STOCHASTIC ACTIVATION OF A DNA DAMAGE RESPONSE CAUSES CELL-TO-CELL MUTATION RATE VARIATION

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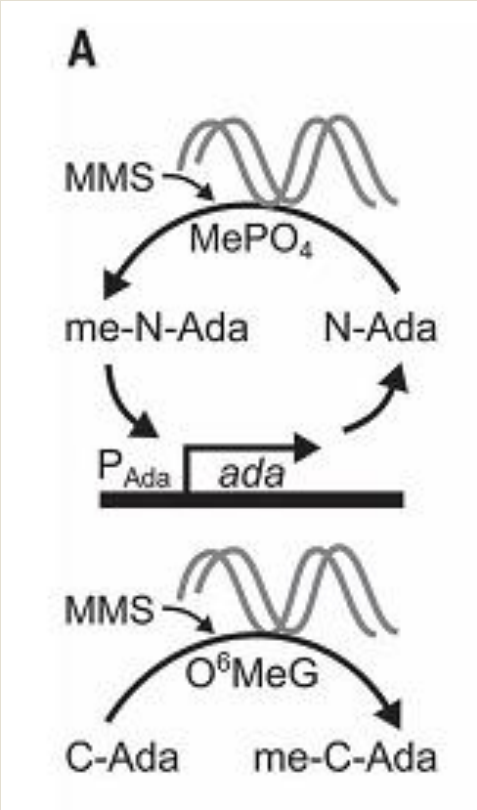
Question?

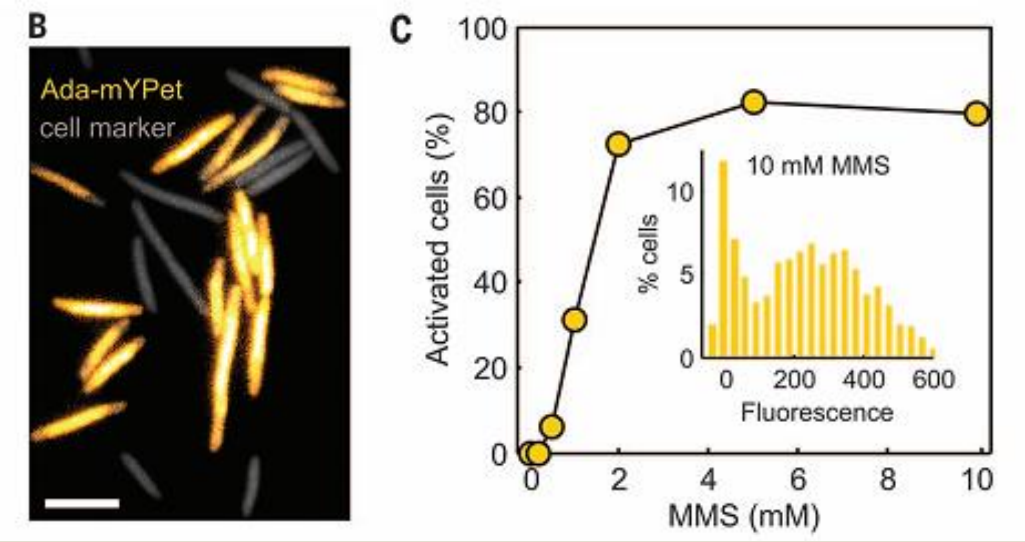
- Most damage events are reversed by active repair systems, but the ones that escape repair can cause cell death or mutations. An intriguing question is what causes those failures.
  - Specifically, the classic perspective suggests that failures to repair reflect the intrinsic error rate of the repair enzymes, for example, because of the random search for lesions.
  - Alternatively, most failures could occur in an error-prone subpopulation of cells (3, 4) in which repair is compromised by fluctuations in the abundances of the repair proteins

