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A dual fluorescence protein expression system detects cell cycle dependent protein noise

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ABSTRACT

Inherently identical cells exhibit significant phenotypic variation. It can be essential for many biological processes and is known to arise from stochastic, 'noisy', gene expression that is determined by intrinsic and extrinsic components. It is now obvious that the noise varies as a function of inducer concentration. However, its fluctuation over the cell cycle is limited. Applying dual colour fluorescence protein reporter system, Cyan Fluorescent Protein (CFP) and Yellow fluorescent protein (YFP) tagged multi-copy plasmids, we determine variation of the noise components over the phases in *lac* promoter induced by Isopropyl β-D-1-thiogalactopyranoside (IPTG) and in presence of additional Magnesium, Mg^{2+} ion. We, also, estimate the how such system deviates from observations of single-copy plasmid. Found 25 % difference between multi-copy system and single-copy system clarifies that observed noise is considerable and estimates population behaviour during the cell cycle. We show that total variation in cells induced with IPTG is determined by higher extrinsic than intrinsic noise. It increases from Lag to Exponential phase and decreases from Retardation to Stationary phase. By observing slow and fast dividing cells, we show that 5 mM Mg^{2+} increases population homogeneity compared to 2.5 mM Mg^{2+} in the environment. The experimental data obtained using dual colour fluorescence protein reporter system demonstrates that protein expression noise, depending on intra cellular ionic concentration, is tightly controlled by phase of the cell.

1. Introduction

Stochasticity, or noise, in gene expression is present in both prokaryotic and eukaryotic cells and originates from the fluctuation of cell components including regulatory molecules [1–4]. It has been reported that the magnitude of the 'noisy' expression is changing with respect to inducer concentration [1,5], mode of activation [6], time of activation [7] and sample size [8]. However, there is limited information available about noise changes in the gene expression during the cell cycle. Here, we are interested to monitor the noise in *E. coli* over the phases and to estimate the effect of regulatory molecules. Noise (η_{tot}), measured as standard deviation divided by the mean, can be divided into two components [1]. First, Intrinsic component (η_{int}) typically derives from individual behaviour of the genes in homogenous population [1,3,9]. Second, extrinsic component (η_{ext}) arises from fluctuation of the gene expression rates determined by constant variation of controlling molecules [1,3,9]. It is now being realized that the magnitudes of both components affect population diversity under changing environment [10,11] and, thus, pre-defines population development.

The ratio of intrinsic and extrinsic components in populations under various experimental conditions is widely-studied by the dual colour fluorescence protein reporter system containing single-copy gene [1,12]. Usage of a single-copy gene allow for eliminating the biased partitioning of plasmid during the cell divisions. While most of the genes are present as a single-copy in chromosome of the *E. coli* some of them depict multi-copies property e.g. 16S rRNA –encoding gene. We are interested to estimate the level noise changes using multi-copy plasmids. We used dual colour fluorescence protein reporter system with multi-copy plasmids expressing Fluorescent Protein (CFP) and Yellow fluorescent protein (YFP). Knowing pros and cons of such a system we

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quantify the noise components over the phases using *lac* promoter activated by IPTG [13–16]. Additionally, we evaluate the effect of one of the most important regulatory element, Mg^{2+} as it is shown to affect transcription dynamics [17–19] and mRNA stability [20–23] that determine the noise.

Our results show that extrinsic component is generally higher than intrinsic over the phases in cells induced with 1 mM IPTG. Both noise components increase from Lag to Exponential phase and then decrease from Retardation to Stationary. Interestingly, throughout the divisions, intrinsic component responsible for the cell to cell diversity decreases when additional 5 mM Mg^{2+} is present in the environment. Observed fluctuation of the noise components may display response of the population with respect to additional Mg^{2+}. It, also, quantifies how population of the cells changes its homogenous state under altering environment during the cell cycle. We find that noise levels differ between single- and multi-copy genes approximately by 12 %. Yet, our results correlate with previously reported levels of both noise components [1]. Therefore, it implies that observed noise levels are close to the true values and, thus, is considerable for the quantification of the noise changes over the phases in *E. coli* cells.

