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# Gene Expression Probing in Live Cells, at Each Time One Protein Molecule

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**Key words:** Detection, exhibit, molecules, individual, decayed

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**Abstract:** In this research, we observed real-time production of single protein molecules in individual Escherichia coli cells using the fluorescence technique and the detection by localization method. They found that protein molecules are produced in burst, the number of protein in each burst varies and the burst exhibit particular temporal spreads (1). Although, they found that the burst happen arbitrarily, they decayed with time and the most important was that each gene expression burst results from one mRNA molecule. So, this research opened new avenues for many single molecule researches.

## INTRODUCTION

Gene expression, a central process to all life, is stochastic because most genes are existed in one or two copies per cell. In addition, the central dogma of molecular biology said that DNA is transcribed into mRNA which is then translated into protein. This statement comes with large populations of cells and molecules. In order to understand how the mechanism of central dogma is work they tried to probe gene expression at the single-molecule level by real-time monitoring of protein production in live cells. And they were able to report direct observation of single protein molecules as they are generated and for the first time, one at a time in a single live *E. coli* cell, providing a measurable explanation about gene expression[1].

They got much information from both the distribution of expression levels among a cell population and the temporal evolution of a single cell by using fluorescent reporters[2]. In this study we are going to explain this research in additional to brief talk about the physical, biological techniques that they used.

**Gene expression:** Before we start to learn about the biological and physical techniques we have to learn about the base of this research which is the gene expression. What is this process and why we interest to learn about. Gene expression is the process of using the information from the gene to produce proteins like ribosomal RNA (rRNA), transfer RNA (tRNA) or small nuclear RNA (snRNA) genes the product is a functional RNA. This process is used by the all living organisms starting from bacteria and viruses ending on human. Gene expression has many steps containing transcription, RNA splicing, translation and post-translational modification of a protein.

### Biological and physical techniques

**Fluorescence technique:** It is a Biophysical method that exploits the phenomenon of fluorescence to examine and analyze protein-protein, protein-nucleic acid, ligand-receptor and ligand-lipid interactions. These techniques are also useful in the study of protein conformation and orientation as well as diffusion and binding constants[3].

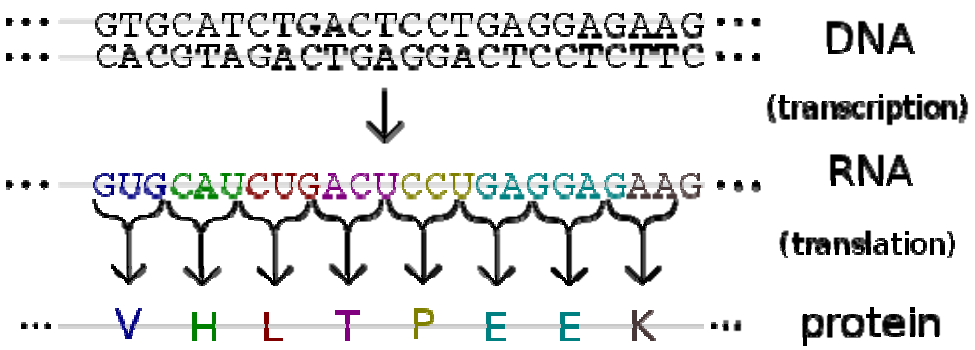


Fig. 1: Genes are expressed by being transcribed into RNA and this transcript may then be translated into protein[2]. Show also Calojets, jets created by the calorimeter output also Genjets, jets created by stable simulated particles. The missing energy is repeated by the dashed line

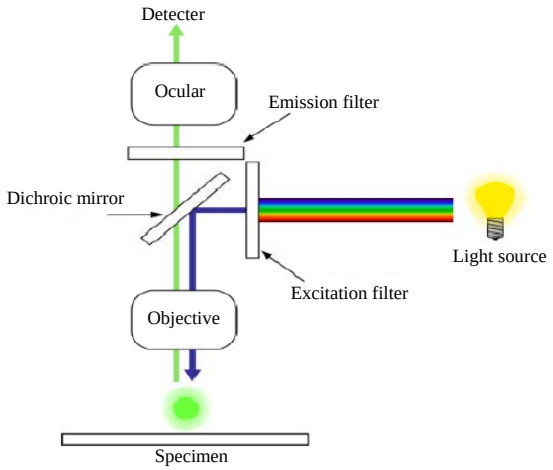


Fig. 2: Epi-fluorescence diagram

There are many fluorescence techniques that used in biological researches one of that methods is using fluorescence reporter.