Subjects



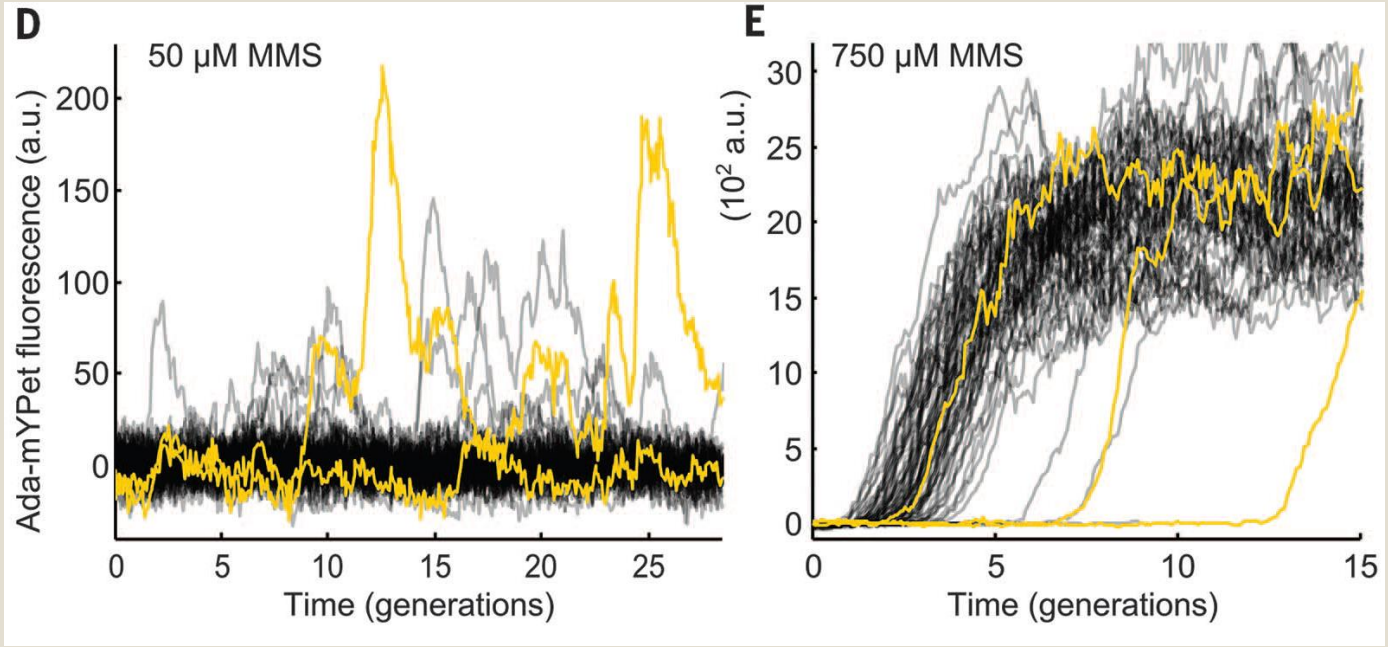
- The Ada protein functions not only in the direct repair of alkylated （烷基化） DNA but also as the transcriptional activator of the adaptive response (Fig. A).
- Specifically, ada expression is induced by methylated Ada (meAda) after irreversible methyl transfer from DNA phosphotriester （磷酸三酯） and O^6MeG lesions onto cysteine （半胱氨酸） residues of Ada.
- Because Ada is present in low numbers before damage, this positive-feedback gene regulation may amplify stochastic fluctuations and create cell-to-cell heterogeneity in the repair system.