2. Results

2.1. Principles of noise

Dual colour fluorescence protein reporter system includes expression of two reporter proteins [1,3]. We used the system expressing *CFP* and *YFP* proteins under control of *lac* promoter in *E. coli*. The *lac* promoter was activated from both upstream and downstream regions. It reduces the systematic variation in the gene expression of *lac* promoter but still possesses essential stochastic difference [1]. Assuming that fluorescent proteins are evenly distributed in individual cells, population would show essential independence and co-expression of noise identifications. The schematic representation distinguishes cells with no intrinsic noise as changes of two reporters correlate in a perfect manner over the phases and amount of both proteins is equivalent in the single cells (Fig. 2A). However, in nature cells likely exhibit uncorrelated fluctuation of two reporters over the cell cycle that results in uneven distribution of both reporters. It increases the level of intrinsic noise and, consequently, cell to cell diversity.

2.2. Distinguishing the noise classes

For measuring the noise, cells containing reporter plasmids were grown in LB medium. Expression of CFP and YFP fluorescence were detected under the microscope. Processing of the acquired images allow for quantification of fluorescence intensities that indicate presence of both intrinsic and extrinsic noise components as both fluorescence intensities vary in repressed wild type *lac* promoter (Fig. 3A). With addition of 1 mM IPTG reduced variation between expressions of both fluorescent proteins indicates the level of cell to cell diversity (Fig. 3B). When 2.5 mM Mg^{2+} is added to the medium variation between fluorescence intensities of both reporters' further decreases (Fig. 3C) that correspondingly reduces intrinsic component. It would apparently diminish variation between individual cells and lead to more homogeneity population, though not as high as population induced by 1 mM IPTG. Presence of 5 mM Mg^{2+} in the medium increases fluorescent variations and, thus, intrinsic component (Fig. 3D) that creates high difference between individual cells, leading to more heterogeneous population. Observed changes in expressions of both reporters suggests that homogeneity of the population is higher in presence of 2.5 mM Mg^{2+} than 2.5 mM Mg^{2+}, though not higher than in cells induced by 1 mM IPTG. We are further interested to understand these changes by estimating the levels of the noise components over the cell cycle.

Table 1
Noise measurements in cells induced with 1 mM of IPTG. Standard errors were calculated from two biological and 2 technical repeats of the experiment.

Noise			
Phases	Mean intrinsic noise (η_{int})	Mean extrinsic noise (η_{ext})	Mean total noise (η_{tot})
Lag	0.030 ± 0.03	0.151 ± 0.02	0.154 ± 0.03
Acc	0.002 ± 0.02	0.052 ± 0.007	0.052 ± 0.03
Exp	0.128 ± 0.01	0.285 ± 0.01	0.312 ± 0.02
Ret	0.004 ± 0.02	0.06 ± 0.008	0.0602 ± 0.03
Sta	0.004 ± 0.02	0.548 ± 0.006	0.549 ± 0.02

Table 2
Noise measurements in cells induced with 1 mM of IPTG and presence of 2.5 mM of Mg^{2+} in the environment. Standard errors were calculated from two biological and 2 technical repeats of the experiment.

Noise			
Phases	Mean intrinsic noise (η_{int})	Mean extrinsic noise (η_{ext})	Mean total noise (η_{tot})
Lag	0.073 ± 0.01	0.222 ± 0.01	0.234 ± 0.02
Acc	0.034 ± 0.006	0.142 ± 0.01	0.146 ± 0.01
Exp	0.056 ± 0.01	0.165 ± 0.006	0.174 ± 0.01
Ret	0.068 ± 0.01	0.182 ± 0.009	0.195 ± 0.01
Sta	0.088 ± 0.004	0.197 ± 0.006	0.215 ± 0.007

2.3. Dynamics of the noise components over the phases

We plotted CFP versus YFP fluorescence intensities in the single cells to estimate the level of intrinsic and extrinsic components as both of them make orthogonal contributions to the total noise, so that relationship of sum of squared intrinsic and extrinsic components equalled to squared total noise is applicable [1,24]. We found that the level of extrinsic component is generally higher than the level of intrinsic in cells induced with 1 mM of IPTG (Fig. 4) and is closer to the total noise (η_{tot}) over the phases (Table 1).

However, it does not mean that cell to cell variability is determined by the extrinsic noise alone as its dynamics should be explained by both noise components [1]. Besides, we imply that the ratio of the noise components to the total cell to cell variation gives more realistic view when is measured as a function of cell phases and not as function of inducer concentration as has been widely investigated previously [1,24]. Thus, when monitoring the noise components over the phases we found that they are increasing from Lag to Exp phase and decreasing from Ret to Sta phase (Fig. 4). It indicates that fluctuation of both noise components is affected by the cell division time [3,25].

