# Adaptive Response In pKZ1 Mouse Prostate After Very Low Dose X-Irradiation

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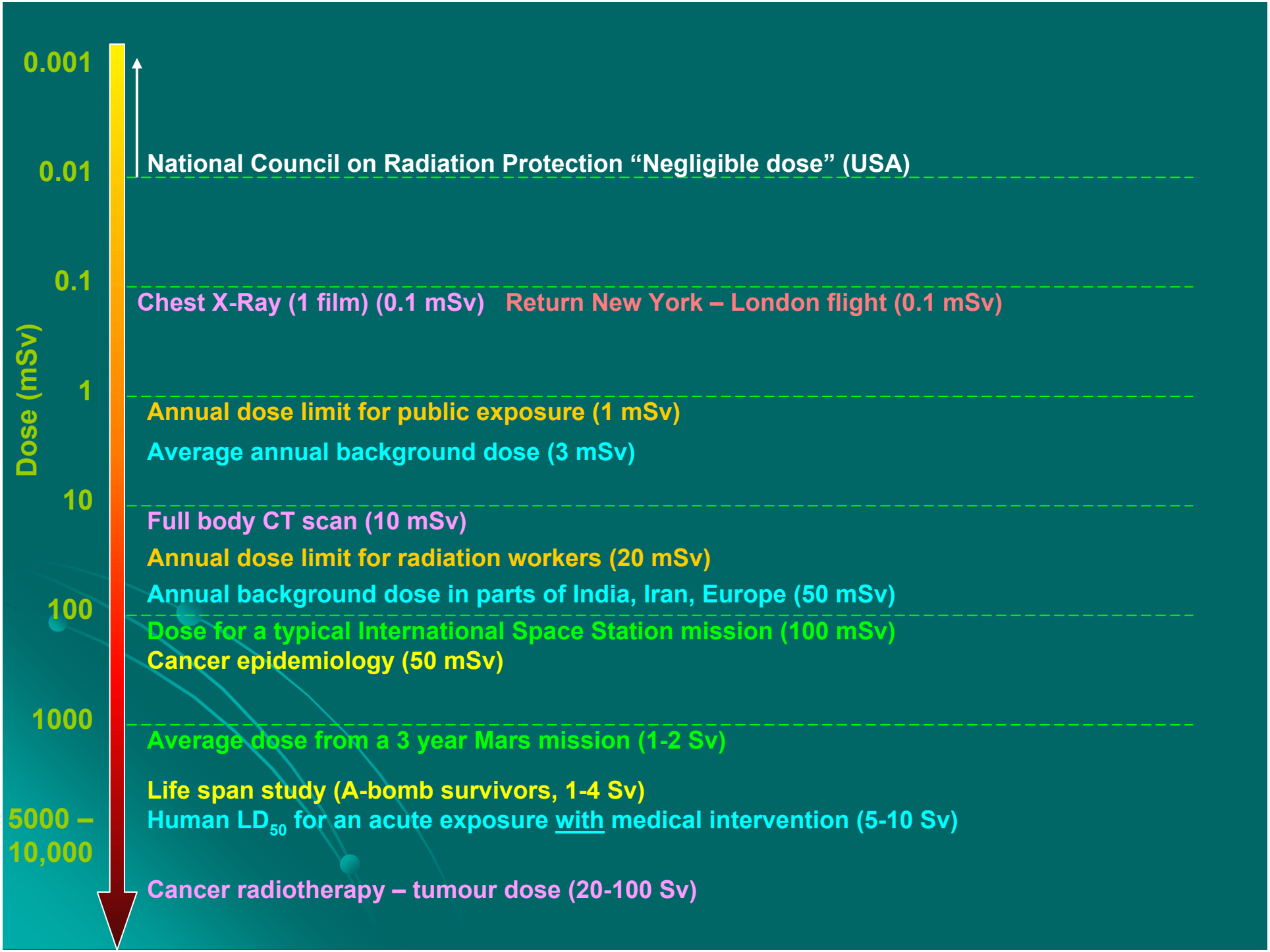
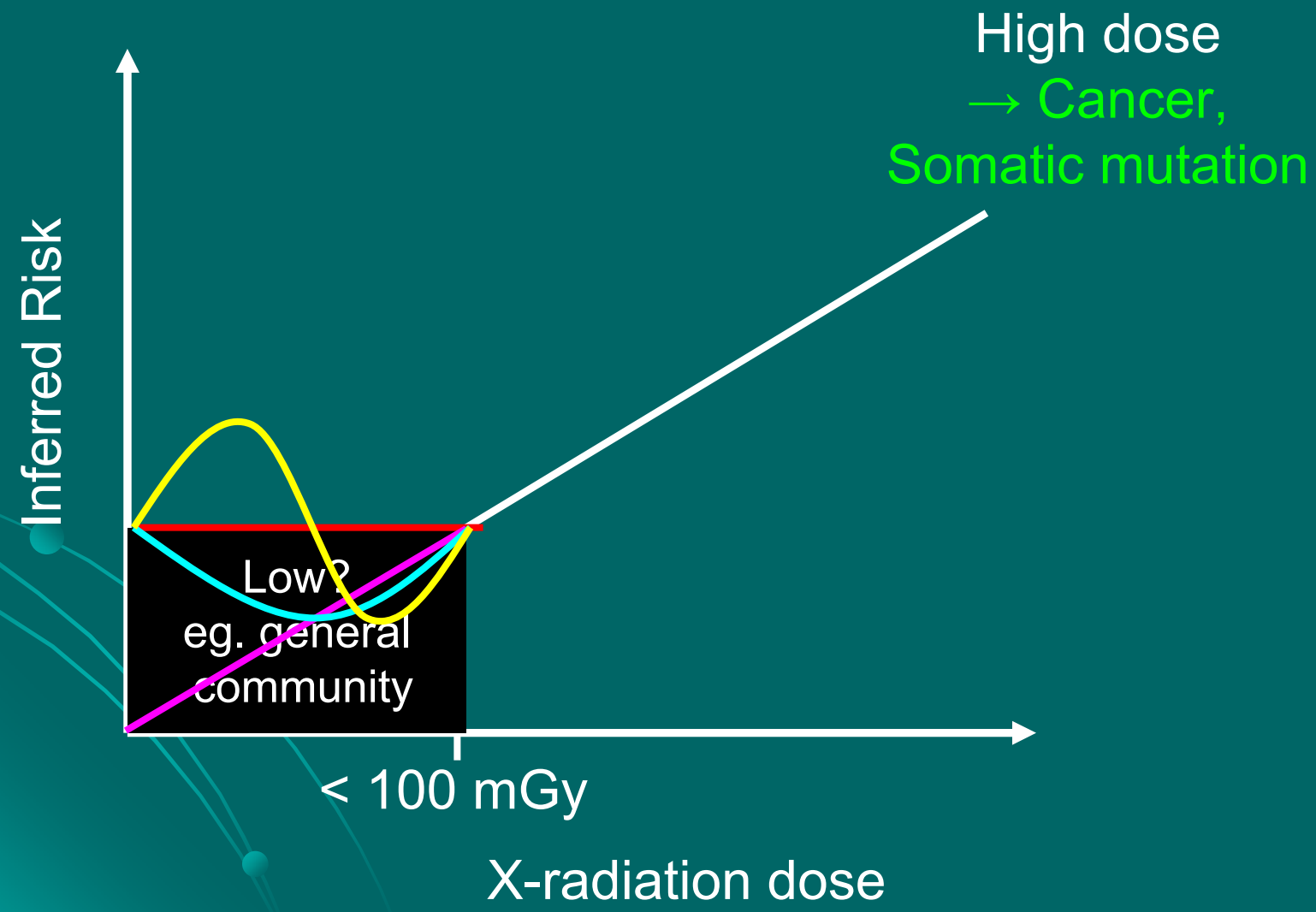

