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Review Article

Nucleoids Dynamic in *Escherichia coli*: A Growth Phase Dependent Process

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Bacterial DNA compacts in nucleoid bodies. The organization of nucleoid body depends on the association of genomic DNA with a numbers of histone-like proteins. The relax nucleoids organization in rapidly growing *Escherichia coli* cells associate with six major proteins, Fis, HU, Hfq, H-NS, StpA and IHF, but at stationary phase the nucleoids further tightly pack with Dps. The final steps of compact nucleoids formation occurs with association of MukBEF complex - a bacterial condensin. The change of nucleoid proteins composition in stationary phase accompanies compact DNA organization and genes silencing. Thus, compact nucleoid organization and gene silencing may be crucial for cell survival in stationary phase.

Keywords: *Escherichia coli*, Nucleoid body, Nucleoid proteins, Nucleoid compaction, Condensin

Introduction

In the nucleus, the genomic DNA of eukaryotic cells organizes into nucleosomes as compact molecules in association with histone proteins. In bacteria such organized nucleosome structure is absent, instead, the bacterial DNA organizes into nucleoid body in association with a sets of specific proteins[1-3]. The 4.7 Mbp DNA (1.5 mm long) of *Escherichia coli* packs in a highly ordered nucleoid sturcture of 1 mm long in association with 10-20 DNA binding proteins[4-10]. The most abundant proteins participte in nucleoid formation are Dps (DNA-binding protein from starved cells), Fis (factor for inversion stimulation), Hfq (host factor for phage Q_β replication), H-NS (histone-like nucleoid structuring protein), HU (heat-unstable nucleoid protein), IHF (integration host factor), MukB (partitioning of sister chromosome), and StpA (suppression of *td*$^-$ phenotype A)[11-37]. However, at present the knowledge on the molecular organization of *E. coli* nucleoid is inadequate, because of insufficient evidence on the molecular structure and composition, and its growth phase dependent variation. This review summarizes the growth phase-dependent variations in the structure and protein composition of *E. coli* nucleoid. Fluctuating patterns of nucleoid proteins and a possible compaction process of genomic DNA into the nucleoids are discussed.

Nucleoid Proteins in *E. coli* Cells

In dynamic transition of relax to compact nucleoid formation during the change of growth phase from exponential to stationary depends on sequential participation of a numbers of nucleic acid binding proteins. Some of these directly bind with DNA engaging them in structural change in nucleoid formation, and the other group alters the transcription or translation, thus bringing the morphological change in *E. coli*[38]. The major DNA binding proteins those involve in growth phase dependent nucleoid organization and gene expression in *E. coli* are discussed.

Dps

Dps (DNA-binding protein from starved cells) is a starvation or oxidative stress inducible 19 kDa DNA-binding protein in *E. coli*[11] and forms a dodecameric complex[39]. Dps is an abundant nucleoid protein in *E. coli* (Table 1). The purified Dps binds DNA without sequence specificity[14] and classifies under bacterial nucleoid-associated protein family including HU, H-NS, IHF and Fis[7,16,39]. The nucleoid DNA turns into a more compact configuration after binding with Dps[16,39-40]. In exponential phase, Dps exists about 6,000 molecules per cell and gradually increases up to about 180,000 molecules per cell at late stationary phase[16,32].

Fis

Fis (factor for inversion stimulation) is a small basic DNA-binding protein in *E. coli* originally identified as a factor for site-specific DNA recombination[41] and participates in transcription of the growth-related genes and DNA replication[42]. Fis protein level depends on growth phase of *E. coli*, and in exponential phase it estimates ~60,000 molecules per cell, but becomes undetectable in the stationary phase (Table 1). The synthesis of Fis stops at stationary phase, resulting decrease of intracellular level by 500 to 1,000 fold[16-17].

