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Cell lysates and egg white create homeostatic microenvironment for gene expression in cell-free system

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ABSTRACT

Homeostasis widely exists in living systems, and plays essential roles for maintaining normal physiological activities, enabling to preserve their functionalities against variations. Gene expression is a crucial process that allows cells to produce the necessary protein, giving cells the flexibility to adapt to variations. Herein we study homeostasis of gene expression in cell-free system. Heat-inactivated cell lysates and egg white are utilized to create homeostatic microenvironment. Results show that both in cell lysates and egg white, gene expression is maintained at relatively stable levels upon variations including gene amount, magnesium ions and temperature. Our work presents a nascent concept and experimental evidence for the homeostasis in cell-free systems, and provides implication for living systems.

1. Introduction

Homeostasis plays essential roles for maintaining normal physiological activities in living systems [1–5]. It is the tendency to regulate and maintain the internal environment in a stable state. This unique feature enables the living systems to preserve their functionalities against variations. For example, as the basic units of modern life, cells have developed multiple regulatory mechanisms to meet the requirements necessary to maintain cellular homeostasis. The microenvironment inside cells is a complex and highly crowded solution, which contains many biomolecules such as proteins, nucleic acids, and small molecules to sustain all essential processes of life. In general, 20–30% of the interior volume inside a cell of *Escherichia coli* is occupied by macromolecules and the total concentration of proteins and RNA is 300–400 g/L [6,7]. Cells keep homeostatic status in this viscous liquid or hydrogel-like microenvironment. Such viscous rather than liquid microenvironment has been demonstrated as the crowding effect. Crowding effect is elucidated to enhance biochemical reaction performance by influencing reaction equilibria and rates, as well as affect protein folding and aggregation [8–10], binding of small molecules [11], interaction with nucleic acids [12], enzymatic activity [13], and protein-protein interactions [14,15]. Crowding also plays essential role in the formation of condensed DNA and nucleus assembly inside the cells [16]. Moreover, the crowding effect has been considered as the one of the critical factors that regulates cell volume and cell growth, even for cellular evolution [17–20]. Rather than the reported crowding effect, we herein find a new homeostasis effect of these biomolecules (Scheme 1). We tested the homeostasis effect in a cell-free gene expression system, a valid simplified model of cells. Cell-free synthetic biology has emerged as an important approach which can engineer biological building blocks without using living cells and provides simpler and faster platform in an open manner than cell system [21]. Cell-free system has also been explored in the new field of *in vitro* synthetic biology, allowing for the engineering of biomolecular systems with cell-like behaviors. We utilized heat-inactivated cell lysates to create the homeostatic environment, which more resembled the realistic conditions of the microenvironment inside cells. Egg white was used as the second homeostasis reagent which contained high percentage of macromolecules, most of which were proteins such as ovalbumin, ovoglobulin and lysozyme [22,23].

2. Methods

2.1. Preparation of E. coli lysates

E. coli strain BL21 was used to prepare *E. coli* lysates. The *E. coli* cells were grown in 2 × YTPG medium at 37 °C until OD_{600} reached 1.8. Then cells were collected by centrifugation at 4 °C, and washed by ice-

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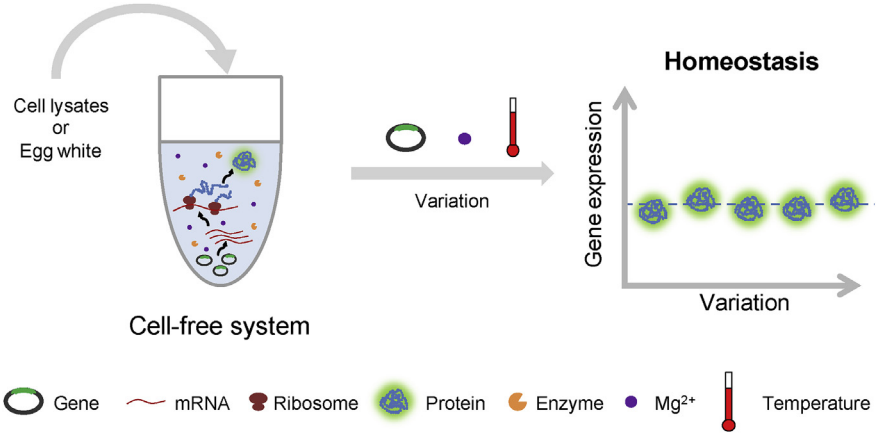

