

Usher et al

① cDNA libraries from killifish *O. latipes* & *O. melanogaster*
- in pBLUESCRIPT

from cell line

from 2-14h old embryos

↓ electroporation

E. coli SY2 (phr::Cmr *uvrA*::Kmr *recA*::tetr)

↓ plate on LB w/ Kan, Tet, chlor, amp (vector?)

↓ 0.12 J/m² UV = 254

↓ 5' white fluorescent lamps (w/ filter to remove short wavelengths & heat)

37° x 12 hrs

↓ recover colonies

↓ isolate plasmids

1.9 kb insert in *O. latipes*
2.1 kb insert in *O. melanogaster*

↓ sequence

② align sequence of goldfish

↓ degenerate primers

↓ PCR from *P. tri-dactylis* cell line cDNA

↓ product

↓ sequence

↓ RACE to get ends

repeat 2x

Ahmad & Cashmore

H44 = mutants of *A. thaliana* impaired in blue-light-dependent inhibition of hypocotyl elongation

- = T-DNA insertion of H44
- = rescued flanking region
- = screened cDNA library
- = pulled out one clone
- = most similar to long-wavelength photolyases

⟨ *A. thaliana* photoactivation is ^{primarily} short wavelength type ⟩

● Todo et al Nature 361:371 1993. *Drosophila* 6-4 photolyase

uv irradi DNA

↓
get mobility

w/ crude extract

band 1 = not affected when tx DNA w/ *E. coli* phr
band 2 = is affected when tx DNA w/ *E. coli* phr

- band 3 disappears when illuminate mixture

- 6-4 Antibody binds DNA except when tx w/ band 1 + light

- cuts at TT or TC

Fluorescent lamp

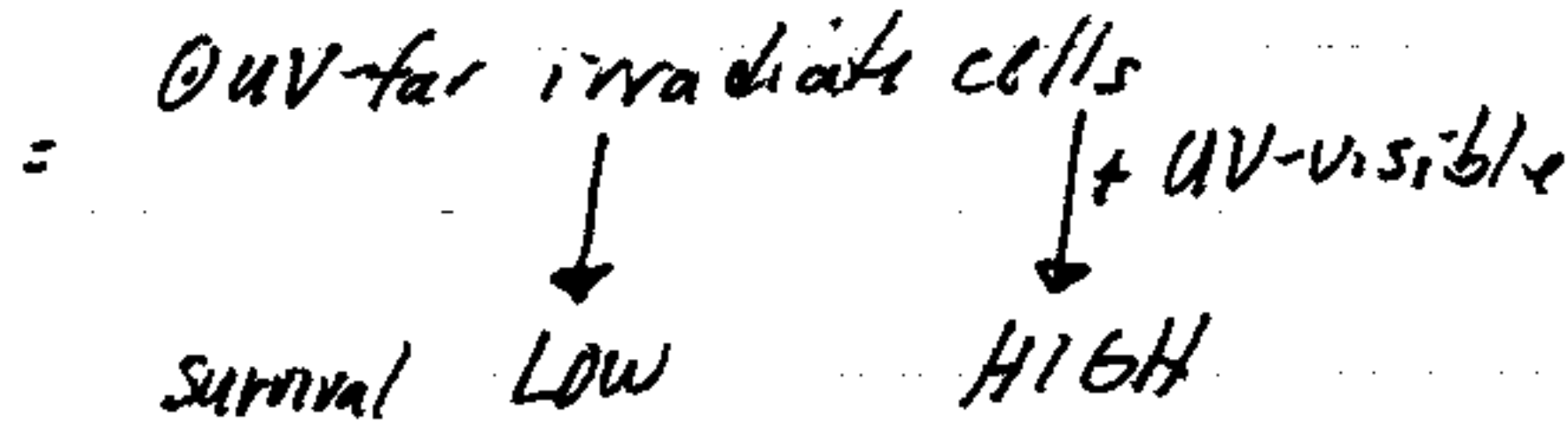
OTHER PHOTOLYASES/SIMILAR

HY4 - *Athaliana* blue light receptor has sequence similarity to microbial photolyases Ahmad & Cashmore 1993

G-4 Photolyases *DROSOPHILA* TOU et al 1993

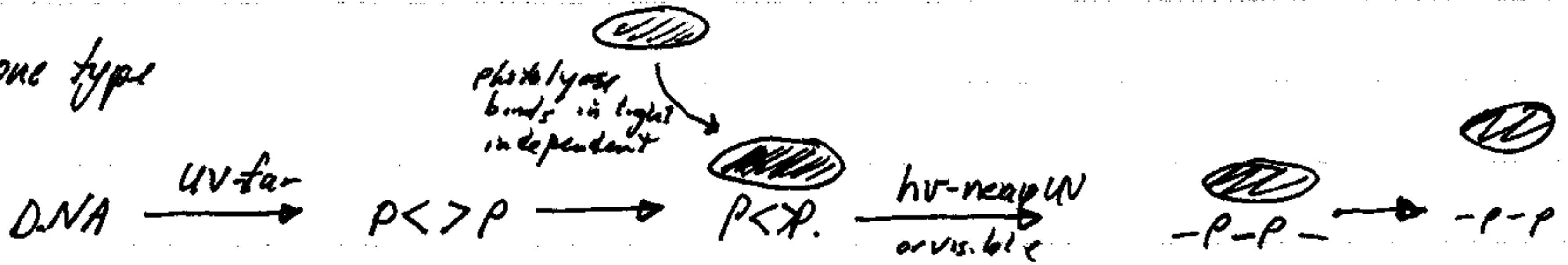
INTRO

Photoreactivation = prevention of the effects of far-UV (200-300 nm) by subsequent or concurrent exposure to near UV-visible light (300-500 nm)



by RUPERT ET AL
Mechanism - one type

FIG 1



Comparisons between species

MICROBIAL PHOTOLYASES

FIG 2

- two classes of cofactors

FAD - cleavage

absorption spectrum light absorb - ^{S₁} METHENYLtetrahydrofolate OR 8-HYDROXY-DEAZARIBOFLAVIN

peak = ~300 nm
FOLATE CLASS

FIG 3

- E. coli
- S. cerevisiae
- N. crassa
- B. firmus
- S. typhimurium

DEAZAFLAVIN

- A. nidulans
- S. griseus
- Scenedesmus acutus
- ? - M. thermoautotrophicum
- H. cutirubrum