**Fluorescence reporter:** In molecular biology, a reporter gene (often simply reporter) is a gene that researchers attach to a regulatory sequence of another gene of interest in bacteria, cell culture, animals or plants. Certain genes are chosen as reporters because the characteristics they confer on organisms expressing them are easily identified and measured or because they are selectable markers. Reporter genes are often used as an indication of whether a certain gene has been taken up by or expressed in the cell or organism population[2]. Reporter genes can also be used to assay for the expression of the gene of interest which may produce a protein that has little obvious or immediate effect on the cell culture or organism. The most popular reporters for monitoring gene expression in live cells are Green Fluorescent Protein (GFP) and its derivatives such as Yellow Fluorescent Protein (YFP) (24-26). We use a YFP variant, Venus as the reporter[1]. In this research they used reporter technology.

**Reporter technologies:** It is the technique that used to determine the molecular processes of interest in vivo and then and their existence using fluorescence. There are two strategies for using reporter: first is the direct method which is related to fluorescent probe that targets a specific such as a receptor or an enzyme. This method is characterized as active or achievable and each one has its characteristics. Second is indirect method which developed from corresponding in vitro reporting assays and is well suited to study gene expression and gene regulation like FRET and PET[3]. In order to image the florescence signal, fluorescence microscopies are used in this specific research they were used Epi-fluorescence microscopy.

**Epi-fluorescence microscopy:** A fluorescence microscope is an optical microscope that uses fluorescence and phosphorescence instead of or in addition to, reflection and absorption to study properties of organic or inorganic substances. The "fluorescence microscope" refers to any microscope that uses fluorescence to generate an image whether it is a more simple set up like an epi-fluorescence microscope or a more complicated design such as a confocal microscope, which uses optical sectioning to get better resolution of the fluorescent image.

In this research they used epi-fluorescence design shown in Fig. 1 and 2. Light of the excitation wavelength is focused on the specimen through the objective lens. The fluorescence emitted by the specimen is focused to the detector by the same objective that is used for the excitation which for greatest sensitivity will have a very high numerical aperture. Since most of the excitation light is transmitted through the specimen, only reflected excitatory light reaches the objective together with the

emitted light and the epi-fluorescence method, therefore, gives a high signal to noise ratio. An additional barrier filter between the objective and the detector can filter out the remaining excitation light from fluorescent light.

**Time-lapse microscopy:** It is time-lapse photography applied to microscopy. Microscope image sequences are recorded and then viewed at a greater speed to give an accelerated view of the microscopic process. It is used to observe the microscopic object over time specially in cell biology to monitor cultured cells[4].

**The research**
**Materials**
***E. coli* strains construction:** In this research the researcher used *E. coli* K-12 strain SX4 which express tsr-venus a chimeric protein that produced by fuse the tsr gene to the N-terminal of *Venus* together with a kanamycin drug resistance marker was incorporated into the lacZ locus, replacing the native lacZ gene from the start codon ATG and leaving intact the *lac* promoter and associated gene expression regulation elements such as the CAP protein binding site, lac promoter, lac operator (O1 and O3) and *lac*Z ribosome binding site.

**Growth conditions and media:** The E. coli cells growing media were M9 glucose enhanced with amino acids, vitamin and suitable antibiotics (kanamycin: 35 μg $mL^{-1}$, ampicillin 50 μg $mL^{-1}$) for a night. That culture was re-inoculated into fresh media of M9 (1:200) until OD600 reaches 0.2. During microscope research the cells were then washed twice in fresh media and re-suspended in 20 folds dilution.

## MATERIALS AND METHODS

In this research the researcher used a protein that called Venus as a reporter gene because it is a fast maturing protein. They used a general strategy that known as detection by localization which is one of the methods that used to measure the single-molecule fluorphores in the live-cell[1] (Fig. 3 and 4).

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Localizing means immobilize the fluorescence protein on the membrane of the e-coli cell so they could success in getting a single molecule. To do that they built a fluorescent protein reporter *tsr-venus*that has the membrane localizing sequence (Fig. 5). They detect the fluorescence signal separately from the cytoplasm and membrane protein because of the difference between their diffusion where the protein molecules inside cytoplasm spread very fast whereas protein molecules inside membrane spread much slower, so it is easier to detect signal from the membrane protein.