Results



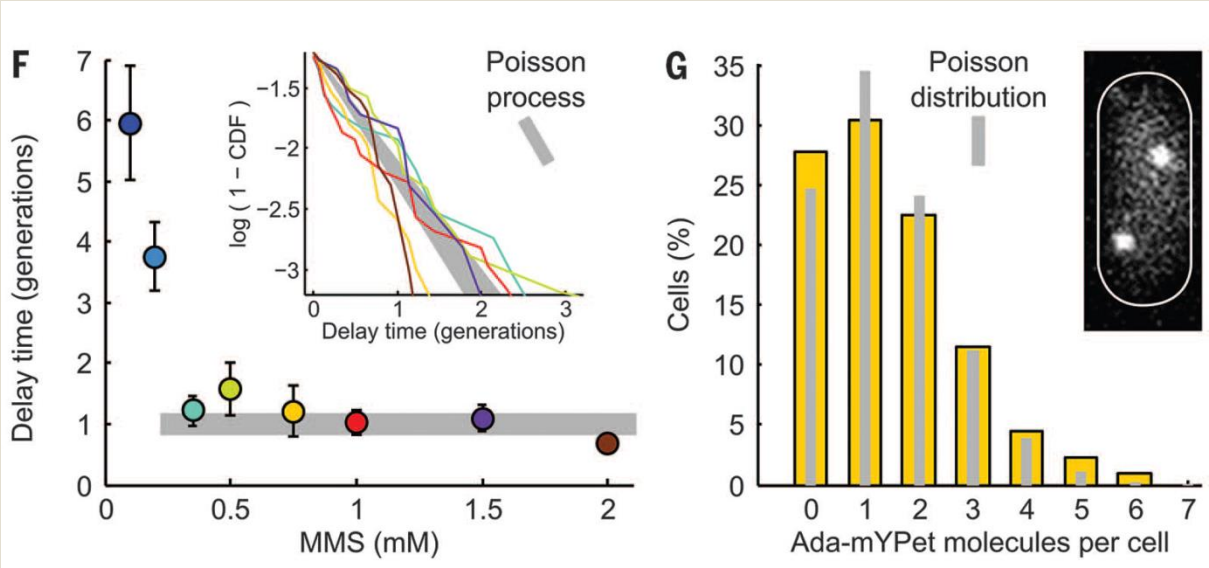
- B:
  - Ada-mYPet fluorescence (yellow) in cells treated with 10 mM MMS (甲磺酸甲酯，烷化剂) for 1 hour.
- C:
  - 20% of the cells did not respond at all, even at saturating doses of MMS.

Results



- At low-to-intermediate MMS concentrations (<200 mM MMS), cells showed random unsynchronized pulses of Ada expression (Fig. 1D).
- At higher MMS concentrations, most cells rapidly induced a persistent and uniform response (Fig. 1E). However, 20 to 30%of cells were lagging even at saturating MMS.

Results



- Late-responding cells activated the response with a Possion rate of once per cell cycle. (Fig. F)
- Distribution of ada protein in live cells without MMS. (Fig. G)

Possion distribution

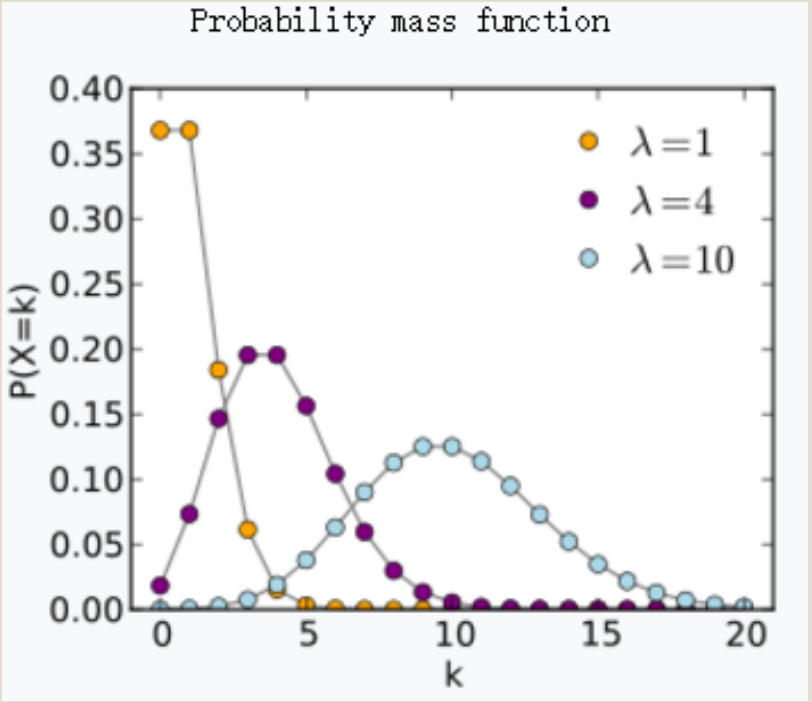
$$P(k \text{ events in interval}) = \frac{\lambda^k e^{-\lambda}}{k!}$$

$$P(k \text{ overflow floods in 100 years}) = \frac{\lambda^k e^{-\lambda}}{k!} = \frac{1^k e^{-1}}{k!}$$