2.4. Effect of Mg^{2+} ions to the noise level in single cells

It is known that ionic strength affects the noise in cell population. Mg^{2+} is one of the most important regulatory element that determines the stability of the transcriptional process [20,21,26,27] and affects the

Table 3
Noise measurements in cells induced with 1 mM of IPTG and presence of 5 mM of Mg^{2+} in the environment. Standard errors were calculated two biological and 2 technical repeats of the experiment.

Noise			
Phases	Mean intrinsic noise (η_{int})	Mean extrinsic noise (η_{ext})	Mean total noise (η_{tot})
Lag	0.043 ± 0.01	0.147 ± 0.02	0.153 ± 0.02
Acc	0.112 ± 0.004	0.222 ± 0.008	0.249 ± 0.01
Exp	0.046 ± 0.01	0.155 ± 0.01	0.161 ± 0.02
Ret	0.031 ± 0.02	0.135 ± 0.01	0.138 ± 0.02
Sta	0.126 ± 0.003	0.228 ± 0.01	0.261 ± 0.007

noise level [28]. To study its effect to the noise level, we add two different extracellular concentrations. We measure degree of the noise fluctuation in slow dividing (Lag, Retardation and Stationary phases) and fast dividing cells (Acceleration and Exponential phases) with respect to 2.5 and 5 mM of Mg^{2+} and compare it to the results observed under presence of 1 mM IPTG (Fig. 5). Generally, cell to cell variation (η_{tot}) increases with the addition of Mg^{2+}. When 5 mM Mg^{2+} is added to the medium, mean of intrinsic component is decreased in slow dividing cells compared to cells having 2.5 mM Mg^{2+} extracellular. It indicates that population of slow dividing cells increases homogeneity state when higher Mg^{2+} is added to the environment (Fig. 5A, Tables 2 and 3). Notably, in both experiments slow dividing cells still have higher intrinsic noise than in presence of 1 mM IPTG that reveals effect of extracellular Mg^{2+} as additional source of population diversity.

In Acceleration phase, fast dividing cells show increased level of noise components and cell to cell variation (η_{tot}) in presence of 5 mM Mg^{2+} (Fig. 5B, Table 3). Compared to Acceleration phase, fast dividing cells in Exponential phase decrease intrinsic and extrinsic components under presence of 5 mM Mg^{2+} and increase them with presence of 2.5 mM Mg^{2+} (Fig. 5B, Tables 2 and 3). Observed fluctuation of noise components display the behaviour of fast dividing cells directed to increase population homogeneity when 5 mM Mg^{2+} compared to 2.5 mM Mg^{2+} experiment. Nevertheless, addition of Mg^{2+} to the cell environment increases population diversity compared to cells induced with IPTG alone. By evaluating such dynamics of the noise components, we conclude that presence of extracellular 2.5 mM and 5 mM Mg^{2} extracellularly oppositely affects population homogeneity over the cell cycle. Predictably, presence of higher Mg^{2+} concentration would increase population homogeneity more compared to presence of lower Mg^{2+} concentration in the environment.

3. Discussion

It was shown that the noise pre-defines stochastic behaviour of the cell populations [1,9,10,29,30]. Stochasticity of the gene expression was studied widely with respect to the inducer concentration [1,5], mode of activation [6], time delay of activation [7] or sample size [8]. However, limited information is available for the noise fluctuation over the cell phases. Here, we monitor fluctuation of the noise components over the phases in repressed wild type *lac* promoter. We observe that extrinsic component is generally higher than intrinsic during the cell cycle that estimates the level of cell to cell diversity [31]. We, also, find that both noise components increases from Lag to Exponential phase and decreases from Retardation to Stationary phase, when cells are induced with 1 mM IPTG. Moreover, we evaluate effect of additional 2.5 mM and 5 mM Mg^{2+} onto the level of the noise components and found that ionic strength determines gene expression rates in lac promoter [26,28,34]. Interestingly, addition of higher Mg^{2+} in the environment decreases the cell to cell variation compared to presence of lower concentration over the phases. Taking into account that division rate varies from phase to phase it appears to be beneficial for the cells to have higher concentration of free ions in the environment.

It was shown that during transcription and translation magnesium affects the synthesis of mRNA and proteins, respectively [30,31]. However, the information of how different concentrations of extracellular Mg^{2+} affect cell-to-cell variability during cell divisions in prokaryotes is limited. Taking it into account, the present results provide new insights into the role of extra-cellular Mg^{2+} demonstrating how it regulates the gene expression noise over the phases.

