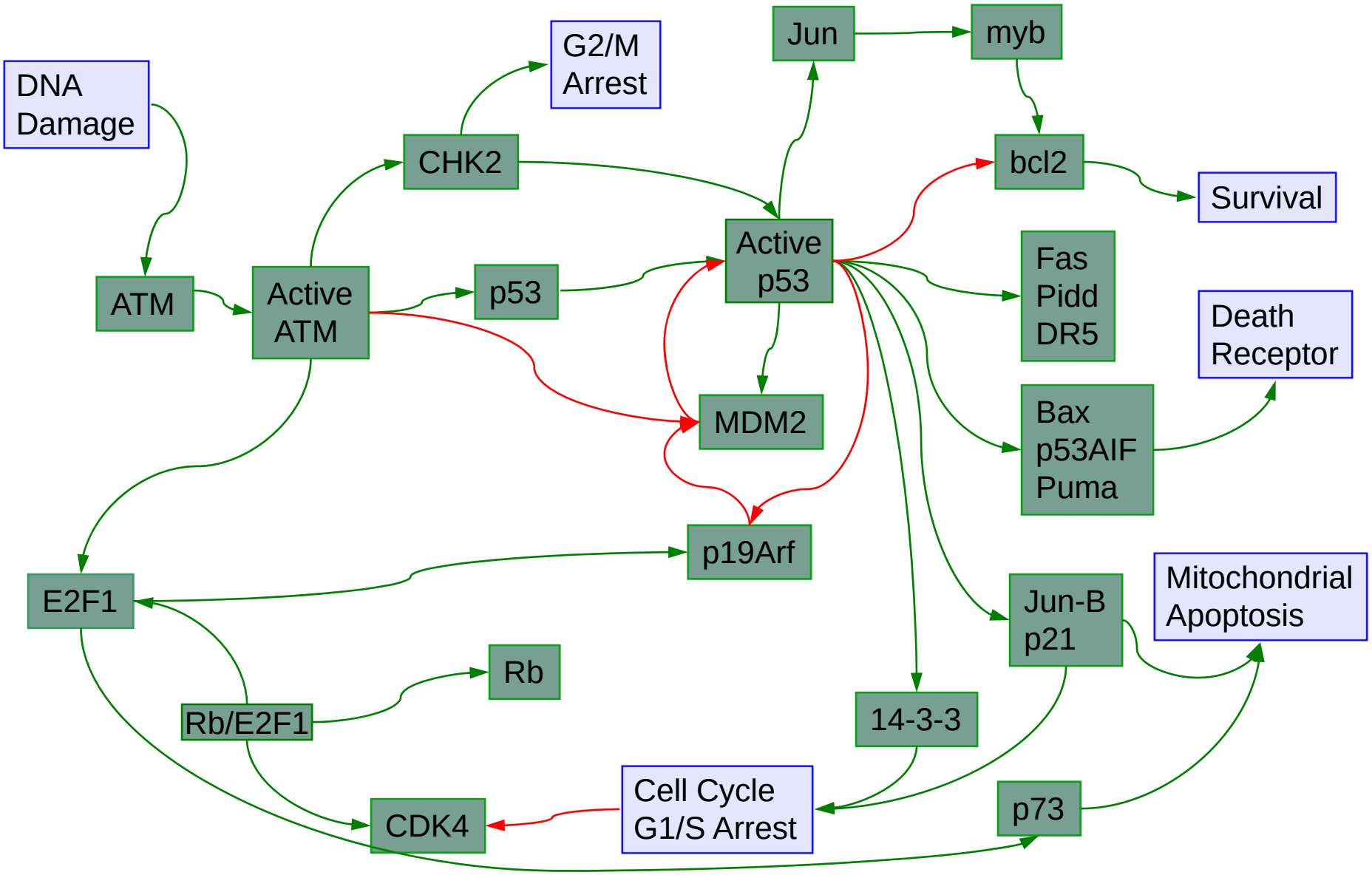
DNA double strand break repair dynamics

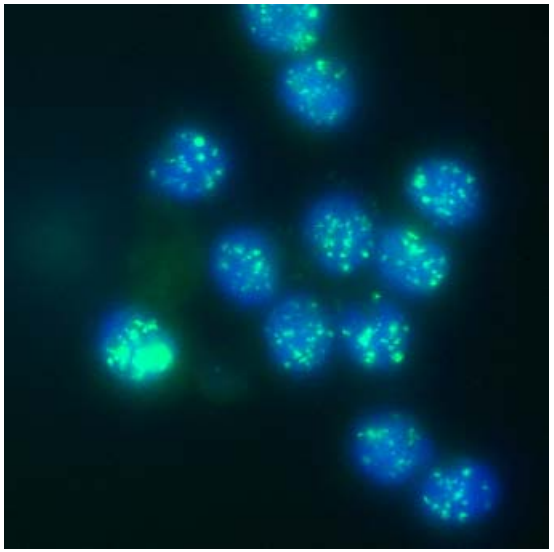
Martino Barenco

The p53 network

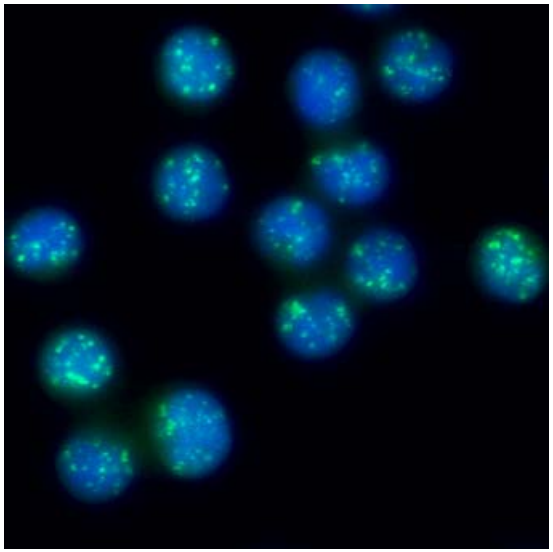


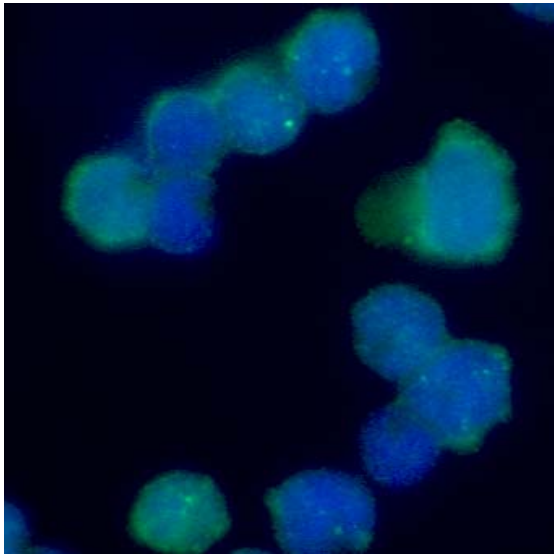
H2AX assays, MOLT4's 500mGy

+30'

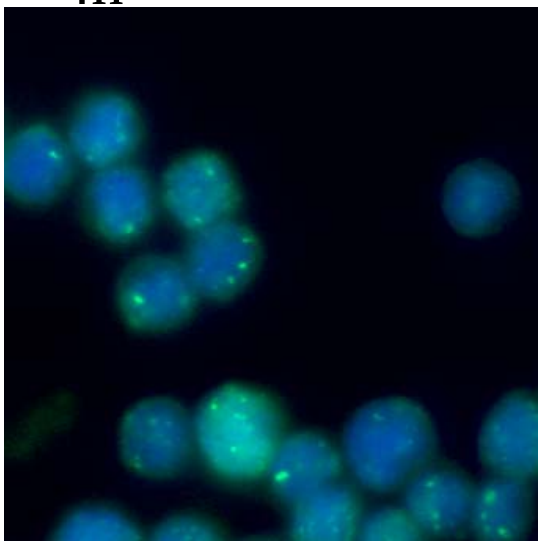


+1h

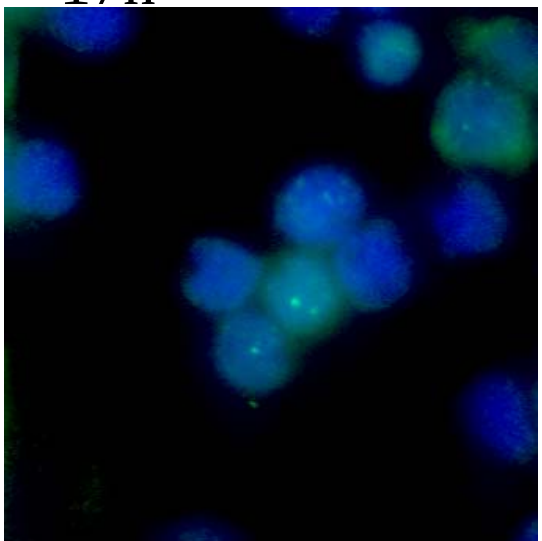




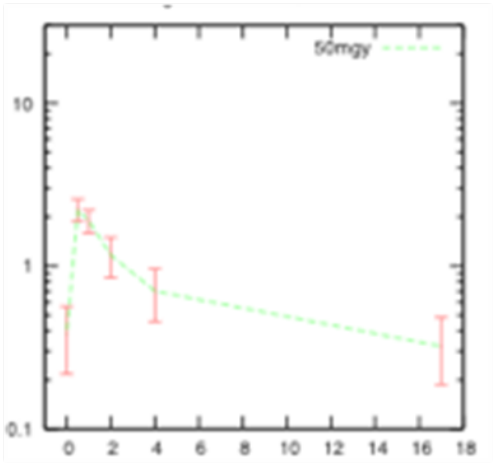
+4h