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Table 1. *General properties of the nucleoid proteins in Escherichia coli*

Protein	Molecules/cell		Native protomer	DNA target	Additional functions	References
	Exponential phase	Stationary phase				
Dps	6,000-8,000	180,000-200,000	Dodecamer	Non-specific	Stress/oxidative DNA damage protection	14,16,32
Fis	40,000-60,000	Almost absent	Homodimer	ctcaaattataatcg	DNA replication, recombination	14,16,17
Hfq	30,000-60,000	10,000-20,000	Hexamer	Curved	Replication of phage Q_β	14,16,29
H-NS	10,000-20,000	4,000-8,000	Dimer or	Curved	Site-specific recombination	12,14,16
HU	30,000-55,000	10,000-17,000	Heterodimer HU-1, HU-2	Kined gapped, 3 or 4-way	DNA replication, recombination junctions	14,16
IHF	6,000-15,000	30,000-55,000	Heterodimer IHFa, IHFb	tctaacgcattgatt	Recombination	14,16,18
StpA	13,000-25,000	5,000-10,000	Dimer or oligomer?	Non-specific	RNA chaperon	12,14,16

Hfq

Hfq (host factor for phage Q_β replication, HF-I), a nucleic acid-binding protein, recognizes as host factor involving in replication of phage Q_β RNA[28-29]. Hfq preferentially binds to curved-DNA sequence in a non-specific manner[14]. Hfq found ~55,000 molecules per cell in exponential phase (Table 1) and assembles in nucleoid body and ribosomes[16,28]. Hfq controls the translation of the σ factor gene *rpoS* and the DNA repair gene *mutS*[43-44].

H-NS

H-NS (histone-like nucleoid structuring protein) is a well-characterized nucleoid-associated protein repressing global transcription that affects more than 100 genes or operons in *E. coli*[12]. The number of H-NS molecules reaches ~20,000 per cell in exponential phase, but decrease to 40% at late stationary phase[16]. Remarkably, the pattern of H-NS growth-dependent variation is similar to those of Hfq, HU and StpA (Table 1).

HU

HU (heat-unstable nucleoid protein) is considered as a prokaryotic homologue of eukaryotic histones[19], but the sequence comparison indicates that HU is more analogous to the eukaryotic high morbility group (HMG) proteins[45]. HU exists in solution as a heterodimer consisting of two similar subunits. Like Hfq, HU is also associated with ribosomes[46]. About 30,000 to 55,000 HU molecules may exist in the exponentially growing cell of *E. coli* W3110. HU dimers may associate every 300-400 bp of the *E. coli* genome under the saturation condition. Upon entry into stationary phase, the HU level gradually decreases to less than one third of the maximum level in late stationary phase[16].

IHF

IHF (integration host factor), the most abundant sequence-specific DNA-binding proteins in *E. coli*, involves in the integration of phage λ DNA into host DNA[14,47-48]. Currently, IHF recognizes as a global transcription regulatory factor for many genes[49]. IHF may exist ~12,000 molecules per cell in exponential phase and the number increases about 5-fold in early stationary phase[16,18]. At the transient state, IHF becomes the second most abundant protein, suggesting IHF plays crucial roles in transitional conversion of nucleoids structure from more relax to compact form during the phase transition of cell growth.

MukB

MukB (partitioning of sister chromosome), a 177-kDa protein, involves in partition of the bacterial chromosome. An estimated ~150 molecules of MukB are present per cell[30]. The *mukB* mutation causes unfolding of the nucleoid[50]. In addition, the domain structure of MukB resembles eukaryotic and bacterial structural maintenance of chromosome (SMC) proteins that interact with topoisomerases in fission yeast and *Drosophila*[51].

StpA

StpA (suppression of *td*$^-$ phenotype A) is a multi-copy suppressor of a *td*$^-$ phenotype of phage T4[52]. The growing *E. coli* W3110 cells contain ~25,000 molecules of StpA whereas in stationary phase have ~7,000-8,000 molecules[16]. The sequence identity (58%) and growth-dependent variation of StpA and H-NS suggests that these two proteins perform overlapping functions. Indeed, both proteins have similar sequence recognition specificity and DNA-binding affinity[14].

Compact and Stress Resistant Stucture Formed by *E. coli* at Stationary Growth Phase

At stationary phase, *E. coli* alters the structures of cell wall, cytoplasm and nucleoid as well as the number of cellular components (Figure 1) and cells become highly resistant to a variety of stresses. The cell volume, and the chemical composition and structure of cell envelope containing the outer membrane, cell wall, and cytoplasmic or inner membrane drastically changes when *E. coli* enter in stationary phase. There is an increase in

cardiolipin and cyclopropyl fatty acid derivatives at the inner membrane (Figure 1)[53-54]. Stationary phase cells also have a higher protein/lipid ratio in the membranes, which makes them less prone to lateral phase separation and a higher degree of crosslinking among membrane proteins[55-56]. The cell wall become thick in stationary phase containing 4-5 layers of peptidoglycan compared to 2 or 3 layers in exponential growth, contributing stability of the envelopes of stationary-phase cells[56].