Observed increased extrinsic noise may arise also from uneven partitioning and plasmid replication during the cell divisions [32]. Even though, multi-copy plasmids distribute randomly during cell division, copy number remains high and, thus, plasmid-free cells arise only rarely [33]. It was experimentally confirmed that variation in a copy number is minimized by plasmid-encoded control circuits, implying efficient correction of any deviation under most circumstances [33]. Stability of the multi-copy plasmids is determined not only by cell division and regulation of the cell state but also by multimer resolution system presented within the *E. coli* chromosome [34]. This natural system allows for avoiding of plasmid cluster formation, multimerization, by converting multimers to monomers [33]. Described mechanisms assist for plasmid maintenance during the cell divisions. Nevertheless, our noise measurements deviate from the noise values reported by Elowitz et al. [35]. This difference, however, may appear not only from different plasmid copy number but also from the different *E. coli* strains and induction used in both experiments. We, also, found that observed mean extrinsic noise in cells induced with IPTG is 9 % higher than the maximum level of extrinsic component shown by Elowitz et al. [35]. Notably, observed noise overestimation falls into the range of calculated difference between multi- and single-copy gene expressions (see Methods). It implies that observed noise levels are close to the true values and, thus, might be considered for the quantification of the noise changes over the phases in *E. coli* cells.

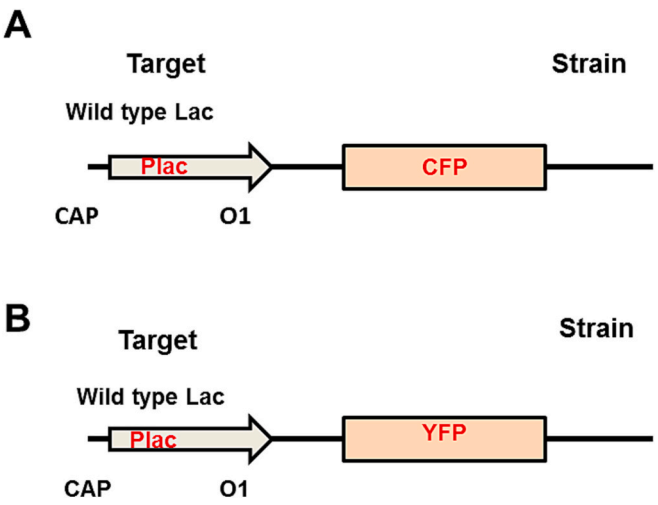

Fig. 1. (A) The target gene coding region for cyan fluorescence protein (CFP) controlled by lacO1 in vector pAK400c with a ColE1 origin. (B) Identical as in (A) and the target gene coding region for yellow fluorescence protein (YFP).

Overall, these results establish qualitative basis for modelling population behaviour during the cell cycle and under changing environment. In future perspective, it can be expanded to study 'noisy' response of the cell population to absence of the Mg^{2+} as well as presence or absence of other regulatory molecules. Studying fluctuation of the noise components in reaction network would further uncover its biochemical origins.

4. Material and methods

4.1. Plasmids, strains and media

Dual colour sensor system was used to study stochasticity of the gene expression (Fig. 1). For this *E. coli* Dh5α-PRO strain was co-transformed with two multi-copy plasmids: 1) pAK400c expressing Citrine-Yellow fluorescent protein (YFP) was controlled by wild type promoter with the binding site for CAP in the upstream and Operator 1 binding site for lac repressor in the downstream. 2) Identical vector system as above with Cerulean-Cyan fluorescence protein (CFP) [38] (generously provided by Prof., Matti Karp, Tampere university of Technology). Both vectors consist of ColE1 origin that provide in average 15 plasmid copies per cell [36]. Target vectors containing *E. coli* strains were grown in liquid LB medium composed of 10 g/L of tryptone (T7293-Sigma Aldrich-USA), 5 g/L of Yeast extract (MC 001-LabM-UK) and 10 g/L of NaCl (S3014-LabM-UK). Antibiotics were also used to grow the cells according to the respective antimicrobial-resistance. For the comparison

