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Simultaneously Monitoring Gene Expression Kinetics and Genetic Noise in Single Cells by Optical Well Arrays

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An optical fiber based well array platform was used for simultaneous, dynamic gene expression monitoring from hundreds of individual live *Escherichia coli* cells carrying promoter−fluorescent reporter gene fusions. High information content about gene expression kinetics and cell-to-cell gene expression variability can be collected from a single experiment. These data are invaluable for investigating gene regulation and gene networks as well as for systems biology applications.

The living cell is a complex system in which thousands of biochemical processes occur simultaneously.[1] This complexity leads to large stochasticity in the rates and timing of gene expression processes among individual cells in a clonal population[2−4] and results in noise among cell populations. Genetic noise provides information about the structure of gene regulatory circuits[5−8] and can help to predict, understand, and ultimately design gene network functions.[3,9−15] To investigate genetic noise, it is necessary to monitor gene expression in many individual cells simultaneously.[16] Recently, flow cytometry[17] was used to measure genetic noise in prokaryotic[3] and eukaryotic[4] cells. Flow cytometry can provide only a single measurement at a single time point of a sample from a cell population, and therefore, it cannot provide continuous gene expression and genetic noise analysis of the same individual cells in a population over time. In this paper, we demonstrate the use of an optical fiber based cell array platform[18] to overcome these limitations and to make the first measurements of the dynamics of genetic noise.

The cell array platform is based on placing cells in an array of microwells (10^7 microwells/cm^2 for bacterial cell array) etched into the face of an optical fiber bundle. The microwells were sized such that each microwell holds only one cell.[18,19] Isotropic fluorescence signals from individual cells at the distal end of the fiber bundle are transmitted back through the fiber bundle to the proximal end and captured by a charge-coupled digital (CCD) camera. The signal transmitting process is highly efficient, enabling sensitive measurements of every individual cell's response.[20] Each optical fiber in the bundle has its own independent light pathway, allowing for thousands of individual cells to be monitored simultaneously with both spatial and temporal resolution.

EXPERIMENTAL SECTION

Strains and Media. *Escherichia coli* strains MG1655+pUA2699 and MG1655+pUA0344 containing a low copy number pSC101 plasmid carrying the gene fusion *recA::gfp*[21] and *lacZ::gfp*,[22] respectively, were inoculated overnight in M9 minimal medium (Becton Dickinson, Le Pont de Claix, France) supplemented with 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, 0.4% glycerol, 0.1% casamino acid (Fisherbiotech, Fair Lawn, NJ), and 50 μg/mL kanamycin sulfate (Fisher Scientific, Fair Lawn, NJ) at 37 °C in an incubator shaker (New Brunswick Scientific, Edison, NJ). Fresh cultures were prepared by diluting the overnight culture 1:50 and incubating at 37 °C until the OD_{600} reached 0.1 as measured using Beckman DU 530 Life Science UV/visible spectrophotometer (Beckman Coulter, Inc., Fullerton, CA).

Cell Array Fabrication and Measurements. Etched imaging fiber bundles containing 3.1-μm-diameter microwells (Illumina, San Diego, CA) were used to fabricate the individual *E. coli* cell arrays as described previously.[20] Aliquots (10 μL) containing ∼10 000