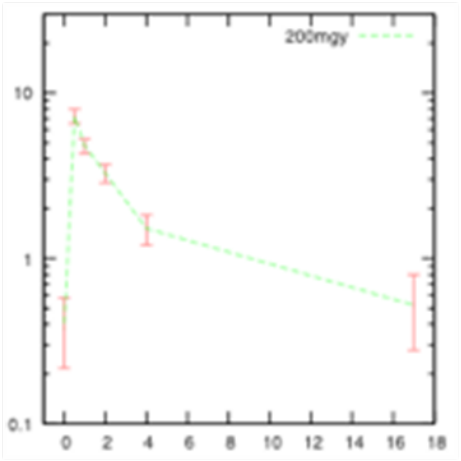


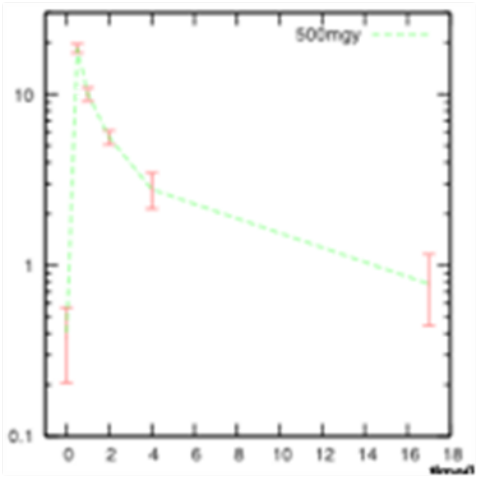
+17h



Average number of DSB count (various doses)







- Initial damage is ~ proportional to the dose
- DSB decay is ~ exponential

Modeling attempts in the litterature

Two lesion kinetic model

7. Stewart RD: **Two-lesion kinetic model of double-strand break rejoining and cell killing**. *Radiat Res* 2001, **156**(4):365-378.

