COMMENTARY

Single-cell mutagenic responses and cell death revealed in real time

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When musing about evolution, the physician and scientific essayist Lewis Thomas wrote, "The capacity to blunder slightly is the real marvel of DNA. Without this special attribute, we would still be anaerobic bacteria and there would be no music" (1). Understanding how DNA mutations arise in cells, either spontaneously or after damage, has been a central question for scientists since the discovery of the structure of DNA over six decades ago. Since that time, significant progress on defining how DNA is damaged by processes external and internal to the cell has been made. Furthermore, several DNA repair pathways have been investigated at the molecular level and have been shown to reverse these genomic injuries, often including feedback mechanisms that up-regulate repair once severe damage is detected. In recognition of this significant mechanistic research in the field of genome stability, two important prizes were awarded in 2015: the Lasker Award to Stephen Elledge and Evelyn Witkin, and the Nobel Prize in Chemistry to Tomas Lindahl, Paul Modrich, and Aziz Sancar. Mismatch repair (MMR), worked out in detail by Modrich (2) and his colleagues, is comparable to the backspace button on our computers to correct typing errors. This process, conserved through all kingdoms of life, acts to eliminate replication errors that occur when a DNA polymerase places the wrong nucleotide triphosphate opposite its appropriate template or opposite a damaged nucleobase, diminishing DNA replication fidelity. The newly synthesized strand is marked by its temporary lack of methylation and it is this feature that allows MMR to fix the newly introduced errors. In *Escherichia coli*, MMR is mediated by three key proteins: MutS, MutL, and MutH. MutS recognizes the mismatch and loads multiple copies of MutL next to the lesion. These molecules translocate along the DNA to locate the MutH endonuclease, which is itself searching for hemimethylated GATC sequences. The resulting interaction triggers the next phase (excision-resynthesis) of repair, which corrects the mismatch. Although textbook renditions of these pathways appear clear and organized, life is dynamic and chaotic, with heterogenous expression of proteins in different cells. To make sense out of chaos, Stephan Uphoff, in a rare single-author article (3), offers a glimpse of how individual cells adapt to deal with DNA damage and how mutations arise in bacterial cells. Uphoff has elegantly applied the simple and accessible technologies of microfluidics and fluorescence microscopy to observe the binding of a yellow fluorescent protein-tagged (mYPet) MMR protein, MutL-mYPet, to replication errors arising in individual bacteria in real time. The binding of this protein to mismatches serves as a proxy for the accumulation of mutations in dividing cells. Uphoff has revealed that mismatches arise stochastically at different rates in individual cells, and that mutagenesis depends upon both the preexisting

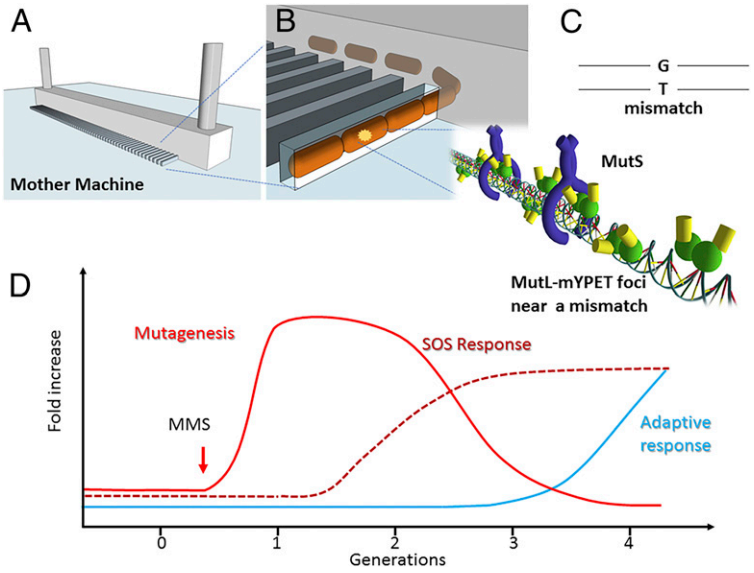

Fig. 1. Watching mutagenesis and cell death in real time. (*A*) Schematic of the "mother machine." (*B*) *Inset* shows mother and daughter cells flowing out of the capillary. (*C*) Mismatches appear as foci of MutL-mYpet; tracking of the cell and induction of the adaptive or SOS responses can be followed by the induction of a fluorescent reporter. (*D*) Chronology of mutagenesis and responses to genotoxic stress.