Dose (mSv)
0.001
0.01
0.1
1
10
100
1000
5000 – 10,000
National Council on Radiation Protection "Negligible dose" (USA)
Chest X-Ray (1 film) (0.1 mSv)
Return New York – London flight (0.1 mSv)
Annual dose limit for public exposure (1 mSv)
Average annual background dose (3 mSv)
Full body CT scan (10 mSv)
Annual dose limit for radiation workers (20 mSv)
Annual background dose in parts of India, Iran, Europe (50 mSv)
Dose for a typical International Space Station mission (100 mSv)
Cancer epidemiology (50 mSv)
Average dose from a 3 year Mars mission (1-2 Sv)
Life span study (A-bomb survivors, 1-4 Sv)
Human LD50 for an acute exposure with medical intervention (5-10 Sv)
Cancer radiotherapy – tumour dose (20-100 Sv)

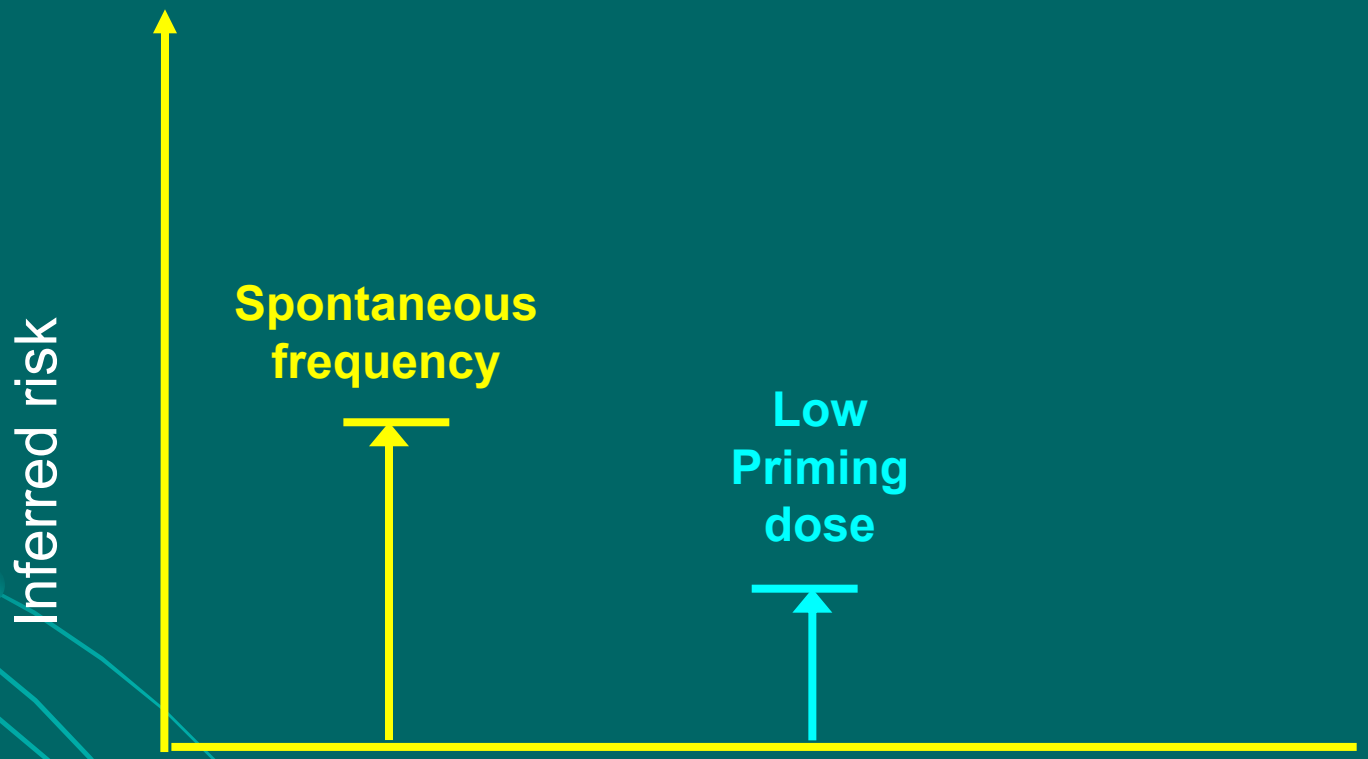
# Models of risk estimation



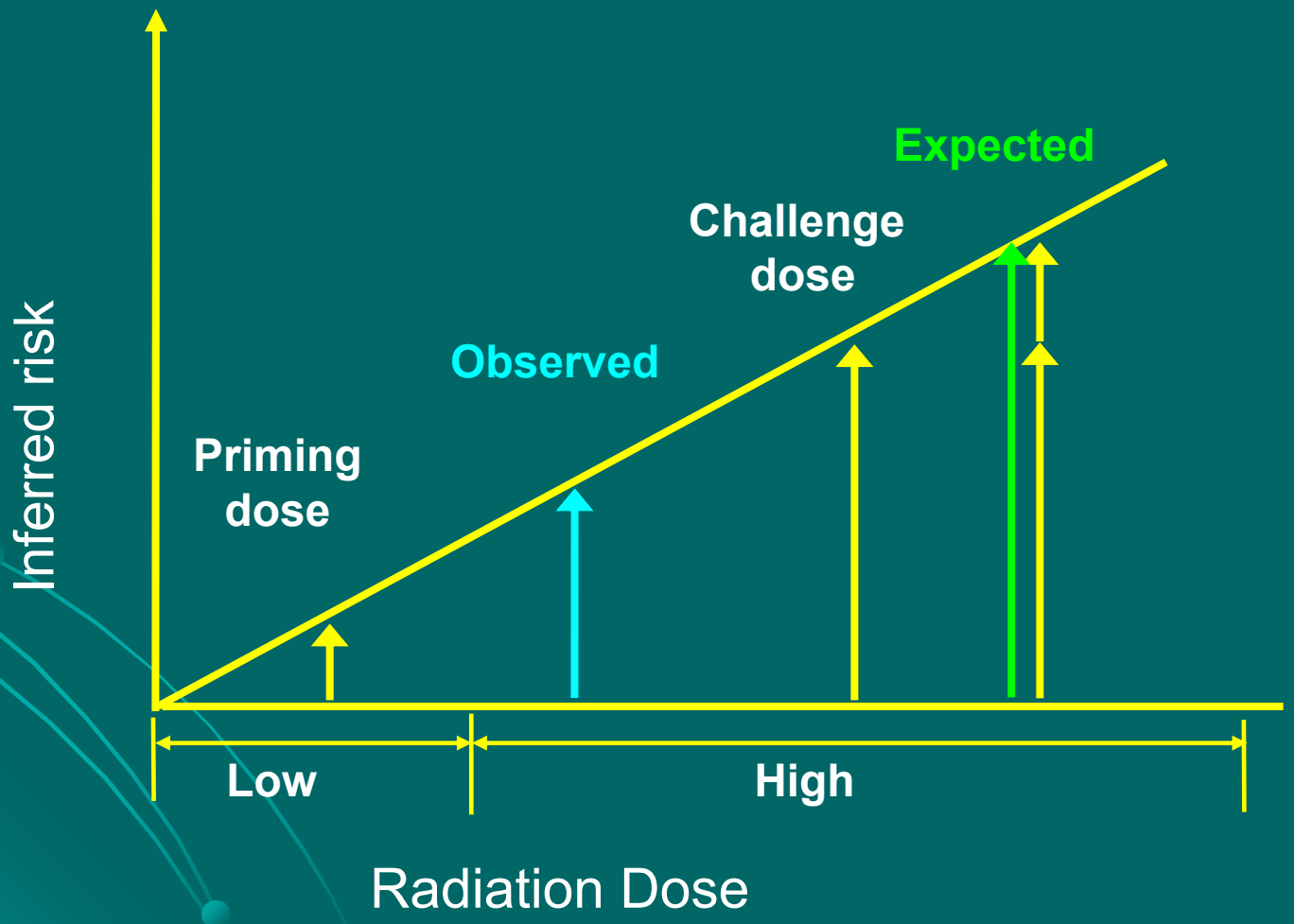
# Adaptive Response

- Response to a stress such as radiation which results in a lower than expected biological response
- One or Two-dose studies
- Priming doses low (1-500 mGy)
- Time between priming and challenge up to 24 hours
- Endpoints include chromosome damage, mutation, DNA damage, cell transformation and cell killing
  - Adaptive response for increased lifespan in tumour-prone mice or for latency of acute myeloid leukemia in normal mice (Mitchel et al, 1999; 2004)
  - A single low priming dose can induce an adaptive response for neoplastic transformation *in vitro* (Redpath and Antoniono 1998)

# One-dose adaptive response



# Two-dose adaptive response



# Mutation assays

- Cancer is important end-point of radiation damage
- Low radiation doses require an extremely large number of mice for statistical significance