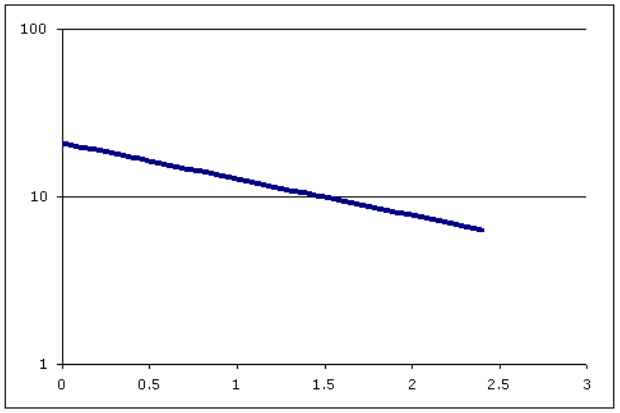
Variable Repair Half-Time Model (VRHT)

17. Foray N, Monroco C, Marples B, Hendry JH, Fertil B, Goodhead DT, Arlett CF, Malaise EP: **Repair of radiation-induced DNA double-strand breaks in human fibroblasts is consistent with a continuous spectrum of repair probability**. *Int J Radiat Biol* 1998, **74**(5):551-560.
18. Foray N, Badie C, Alsbeih G, Fertil B, Malaise EP: **A new model describing the curves for repair of both DNA double-strand breaks and chromosome damage**. *Radiat Res* 1996, **146**(1):53-60.

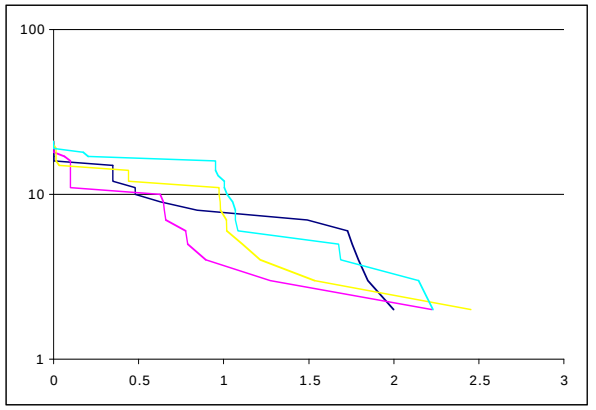
- Deterministic models: only **average** number of DSB in a population is being modeled
- Reason: H2AX assays had not been devised then.
- Hypothesis: Repair time of a given DNA DSB does **not** depend on the cell environment (ie how many other strand breaks there are).

Deterministic vs Stochastic modelling

Deterministic



Stochastic



Deterministic model: evolution of 1 single value eg population average of something.

Stochastic model: description at the individual cell level, either:

- Numerical simulations of a large number of individuals
- “Solve” the model theoretically ie describe evolution of probability distribution (not always possible).

The VRHT model in a stochastic context

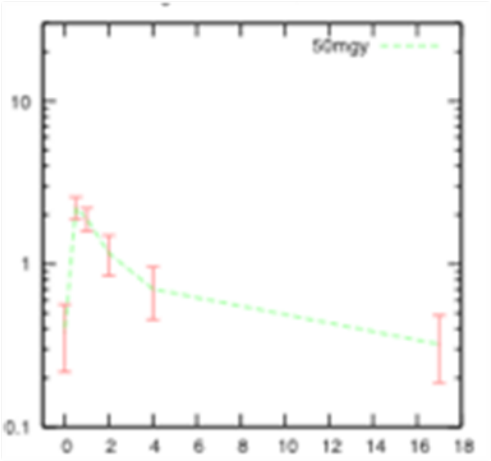
- In this model, the time to repair can be variable, depends on the type of lesion, and nothing else
-Markovian creation of DSBs and/or Poisson-distributed pulses
-With this hypothesis:

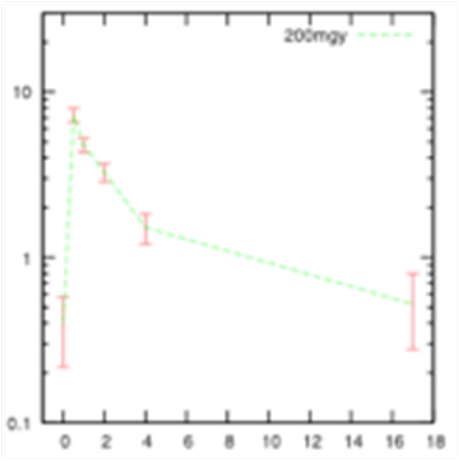
> Can prove that the distribution of DNA DSBs in a Population of irradiated cells has to be **Poisson.**

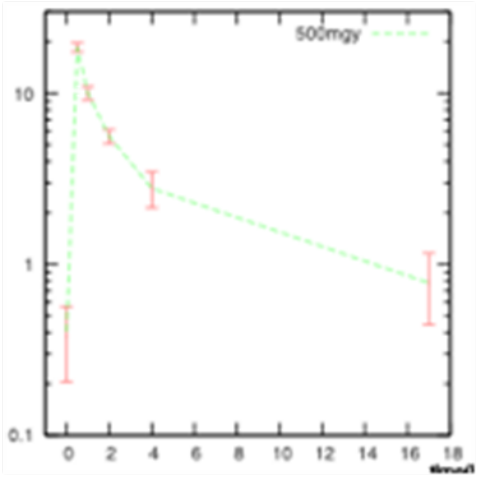
In other words, the observed Variance/Average* should not be different from 1.