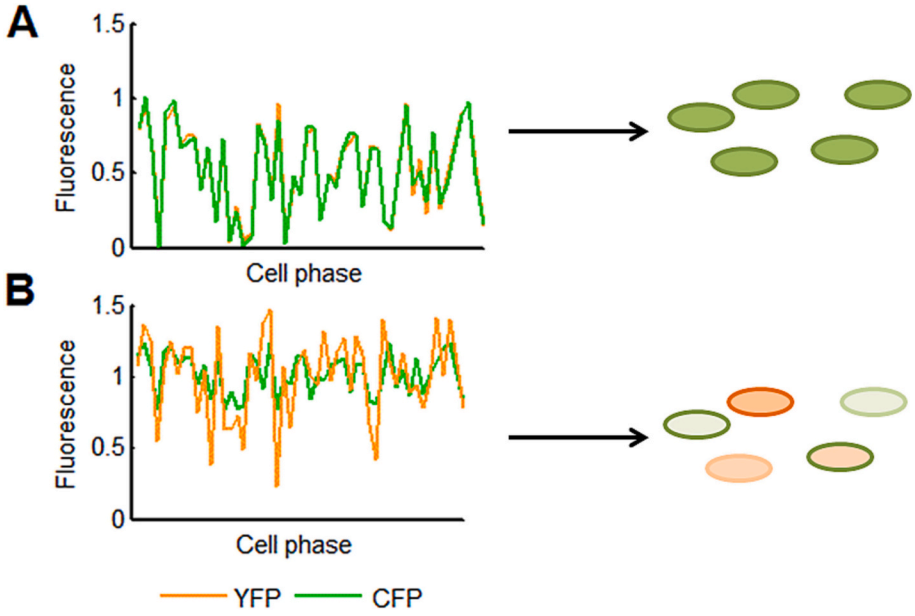

Fig. 2. Schematic representation of the dual colour fluorescence protein reporter system: cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP) controlled by same promoter A) equivalent expression of both proteins over the time affecting single cells to be identical that appear in absence of intrinsic noise, though extrinsic noise is present B) variations in gene expression rates of both proteins result in different amount of proteins per single cells caused by rising of intrinsic noise.

of gene expressions from multi-copy plasmid and single-copy plasmid we used $P_{lac/ara\text{-}1}$-mRFP1-96bs, a bacterial artificial chromosome.

Dh5α-PRO strain genotype as follows: deoR, endA1, gyrA96, hsdR17 (rk-mk+), recA1, relA1, supE44, thi-1, Δ(lacZYA-argF)U169, Φ80δlacZΔM15, F-, λ-, PN25/tetR, PlacIq/lacI, and SpR. Frag1A: F-, rha-, thi, gal, lacZam, ΔacrAB::kanR, PN25/tetR, PlacIq/lacI, and SpR. Frag1B: F-, rha-, thi, gal, lacZam, PN25/tetR, Placiq/lacI, and SpR. The PN25/tetR, Placiq/lacI, SpR cassette was transferred from DH5αPRO to Frag1 to generate Frag1B by P1 transduction. The ΔacrAB:kanR cassette was transferred from KZM120 to Frag1B to generate Frag1A.

4.2. Cell phase determination

Generation time of *E. coli* DH5a-PRO strain was calculated to determine the phases of the cells. Cells were grown overnight in an orbital shaker (Labnet) at 30 °C with aeration at 250 RPM. Following overnight culture, cells were diluted in fresh media to reach an optical density (OD_{600}) of 0.05 using a spectrophotometer (Ultraspec 10-Amersham Biosciences) and grown at 37 °C at 250 RPM. Cell growth was measured every 30 min and OD_{600} values were used to calculate the generation time as described in earlier literature [37]. Cell phases were defined as suggested from Jacob Monod study based on the state of the cells division time, i.e. lag, acceleration, exponential, retardation and stationary phases [38].

4.3. Gene activation and Mg^{2+} study

To study the regulation of wild type lac promoter (P_{lac}) over the phases, cells were induced in the state of particular phase. To detect the single molecules the reporter gene was activated with 50 ng/mL of anhydrotetracycline (aTc) (lot number 2-0401-001, IBA GmbH, Germany) for 20 min incubation. Full induction of the target P_{lac} was achieved by the activation of downstream promoter region with the addition of 1 mM IPTG (L6758-Sigma Aldrich-USA) and upstream promoter region by endogenous cAMP [39]. In the population study, to observe fluorescent intensity, cells were incubated with inducers for 15 min depending on induction scheme in each phase. The cells were observed under microscope immediately after the division time of every phase. Addition of IPTG in total population experiment was used as a control. To study the effect of Mg^{2+} onto fluctuation of the noises over the phases we have added 2.5 and 5 mM solution of $MgCl_2$ to the cells in 15 min prior to the division.