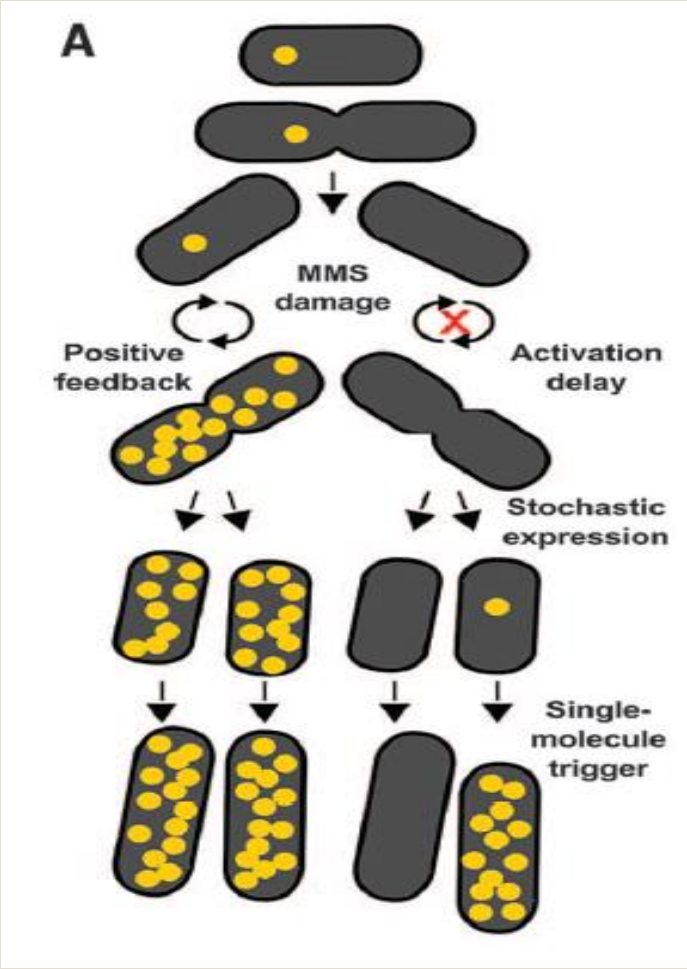
$$P(k = 0 \text{ overflow floods in 100 years}) = \frac{1^0 e^{-1}}{0!} = \frac{e^{-1}}{1} = 0.368$$

$$P(k = 1 \text{ overflow flood in 100 years}) = \frac{1^1 e^{-1}}{1!} = \frac{e^{-1}}{1} = 0.368$$

$$P(k = 2 \text{ overflow floods in 100 years}) = \frac{1^2 e^{-1}}{2!} = \frac{e^{-1}}{2} = 0.184$$