- Neoplastic transformation is a marker for tumourigenic potential
  - But it is *in vitro*
- Mutation is a surrogate measure for cancer
- Transgenic mouse mutation models are *in vivo* systems which require less mice and can quantify a marker for radiation-induced damage

# Mutation and Cancer

- X-radiation can induce DNA damage, including DNA double and single strand breaks
- Repaired by recombination repair
  - inversions, deletions, or repaired to original sequence
- Inversions and deletions are important in cancer
- Sensitive assays which measure the effects of X-radiation are essential to characterise the mutagenic effects of low dose radiation exposure

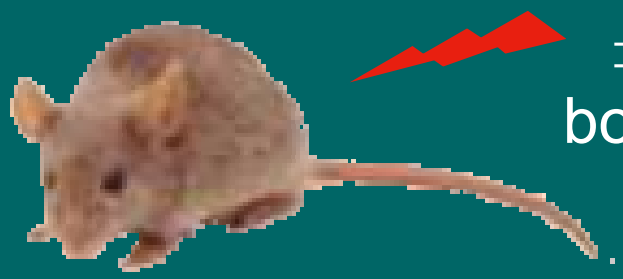
# Double strand break (DSB) repair

- Current knowledge of DSB repair in mammals is based on high dose radiation in higher eukaryotes
- DSBs repaired by homologous recombination (HR, error free) or non-homologous end-joining (NHEJ, error-prone)
  - Rejoining phase of NHEJ uses the same enzymes as V(D)J recombination in lymphocytes
- Most radiation induced DNA DSBs are repaired by NHEJ after high dose irradiation

(Hinz *et al.*, 2005; Marcon *et al.*, 2000; Rothkamm *et al.*, 2001; Takata *et al.*, 1998)

# The pKZ1 Transgenic Mouse

# Experimental Protocol

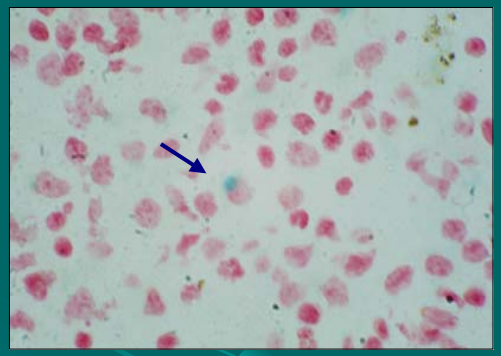


± Acute whole body X-irradiation

3 days

Spleen

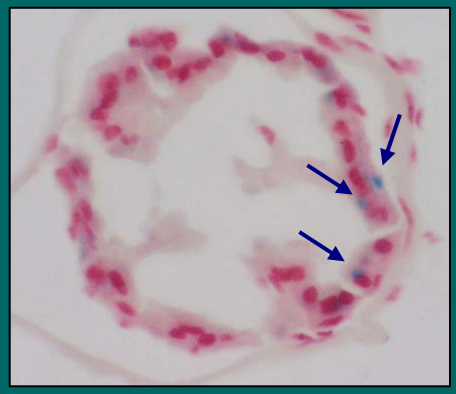
Prostate



Cut frozen sections

Fix and stain with X-gal

Code slides



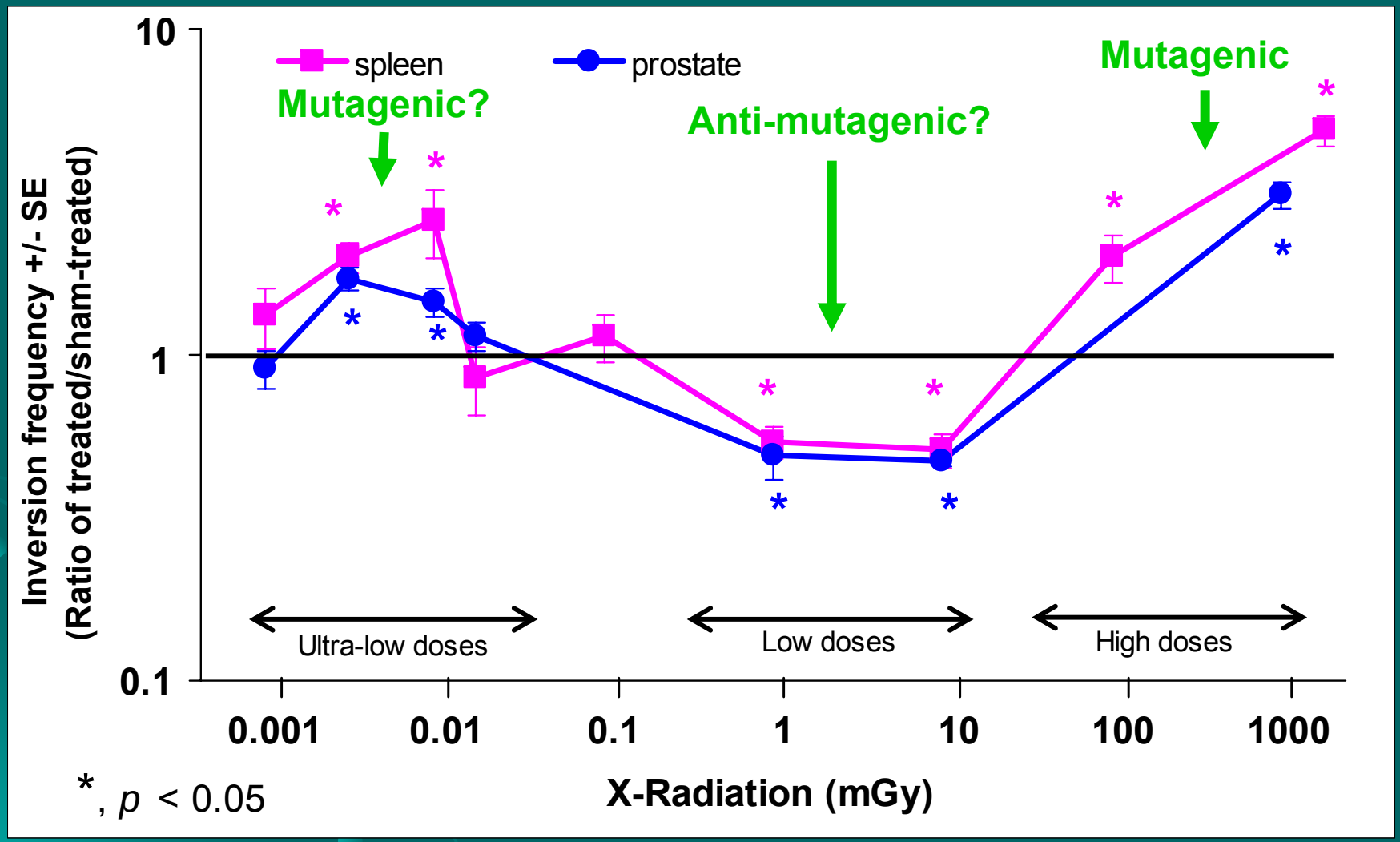
Number of blue staining cells / Total number of spleen cells