Scheme 1. Cell lysates and egg white create homeostatic microenvironment for gene expression in cell-free system. When heat-inactivated cell lysates or egg white was added into cell-free system, gene expression remained homeostasis upon variations.

cold MilliQ water for three times. Next the cells were disrupted by sonication, and collected as *E. coli* lysates. Then *E. coli* lysates were divided into small aliquots, heated at 100 °C for 10 min to inactivate enzymes remained, frozen in liquid nitrogen, and stored at −80 °C.

2.2. Preparation of egg white

Egg white was isolated by pipette from freshly purchased egg, and then frozen in liquid nitrogen and vacuum freeze dried. Subsequently, the egg white powder was dissolved in MilliQ water and stored at −80 °C.

2.3. Gene expression in cell-free system

The crude cell lysate for cell-free system were prepared from *E. coli* strain BL21. The cells were grown at 37 °C in 2 × YTPG medium. When OD_{600} reached 1.8, cells were collected by centrifugation, then thoroughly dissolved in ice-cold 20% sucrose solution and incubated for 10 min. Next, cells were collected and washed by ice-cold MilliQ water for four times to remove periplasmic protein. The cell pellets were subsequently suspended in buffer containing 10 mM Tris–acetate buffer (pH 8.2), 14 mM magnesium acetate, 60 mM potassium glutamate, and 1 mM dithiothreitol (DTT) and disrupted by sonication, followed by centrifugation (30,000 × *g* for 30 min). The supernatant was collected as crude cell lysate, then divided into small aliquots, frozen in liquid nitrogen, and stored at −80 °C.

Reaction mixture of cell-free system for gene expression contained crude cell lysate and reaction buffer consisted of 50 mM HEPES (pH 8.0), 90 mM potassium glutamate, 0.75 mM cAMP, 0.33 mM NAD, 0.26 mM coenzyme A, 0.068 mM folinic acid, 0.2 mg/mL E. *coli* total tRNA mixture, 1.5 mM each of 20 amino acids, 3 mM GTP, 1 mM ATP, CTP, and UTP each, 0.66 mM spermidine, and 20 mM 3-phosphoglyceric acid (3-PGA). Plasmid, magnesium glutamate, T7 RNA polymerase were added into reaction mixture to initiate gene expression. Different concentrations of heat-inactivated *E. coli* lysates or egg white were added as well. A 6 h kinetic read was performed in microplate reader (Biotek Synergy H1), with fluorometric reads at 488 nm of excitation and 520 nm of emission every 5 min. Reactions were incubated at 30 °C with 5 s shaking at 282 cpm. The 96-well microplate was covered with film to prevent evaporation.

2.4. Construction of genes

All enzymes used for the construction of pRSET-eGFP and pRSET-YFP were purchased from New England Biolabs, USA. The correct band after double enzyme digestion of eGFP or gene and pRSET vector was cut from an agarose gel and purified, following ligation at 16 °C overnight by T4 DNA Ligase. The ligation product was transformed into Trans1-T1 cells (Transgen, China), and the cells were cultivated on ampicillin agar plates at 37 °C overnight. After sequencing, correct colonies were propagated for plasmid extraction using a TIANprep Mini Plasmid Kit (Tiangen, China).

