Introduction

Damage tolerance

Damage detection

Conclusions
The Great East Japan Earthquake

Fukushima 1 nuclear power plant

▲ the left is Unit 1, the right is Unit 2
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▲ The map of the radioactive pollution
Existing dosimeter

- High cost
- Not necessarily biologically-relevant
- Numbers reported infrequently
- Not very useful

Bio-dosimeter (our concept)

- Low cost
- Reports biologically-relevant dosage
- Quick response after exposure
- More useful
DNA repair

DNA damage by radiation

Damage tolerance

SOS promoter activation

Damage detection

Survived!

Let’s warn the humans!
Further development

Last year

DNA damage \Rightarrow induced by UV
Strains \Rightarrow DH5\alpha

This year

DNA damage \Rightarrow induced by various types
Strains \Rightarrow Rosetta
The effects of radiation

Radiation can cause

Production of Reactive oxygen

Breaks of chemical bonds

Hydrogen peroxide

Mitomycin C
Why have so high tolerance?

Because of...

very efficient DNA damage repair system
DNA repair system

1. **Irradiation** → **Damaged DNA**
   - **RecA** binds ssDNA
   - **Activated RecA** induces
   - **PprM** regulates

2. **RecA** binds ssDNA → **Activated RecA**
   - **LexA2** protease activity
   - **Unknown protein** regulates

3. **PprM** enhances pprA promoter activation

4. **End-joining Repair**
   - **PprA** binds/catalyzes ligation
   - **Blunt end**
Damage tolerance parts

**Single parts**

- LacI promoter

**Combination parts**

- LacI promoter
- CDS1
- LacI promoter

CDS = PprI, PprA, PprM, RecA
Tolerance assay

Damaged by chemical agents

Transformed *E. coli*

1. Incubate
2. Induction with IPTG
3. Incubate at 37°C for 16 hours
4. Wrap with aluminum foil
5. Plate onto agar
6. Air dry
7. Medium exchange
8. Induction with IPTG (Mitomycin C or H$_2$O$_2$)
9. Count number of colonies formed
Tolerance assay: MMC

Effects of single tolerance parts

Viable fraction (relative to control)

- **PprI**
- **PprA**
- **PprM**
- **RecA**
- **Wild type**

10 ng/ul

20 ng/ul
How MMC damages DNA

MMC can introduce

Interstrand cross-links
Double-strand breaks etc.

bind with DNA

Damaging!
Tolerance assay: MMC

Effects of single tolerance parts

Viable fraction (relative to control)

PprI  PprA  PprM  RecA  Wild type

10 ng/ul 20 ng/ul
Tolerance assay: MMC

Effects of combining tolerance parts

Viable fraction (relative to control)

- PprM
- RecA
- PprM+PprA
- PprM+RecA
- PprM+RecA
- Wild type

10 ng/ul
20 ng/ul
How $\text{H}_2\text{O}_2$ damages DNA

It hurts...
not only

highly reactive!

$\text{H}_2\text{O}_2$

but also

DNA

Cell membrane

cytoplasm

etc.
Tolerance assay: $\text{H}_2\text{O}_2$

**Effects of single tolerance parts**

- **PprI**
- **PprA**
- **PprM**
- **RecA**
- **Wild type**

Viable fraction (relative to control)

- 3 mM
- 6 mM
Tolerance assay: H$_2$O$_2$

Effects of combining tolerance parts

<table>
<thead>
<tr>
<th></th>
<th>Viable fraction (relative to control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pprl</td>
<td>1.00</td>
</tr>
<tr>
<td>RecA</td>
<td>0.75</td>
</tr>
<tr>
<td>Pprl+PprA</td>
<td>0.90</td>
</tr>
<tr>
<td>Pprl+PprM</td>
<td>0.85</td>
</tr>
<tr>
<td>Pprl+RecA</td>
<td>0.90</td>
</tr>
<tr>
<td>Wild type</td>
<td>0.60</td>
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</tbody>
</table>

3 mM

6 mM
What we discovered

- PprM appears to protect against DNA damages induced by Mitomycin C
- PprI alone does not secure against Mitomycin C
- *D. radiodurans's* RecA conferred resistance to any mutagen
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- Conclusions
DNA damage

Responsive promoter

Reporter genes

Color
Detection: SOS promoter

DNA damage

Repressed
LexA

SOS box in SOS promoter

Active!
Cleavage of LexA

Expression of SOS genes

RecA activation

SOS box
FPP (no color)  \[\text{E. coli produce}\]

\[\text{Synthetic pathway via CrtEBI genes}\]

Lycopene (Red)
it remains unclear in the quantitative relation between promoter activity and color intensity.
Promoter evaluation device

Firefly luciferase

SOS promoter

Luminescence

RPU = Firefly luminescence

const. promoter

Renilla luciferase

Luminescence

Renilla luminescence
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We conducted a survey in conjunction with the KIT-Kyoto and Ehime-Japan team, asking questions related to both synthetic biology and radioactivity.
In this project

- We assayed *D. radiodurans* proteins for the effects on DNA damage
- We surveyed the public perception of radiation and synthetic biology.
Future work

What we can further do

- quantifying the measurement by the "Bio-dosimeter"
- building a complete Bio-Dosimeter
- testing the response of the SOS promoter to various types of ionizing radiation
Acknowledgement

Prof. Takeshi Todo and all the members of Medicine Lab at Osaka University for lending us lab space and putting up with our intrusion.

Dr. Issay Narumi of Ion Beam Mutagenesis Research Group at the Japan Atomic Energy Agency for supplying us with *D. radiodurans* genomic DNA as well as helpful information and advice concerning the RecA promoter.
THE END

Thank you for your attention!
The effects to MMC

Viable fraction (relative to control)

10 ng/ul

20 ng/ul

Pprl  PprA  PprM  RecA  Pprl+PprA  Pprl+PprM  Pprl+RecA  PprA+PprM  PprA+RecA  PprM+RecA  Wild type
The effects to H₂O₂