*(NB: if Average=Variance=0 then we say ratio=1.)

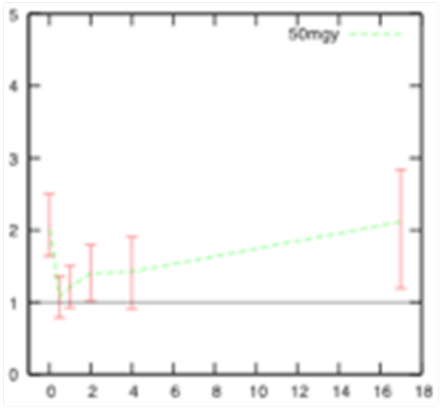
Results (MOLT4 cells)

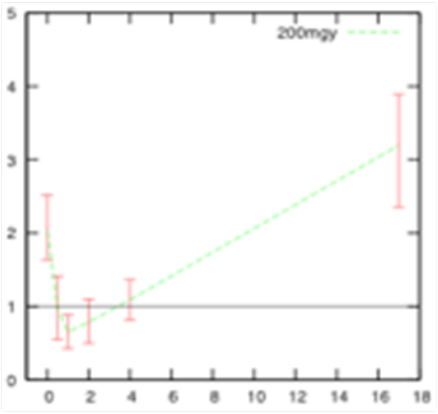


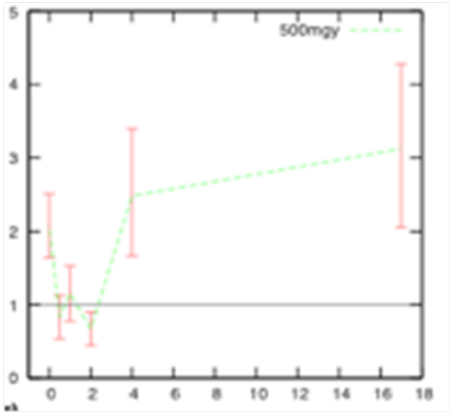




Variance/Average Ratios

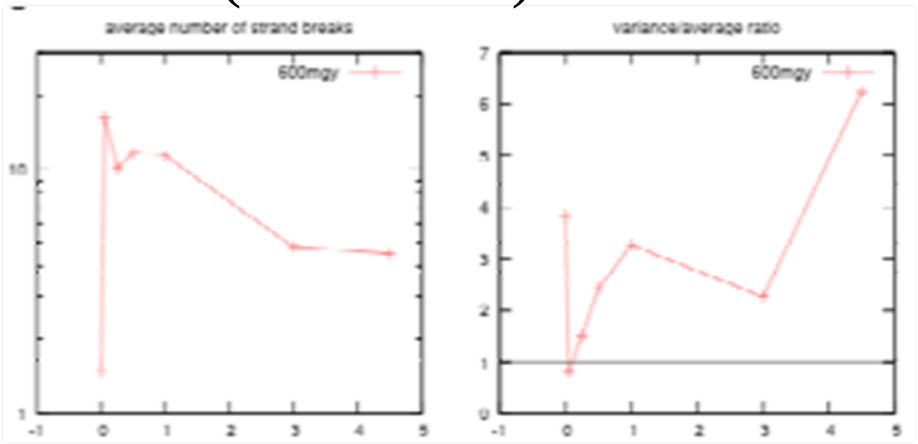






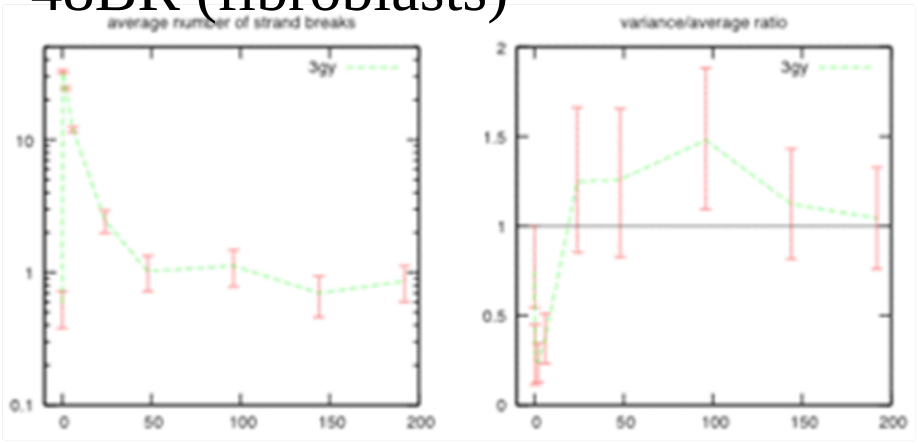
Results (other types of cells)