Inversion frequency

Number of blue staining cells / Total number of luminal epithelial cells

Inversion frequency

# Inversion frequency in pKZ1 spleen and prostate after single whole body X-irradiation

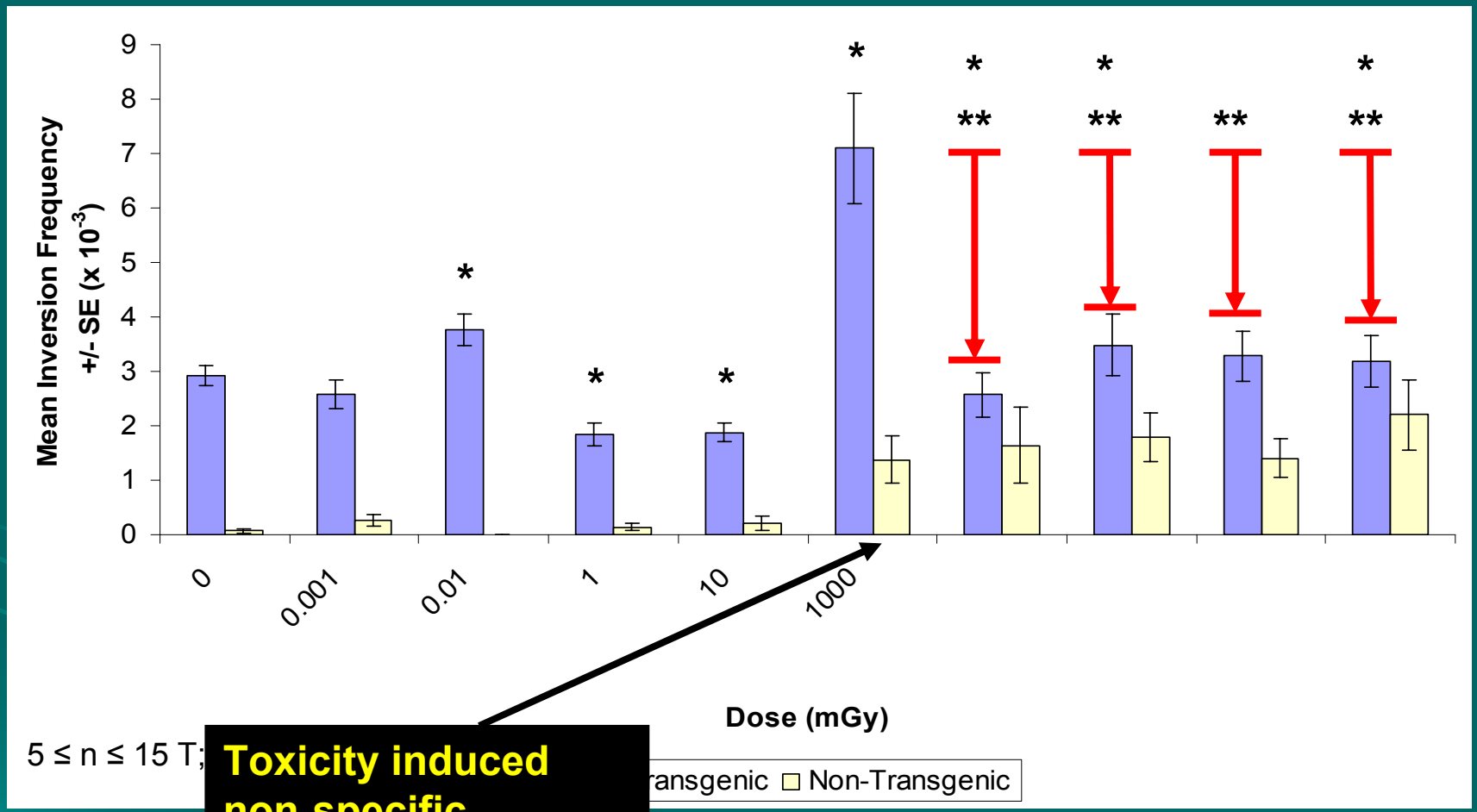



Hooker *et al*, *Radiat Res*. 2004; Zeng *et al*, submitted

# Adaptive response experimental protocol

- Experimental groups:
  - Priming
  - Challenge
  - Priming + Challenge
  - Sham-treated
- Time between priming and challenge 4 h
- Prostate isolated 3 days after X-irradiation

# Chromosomal inversion adaptive response in pKZ1 prostate




5 ≤ n ≤ 15 T;

**Toxicity induced non-specific staining (mammalian β-gal)**

*: statistica…am-treated Vs treated]