**Detect real-time gene expression:** There are many steps in this research to detect the real time. Taking fluorescence images of *E. coli* cells using epi-

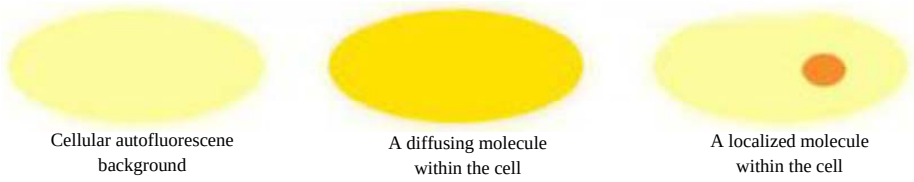


Fig. 4: Detection by localization

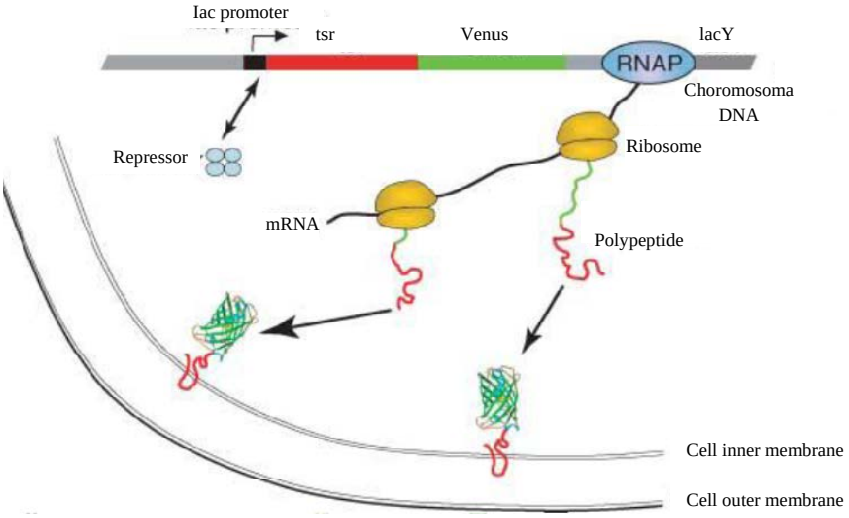


Fig. 5: Scheme of live-cell observations of gene expression

fluorescence microscope (Olympus IX71) whose laser beam 514 nm and power density 0.3 kW/cm^2. Then using a 60x microscope objective (NA = 1.45, Olympus) images were taking each 3 min to monitor the real time gene expression. Using Differential Interference Contrast (DIC) images are taking to track the dividing cell. The fluorescence signal was collected with a cooled charge coupled device CCD camera. All the images that collected were controlled by Meta Morph image. After 100 msec the fluorophores were photo-bleached using 1100 nm laser exposure of 0.3 kW/cm^2. In order to insulate the diffraction-limited spots of fluorescent fusion protein molecules inside cells, the images were processed by thresholding. They integrated over 9 pixels around the maximum intensity to determine the fluorescence spot. From those steps they were able to record movies of growing *E. coli* cells to study the real-time expression from the *lac* operon in repressed state in real-time Fig. 3.

## RESULTS AND DISCUSSION

**Time trace evidence and data analysis:** After doing the research the researcher noticed that the protein molecules are generated in bursts that showed specific time-based spreads. In addition they found that the number of protein that generated in bursts are differs from burst to burst. That evidence produced many questions: Do these gene expression bursts occur randomly in time? How many mRNA molecules are responsible for each gene expression burst under the repressed condition? What is the distribution of the number of protein molecules in each burst? And what is the origin of the temporal spread of the individual bursts.

In order to answer the first question they used the data of the distribution of number of gene expression burst per cell cycle for all cells Fig. 6a. And by fitting function they found that the best fit was Poisson distribution function that explains that the gene expression bursts arise randomly and are uncorrelated in time. In addition they found that there is weak dependence on burst frequency in each cell cycle. That weak dependent probably come from the increasing of the number of the gene copy that related to the replication of the DNA while growing. The average number of burst that they found was $n_{burst}$ = 1.2/cell cycle this gives the average time to be 46 min between two burst. If we compare the result that the researcher got in vitro (20-50 min) is very different.