The transcription of each set of genes under specific growth-condition by DNA-dependent RNA polymerase depends on the presence of one of the seven σ subunits plus core enzyme. The synthesis of σ^S is regulated by ppGpp and H-NS[57-58]. The level of σ^S increases and selective activation of $E\sigma^S$ holoenzyme may occur due to accumulation of trehalose in stationary growth phase[59-60]. Decreasing linking number of supercoiled DNA in stationary phase enhances the activity of σ^S holoenzyme[61-62].

Translation machinery adapts in stationary phase by forming translationally incompetent 100S ribosome, in which Rmf (ribosome modulating factor) is involved. When stationary phase culture transfer into fresh medium, the ribosome dimers, 100S, are converted into 70S monomers within a few minutes[63]. *E. coli rmf*$^-$ mutant cannot survive in stationary phase, implicating ribosome dimerization is presumably essential for stationary phase survival[64-65].

Mechanisms of Nucleiod Body Formation in *E. coli*

Bacterial nucleiod is continously remodleed by the defined sets of genes expression to fit the needs of the physiological state of growth of it. In *E. coli* nucleoid body formation and compaction, is growth phase dependent process, and the most striking changes occur during the phase switching from exponential to stationary phase (Figure 2). Similar compaction of DNA is observed in other microorganisms *Listeria monocytogenes* and *Lactobacillus plantarum*[66-67]. In exponential phase *E. coli* DNA form less compact supercoiled nucleoid than those in the stationary phase, possibly due to presence of molar excess of Fis over Dps (Figure 1 and 2).

Nucleoid Compaction

In *E. coli*, several factors involve in the compaction of chromosomal DNA into the nucleoid. The size of compacted nucleoid was estimated by measurement of the areas of the fluorescence images of individual nucleoids, with a user-independent thresholding procedure[68]. The average thresholded area of compacted nucleoids is 1.4 μm^2. Whereas the average area for the expanded nucleoids is 2.8 μm^2 as released by the low salt-spermidine procedure[38,69]. Nucleoids in rapidly growing cells are complex structures with multiple genome equivalents of DNA at different stages of replication associated with large amounts of proteins and other ligands[16,69-72]. Model studies indicate that changes in these levels of compaction can have important effects upon the ability of the DNA to function in a wide variety of enzymatic and chemical reactions[68]. Many reactions related to DNA are accelerated by compaction[68,73-75], but the rate of transcription decreases[59]. The current review is part of an attempt to better understand the factors that control DNA compaction. A variety of DNA-compacting factors have been suggested[3,19,68,71,76-78].