**: statistic…laptive Vs 1000 mGy]

p<0.05, 2-tailed Mann-Whitney U test

Day *et al*, submitted

Adaptive response

↓

pKZ1 mice

↓

**Ultra-low or low**
(0.001 – 10 mGy)
priming X-irradiation

↓

**High** (1000 mGy)
challenge X-irradiation

↓

**Adaptive response**
for DNA inversions

"Reverse" adaptive response

↓

pKZ1 mice

↓

**High** (1000 mGy)
priming X-irradiation

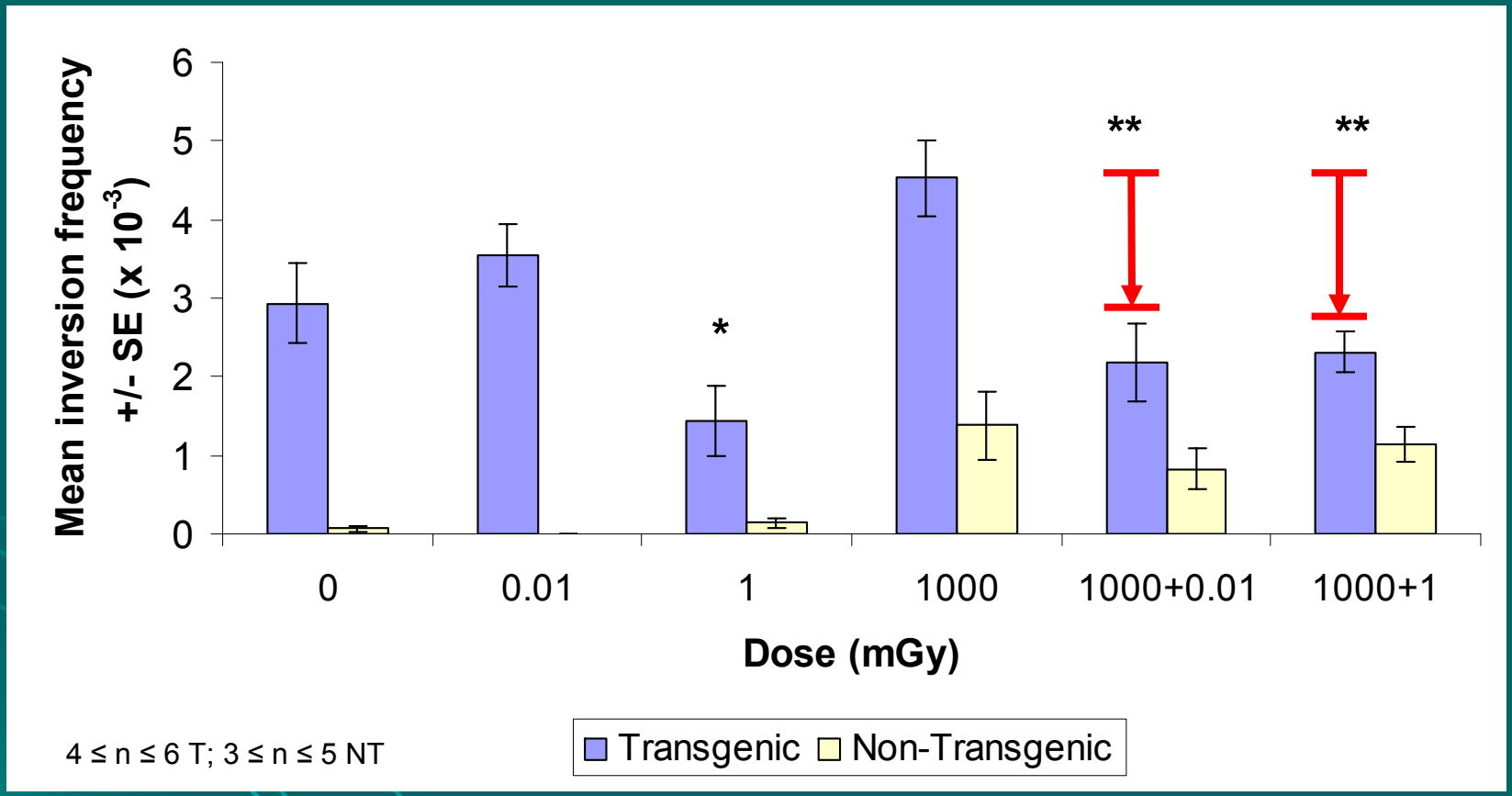
↓

**Ultra-low or low**
(0.01 – 1 mGy)
challenge X-irradiation

↓

**????**

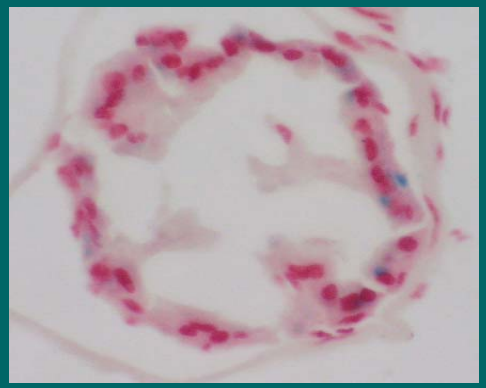
# “Reverse” chromosomal inversion adaptive response in pKZ1 prostate



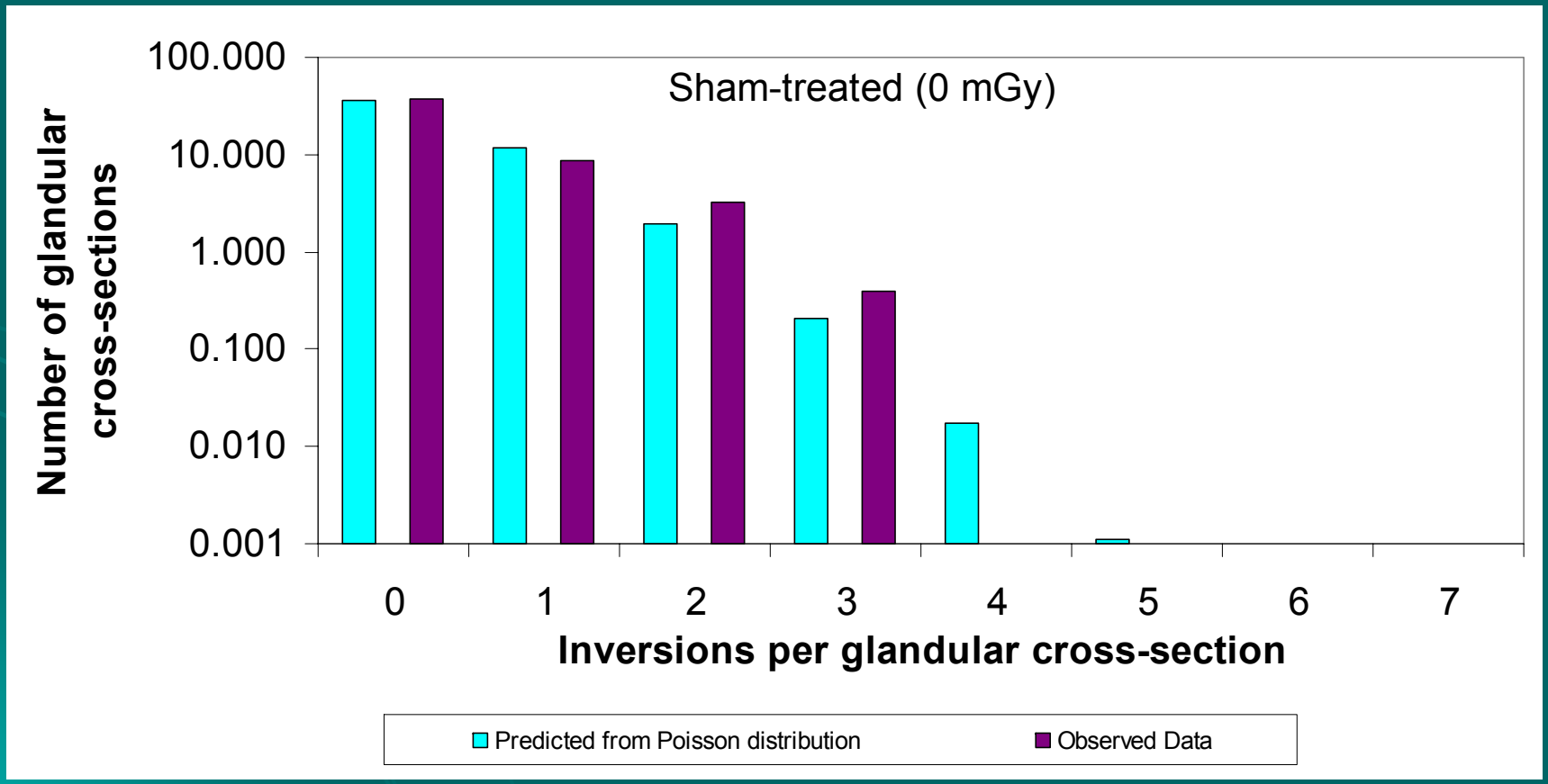

*: statistically significant, T-NT [sham-treated Vs treated]