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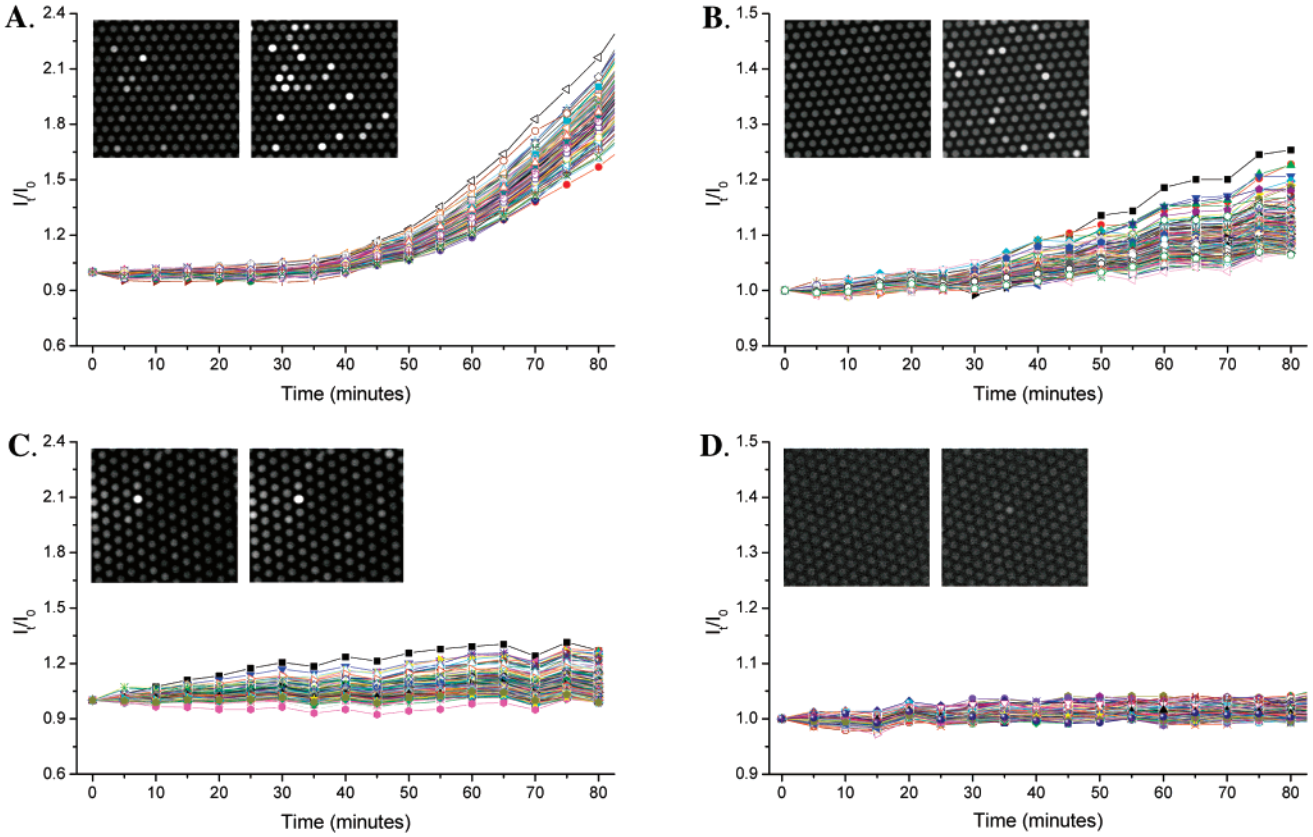

Figure 1. Monitoring *lacZ* and *recA* promoter activity in multiple individual cells. Fluorescence signals are expressed as the fluorescence intensity increase ($I_t/I_{0)}$. (A) Individual *recA::gfp* cells from an array exposed to 10 μg/mL MMC. (B) *recA::gfp* cells in the control array incubated only with medium. (C) Individual *lacZ::gfp* cells in an array exposed to 5 mM IPTG. (D) *lacZ::gfp* cells in the control array incubated only with medium. Approximately 200 cells from each array are shown. Fluorescence images from a small portion of the imaging fiber based cell array corresponding to each experiment are shown in the insets. In each inset, the left panel shows the fluorescence image at time 0 and the right panel shows the image after 80 min.

cells from overnight or fresh cultures were loaded into the array. The cell array was then mounted on an epifluorescence microscope (model BX61, Olympus America Inc., Melville, NY). Fluorescence images were acquired every 5 min from the proximal end of the fiber by a CCD camera (Orca-ER, Hamamatsu). The fluorescence signals (ex 480 nm/em 520 nm) were measured by IPlab software (Scanalytics, Fairfax, VA) with a 500-ms acquisition time.