3. Results and discussion

3.1. Non-homeostatic gene expression in conventional cell-free system

Gene expression is one of the essential biological processes for all living organisms, which involves multistep reactions of transcription and translation [24]. In cell-free system, crude cell lysate which contained the necessary translational machineries (including ribosomes, tRNAs, and aminoacyl tRNA synthetases) was used to drive gene transcription and translation, with the addition of gene template, energy source, amino acids, nucleotides, cofactors, and salts. In conventional cell-free system, gene expression was generally carried out in the diluted solution, without massive biomacromolecules that existed in the real microenvironment of cell. In this circumstance, the expression of gene was easily affected by variations, showing the non-homeostatic effect (Fig. 1). When one of the elements changed, gene transcription and translation were subsequently influenced, leading to the fluctuation of the yield of gene expression. The plasmid encoded with enhanced green fluorescent protein (eGFP) was used as the template of gene expression. Gene was transcribed into mRNA with the catalysis of T7 RNA polymerase. As one of the substrates in gene expression, plasmid concentration affected the final production. With the increase of gene concentration from 1 ng/μL to 6 ng/μL, the yield of eGFP for 6 h increased at first, then reached the maximum at 4 ng/μL, and reduced subsequently (Fig. 1a).

Magnesium ion was one of the key factors that affected the activity of enzymes that involved in gene expression. Enzymes required the presence of magnesium ions for their catalytic action, such as RNA synthesis using nucleotides as ingredients. Moreover, magnesium ion was indispensable for adenosine triphosphate (ATP), which was the main source of energy in gene expression. ATP must be bound to magnesium ion in order to maintain biologically active. We tested gene expression under the variation of the concentration of magnesium ions. Results shows that eGFP expression has variable production with different concentrations of magnesium ions (Fig. 1b). With the concentration range of 12 mM–22 mM of magnesium ions, the eGFP production varied. When the concentration of magnesium ions was 18 mM, eGFP expression was higher than that of other concentrations, reaching the peak. When the concentration of magnesium ions was low as