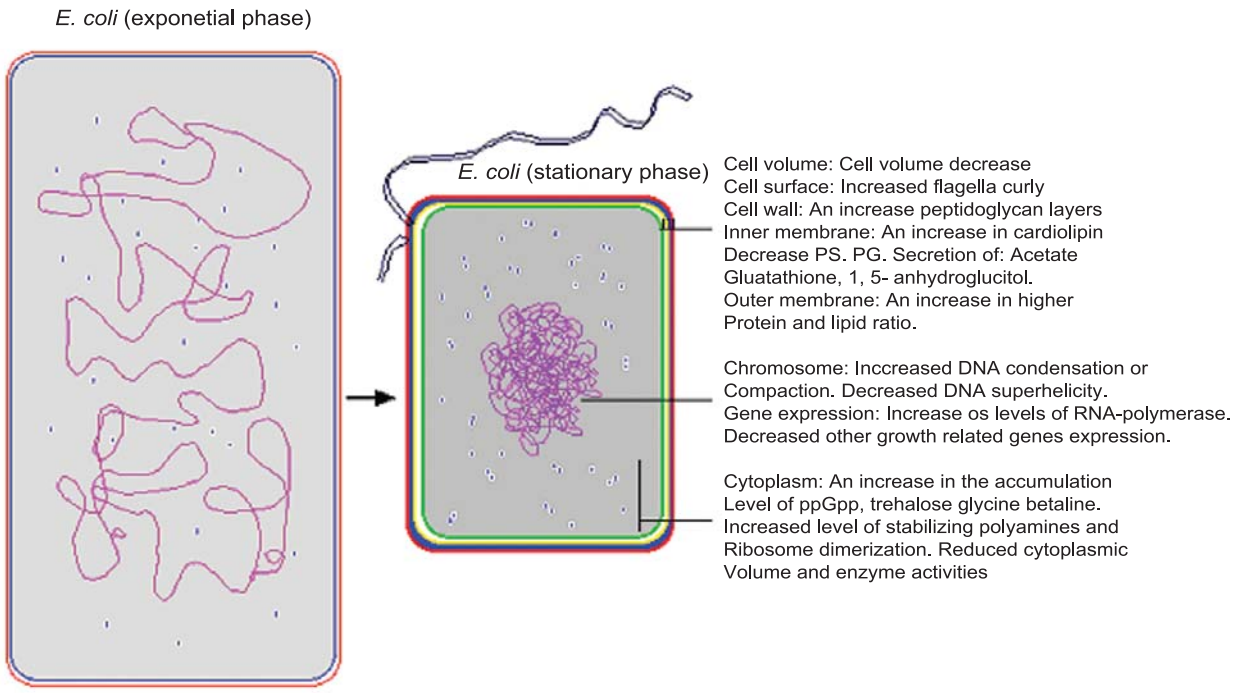

Figure 1. *Overall changes in Escherichia coli cell entering into stationary growth phage.*

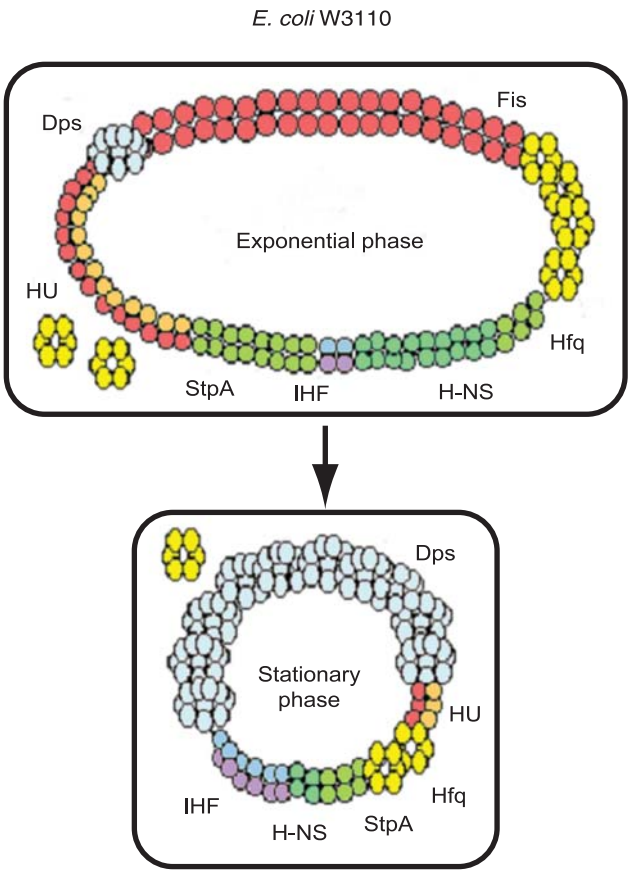

Figure 2. *Schematic representation of protein composition ans structure of Escherichia coli nucleoids and their variations in two different growth phases. The intracellular concentrations of nucleoid proteins in E. coli were converted into the number of molecules per cell. All the nucleoid proteins are assumed to be associated with the nucleoid. The nucleoid structure in stationary phase is more tightly compacted than that in exponential phase. The oeder of accumulation level in exponentially growing E. coli is: Fis (27%) >Hfq(24%) >HU(23%) >StpA (10%) >H-NS (7%) >IHF (6%) >Dps (3%). The major protein components of nucleoid change from Fis, Hfq and HU in the exponential phase to Dps in the stationary phase. The order of abundance in the stationary shase is: Dps (70%) >IHF (15%) >HU (5%) >Hfq (5%) >StpA (3%) >H-NS (2%) >Fis (0%).*