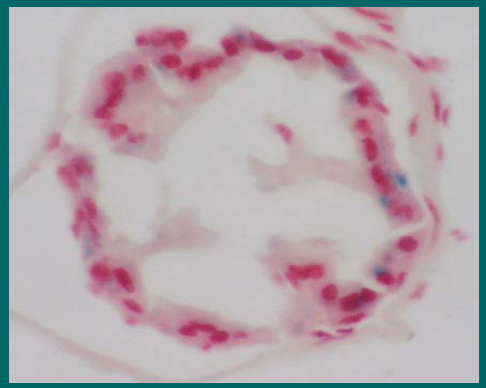
**: statistically significant, T-NT [adaptive Vs 1000 mGy]

$p<0.05$, 2-tailed Mann-Whitney U test

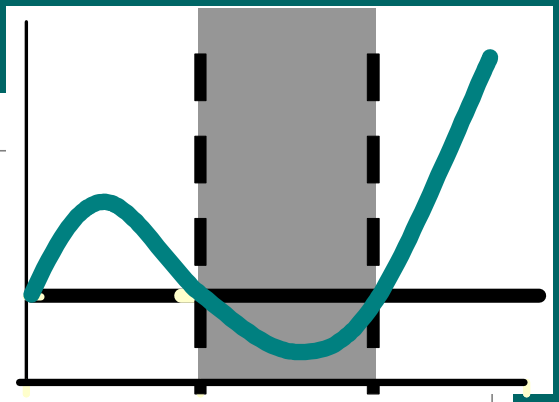


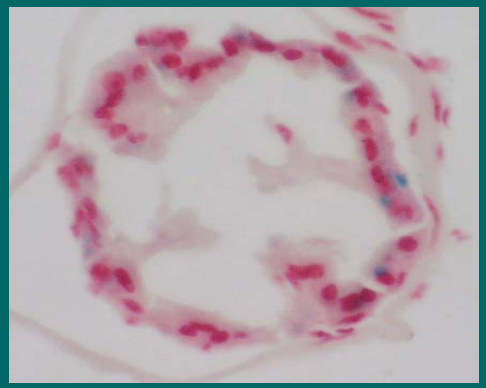
# Distribution of inversions in glandular cross-sections – sham-treated



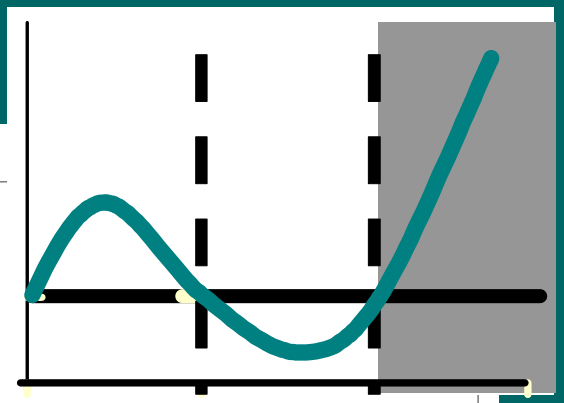


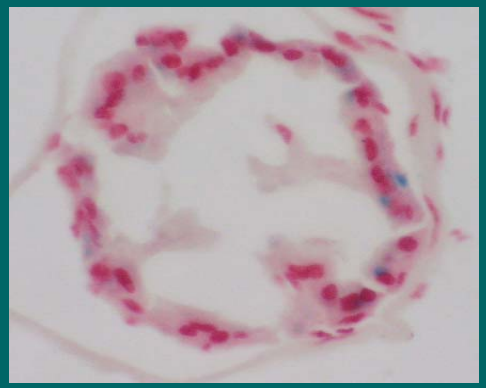
# Distribution of inversions in glandular cross-sections – low dose irradiation