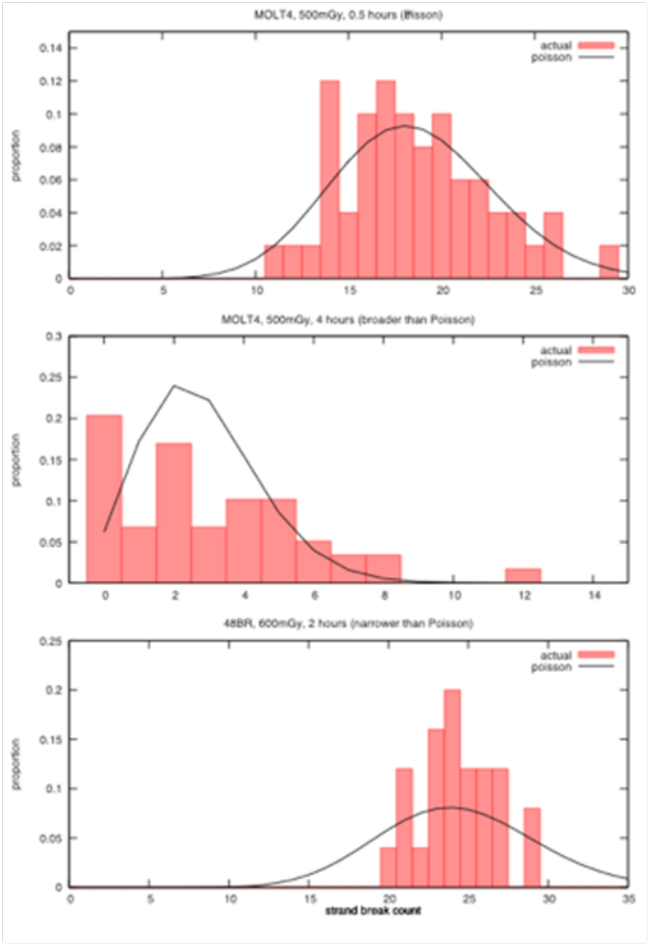
IMR90 (fibroblasts)



12. Rogakou EP, Boon C, Redon C, Bonn er WM: **Megabase chromatin domains involved in DNA double-strand breaks in vivo**. *J Cell Biol* 1999, **146**(5):905-916.

48BR (fibroblasts)





So far:

-Models such as VRHT, are good at describing the average DSB count.
-But distributional features, such as the variance are poorly described.
-Try to add extra feedback loops to the model

feedback loops

Negative feedback loops

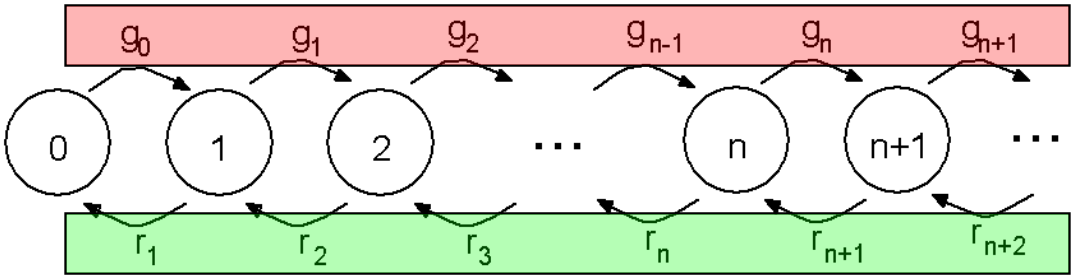
- Have a stabilising/centripetal effect (in both deterministic and stochastic systems).

Positive feedback loops:

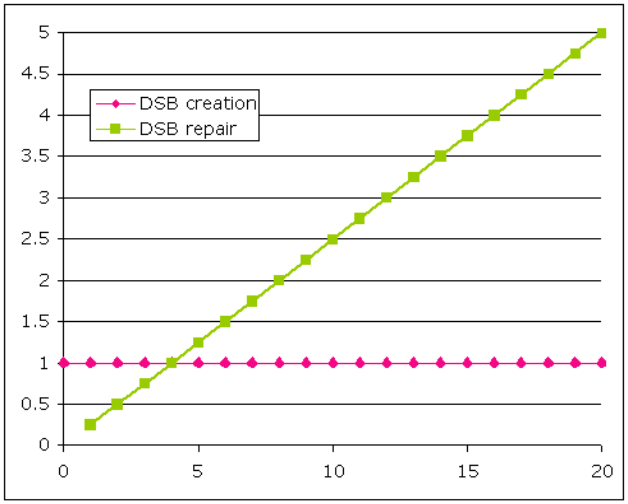
- Have a centrifugal effect
- e.g. In deterministic systems, postive feedback loops are required for multistationarity (2 or more equilibrium states).