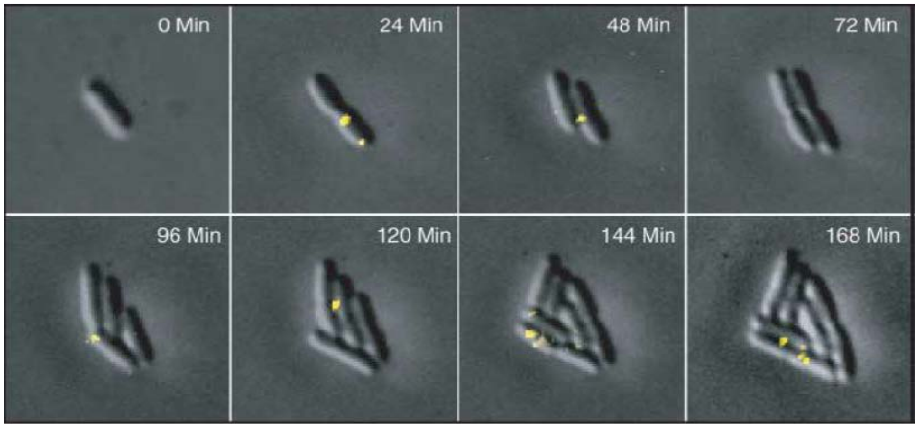


Fig. 6: Sequence of fluorescent images (yellow) overlaid with simultaneous DIC images (gray) of *E. coli* cells expressing Tsr-Venus. The eight frames are from time-lapse fluorescence movie S1 taken over 195 min with 100-ms laser exposures (0.3 kW/cm2) every 3 min

In the second question they were try figure out was how many mRNA are responsible for each gene expression, so they determined the average number of mRNA molecules per burst (m) according to:

$$m = n_{mRNA}\,\tau_{cell}/\tau_{burst}\,\tau_{mRNA}$$

Where:

$n_{mRNA}$ = The steady-state abundance of Tsr-venus mRNA molecules averaged over a cell population
$\tau_{cell}$ = The average cell division time
nburst = Tthe average number of expression bursts per cell division cycle
$\tau_{mRNA}$ = The cellular lifetime of the tsr-venus mRNA

Using the calculation real-time reverse transcription polymerase chain Reaction (RT-PCR), they got $n_{mRNA} = 0.037\pm0.013$, $\tau_{mRNA} = 1.5\pm0.2$ minfrom those result that they got from RT-PCR assay they prove that under the repressed condition each gene expression burst results from one mRNA molecule which suggests that Lac repressor quickly rebinds the exposed operator region of DNA, allowing transcription initiation of one mRNA molecule (Fig. 7).

Cellular lifetime of tsr-venus mRNA. The decay of the tsr-venus mRNA (squares) is fitted with a single exponential function (solid curve) which yields an mRNA degradation time constant of 1.5±0.2 min.

After they proved that the burst gene expression bursts arise randomly and one mRNA is responsible for one gene expression, they found that protein copy numbers in the bursts follow an exponential distribution as shown in Fig. 6b which means a geometrical distribution for integer n which come from stochastic cellular lifetime of an mRNA molecule with mean =1.5 min.

Finally, the temporal spread of the expression bursts can be categorized from the autocorrelation function of the fluctuation in protein expression that calculated from about 30 cell images from 15 movies Fig. 6c.

$$C^{(2)}(\tau) = (s/1\text{-}p)\left[1+(k/s)\exp(-k\tau)\right]$$

Where:

$\rho$ = The probability of the ribosome binding
s = Average rate of expression burst
$\kappa$ = Rate constant of Tsr-Venus assembly process

Each exponential gives $C^{(2)}(\tau) = 0.7 \pm 2.5$ min identical to the average spread of the stochastic arrival times of fluorescent reporter proteins within a burst but that is regardless of the fact that the polypeptides are generated within the short lifetime of an $\tau_{mRNA}$ =1.5 min as they have found before.

By answering those question based on what they have got they conclude that the stochastic process contain low number of biomolecules copy and their work was too difficult in the technology that they used but what they have got was the first step for the other to try to enhanced single molecule technology because it is the base of the other complex biological processes.

**Modern single molecule researches:** In 14 April 2006, X. SunneyXie*, Ji Yu, Wei Yuan Yang In their research Living cells as test tubes used the probing of biochemical reactions in living cells technology to detect and track a particular protein with single-molecule sensitivity. Then studying gene expression, active transport and lipid metabolism.