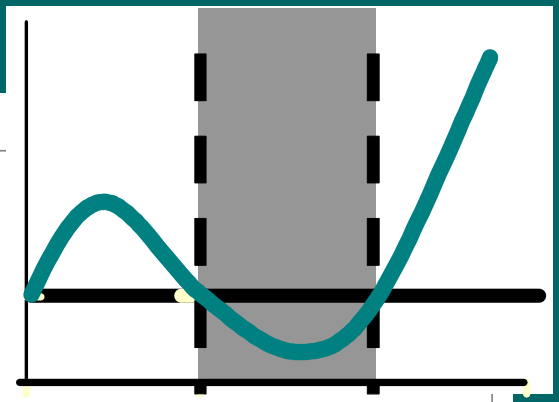


# Distribution of inversions in glandular cross-sections – high dose irradiation

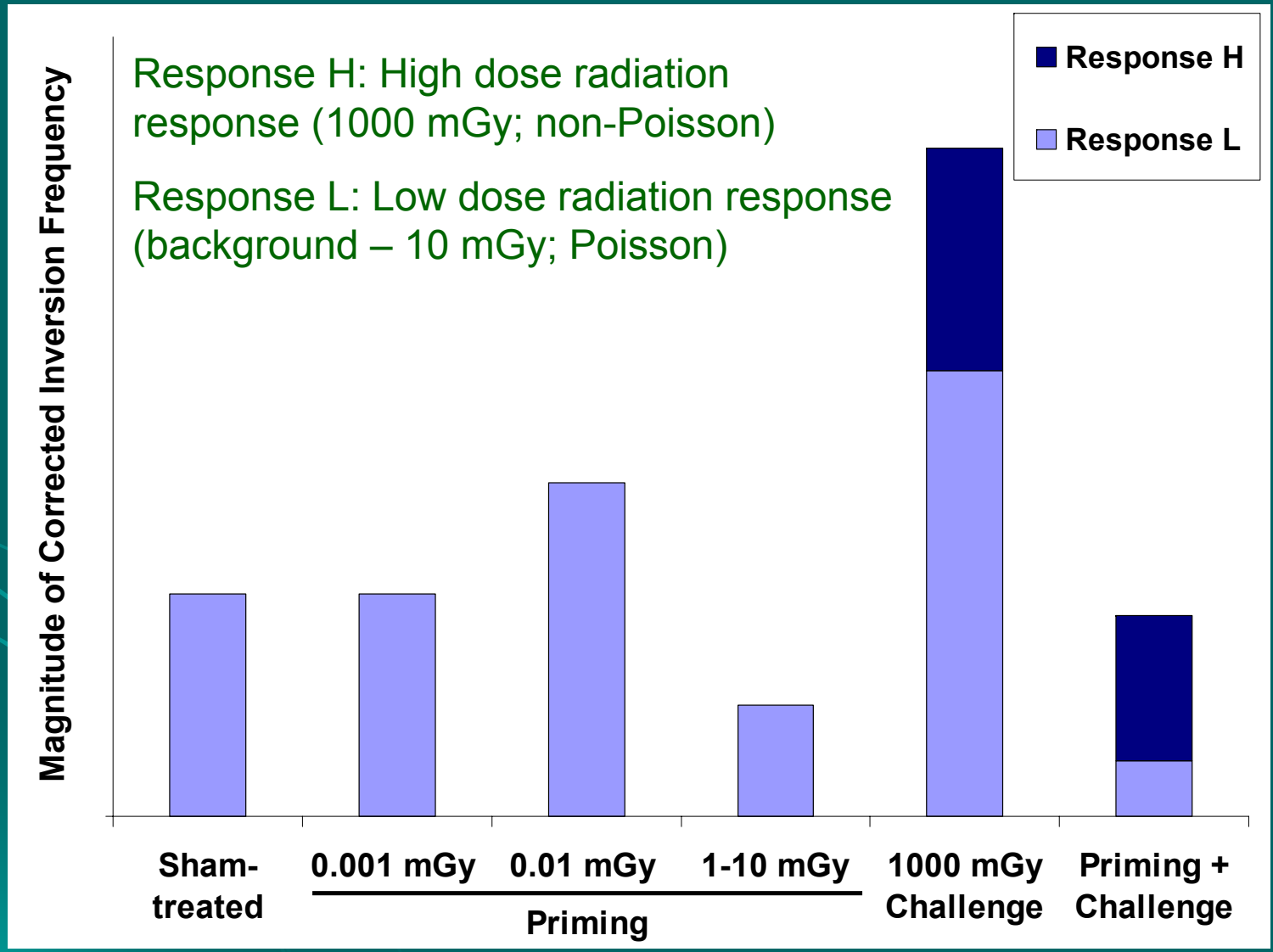




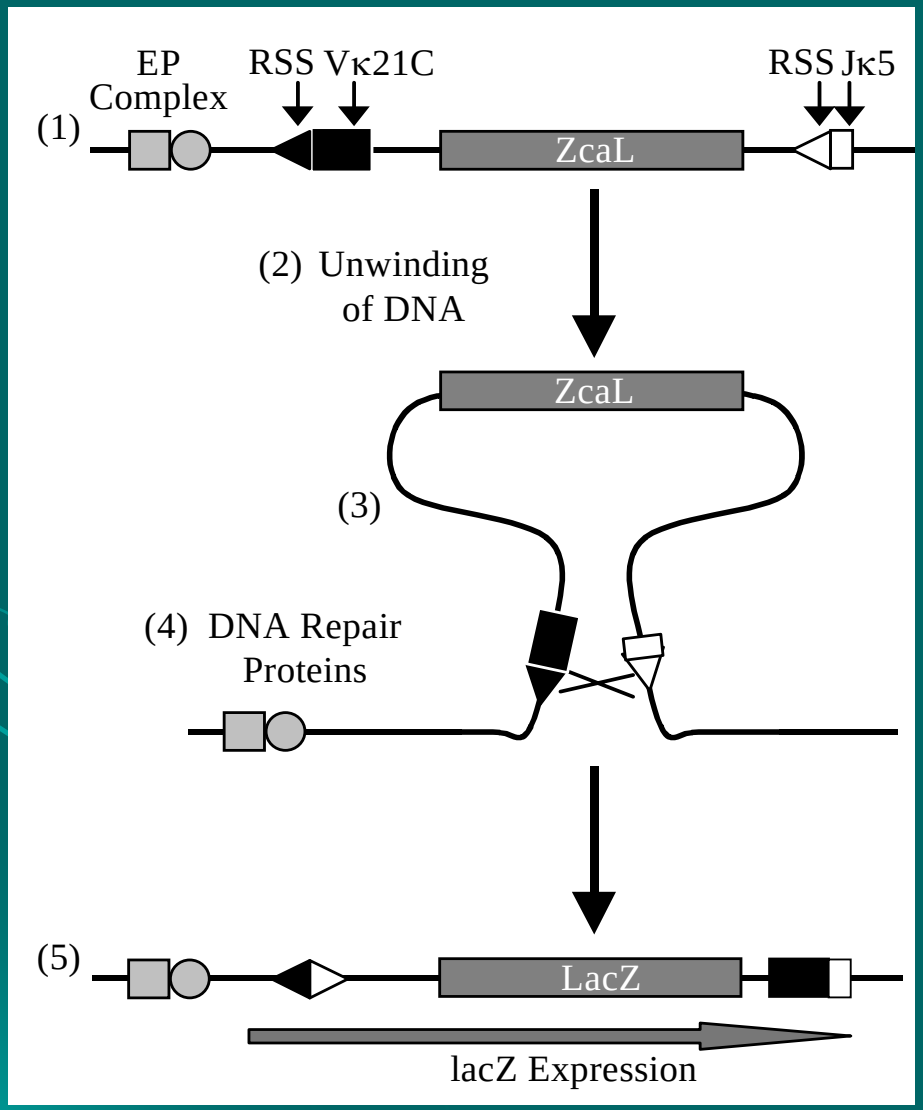
# Distribution of inversions in glandular cross-sections – low then high irradiation



# Hypothetical model for mechanism of adaptive response in pKZ1 prostate



# Proposed mechanism of inversion in pKZ1 transgene

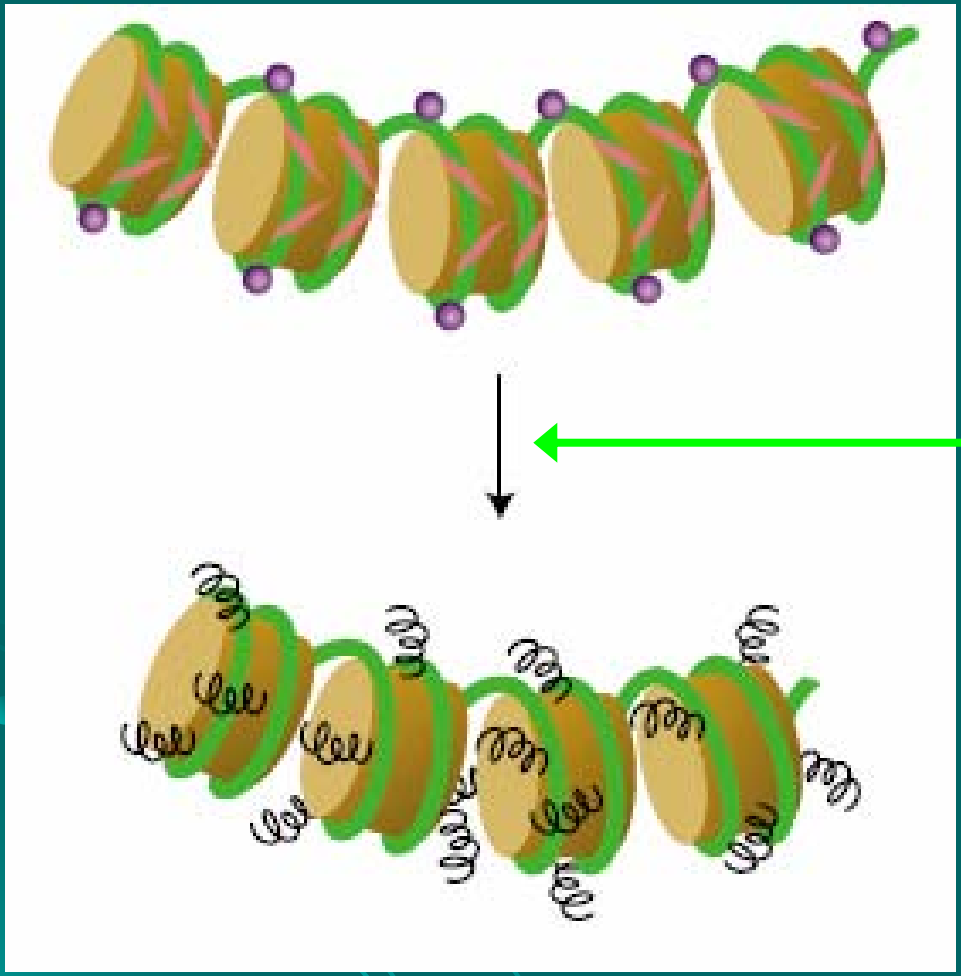



Demethylation?

Chromatin remodelling?