Stochastic model: Birth and death process

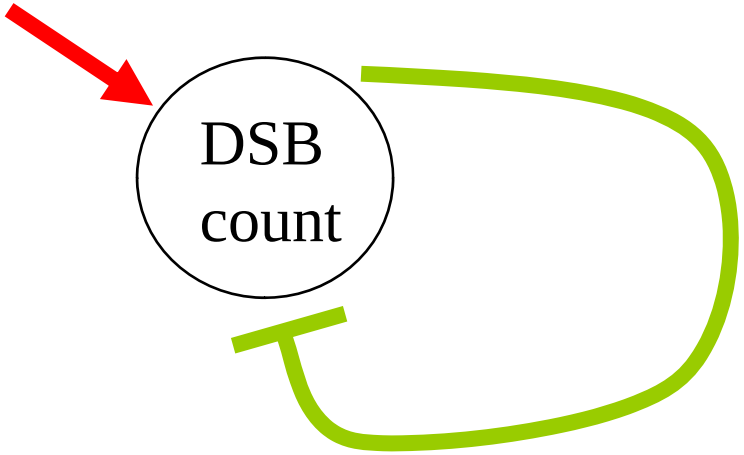
Individual cell model, variable = DSB count



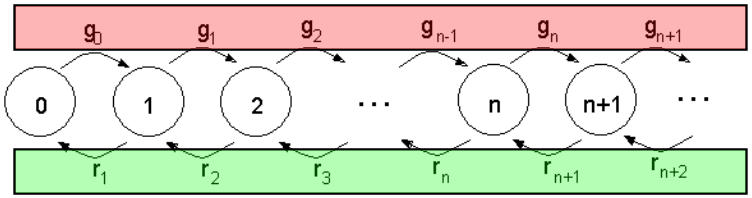
VRHT-type model



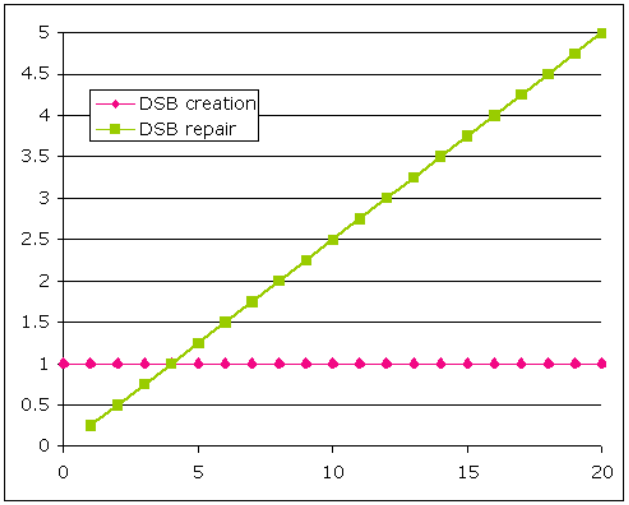
Repair rate for individual DSB does not depend on DSB count



Master equation



$$\frac{d}{dt}p_i(t) = \begin{cases} p_1(t)r_1 - p_0(t)g_0 & \text{for } i = 0 \\ p_{i-1}(t)g_{i-1} + p_{i+1}(t)g_{i+1} - (g_i + r_i)p_i(t) & \text{for } i > 0 \end{cases}$$



Define rates as a function of the state Index

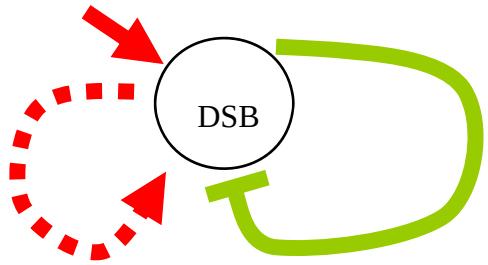
e.g.

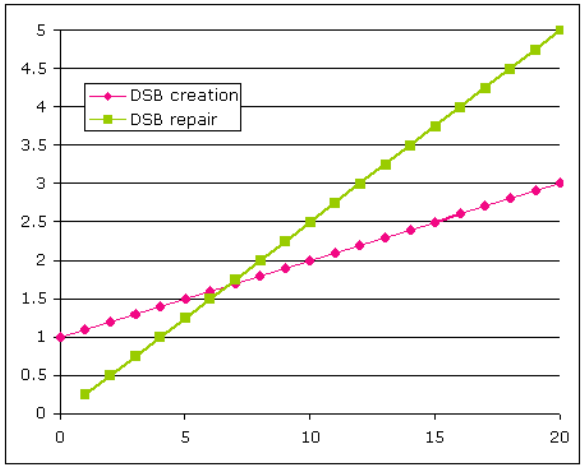
$R_i = r(i) = Di$

$g_i = g(i) = a$

Adding feedback loops

A positive one on The DSB creation Side.

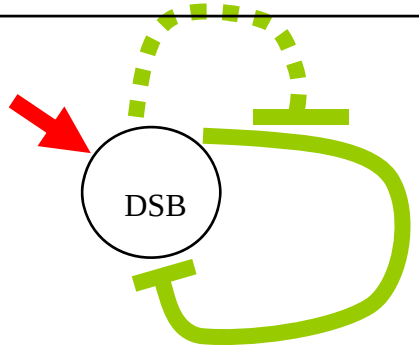


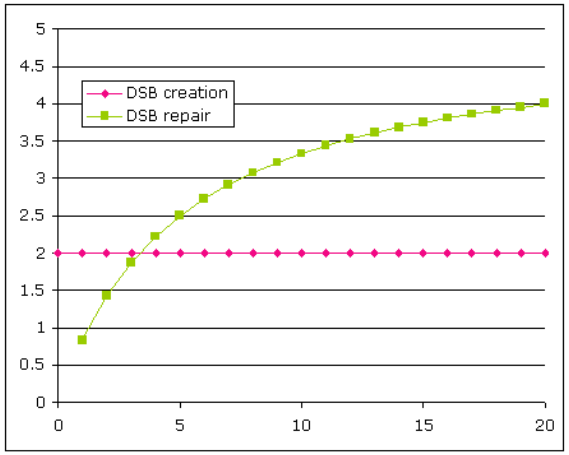


$g_n = a + bn$

$r_n = Dn$

A positive one on The DSB repair Side.

