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The Digital Character Of SOS System

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Living cells use regulated genetic networks to respond to changes in their environment. Studying the dynamic response of these networks at the level of individual cells provides access to system properties which may be masked in studies of cell populations. Here, we present a study of the regulation of a well-characterized bacterial network, the SOS system, in individual *Escherichia coli* cells. This system was the first bacterial DNA damage-induced regulatory network to be identified.

Studies of the SOS system in the last 30 years provided detailed information on its components and the interactions between them (*1,2*). This network includes more than thirty genes in *E. coli (3,4)*, which carry out diverse functions including nucleotide excision repair, translesion DNA replication, homologous recombination and cell division arrest. The network is controlled by a master repressor, LexA, which down-regulates itself and the expression of the other SOS genes (Fig. 1A). While the sequence of events that lead to the induction of the response *(5)* has been well studied, less is known about the regulation after induction, and how the response is shut off.

In this study, the dynamics of SOS induction was investigated at high temporal resolution in individual cells and in bacteria cultures. The promoter activity of a LexA-repressed promoter *(recA or umuDC)* was monitored using low-copy plasmids in which the promoter under investigation was fused to the green fluorescent protein (GFP) gene. The accumulation of GFP in a cell is proportional to the rate of transcript production from the promoter *(6)*.

Time-lapse fluorescence microscopy was used to measure the fluorescence intensity and size of individual bacteria after UV irradiation (Fig 1B-C). Alternatively, multiwell fluorimetery was used in measurments of bacteria cultures. From these measurments the promoter activity (PA) could be

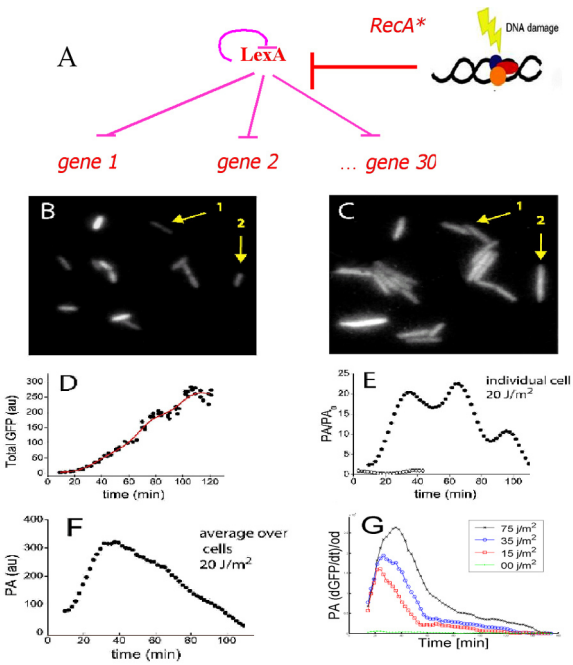

Fig. 1 *(A) The SOS genetic network. (B and C) Snapshots of AB1157 E. coli cells with GFP reporting for the RecA promoter(6), (B)8 min and (C) 70 min after irradiation with a UV dose of 10 J/m². Cell 1 divides whereas cell 2 undergoes filamentation. The integration time corresponding to B is ten times that for C. (D) Total GFP as a function of time in an individual cell irradiated at 20 J/m². (E) Normalized promoter activity PA/PA$_0$ as a function of time for the same cell as in D. PA/PA$_0$ measured on a typical unirradiated cell is also shown (empty circles). (F) Average PA over all 23 cells in an experiment at 20 J/m². (G) Normalized promoter activity (dGFP/dt)/od as a function of time for bacteria culture.*

calculated, either as an average value in culture experiments, or for every individual bacteria in the microscopy experiments. Bacteria culture irradiated with different levels of UV gave a typical single peak in which promoter activity increases during the initial phase of the SOS response, and decreases

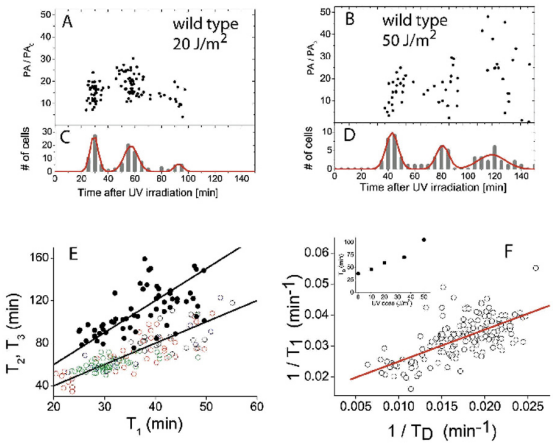

Fig. 2 *Quantitative analysis of the oscillatory behavior. Peak promoter activity is plotted as function of peak time for individual cells irradiated with a UV dose of: (A) 20 J/m^2, (B) 50 J/m^2. (C-D) Histograms of peak times corresponding to (A-B), and fits to the histograms with the sum of three gaussians (red line). (E) Time of the second (T_2, empty symbols) and third (T_3, full symbols) peaks in promoter activity as a function of the time of appearance of the first peak (T_1). Each point corresponds to an individual cell. Full lines, $T_2=2T_1$ and $T_3=3T1$ are shown as a guide to the eye. (F) $1/T_1$ as a function of the doubling rate $1/T_D$ of individual cells irrespective of dose. A linear fit to the data (red line) yields a slope of 1.0±0.1. Inset: Cell doubling time T_D grows as a function of UV dose.*

to normal levels after the response is shut off, as was shown before *(6)* (fig 1G). The characteristics of the SOS response in culture correspond to an analog response, where the level of induction grows in response to increased levels of damage. Analog circuit can be created by an open feedback loop, in this case RecA, cleaving LexA after damage.

The response of single cells is highly structured, in contrast to the single-peaked response found in culture. Three peaks are typically observed within the duration of the experiments, with the typical number of peaks increasing with damage level.

We plot in Fig. 1D-F the amplitude and time of the PA peaks for each and every cell in the experiments. The data form distinct clusters, characterized by narrow variability in peak timing but larger variability in PA values. To further characterize the timing of the peaks, we plot in Fig. 2A-D the corresponding histograms of peak times, irrespective of their amplitude. The histograms exhibit three narrow peaks (std/mean <~ 10%), showing that peak timing is very accurate among different cells under the same UV dose. In contrast, the variability of peak PA values is larger (std/mean ~ 25%). A pronounced positive correlation is found between the first peak (T_1) and the second peak (T_2) (and also between T1 and T3) in the same cells (fig 2E-F). The ratio $\langle T_2/T_1 \rangle$ averaged over all 132 cells measured is 1.98 ± 0.02, (mean ± SE), whereas $\langle T_3/T_1 \rangle$ = 2.98 ± 0.06 for the 60 cells that show a third peak.

The increase in the number of peaks correlates with an increase in UV dose (or DNA damage), suggesting that the system has a mechanism of checking the precision of the repair. The exact peak timing ratio of 1:2:3 suggests that the response is controlled by a clock that measures the time elapsed since the detaction DNA damage. Mutation in the UmuD gene causes the loss of the time precision characterizing the response.

A number of proteins of the SOS network including DinI, the recombination system and others SOS protein are known to compete with LexA for the activated RecA* filament. This competition may lead to modulation of LexA cleavage, and hence checkpoints by closing a negative-feedback loop

Our experiment on single cells shows for the first time that the SOS response shows an oscillatory/digital feature. This feature was masked in experiments carried on bacterial culture, where average properties were measured.

Selected Publications

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