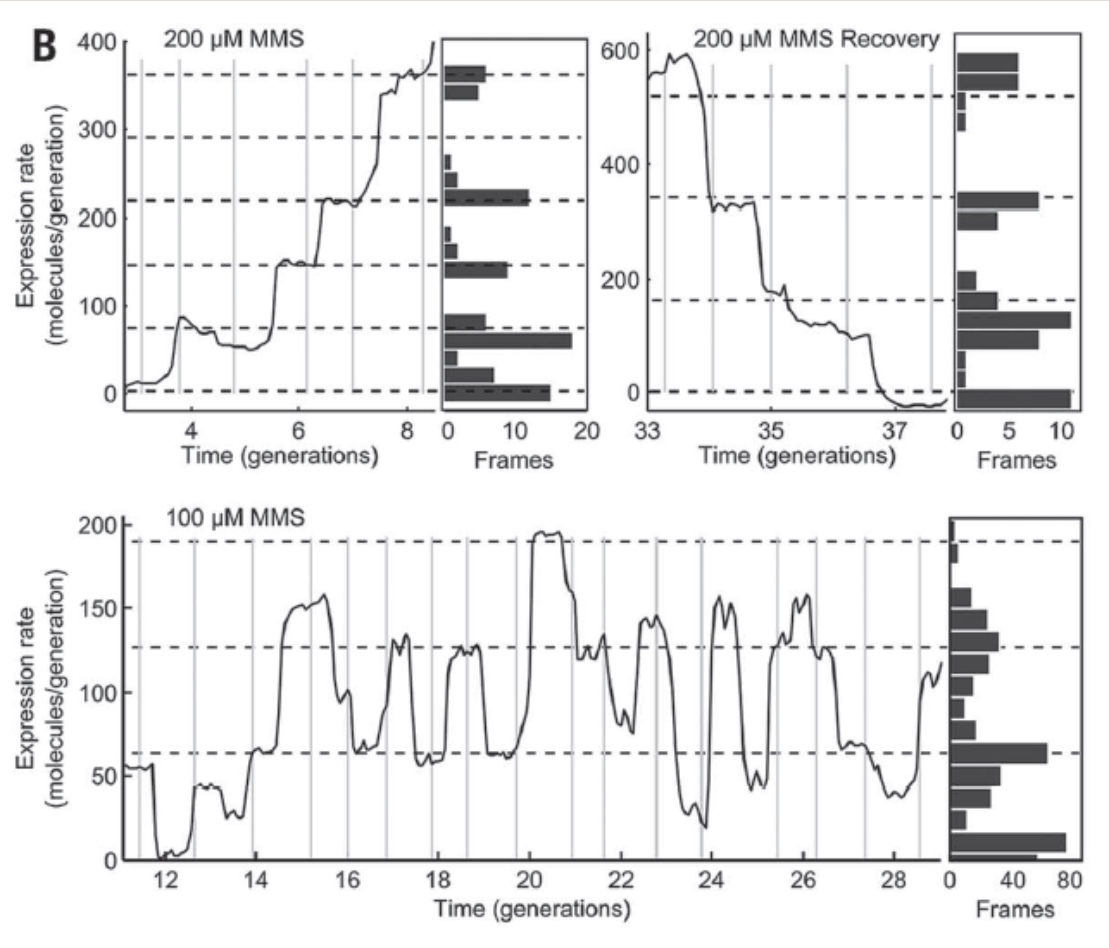


Results



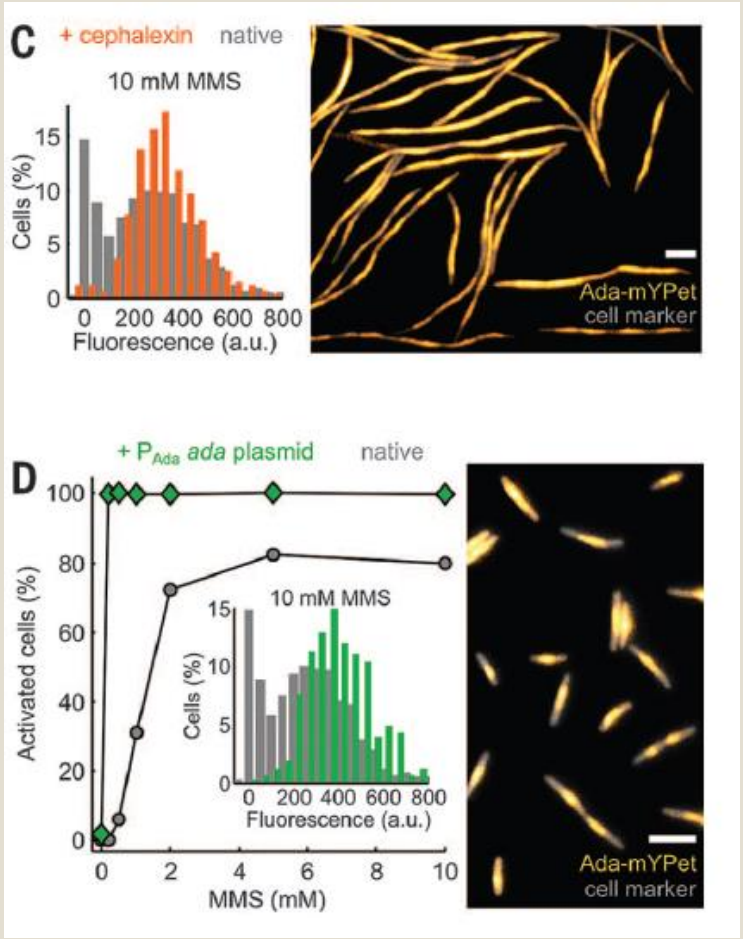
- Most cells reliably launch the response with just one or two Ada molecules to sense the damage and to induce ada expression.