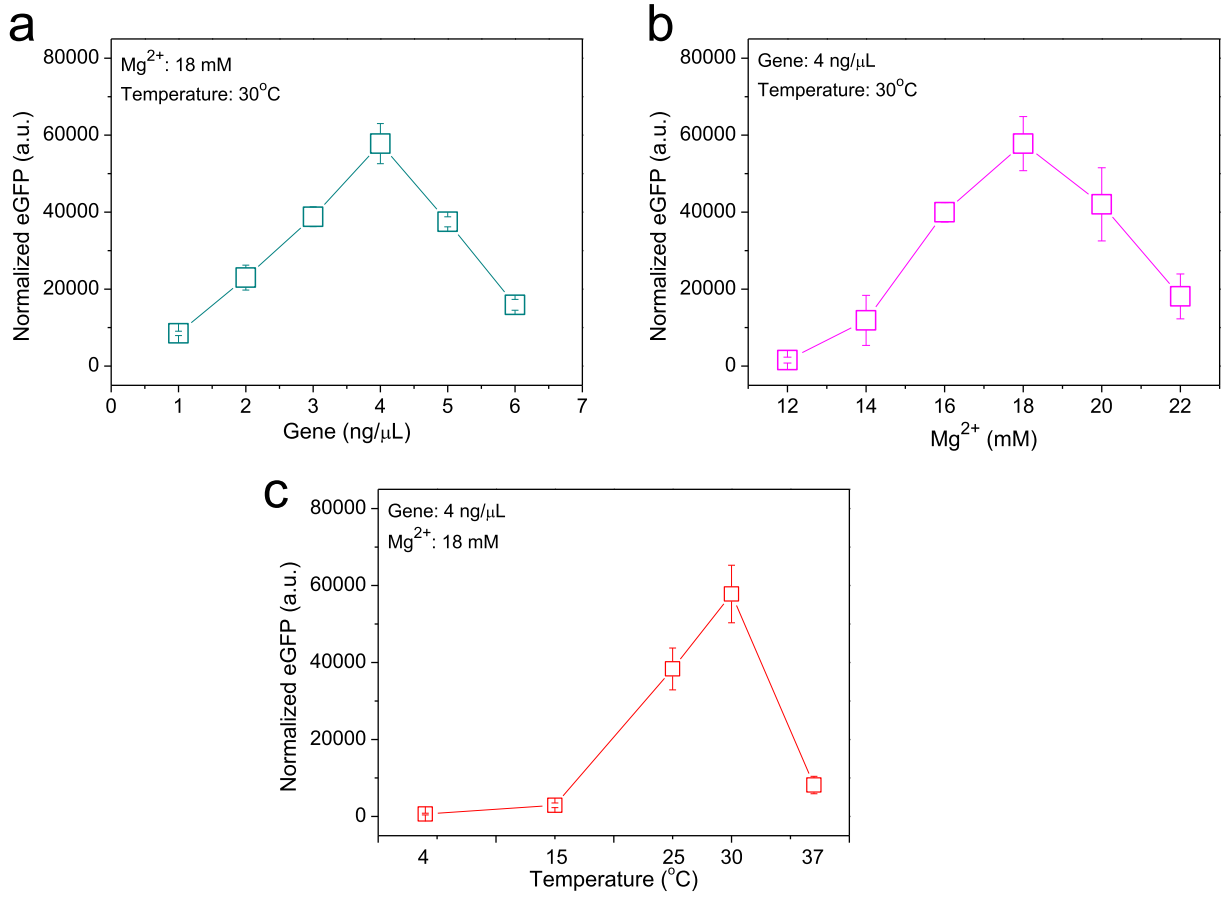

Fig. 1. Non-homeostatic gene expression affected by variations in conventional cell-free system. Gene expressions of pRSET-eGFP with different concentration of gene (a), different concentration of magnesium ions (b), and different temperature (c).

12 mM, the fluorescent intensity of eGFP was approximately zero. Under other concentrations of magnesium ions, the eGFP production also fluctuated with different levels.

Since environmental factors influenced gene expression as well, temperature was one of the variations we investigated in conventional cell-free system (Fig. 1c). The tendency was similar to that of concentration of gene and magnesium ions. When the temperatures were 4 °C and 15 °C, gene of eGFP barely expressed. At 30 °C, the production of eGFP reached the maximum value. In conventional cell-free system, gene expression occurred in the diluted solutions, without macromolecules around. When there are variations in the system, such as concentration of gene, concentration of magnesium ions, and temperature, transcription and translation were severely influenced, showing the non-homeostasis of gene expression.

3.2. *E. coli* lysates created homeostatic microenvironment in cell-free system

The most commonly used crowding agents to simulate the cellular conditions are synthetic polymers, for example, polyethylene glycol (PEG), ficoll, dextran and polyvinyl pyrrolidone (PVP). Such synthetic polymers at high concentrations exhibited macromolecular crowding effects and stabilized proteins, increased ribozyme activity, affected protein-protein associations and promoted protein aggregation [25]. However, these artificial agents were non-biological and compressed at high concentrations owing to self-crowding, revealing less physiologically relevant information [26]. PEG can interact with proteins and form coacervates at high concentrations, and in the presence of inorganic salts, which may lead to spatial heterogeneity in cells [7]. Considering that highly concentrated bacterial cell lysates, such as *E. coli* lysates, are physiologically more relevant, which provide more heterogeneous surfaces and more closely mimic the soft interactions of the cytosol than synthetic polymers, we herein used the heat-inactivated cell lysates of *E. coli* to study the homeostasis effect of cell lysates on gene expression. The *E. coli* lysates consisted of the enzymes that involved gene expressions. In order to eliminate the influences of enzymes, we heated lysates at 100 °C to inactivate the enzymes. *E. coli* lysates were incorporated into the cell-free system. We demonstrated the gene expression of eGFP with the addition of *E. coli* lysates in the presence of the variations, including concentration of gene, concentration of magnesium ions, and temperature (Fig. 2). After *E. coli* lysates were added in the cell-free system, eGFP production was much more stable upon variations. For instance, when the concentration of *E. coli* lysates was 100 mg/mL, with different concentrations of gene, all the fluorescent intensity of eGFP expression were around 20000 a. u. (Fig. 2b), showing the homeostatic effect of *E. coli* lysates. When the concentration of magnesium ions varied, eGFP expression kept consistent upon the addition of *E. coli* lysates (Fig. 2c&d). When temperatures were varied, the trend of gene expression was similar to that of concentration of gene and magnesium ions (Fig. 2e&f). Upon the concentration of *E. coli* lysates increasing, the eGFP expression range declined from 0 to 80000 a. u. to 0–40000 a. u. Above all, with the addtion of *E. coli* lysates, gene expression remained relatively consistent upon the variations including gene concentration, magnesium ions concentration, and temperature.