Induced and Noninduced Cell Array Experiments. To begin the gene expression experiments, medium was introduced to the array and fluorescence signals were immediately measured at predetermined time intervals (5 min). M9 medium was used for control experiments, and M9 supplemented with 10 μg/mL MMC or 5 mM IPTG was used for induction experiments. The concentrations that fully induce *lacZ* and *recA* promoters were obtained from cell array experiments with different concentrations of IPTG (data not shown) or MMC,[20] respectively. Because the noninduced *lacZ* cells signals are very low, we verified the live cell's location by exposing the cell array to 5 mM IPTG at the end of the control array experiments.

The simple cell immobilization procedure and the physical structure of the well arrays provide easy access to medium enabling the cells to be kept alive and functioning over a long period of time. In a previous study, we monitored *recA* promoter activity in the MG1655+pUA2699 cell strain and obtained dose−response relationships to multiple inducers that were comparable to conventional cell culture based microtiter plate assays.[20] In addition, we monitored the *lacZ* promoter activity in the MG1655+pUA0344 cell strain over 8 h with our well arrays and found that GFP accumulated continuously in the cells over the entire course of the experiment (data not shown). Cell division is avoided because the cells are confined in the microwells, they are provided with only minimal medium, the carbon source (glycerol) is not readily metabolized, and the cells are maintained at ambient temperature, below their optimal growth temperature.

Data Analysis. The acquired images were analyzed, and the intensity levels from each cell-containing microwell were determined. Fluorescence intensity was recorded in arbitrary units and expressed as percentage increase I_t/I_0 (Figures 1 and 2). I_t denotes the fluorescence intensity at time t; I_0 denotes the fluorescence intensity right before applying the inducer. Noise for each time point was expressed as variance (the square of standard deviation (SD)) divided by the mean (av) and then multiplied by 10^4 [$(SD)^2/av \times 10^4$]. To compare the noise of both *recA* and *lacZ*, gene noise values were plotted versus expression levels (Figure 3). The maximum expression level was set to 1.2 because this was the average value obtained at 80 min in the *lacZ* cell array experiments (Figure 2B).

RESULTS AND DISCUSSION

Simultaneously Monitoring Genetic Activity in Multiple Single Cells. The optical imaging fiber based single bacterial cell