Results



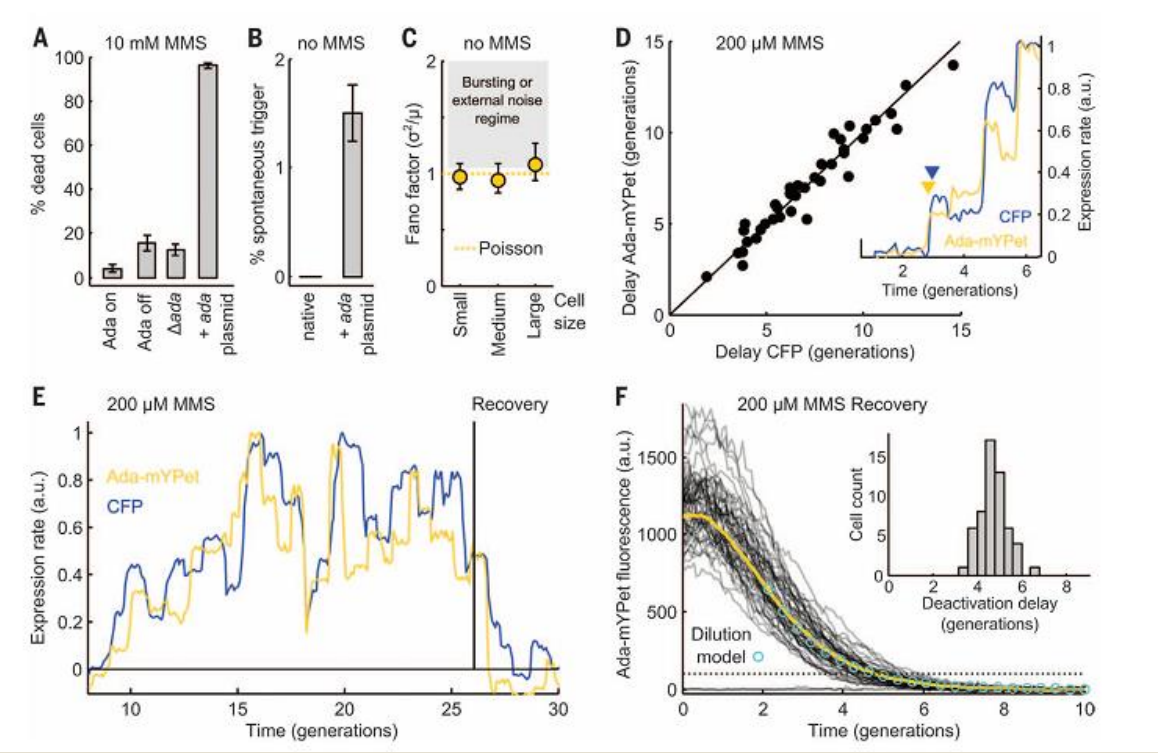
- Sections of time traces showing distinct steps in Ada-mYPet expression rates during response activation upon 200 mMMS treatment, deactivation after MMS removal, and stochastic activation and deactivation transitions with 100 mM MMS.
- Vertical lines indicate cell divisions. Histograms show number of frames spent in the expression rate states.