3.3. Egg white created homeostatic microenvironment in cell-free system

Egg white contains high concentration of macromolecules with about 40 different proteins, which are more than 12% by weight. The highly concentrated macromolecules present egg white the

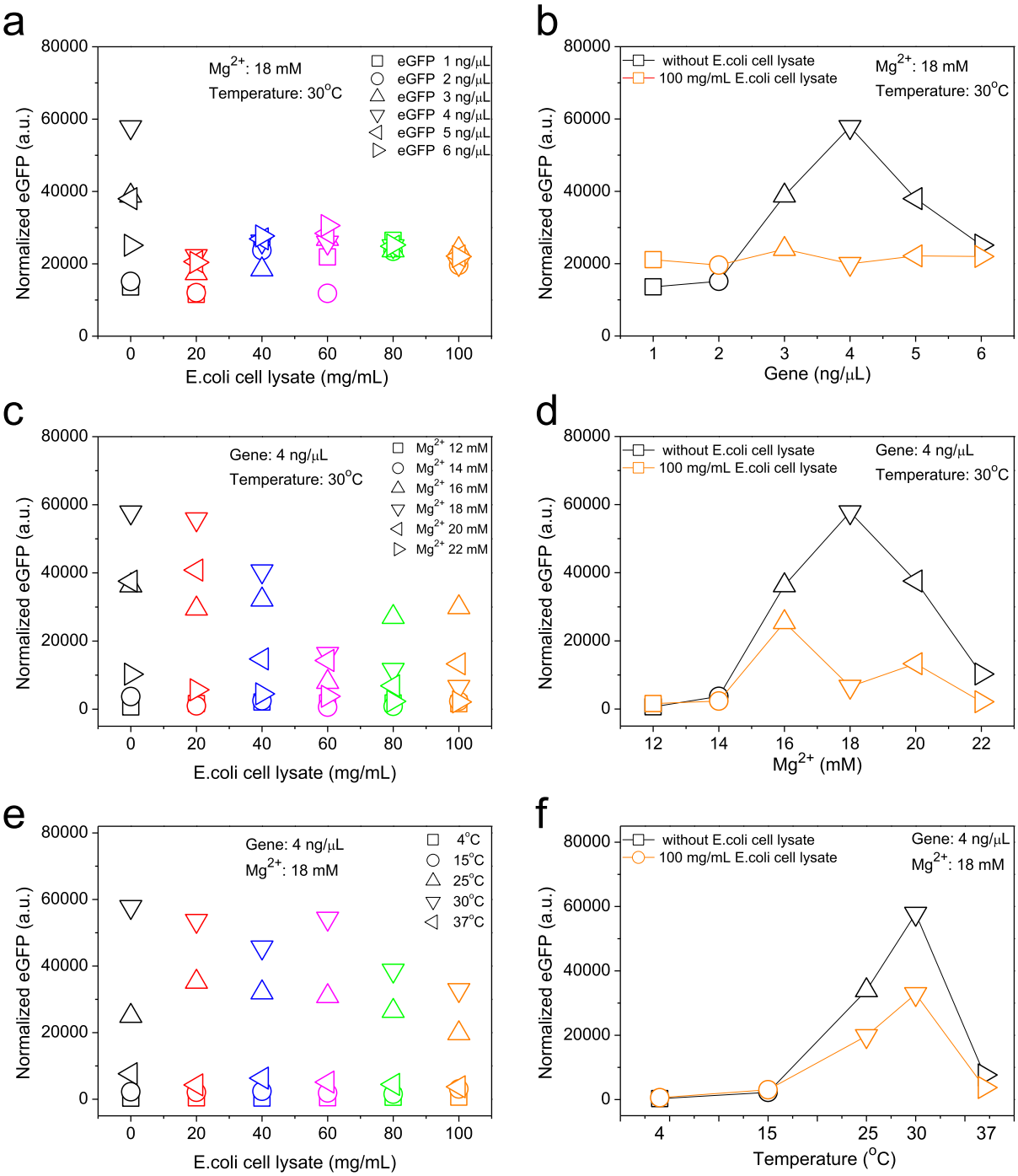

Fig. 2. *E. coli* lysates create homeostatic microenvironment for gene expression in cell-free system. Gene expressions of pRSET-eGFP with different concentrations of *E. coli* lysates with different concentration of gene (a,b), different concentration of magnesium ions (c,d), and different temperature (e,f).