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levels of DNA damage-processing proteins in the cell, and that cell's ability to up-regulate its own repair responses.

DNA damage that leads to mismatches can be caused by treating cells with the alkylating agent methylmethane sulfonate (MMS), which donates a methyl group to the N7 position (~80%) of guanine or to adenine at the N7 (~2%) or N3 (10%) positions. While donation to other positions occurs substantially less frequently, it includes the O6 position of guanine (O6meG), which pairs equally well with T or C, and consequently is highly mutagenic. N7-guanine adducts are unstable and spontaneous hydrolysis of the glycosidic bond generates abasic sites; as with methyl-N3-adenine lesions (3mA), these are strong blocks to replicative DNA polymerases and are repaired by the base excision repair pathway. Visualizing mismatches in cells was pioneered by Elez and Radman (4–6), who developed a general method for observing foci of a fluorescently labeled MMR protein (MutL) at sites of mispaired DNA in actively growing cultures of *E. coli*. As shown in Fig. 1, Uphoff (3) used a similar approach with a microfluidic device known as the "mother machine." This device has multiple channels wide enough to accommodate one bacterial cell at the end of each channel and cell division pushes daughter cells along the channel, out into the media (7). This flow cell was coupled to a fluorescent microscope equipped with an sCMOS camera to create time-lapse movies of the tagged proteins. Building on his previous work (8), Uphoff (3) used automatic tracking of actively growing MMS-treated *E. coli* cells and followed the appearance of MutL-mYPet foci over several generations. After a rapid rise in mismatched bases (within 20 min), there was an exponential decay of these mismatches that returned to base line within two to four generations, depending on the initial dose of MMS. *E. coli* cells mount two general types of response to DNA damage: the adaptive response and the SOS response. The adaptive response was first described by Leona Samson and John Cairns, who found that cells exposed to a low dose of an alkylating agent could withstand a subsequent onslaught of much higher concentrations of the same or different alkylating agent (9). It was later shown that this response was mediated by a specific genetic region in *E. coli* under the control of a unique protein, Ada, which directly reverses O6meG lesions through a methyltransfer-suicide reaction. In a phoenix-like transformation, methylation of Ada blocks further rounds of transferase activity but then transforms this protein into a transcriptional activator for itself and two other repair proteins: AlkB (dioxygenase/demethylase) and AlkA (3mA glycosylase). The *alkA* gene can be induced 100-fold after exposure to an alkylating agent, which complements the constitutive expression of another such enzyme, Tag (3mA DNA glycosylase).

Uphoff (3) used a fast-maturing CFP under the control of the Ada promoter to define the timing of the adaptive response. At the single-cell level, he clearly established that Ada levels are inversely proportional to the production of mismatches; the stochastic induction of Ada helps to ameliorate the mutagenic potential of MMS. The power of the single-cell approach used here was validated by the correlation between the initial level of Ada expression and the MMS response time. Uphoff notes that cells lacking Ada protein before MMS treatment needed to go through several generations before they expressed sufficient Ada to lower the mutagenesis rate. Alternatively, cells already expressing Ada showed a rapid response to initiate a fast decline in mutagenesis. Furthermore, Uphoff shows that cells in which the *ada* gene was deleted showed no decline in mutagenesis, but the accumulation of mutations was capped. To test the hypothesis that this mismatch accumulation rate was affected by repair, Uphoff deleted the constitutive repair proteins Ogt (O6meG DNA methyltransferase) and Tag, affecting both the number of mismatches and the timing of the mutational wave. Ogt reverses the mutagenic O6meG lesions, which if unrepaired cause O6meG-T mismatches, whereas Tag works on the replication blocking 3mA adducts so that mismatches arise from these lesions more slowly. Finally, Uphoff showed that deleting AlkA, the inducible 3mA glycosylase, resulted in a slower increase in mismatches that did not show any decline over as many as six generations. Using the mother machine, Uphoff could also provide a chronology for the contribution of DNA damage to cell death. He found Tag was responsible for the survival of ~25% of the cells that failed to mount an inducible Ada response, and the induction of AlkA was the key component of the Ada response that contributed significantly to cell survival.