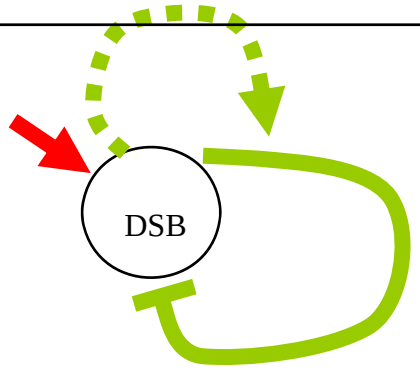


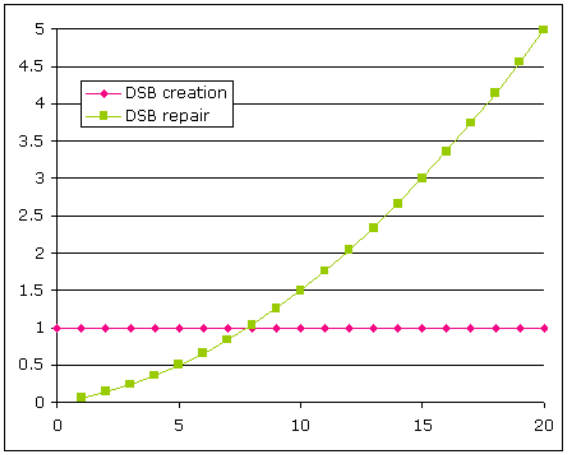


$g_n = a$

$r_n = Dn\dfrac{k}{k+n}$

A negative one on The DSB repair Side.

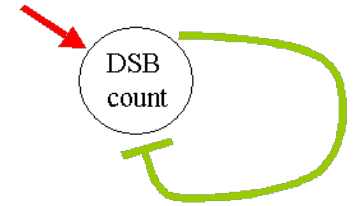


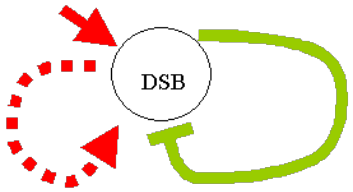


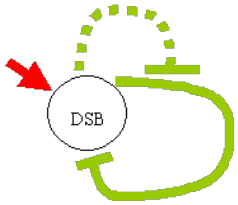
$g_n = a$

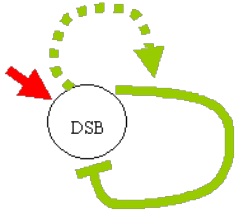
$r_n = Dn + D_2 n^2$

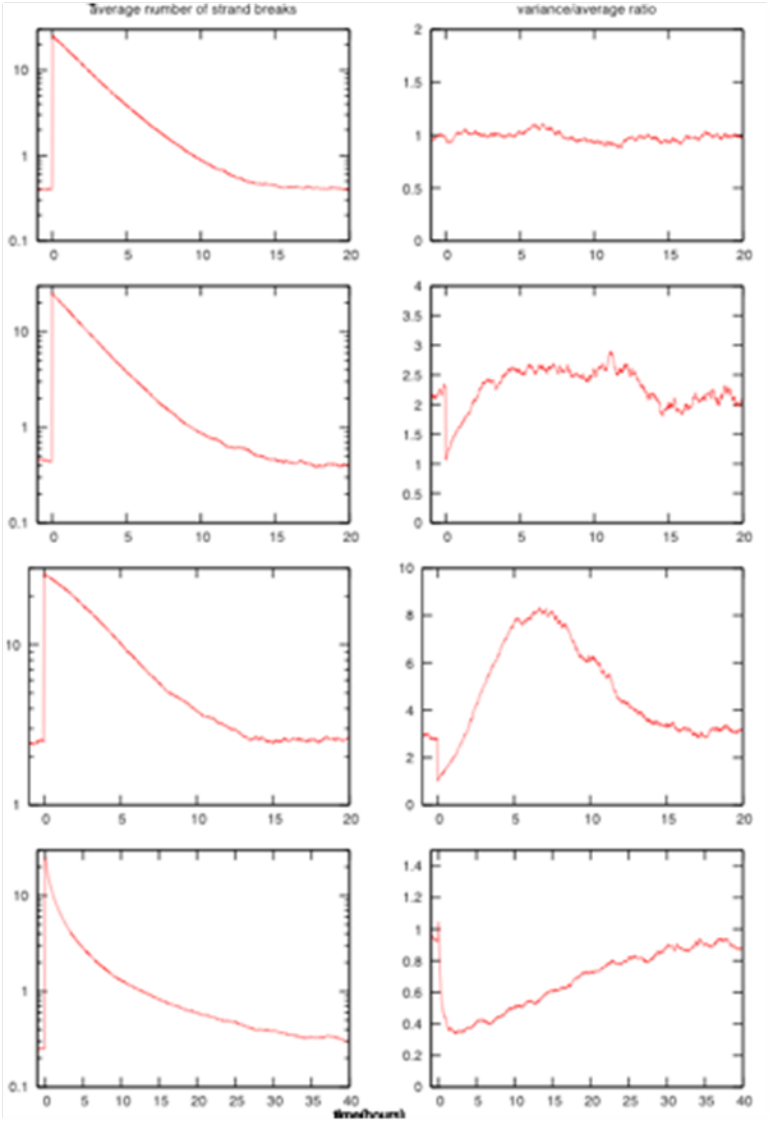
Effect of extra feedback loops on DSB distribution dynamics