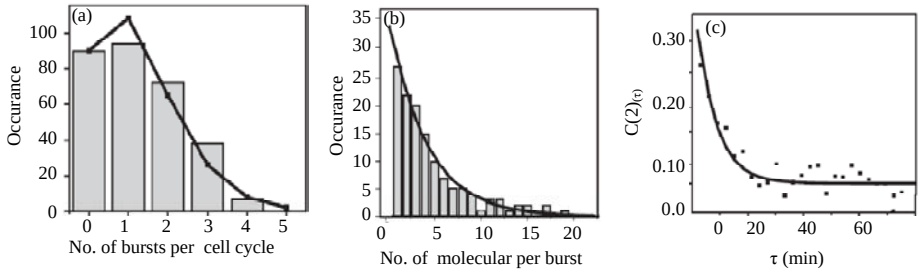


Fig. 7: Statistical analyses of the protein production time traces. (A) Histogram of the number of expression events per cell cycle. The data fit well to a Poisson distribution. (B) Distribution of the number of fluorescent protein molecules detected in each gene expression burst (C) Autocorrelation function of the protein production time traces

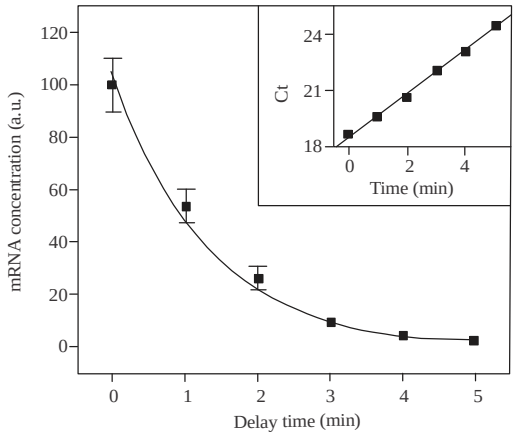


Fig. 8: Cellular lifetime of tsr-venusmRNA. The decay of the tsr-venus mRNA (squares) is fitted with a single exponential function (solid curve), which yields an mRNA degradation time constant of 1.5±0.2 min

About 31 July 2007, Robert J. Silbey in the research New directions in single-molecule imaging and analysis used Optical fluorescence imaging and analysis of single molecules continues to unfold as a powerful way to study the individual behavior of biological systems (Fig. 7 and 8).

January 2008, Ashok A Deniz*, Samrat Mukhopadhyay and Edward A Lemke in their research Single-molecule biophysics: at the interface of biology, physics and chemistry probe the complex behavior of biological molecules, due to their unique abilities to probe molecular structure, dynamics and function, unhindered by the averaging inherent in ensemble researches.

November 2008, Vahid Shahrezaei1 and Peter S. Swain in Analytical distributions for stochastic gene expression they got approximation to calculate the mean, variance and distribution of protein number. They got the same results that first, protein synthesis occurs in geometrically distributed bursts and which are asymmetric and may poorly characterize by their mean and variance. Second, allows mRNA to be eliminated from a master equation description.

Genes Dev. 15, June 2011 in Biological mechanisms, one molecule at a time used the same single molecule technique to understand how the translation of a messenger RNA into its encoded protein by the ribosome.

March 2012, Mol. Biol. Cell Single-molecule imaging of translational output from individual RNA granules in neurons they use single-molecule imaging to count the number of RNA molecules in each granule and to record translation output from each granule using Venus fluorescent protein as reporter.

April 2014,MolSystBiol Dynamics of single-cell gene expression they used the same technique to study the production of individual mRNA molecules and the dynamic of the gens also using gene reporter.

About 17 Jan 2014, David Gomez in Modeling stochastic gene expression in growing cells show how the region of bistability becomes diminished by increasing the effect of noise via a reduced copy number of the regulatory protein. Cell volume determines the region of bistability for different noise strengths. The method is general and can also be applied to other cases where synthesis rates of proteins are regulated and an appropriate analytical description is difficult to achieve 9. 6 June, 2014 J R Soc in Stochastic holin expression can account for lysis time variation in the bacteriophage λ show that stochastic holin expression is sufficient to account for the intercellular LT differences in both wild-type phages and phage variants where holin transcription and the threshold for lysis have been researchally altered.

Finally, our analysis reveals regulatory motifs that enhance the robustness of lysis timing to cellular noise 10. Michael B. Elowitz, Arnold J. Levine, Eric D. Siggia and Peter S. Swain in Stochastic Gene Expression in a Single Cell showed that the intrinsic and extrinsic class of

noise are important in setting cell-cell variation in gene expression, both noise should similarly occur in all intracellular reaction involving small no of reactions. At the same time noise offer the opportunity to generate long-term heterogenety in a clonal population.

## CONCLUSION

From all researches above we can see very clearly that the researchers are very curious in the single molecule techniques for how qualitative measurement and results that could provide. In addition all previous investigations were used bulk-scale methods which measured the average value for crowd of cells that couldn't give rigorous result but give only approximation results. All in all we can say that Ji Yu's research was the starting point for others to float in the sea of single molecule technology.

## ACKNOWLEDGEMENTS

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