characteristics of a closer cell-like microenvironment [22]. We herein used egg white, a natural agent, to create a cellular-mimicking microenvironment and study the homeostasis effect of egg white in cell-free gene expression system. We expressed eGFP in cell-free system with the addition of different concentrations of egg white and with the variations including concentration of gene, concentration of magnesium ions, and temperature. Upon the increasing concentration of egg white, the yield of eGFP in the presence of the variations kept the consistent tendency (Fig. 3), which is similar to *E. coli* lysates. Moreover, with the increasing different concentration of gene, concentration of magnesium ions, and temperature, the fluorescent intensities of eGFP expressed in cell-free system all remained within the range from 0 to 20000 a. u. (Fig. 3b, d, f). In conclusion, gene expression maintained at a consistent level upon the addition of egg white in the presence of different variations, indicating the homeostasis effect of egg white.

We also tested different gene (pRSET-YFP) with the addition of *E. coli* lysate under the variations of concentration of gene (Fig. 4). Results showed that the gene expression kept a similar expression level at 1500 a. u. with 100 mg/mL E. *coli* lysate, which was consistent with the results with eGFP.

E. coli lysate and egg white resembled more realistic in the cellular microenvironment, which was occupied with massive biomacromolecules. In cells, gene expression was an essential and complex process. The cellular microenvironment with massive biomacromolecules