Results



- Because failure to trigger the adaptive response seems to be the result of a complete lack of Ada molecules in a fraction of cells, it should be possible to reduce this fraction with a slight increase in the average abundance of Ada.

Why keeping Ada at low number



- The extremely low abundance of Ada can thus be advantageous to the population as a whole, which implies that the repair system faces a trade-off to repair exogenous alkylation damage without introducing harmful effects.

More—mutation rates

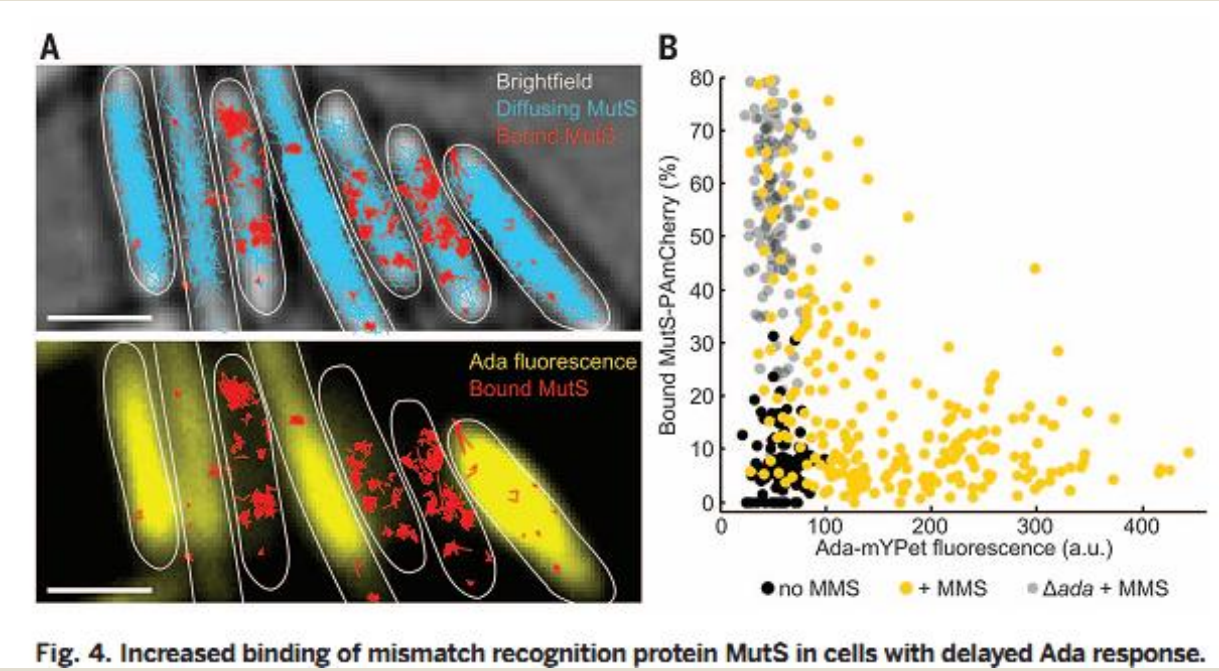


Fig. 4. Increased binding of mismatch recognition protein MutS in cells with delayed Ada response.

- Stochastic activation of the adaptive response therefore leads to an error-prone cell subpopulation that does not efficiently repair DNA alkylation damage and accumulates mutations.

Conclusion

- 主要结论：DNA损伤修复的失败由于：1.不同细胞内修复蛋白的丰度不同，2.修复蛋白随机寻找DNA损伤；DNA修复失败最终导致细胞间突变率的差异。
- 启发
  - 找有探究价值的问题，而不只是增加学术研究的热闹气氛。
  - 研究要有清晰的逻辑思路，精密的实验设计再配合先进的实验手段。
- 研究可能的改进及拓展：
  - 在多细胞生物中是否存在不同细胞类型或相同细胞类型不同细胞间修复蛋白的丰度差异，及其影响
  - DNA损伤修复的失败造成的细胞突变，对酵母的进化（如基因的转录调控）产生的影响。