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The next question Uphoff (3) explores is the dynamics of the SOS cellular damage response, first worked out by Evelyn Witkin for UV light-induced mutagenesis (10). Because many replicative DNA polymerases simply stall when encountering a lesion, it was shown by others, including Walker and colleagues (11) and Woodgate and colleagues (12), that two new DNA polymerases (Pol IV and Pol V) are induced in *E. coli* during SOS to bypass damaged bases. These DNA polymerases are necessarily error-prone to bypass the lesion and are associated with mutation induction. Using a fluorescence reporter for the SOS response, which is under LexA control, Uphoff (3) found that all cells had a low basal expression that rose uniformly to high steady-state levels within two generations following induction. Interestingly, he found that the SOS response was directly correlated with the formation of mismatches. Finally, by following cell survival, Uphoff shows that SOS is essential for survival immediately after MMS exposure, whereas the adaptive response is important for survival after prolonged exposure. Uphoff found that SOS responses were well correlated with hyperinduction of mismatches generated by the fluoroquinolone antibiotic, norfloxacin, likely due to the induction of the error-prone polymerases during SOS. This last experiment harks back to earlier work by Jeffrey Miller and colleagues (13) that showed the passage of wild-type *E. coli* through three selection steps, including antibiotic resistance, resulted in individual bacteria that were defective in MMR. The surprising observation in those studies is that while deletion of either of the two inducible polymerases dinB (Pol IV) or umuD (Pol V) lowered cell survival, they had no effect on the dynamics of mismatch production. These results differ from previous studies showing that Pol IV deletion lowers MMS mutagenesis (14). However, one concern is that the MutL-mYPet reporter may not rapidly identify mismatches inserted across from damaged bases bypassed by these polymerases.

Overall, the Uphoff (3) study nicely defines the dynamics of damage response at the single-cell level and describes how *E. coli* cells reduce (adaptive response) or enhance (SOS response)

mutagenesis. The chronology of events that occur in actively growing bacteria after MMS treatment proceeds to first remove as many lesions as possible with the repair enzymes on hand (constitutive repair), before replication-fork stalling. Second, because cells need to complete the DNA replication cycle to survive, they must induce DNA polymerases (SOS response) that can bypass alkylated bases. Finally, only if genotoxic stress continues will the bacterial cell induce more repair enzymes (through the adaptive response) to help lower mutational load, which is deleterious to the fitness of the organism's lineage (6).

Real-time optical detection of mismatches could be used to help answer a wide range of questions. For example, what are the effects of nutrient deprivation on mutagenesis, first studied by Witkin (15), which she described as the phenomena of mutation frequency decline. This powerful platform could detect other types of damage and subsequent mismatch production: such as, for example, labeling UvrB with one color and MutL with another, which might allow UV-induced mutagenesis to be followed in real time. In the future one can envision an approach where this microfluidic and optical platform could be combined with single-cell sequencing to correlate the frequency of mismatch production with the actual DNA sequence changes that have occurred in one particular cell and its daughters over time. Such studies would be (the Lewis Thomas) music to the ears of clinical scientists studying how antibiotics may drive mutational load, and subsequent antibiotic resistance in bacterial cells.

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