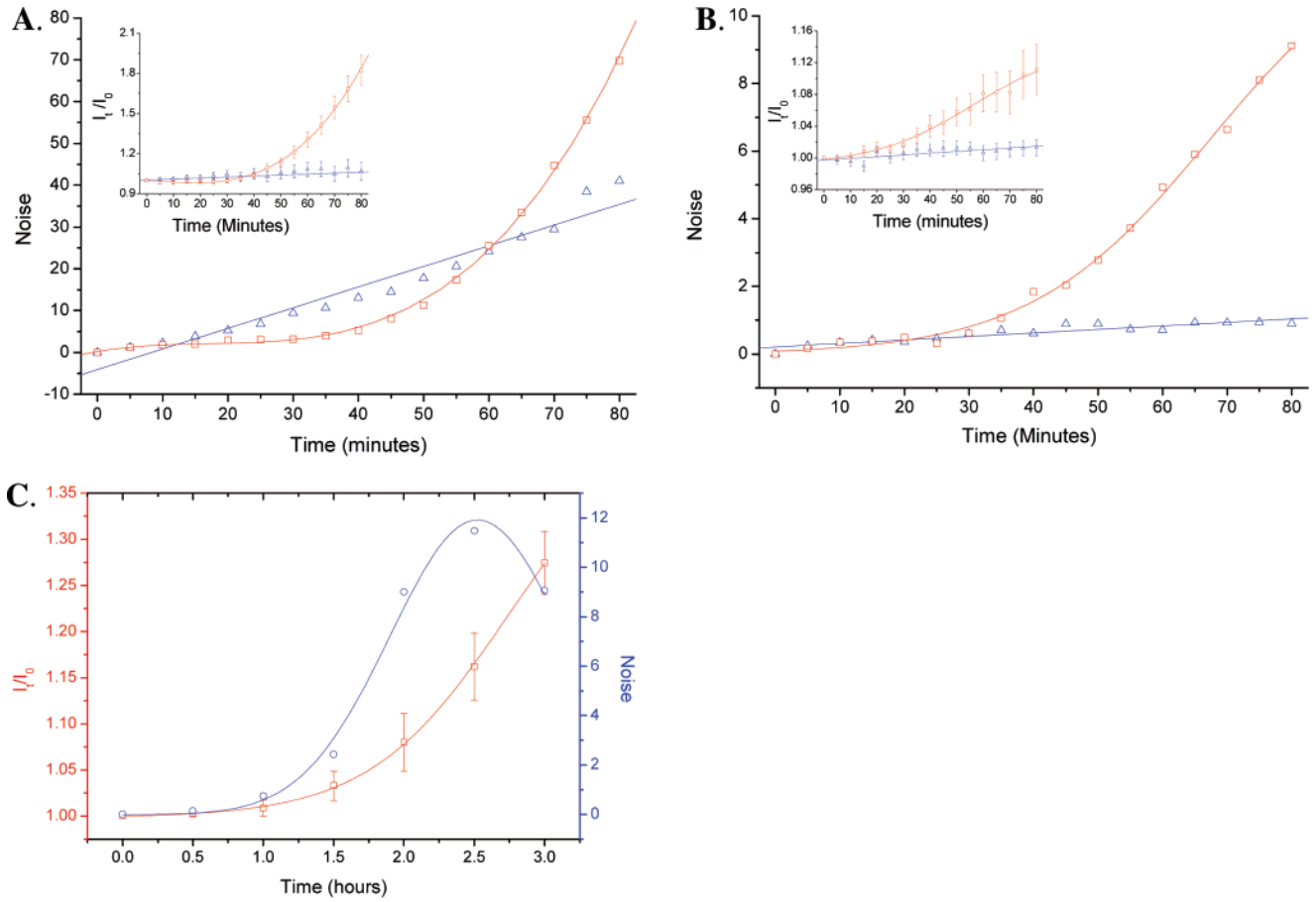

Figure 2. Measuring *lacZ and recA* genetic noise. (A) The noninduced *recA* noise (triangles) shows a linear increase with $R^2 = 0.95$ and slope of 0.4940. The induced *recA* (squares) shows a near-exponential increase. Third-order polynomial fit is included for clarity with $R^2 = 1.00$. Inset 1 shows the average increase in gene expression signals from induced and noninduced cells. Error bars represent 95% confidence. (B) The noninduced *lacZ* noise (triangles) show a linear increase with $R^2 = 0.86$ and slope of 0.0104. The induced *lacZ* noise (squares) shows a more exponential shape increase. Second-order polynomial fit is included for clarity with $R^2 = 1.00$. Inset shows the average increase in gene expression signals from induced and noninduced promoters. (C) Induced *lacZ* noise (circles) shows a decrease in a longer experiment and higher expression levels (squares).

arrays were employed to study *lacZ* and *recA* gene expression kinetics and the associated dynamic change in population noise in *E. coli* strains carrying pSC101 plasmids with *lacZ::gfp* and *recA::gfp* fusions, respectively. Gene expression was measured by acquiring fluorescence images from the array (Figure 1 insets) every 5 min. The fluorescence intensity from each cell-containing microwell correlates to the cell's gene expression level. When the image sequence was analyzed and the increase in intensity from each cell was plotted over time, a graph was obtained showing expression kinetics from many individual cells (Figure 1). To monitor the expression rates, we normalized each cell's intensity level to the first measurement. Such normalization makes the analysis independent of instrumental and array-related variations such as the cell size or its orientation within the microwell.

***E. coli* MG1655(*recA::gfp*) and MG1655(*lacZ::gfp*) Gene Expression Kinetics.** The *recA* expression was monitored in individual cells from an array exposed to the genotoxin mitomycin C (MMC), a strong inducer of *recA*[23] (Figure 1A). A control array incubated without inducer was monitored (Figure 1B). The two inset images in Figure 1A and B show a portion of the cell arrays. The left image was taken at time zero and only background signals were detected. The right image was taken after 80 min. The bright spots represent high GFP fluorescence and correspond to high *recA* expression levels. The variation in transcriptional and translational processes leads to a distribution of *recA*-dependent GFP expression rates. In the control array, lines with positive slopes represent cells with higher expression rates than cells with zero or negative slope in which the GFP degradation is higher than its synthesis rate. The linear increases indicate that the transcription level in each cell is constant and does not change over the course of the experiment. In the MMC-exposed array, all the cells show increased rates of GFP expression. The average single-cell responses are shown in the Figure 2A inset and demonstrate a significant difference between the MMC and the control arrays.