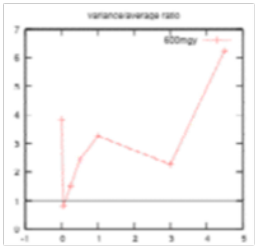


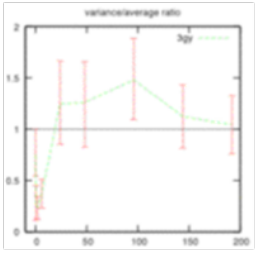






Reality:





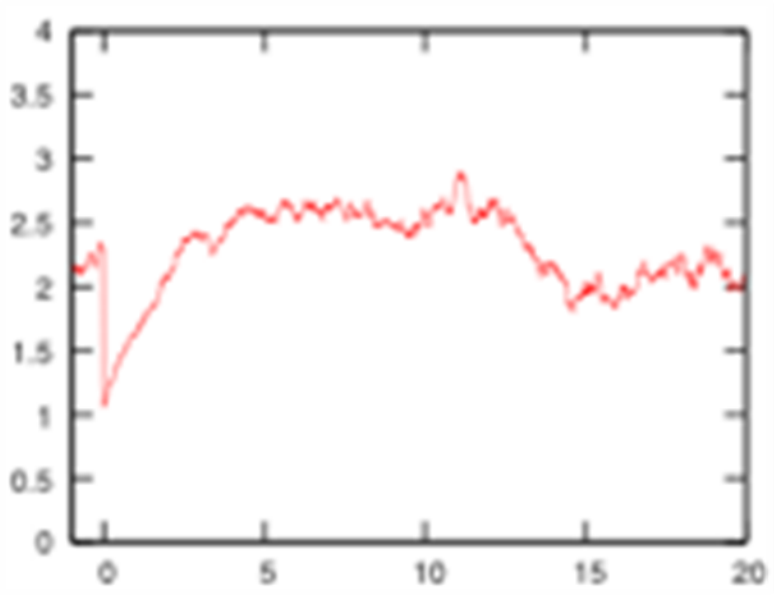
Possible Biological Mechanisms:

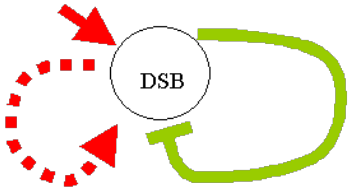
-> bystander-like effect?

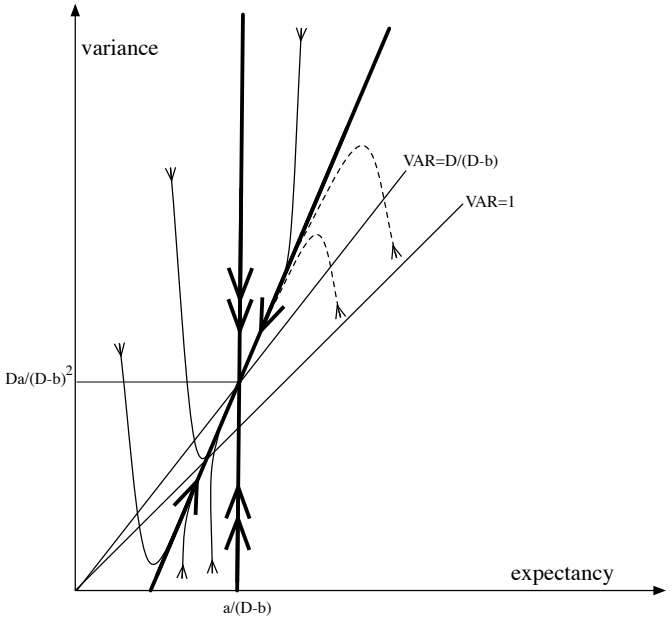
-> enzymatic saturation?

-> extra repair pathways?

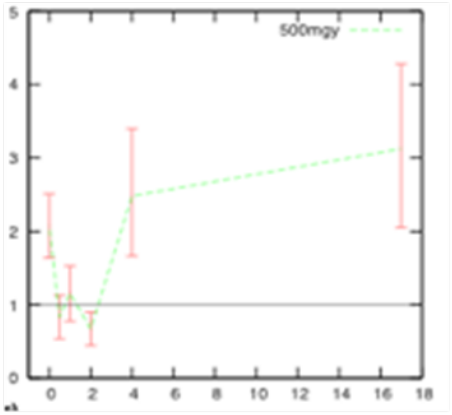
Numerical analytical







Data:



$$\begin{pmatrix} \frac{d}{dt}\mu(t) \\ \frac{d}{dt}\mu_2(t) \end{pmatrix} = \begin{pmatrix} a \\ a \end{pmatrix} + \begin{pmatrix} -(D-b) & 0 \\ D+b & -2(D-b) \end{pmatrix} \begin{pmatrix} \mu(t) \\ \mu_2(t) \end{pmatrix}$$

Verification?

- Ideally, identify molecular pathway
- More realistically: follow individual cells in time and follow the creation/disappearance of *individual DSBs*

Summary + Conclusions

- Existing deterministic model explain well the DSB repair dynamics when observed at the population level (averages only).
- Not so good when considering distribution of DSBs.
- Hypothesis #1: there are feedback loops (ie individual DSB repair dynamics depend on the number of other DSBs in the cell). These may explain some of the distributional features observed.
- Hypothesis #2: Variabilty of something that is being observed can tell something about the underlying dynamics.

Acknowledgements

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