4.4. Single cell fluorescence microscopy

Cells were pelleted by centrifugation at 6000 RPM then suspended in required volume of LB to facilitate the observation of several cells under microscope. Suspended cells were then placed on a microscopy slide between 1 % LB-agarose gel pad and a microscopic cover slip. For the image acquisition of fluorescent intensity of the cells, we used inverted fluorescence microscope (Nikon, Eclipse Ti-E) with 100× N.A. 1.49 oil immersion objective. The microscope is equipped with a hardware autofocus module, motorized z-drive and Nikon's Perfect Focus System to maintain the cells in focus during the image acquisition. Inbuilt microscope software (Nikon, NIS-Elements C) was used to acquire images. Fluorescence was measured using emission filters 515 nm for YFP and 488 nm for CFP. For mRFP1 expression detection we used filter 515 nM. For the real time observation of fluorescent changes, target promoters were induced as described above. The cells were observed under microscope immediate (3 min) after the division time of every phase. Cells were then placed between a gel pad and a cover slip in a microscopic slide during the measurement.

4.5. Image processing

Cells having fluorescent signals were selected from microscopic images (Fig. 3). Using automated methods implemented in Matlab R2013a, cells with fluorescence were detected in the converted to gray-scale images and subjected to the principal component analysis, Kernel Density Estimation and Otsu's threshold methods. Extracted information about the locations, dimensions and orientations of masked cells, intensity of the fluorescence per cell and background [40–42] were used to exclude the outliers with fluorescence intensity greater than three standard deviations from the mean of the population (Table 4).