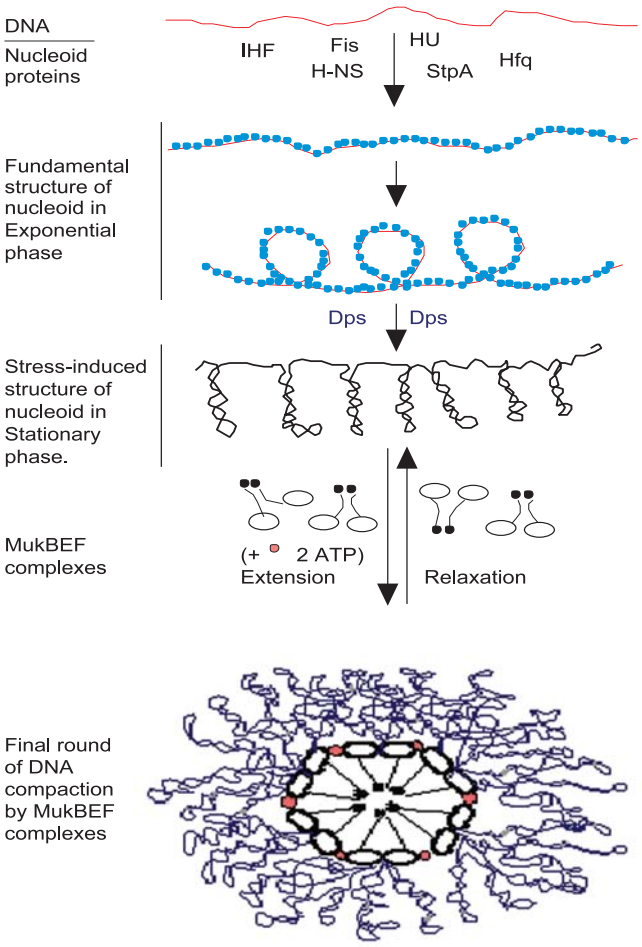

Figure 3. *A model for the DNA compaction mechanism by histone-like proteins (Fis, Hfq, H-NS, HU, IHF, StpA and Dps) and bacterial condensing, MukBEF. The DNA and histone-like proteins form a bacterial chromatin-like structure, nucleoid. The nucleoid, which becomes tightly compacted and forms a coral reef structure with several supercoiled loops in stationary phase, is mainly mediated via Dps. The final round of DNA condensation is mediated via MukBEF complexes (with a central hinge, DNA and two ATPs between adjacent heads are shown), in which 30-kb loops of supercoiled DNA form topological domains between heads of individual helically propagated MukBEF complexes. The bottom part of this figure is adapted from Case et al.*[93].

Growth phase

Stationary phase nucleoids are much more compacted compared to exponential phase nucleoids in intact cells[16]. Sedimentation profiles[38] and atomic force microscopy[40] appearence studies of *E. coli* suggests that nucleoids in stationary phase is condensed more than that of exponential phase. Therefore, chromosome compaction/condensation occurs at the transition step from exponential phase to stationary phase where DNA superhelicity decreases (Figure 3).

Supercoiling

DNA is negatively supercoiled *in vivo*[42,78-80]. Half of the supercoiling controls a number of nucleoid-associated proteins, which have been implicated in the organization of bacterial nucleoid, with additional roles in transcription, recombination, and replication[59]. The remaining half of its supercoiling is introduced into DNA molecules by enzymes called DNA topoisomerases[80-82]. In *E. coli*, the level of supercoiling depends mainly on the activities of DNA TopI (topoisomerase I) and TopII (gyrase) and, to a lesser extent, on that of TopIV[80-81,83]. TopI introduces single strand breaks into DNA molecules and causes their relaxation. Gyrase, an ATP-dependent enzyme composed of two GyrA and two GyrB subunits, makes double-strand breaks and introduces supercoils into DNA molecules. TopIV is an ATP-dependent enzyme that makes double-

strand breaks and contributes to DNA relaxation[82-83]. The level of DNA supercoiling may be regulated by a complex homeostatic control[83-84]. Transcription of *topA*, encoding TopI, increases when the supercoiling level is high, whereas transcription of *gyrA* and *gyrB*, encoding GyrA and GyrB, respectively, increases when the supercoiling level is low[84]. It is well established that the supercoiling level influences the activity of many promoters[82] and that environmental stresses alter this level[61-62,85].