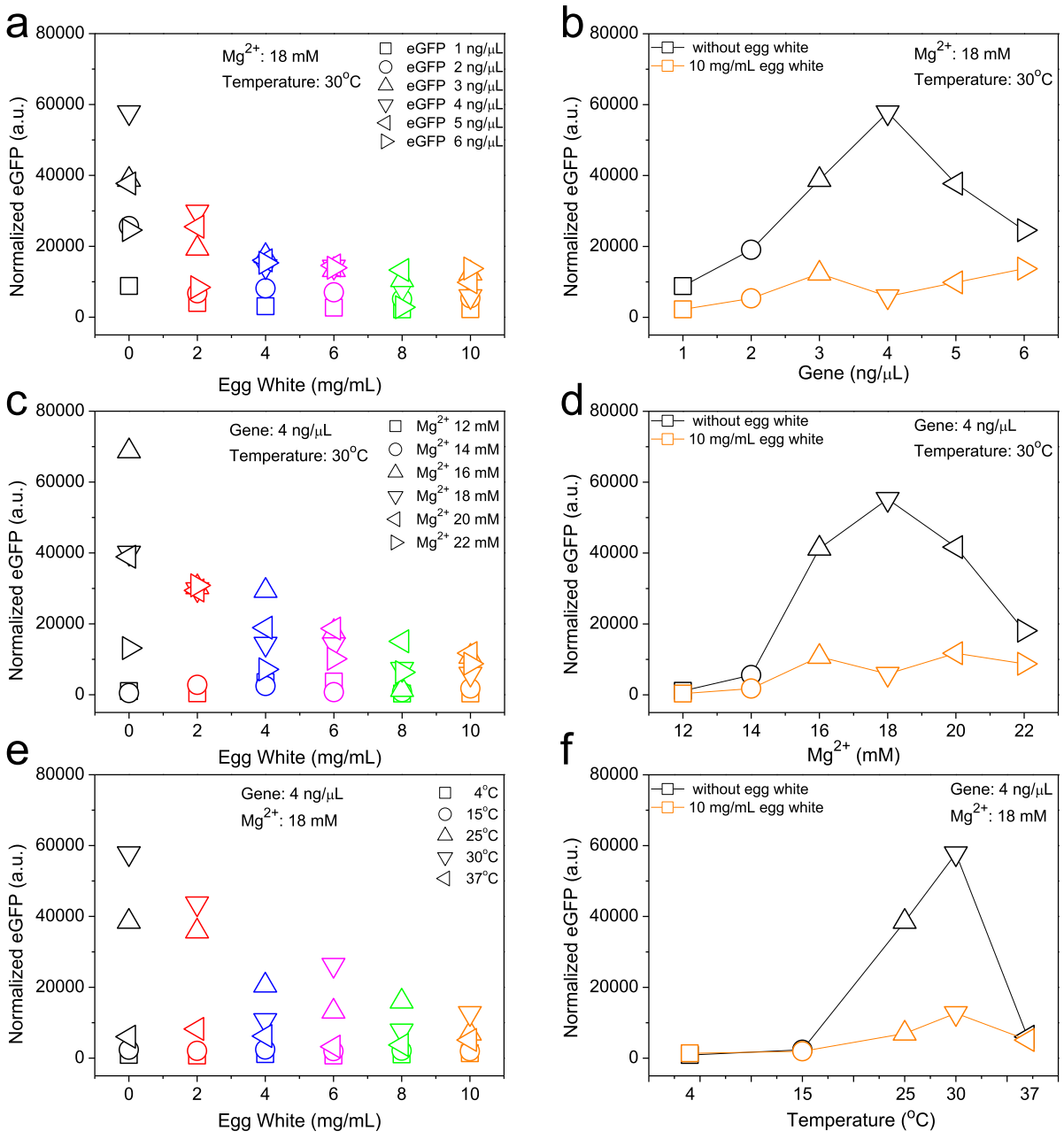

Fig. 3. Egg white creates homeostatic microenvironment for gene expression in cell-free system. Gene expression of pRSET-eGFP with different concentrations of egg white with different concentration of gene (a,b), different concentration of magnesium ions (c,d), and different temperature (e,f).

decreased the mobility of biomacromolecules and provided barriers for diffusion, thus affected the gene expression by increasing the rate constant for transcription and inhibiting translation [26]. The biomacromolecular microenvironment also increased the robustness of gene expression with simulated and experimental evidences under the variations of parameters [27]. As our experimental results indicated that the biomacromolecules in *E. coli* cell lysate or egg white compromised the gene expression with the variations of concentration of gene, concentration of magnesium ions and temperature.

4. Conclusion

Homeostasis is vital for maintaining normal physiological activities in all living systems. Gene expression is one of the fundamental biological processes that maintain homeostasis. *E. coli* lysates and egg white more resembled the realistic conditions of the microenvironment inside cells. Herein, we exploited heat-inactivated *E. coli* lysates and egg white as the natural cellular-mimicking agents, to study the homeostasis effects on gene expression in cell-free system. When there were variations, including gene concentration, magnesium ions concentration, and temperature in the system, *E. coli* lysates and egg white maintained gene expression at consistent level, indicating the homeostasis effects and protecting the gene expression from variations in cell microenvironment.

Conflicts of interest

The authors declare that they have no conflict of interest.

Author contributions

D.Y. and Y.L. designed the study. Y.L. carried out experiments. All

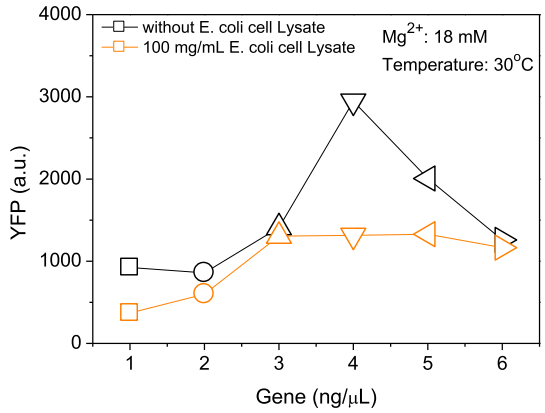

Fig. 4. Gene expression of pRSET-YFP with 100 mg/mL E. *coli* lysates with different concentration of gene.

authors conducted all data analysis and wrote manuscript.

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