In a similar experiment, *lacZ* promoter kinetics in individual cells was monitored in both an array exposed to the *lacZ* inducer IPTG (Figure 1C) and a control array (Figure 1D). In the control array, the raw fluorescence signals (Figure 1D inset) and expression rates obtained from the control array were low (Figure 1D), indicating minimal *lacZ* gene expression levels when the promoter is not induced. The average signal of the cell array exposed to

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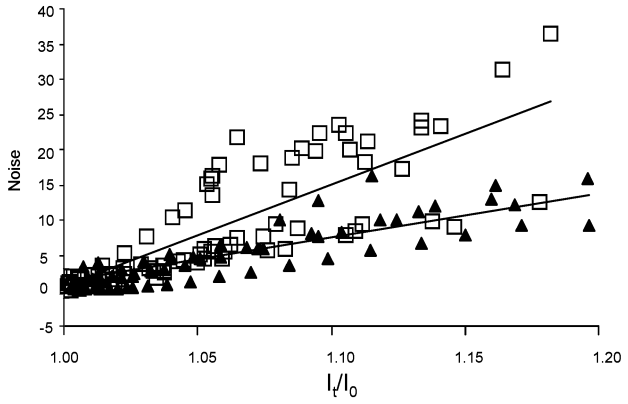

Figure 3. Comparison of noise levels in induced *lacZ* and *recA* gene promoters. The noise values versus expression levels from five independent array experiments of fully induced *lacZ* (empty squares) and *recA* (filled triangles) are shown. Lines fit $y = 143.83x - 143.2$ ($R^2 = 0.67$) for *lacZ* and $y = 62.2x - 60.799$ for *recA* ($R^2 = 0.75$). The maximum expression level was set to 1.2 because this level was the average value obtained from the 80 min *lacZ* cell array experiments (Figure 2B). The smaller slope for *recA* indicates that it is less noisy than the *lacZ* promoter within this expression range.

IPTG was significantly higher than the control cell array (Figure 2B inset).

Kinetics of Population Noise Associated with the Gene Expression. These cell array experiments provide information about gene expression that is not accessible with culture-based assays or other single-cell assay methods (e.g., flow cytometry). First, *continuous* gene expression kinetics can be monitored from hundreds (as shown here) to thousands of individual cells (not shown). Second, information about the *variation*, or genetic noise, in individual cell gene expression rates, can be obtained. Genetic noise is expressed as the Fano factor and is calculated by dividing the signal variance by the signal mean. This calculation is based on the assumption that gene expression levels in individual cells follow a Poisson distribution and that the noise is a measure of the deviation from this distribution.[3,4,6]

Figure 2A shows the noise kinetics of induced and noninduced *recA* promoter obtained from the cell arrays shown in Figure 1A and B. Since the measurements are differential, both constant gene expression rates (noninduced *recA*) and increased expression rates (induced *recA*) will cause an increase in the standard deviation and result in increasing noise over time. The difference in gene expression kinetics and noise between induced and noninduced *recA* cells can be explained by the *recA* gene function. The *recA* gene product, RecA, has two functions in *E. coli*.[24] The first function is as a housekeeping protein, which is part of the homologous recombination process, requiring a certain constitutive expression level. The second function is as a regulatory protein that activates the SOS response, requiring the *recA* gene to be under the control of an inducible promoter but also requiring the presence of some basal levels of RecA. This basal level (~1000 copies/cell) is responsible for the initiation of the SOS response. Since the basal level is not tightly regulated and is based on the "leakiness" of the *recA* promoter, higher variation in basal RecA levels is expected in individual cells. When *recA* is induced (Figure 1C), a high expression level is observed that is accompanied by lower noise compared to noninduced promoter (first 60 min shown in Figure 2A). The initial lower noise may indicate a shift between the noisier constitutive expression mechanism to the more efficient and less noisy *recA* induction mechanism, which is crucial for cell survival. The induction of *recA* depends on a single step, the removal of LexA repressor from the *recA* promoter region by the activated RecA. After the initial induction, GFP begins to accumulate after ~30 min (Figure 2A inset), the number of transcription and translation events increases exponentially, and the noise increases. After 60 min, the noise of induced *recA* becomes higher than noninduced *recA* (Figure 2A).