(Epigenetic effects)

# Chromatin structure



Transcriptionally inactive (inaccessible) DNA wound around de-acetylated histone tails and methylated DNA

Histone deacetylase inhibitor (Trichostatin A, TSA)

or

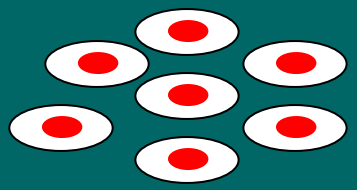
Demethylation (5-aza-2'-deoxycytidine, 5-aza)

Transcriptionally active (accessible) DNA wound around acetylated histone tails and unmethylated DNA

Adapted from (Strathdee & Brown, 2002)

# Epigenetic modification influences pKZ1 inversions *in vitro*

pKZ1 hybridoma cells

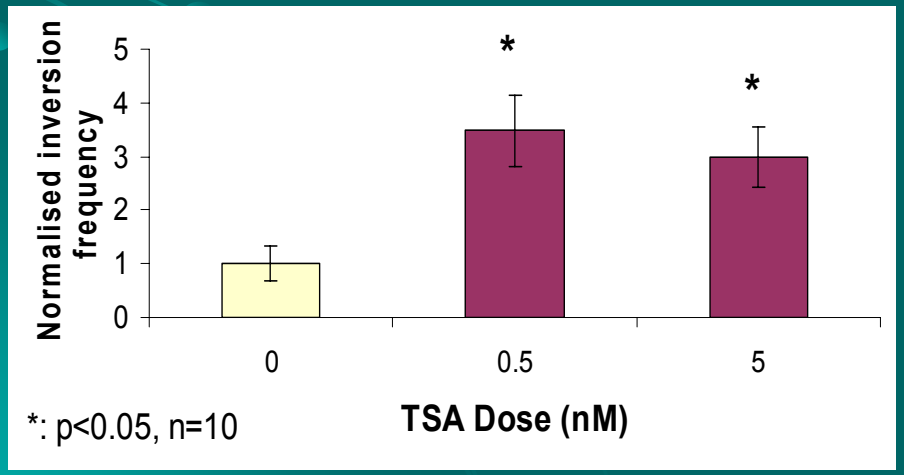


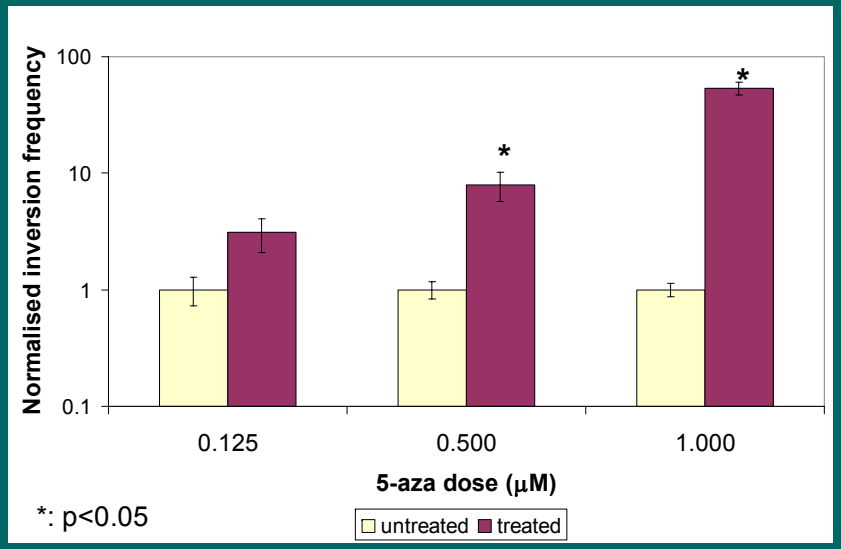
5-aza-2'-deoxycytidine

(CpG demethylase)

Trichostatin A

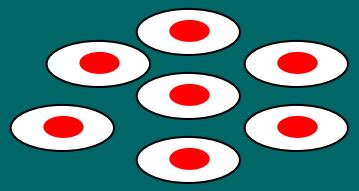
(histone deacetylase inhibitor)



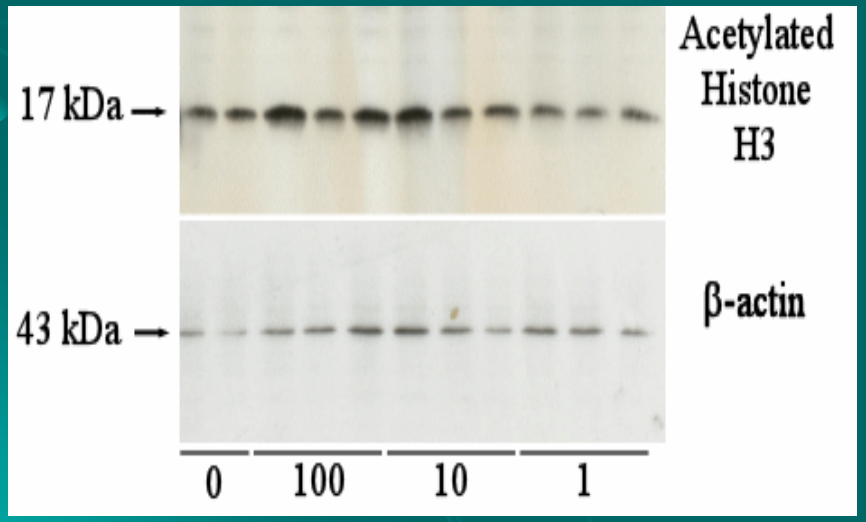



# X-Irradiation and epigenetic modification *in vitro*

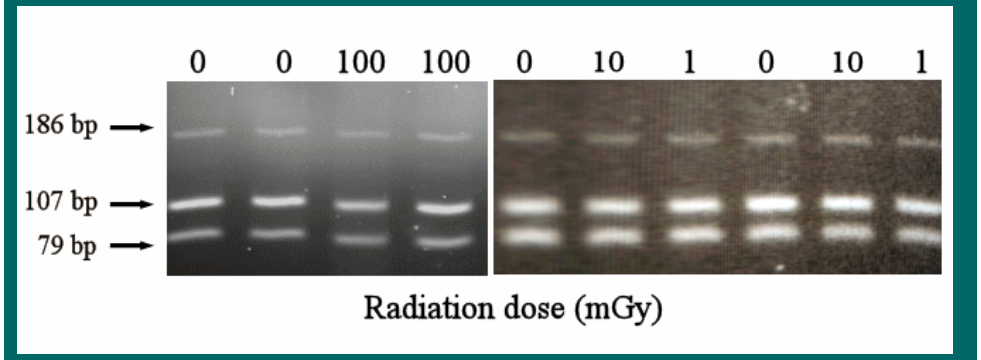
pKZ1 hybridoma cells → 1, 10 or 100 mGy X-rays



Histone acetylation




DNA Methylation (pKZ1 transgene)



# Summary and Conclusions

- Induction of an inversion adaptive response with **priming doses ≥ 3 orders of magnitude lower** than previously reported (0.001 mGy)
  - Radiation doses thought to be too low to have biological significance induce biological effects
- No difference in the magnitude of adaptive response induced by priming doses between 0.001 – 10 mGy
  - Agrees with hypothesis that adaptive response is on-off mechanism
- Adaptive response can cause a **reduction** in the inversion frequency to **below the sham-treated inversion frequency**
  - Largest magnitude of adaptive response reported
  - Overcompensation? Is a reduction below the endogenous inversion frequency good?

# (cont'd)

- Inversion response in pKZ1 prostate **does not follow LNT** for priming + challenge irradiation with a 4 h time interval
  - Implications for risk assessment
- Doses **≤ 10 mGy are protective** against inversions induced by a challenge dose
  - Potential for radioprotection?
- Adaptive response in pKZ1 prostate induced when **first dose** was **higher** than **second dose**
  - Supports different gene expression response for low and high doses
- **Chromatin remodelling** affects pKZ1 transgene recombination
  - Global methylation changes or other histone modifications??

# Acknowledgements

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