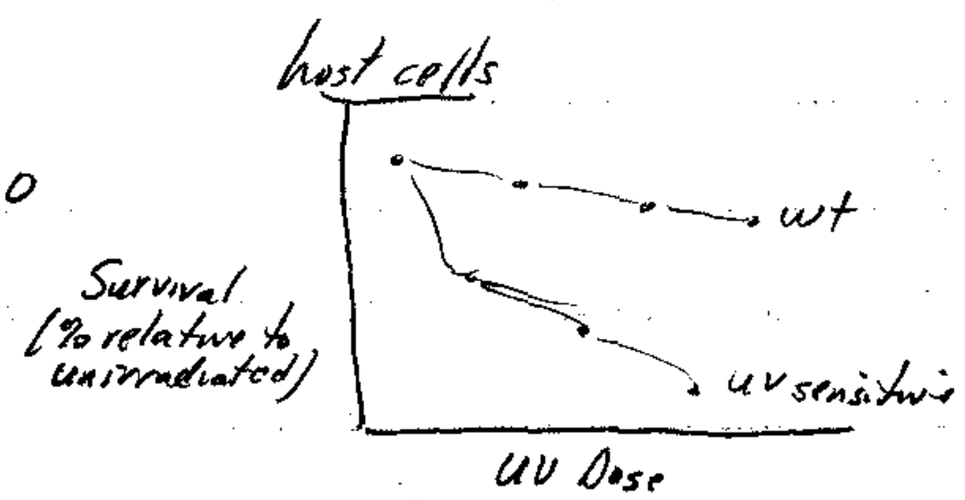
4/20/93

Ann Ganesan - Use of Viruses & Plasm.ds to Study DNA Repair

Why - because you can manipulate DNA

Host cell reactivation

①Ellison et al 1960



- wild type strain repairs bacteriophage DNA

Phage vs. Plasmids

①Phage have own mechanism to insert DNA

②Irradiate ϕx174 DNA

05 - much host cell reactivation
55 - no host cell reactivation
} suggests that most host cell reactivation due to excision repair

③Irrad. plasmid DNA

ⓐ recA mutations = same hsr as wild type

- so recA dependent repair NOT important here

⑥ insert into human cells (use CAT selection)

- XPA ± 0 show lower CAT activity than "normal"

- take plasmid w/ many U's
- allow turnover
- assay plasmid for C/A

Why

① can damage the DNA w/o affecting repair system

② plasmids/viral genomes are small

③ can manipulate plasmids

④ easy to use in cell-free extracts R. Wood

- use radiolabelling to study incorporation
- can compare diff. plasmids on/off UV light
- run on agarose gel
- more radioactivity in UV irrad. plasmids
- can compare plasmids with specific lesions