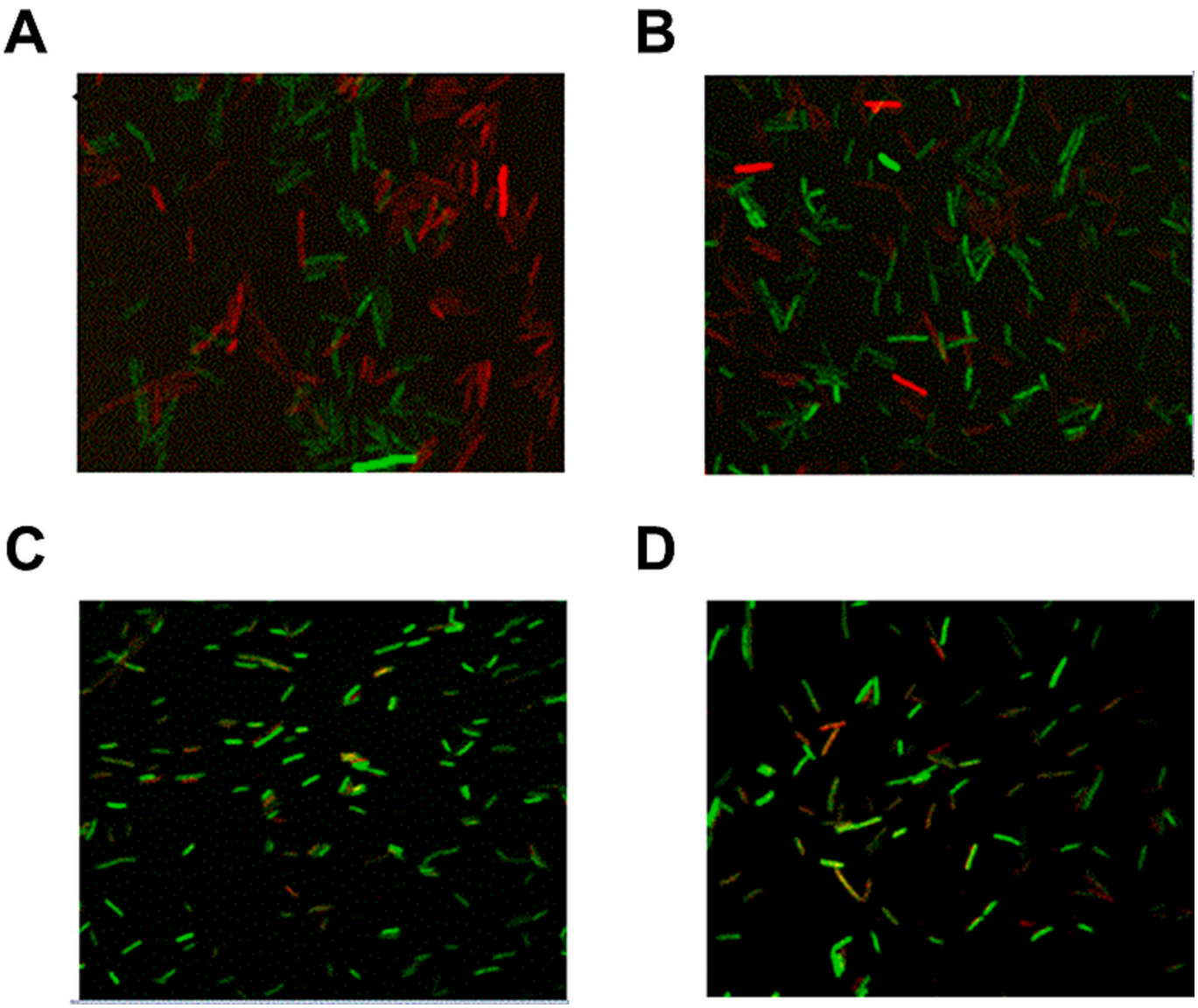

Fig. 3. Dual colour reporter study shows the stochastic gene expression in *E. coli*. CFP and YFP fluorescence images were combined in the green and red channels. (A) In strain DH5α PRO, lacI repressed wild type Lac promoter expresses the different levels of red and green colour indicate significant amounts of intrinsic noise. (B) In the presence of 1 mM IPTG both fluorescent proteins were increased which has reduced the noise (C) as in (B), with the addition of 2.5 mM Mg^{2+}, increased the expression of both fluorescent protein which further increased intrinsic noise. (D) As in (B), with 5 mM Mg^{2+} addition, cell exhibits increased intrinsic and extrinsic than control and 2.5 mM Mg^{2+}.

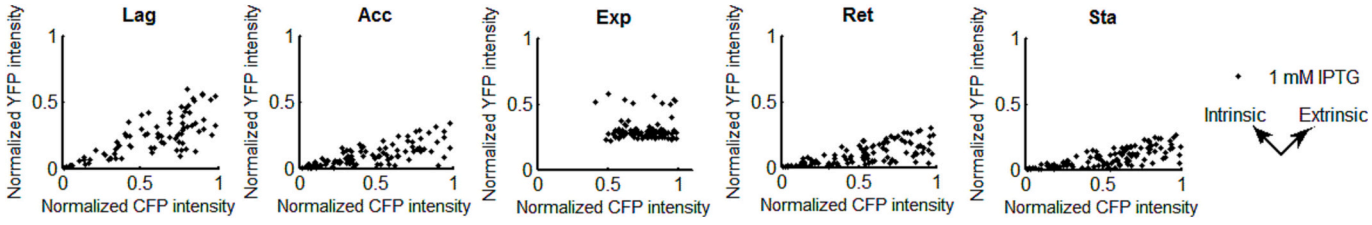

Fig. 4. Quantification of noise components measured by plotting normalized CFP and YFP intensities per single cell induced with IPTG over the phases. Points scattered perpendicularly to the diagonal line where CFP and YFP intensities are equal shows intrinsic noise, points scattered along this line represents level of extrinsic noise. Total cell to cell variability is exponentially decreasing from Lag, Acceleration, Exponential, Retardation and Stationary phases. Intrinsic noise is increase in Acc, Ret and Sta phases. Extrinsic noise increases in Lag and Exp phases still not exceeding intrinsic noise.

4.6. Comparison of gene expression from multi-copy and single-copy plasmid

Cells expressing CFP/YFP (multi-copy origin) were compared to the cells expressing mRFP1 (single-copy origin) in Exponential phase for verification of considerably insignificant difference between protein expressions from single and multi-copy plasmids or null hypothesis. For this purpose, we used Kolmogorov-Smirnov test. In both cases,