The linking number of supercoiled DNA decreases in stationary phase under different physiological conditions including high temperature, oxidative stress, NaCl and extreme pH[61,86-87]. Stationary phase cells may have relaxed plasmids, which are converted to supercoiled structures by gyrase as soon as nutrients become available[86,88]. Stationary phase *rpoS* mutant cells show a bimodal distribution of plasmids and fail to supercoil plasmids after the addition of nutrients, suggesting that *rpoS* plays a role in the regulation of plasmid topology during stationary phase[88]. Dps is also involved in the negative supercoiling of the *E. coli* genomic DNA[38]. Both the RpoS and Dps levels are more than 10-times higher in stationary phase than in exponential phase as mentioned earlier[16,38].

Dps

The tight compaction of nucleoid in stationary phase is mediated by Dps[40]. However, Dps expression in exponential phase is unable to induce the nucleoid compaction, presumably due to the presence of some proteins, which prevent Dps from binding to DNA. Overexpression of Dps induced an intracellular crystalline structure *in vivo*[89-90], and purified Dps proteins were co-crystallized with DNA[89-92]. The highly compacted nucleoids observed in stationary phase appear to have similar characteristics to a biocrystal that is resistant to the detergent treatment[40]. Bacterial cells might protect their own nucleoids from environmental stresses including chemicals by tight compaction. Interestingly, the *dps* mutant cells are very sensitive to environmental stresses[11,31]. The Dps protein is thus crucial for achieving higher ordered structures[16,40,89-91], and for protecting the stresses.

Formation of the coral reef structure might be the first step towards the tight compaction as suggested by Kellenberger and his co-worker[92]. Recent finding suggested that the bacterial condensin, MukBEF complex is involved in compaction of the genome DNA[94]. Condensins are conserved proteins containing SMC (structural maintenance of chromosomes) moieties. It is well characterized that eukaryotic condensins stabilize DNA supercoil *in vitro*[95,97], and prokaryotic condensins affect the supercoiling state of the nucleoid *in vivo*[72,98]. MukBEF contains an ATP-binding domain in the N-terminal region and a DNA-binding domain in the C-terminal region[99]. Mutational inactivation of any of the three *muk* genes results temperature-sensitive growth, and expanded and disordered nucleoids containing cells[100]. A mutation in the TopI gene suppresses the Muk phenotype, probably because the absence of TopI leads to an increased compaction of the nucleoid by the additional negative supercoils[101-102]. The cellular concentration of MukBEF *in vivo* is about one MukBEF per ~30 kb of *E. coli* genome DNA[30]. Because the nucleoid is already condensed by DNA binding proteins and negative supercoiling[3,8,16,59], MukBEF-binding sites that are 30 kb apart on the genome may be trapped in close proximity between adjacent MukBEF heads. Moreover, MukBEF localizes to the center of the nucleoid[34]. Therefore, MukBEF would also be an important contributor to the final round of compaction of the genomic DNA into the folded chromosome with several topological domains (Figure 3)[51,94,102].

Conclusion

We propose two models for growth phase-dependent changes in the structure and protein composition of *E. coli* nucleoids (Figures 2 and 3). Firstly, relaxed form of nucleoids in rapidly growing cells are mainly organized by 6 major proteins, Fis, HU, Hfq, H-NS, StpA and IHF. Secondly, ins stationary phase, the concomitant decreased of FIS and increase of polyamine might be involved in forming compacted nucleoid structure from relaxed DNA. Increase of Dps in molar excess to Fis might re-fold and supercoil the DNA negatively. Both of these process occur simultenously. Then, the negative super-coiled structured DNA folded back and Dps further deposited tightly on the folded back structure. Thus, a highly compact nucleoid form of coral reef structures with several supercoiled domains in the stationary phase of growth is formed. The final step of nucleoid compaction occurs in the presence of MukBEF complex which binds and induces further condensation of dsDNA, thereby creating topologically isolated domains (Figure 3). This remodelling of nucleiod regulates the expression of defined sets of genes to fit the needs of cells under specific growth condition.

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