In contrast to *recA*, the noise of induced *lacZ* was 5 times higher than noninduced *lacZ* after 80 min (Figure 2B). These differences in expression level and noise between induced and noninduced states can also be correlated to the function and regulatory mechanisms of *lacZ*. The *lacZ* gene product, β-galactosidase, metabolizes lactose. The induction of *lacZ* requires the absence of glucose and the presence of lactose (or other synthetic inducers such as IPTG). The gene is highly repressed in the absence of inducer molecules, and therefore, both the basal level and genetic noise are low (Figures 1D and 2B). On the other hand, the presence of inducer molecules initiates a complex induction process that is based on a gene network consisting of multiple genes.[22] In each cell, the induction initiation timing and rates are slightly different and can result in higher noise (Figures 1C and 2B). At higher expression levels (approximately $I_0/I_t = 1.2$), noise starts to decrease (Figure 2C). This finding is in agreement with stochastic gene expression models and also with previous experimental results obtained from measurement of the *lac* promoter noise in *E. coli* cells[2] and the noise of the *GAL1* promoter in yeast.[4]

Figures 1 and 2 show that the most pronounced difference between *recA* and *lacZ* noise is in the noninduced state. Both the expression level and noise of noninduced *recA* are higher than *lacZ*. This difference was verified in three independent control experiments showing that after 80 min *lacZ* noise was 8.5 ± 5.8 and *recA* noise was 40.33 ± 16.4. RecA's functional requirement for basal expression is probably the main cause for its higher noise.

Conversely, since the gene regulation mechanism of *lacZ* is more complex than *recA*, the initial expression of induced *lacZ* gene would be expected to be noisier than the induced *recA* gene. To compare the noise of induced *recA* and *lacZ* genes, the noise values were plotted against the genes' expression levels (Figure 3). Although the array-to-array variation is high, as indicated by R^2 values of 0.67 and 0.75 for *lacZ* and *recA*, respectively, the higher slope value for the *lacZ* best-fit line (143.8) compared to *recA* (62.2) indicates that the fully induced *lacZ* gene is noisier than the fully induced *recA* gene. It is important to note that, apart from the inherent gene expression noise, the fluctuation in pSC101 plasmid number among cells is another potential noise source. The plasmid number in a cell population follows a normal distribution and can range between 3 and 10 per cell;[25] however, the contribution of the variation in plasmid number to the total noise should be similar in each experiment. In addition, the results reported here were calculated on the basis of the relative fluorescence intensity increase (I_t/I_0) so variations in plasmid number should be somewhat mitigated.

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CONCLUSIONS

The results presented in this work demonstrate the value of making single-cell measurements using an array platform for analyzing gene expression kinetics and genetic noise. A simple mathematical analysis was used to analyze the results of induced and noninduced expression in two different systems. To achieve more accurate and in-depth information about the kinetics of gene expression at the single-cell level, more advanced analysis and mathematical modeling are required. Ideally, cells with chromosomal promoter−reporter fusions should be used and the expression kinetics in intermediate induction states should be measured. These improvements would allow this technology to be used to elucidate the genetic mechanism of genes with unknown regulatory mechanisms (e.g., autocatalytic regulation mechanisms, a single control gene, or complex regulatory gene networks) based on genetic noise measurements. This new cell array approach provides a tool for addressing important problems in cell biology including genetic regulatory mechanisms and genetic noise. An advantage of this array format is that, unlike other cell array approaches, our cell arrays provide a simple approach to study isolated individual cells because each cell is separated from its neighbor by a wall. Even though the influence of the neighboring cells may be excluded, this limitation may not be absolute because one can adjust the well-to-well distance in the array, such that cells may be close enough to one another to sense the local microenvironment created by neighboring cells.

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