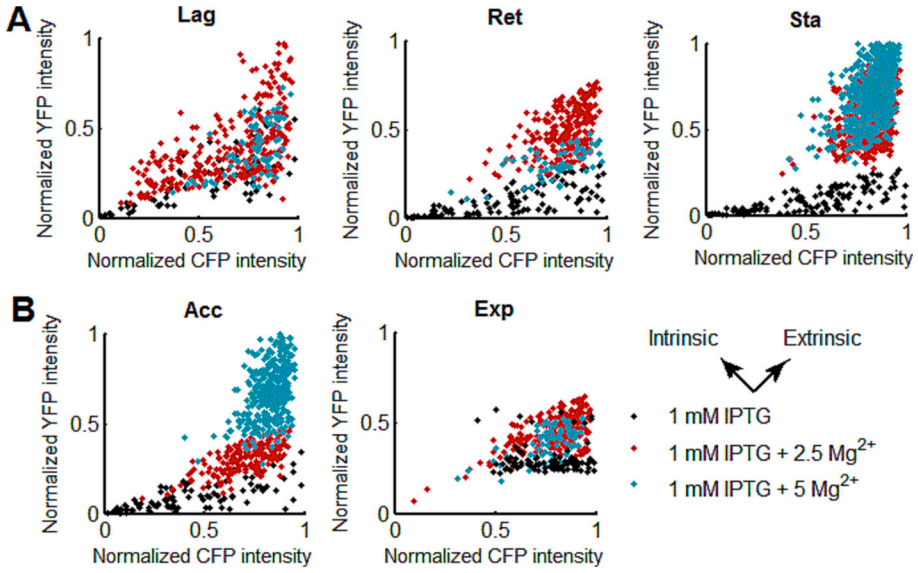

Fig. 5. Noise quantification in cells induced by IPTG with presence of 2.5 and 5 mM of Mg^{2+} over the phases. Addition of higher of magnesium concentration increases total cell to cell variability (total noise) over the phases. A) Group of slow dividing cells includes Lag, Ret and Sta phases with 2.5 mM of Mg^{2+} shows higher extrinsic noise increase and presence of 5 mM of Mg^{2+} increases intrinsic noise. B) Both magnesium concentrations have similar noise effect in fast dividing cells of Acc and Exp phases, though concentration of 5 mM inhibits extrinsic noise causing population to reach cellular homogeneity.

Table 4
Number of processed cells in each experimental condition.

Condition			
Phase	1 mM IPTG	1 mM IPTG +2.5 mM Mg^{2+}	1 mM IPTG +2.5 mM Mg^{2+}
Lag	83	343	77
Acc	103	172	347
Exp	235	243	76
Ret	106	228	117
Sta	126	595	510

$$\eta_{int}^2 = (mean((CFP - YFP)^2)) / (2 * mean(CFP) * mean(YFP)) \quad (1)$$

Schema 1..

$$\eta_{ext}^2 = mean(CFP^2 + YFP^2) - 2 * mean(CFP) * mean(YFP) / mean(CFP) * mean(YFP) \quad (2)$$

Schema 2..

expressions of CFP and YFP expressions tested against mRFP1 expression in individual cells, were consistent with the null hypothesis with *p*-values of 0.1601 and 0.0571 respectively. Nevertheless, expression of reporter proteins from the multi-copy plasmids may originate from uneven partitioning and plasmid replication during the cell divisions. Thus, we also estimate the resulting 'noise' of the multi-copy system in Exponential phase, $\eta_{int0} \sim 0.08$, that approximates possible deviation of the noise from those which would be observed from the single-copy expression. So, observed intrinsic noise can deviate from the true values by a factor $\sqrt{1 \pm \eta_{int0}^2 / \eta_{int}^2}$ or 25.79 % [1].

4.7. Noise definition and data handling

Intrinsic (η_{int}) and extrinsic (η_{ext}) noise classes were calculated by the Schema 1 and 2, where, CFP and YFP are intensities of reporter proteins per cell. Noise classes are treated symmetrically, so that, CFP=YFP in a diagonal. It allows for η_{int} being normalized r.m.s. (root-mean-square) distance from the diagonal where squared sum of both classes represents squared total variation (η_{tot}) (Schema 3). Total variation is defined by Schema 4. If either of the noise components is changed another part controversially affects the total variation.

$$\eta_{int}^2 + \eta_{ext}^2 = \eta_{tot}^2 \quad (3)$$

Schema 3..

$$\eta_{tot}^2 = mean(CFP^2 + YFP^2) - 2 * mean(CFP) * mean(YFP) / 2 * mean(CFP) * mean(YFP) \quad (4)$$

Schema 4..

CRediT authorship contribution statement

Akshaya Murugesan: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Reshod A. Alshagrawi:** Writing – review & editing, Formal analysis. **Ramesh Thiyagarajan:** Writing – review & editing, Formal analysis. **Meenakshisundaram Kandhavelu:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare no competing financial interests.

Data availability

Data will be made available on request.

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