH2AX negative</td>
</tr>
<tr>
<td>365 nm</td>
<td>~ 0.19 μJ</td>
<td>γH2AX negative</td>
</tr>
<tr>
<td>(low power)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>365 nm</td>
<td>~ 0.49 μJ</td>
<td>γH2AX positive</td>
</tr>
<tr>
<td>(high power)</td>
<td></td>
<td>8-oxodG</td>
</tr>
<tr>
<td>405 nm</td>
<td>~7.5 μW</td>
<td>Low power, γH2AX negative</td>
</tr>
<tr>
<td></td>
<td>~16-800 μW</td>
<td>8-oxodG and γH2AX positive</td>
</tr>
</tbody>
</table>
BER or SSBR

AP site

AP Endonuclease

3' OH 5' dRP

PARP

PARylation (●), Recruitment BER

3' OH 5' dRP

Pol β XRCC1 Ligase III

Ligase III

PARP

3' P 5' P

PNKP XRCC1 Ligase III

Pol β XRCC1 Ligase III

PCNA XRCC1 FEN1 Ligase III
Timeline of Repair Events

- OGG-1
- APE-1
- PARP-1
- XRCC1
- Pol β
- PNKP

- Sensing/Signaling
- SSB repair
- DSB signaling

γH2AX
53BP-1
Characterization considerations

Lan *PNAS* 2004


Hanssen-Bauer *DNA Repair* 2012

Gassman *PLoS* 2012
Tale of two wavelengths, 355 and 405 nm

1. Induce single strand breaks or base lesions without a sensitizer

2. Monitor the response of repair proteins to that site of damage
   A. Live cell
   B. Immunofluorescence
Laser microirradiation

Stage Registration !!!!!!!
Recruitment of XRCC1

CHO XRCC1-GFP
15 sec post damage

355 nm

405 nm

2 s

10 s

8 fps

0.5 fps

Mean Intensity of ROI

Time (sec)
Complex break mixture at high powers

8-oxodG

CPD

1 min post-irradiation

10 sec

355 nm

405 nm

2 sec

8 fps

0.5 fps

Nate Holton
Comparing XRCC1 recruitment

~ 19 µJ
CHO XRCC1-GFP

355 nm 2 sec

MEF Xrcc1-GFP

1.5 mW

405 nm 8 fps

CHO XRCC1-GFP

15 s post damage

MEF Xrcc1-GFP

Intensity = Focus Nucleus

Nate Holton
Better separation in between SSB and DSB with 355 nm

Graphs showing relative fluorescence over time for different wavelengths and markers.
Uniformity of DSB

Nate Holton

Uniformity of DSB

\[ \gamma H2AX \]

\[ 53BP1 \]

8 fps 405 nm
SSBR in U2OS XRCC1-GFP

355 nm 2 sec

ROI 2 → ROI 3

~12.35 min
SSBR in U2OS XRCC1-GFP

1 min

5 min

20 min
SSBR in U2OS endogenous
SSBR in U2OS endogenous

355 nm 2 sec

XRCC1  53BP-1  γH2AX  DAPI  MERGE

1 min

5 min

20 min

40 min
SSBR in U2OS endogenous XRCC1

355 nm 2 s

**U2OS XRCC1-GFP**

- **XRCC1**
- **γH2AX**
- **53BP-1**

**Intensity** = \[
\frac{\text{Focus}}{\text{Nucleus}}
\]

Normalized to undamaged cells
355 nm 750 ms U2OS
Endogenous XRCC1 750 ms U2OS

XRCC1

Mean Focus Intensity/Nuclei

γH2AX

53BP-1

Mean Intensity of ROI

Time (min)
SSBR in A549 XRCC1-GFP

A549 XRCC1-GFP

355 nm 2 sec
SSBR in A549 endogenous XRCC1

355 nm 2 sec

![Graphs showing SSBR in A549 endogenous XRCC1 with measurements for γH2AX and 53BP-1 over time.](image)
SSBR in A549 endogenous XRCC1

355 nm  750 msec

XRCC1

\[ \gamma H2AX \]

53BP-1
## Microenvironment influence response

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>XRCC1 response</th>
<th>γH2AX</th>
<th>53BP-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO-K1 (GFP-XRCC1)</td>
<td>Peaks ~ 1 sec, Resolves ~ 8 min</td>
<td>10 min (weak) 40 min</td>
<td>20 min 40 min</td>
</tr>
<tr>
<td>U2OS (GFP-XRCC1)</td>
<td>Peaks ~ 1 sec, Resolves ~12 min</td>
<td>5 min</td>
<td></td>
</tr>
<tr>
<td>U2OS</td>
<td>Resolves ~20 min</td>
<td>5 min</td>
<td>10 min</td>
</tr>
<tr>
<td>A549 (GFP-XRCC1)</td>
<td>Peak~ 1 sec, Resolves &gt; 20 min</td>
<td>5 min</td>
<td></td>
</tr>
<tr>
<td>A549</td>
<td>Resolves ~ 20 min</td>
<td>20-40 min</td>
<td>20 min</td>
</tr>
<tr>
<td>CHO-K1 (GFP-XRCC1)</td>
<td>Resolves within 5 min</td>
<td>40 min (weak)</td>
<td>Not detected</td>
</tr>
<tr>
<td>U2OS</td>
<td>Resolves ~ 15 min</td>
<td>Low levels from 5 min forward</td>
<td>10 min</td>
</tr>
<tr>
<td>A549</td>
<td>Resolves ~ 10 min</td>
<td>Not detected</td>
<td>Very low levels &gt; 10 min</td>
</tr>
</tbody>
</table>
DNA damage signaling may impact recruitment and response

PARP activation levels allow to discriminate between repair pathways to adjust damage mixtures.

ATM-DNAPK-PARP signaling can alter the recruitment of repair factors and markers, like 53BP-1
Summary

• Use laser induced DNA damage to monitor recruitment of BER/SSBR proteins
• Inconsistencies in recruitment, timing, and other interactions may be due to differences in strand break mixtures
• Significant unknowns in how the microenvironment (cell line difference, signaling alterations, germline or somatic mutations) impacts the induction of damage and the resulting repair response
• Best practices for damage induction
  – Multiple markers for repair process of interest and strand breaks
  – Sample damage across a broad window
  – Iterate across multiple cell lines, preferably repair-proficient or wild-type and cancer or repair-deficient cell line
  – Attempt to separate signaling events as much as possible
  – Increase the n of the experiment, whenever possible
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