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Invited Review

Building a complete image of genome regulation in the model organism *Escherichia coli*

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The model organism, *Escherichia coli*, contains a total of more than 4,500 genes, but the total number of RNA polymerase (RNAP) core enzyme or the transcriptase is only about 2,000 molecules per genome. The regulatory targets of RNAP are, however, modulated by changing its promoter selectivity through two-steps of protein-protein interplay with 7 species of the sigma factor in the first step, and then 300 species of the transcription factor (TF) in the second step. Scientists working in the field of prokaryotic transcription in Japan have made considerable contributions to the elucidation of genetic frameworks and regulatory modes of the genome transcription in *E. coli* K-12. This review summarizes the findings by this group, first focusing on three sigma factors, the stationary-phase sigma RpoS, the heat-shock sigma RpoH, and the flagellar-chemotaxis sigma RpoF, as examples. It also presents an overview of the current state of the systematic research being carried out to identify the regulatory functions of all TFs from a single and the same bacterium *E. coli* K-12, using the genomic SELEX and PS-TF screening systems. All these studies have been undertaken with the aim of understanding the genome regulation in *E. coli* K-12 as a whole.

Key Words: *Escherichia coli*; genome transcription; RNA polymerase; sigma factor; transcription factor

Abbreviations: PS-TF, promoter specific-transcription factor; RNAP, RNA polymerase; SELEX, systematic evolution of ligands by exponential enrichment; TF, transcription factor

Introduction

Most of our current molecular-level knowledge of biological systems was obtained using *Escherichia coli* as a model organism in the early development of molecular biology. With the advance in DNA sequencing technology, the complete genome sequence was first determined for two *E. coli* K-12 strains, MG1655 by a US group (Riley et al., 2006) and W3110, by a Japan group (Hayashi et al., 2006). Up to the present time, the complete genome sequence has been determined for more than 1,000 *E. coli* strains. From the complete genome sequence, the complete set of protein-coding sequences on the *E. coli* K-12 genome has been predicted, even though the molecular functions of gene products still remain unidentified for about one third of the predicted genes. In the early stages of research, the analysis of gene expression was performed using exponentially growing *E. coli* under laboratory culture conditions at 37°C and in the presence of sufficient nutrients and oxygen, where only a set of the genes are expressed and the majority of genes remain silent. In nature, however, bacteria are exposed to stressful environments in the absence of sufficient nutrients and oxygen, under various temperature, pH, and osmolality conditions, often being challenged by various chemical stresses, including reactive oxygen species, antibiotics and drugs. Under these stressful conditions, various sets of genes are expressed, which are otherwise silent in laboratory cultures. Bacteria are fortified with various stress response systems for controlling the expression of the genes that are needed for survival under such stressful conditions. Many of these adaptive response pathways are specific, involving a group of regulators and a set of stress-response genes for cell survival. At present, however, the detailed mechanism, as to how such a marked change in the pattern of genome expression takes place, remains unsolved. With regard to an understanding of the whole set of genes

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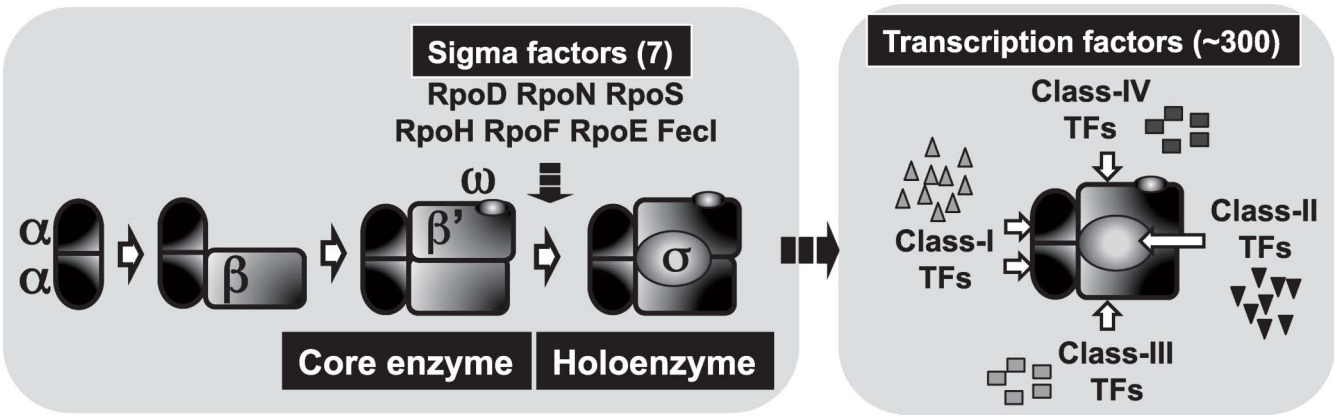

Fig. 1. Functional differentiation of RNA polymerase.

The core enzyme of *E. coli* RNA polymerase (RNAP) is formed by a step-wise assembly of four subunits, 2α, β, β' and ω. Its function is modulated through two steps of protein-protein assembly with seven species of the sigma factor at the first step and about 300 species of the transcription factor (TF) at the second step. Each TF interacts with one of the core enzymes: α-contact class-I, σ-contact class-II, β-contact class-III and β'-contact class-IV (Ishihama, 1992, 1993).

within the genome from a single organism, *E. coli* is recognized as a model organism, because of the huge accumulation of knowledge regarding the physiological functions and regulations of individual genes.

The growing *E. coli* cells contain only about 2,000 molecules of the RNA polymerase (RNAP) core enzyme per genome equivalent of DNA, which is less than the total number of about 4,500 genes, or about 2,500 transcription units, on the *E. coli* K-12 genome. Thus, the pattern of genome transcription is determined by controlling the utilization of a limited number of RNAP among the genes within the *E. coli* genome (Ishihama, 2000, 2010, 2016). Sometime ago, we proposed a model that the pattern of genome transcription is altered through modulation of the gene selectivity of RNA polymerase (RNAP) after interactions with two groups of the regulatory proteins, i.e., seven species of the σ factor (the promoter recognition subunit of RNAP) and a total of about 300 species of the transcription factor (TF), including not only the protein factors but also the nucleotide factors (Ishihama, 2000, 2010, 2012) (Fig. 1). *E. coli* K-12 contains one major house-keeping sigma σ^{70} (RpoD) and six alternative σ factors, i.e., σ^{54} (σ^{N}, RpoN), σ^{38} (σ^{S}, RpoS), σ^{32} (σ^{H}, RpoH), σ^{28} (σ^{F}, RpoF), σ^{24} (σ^{E}, RpoE) and σ^{19} (FecI) (Gourse et al., 2006; Helmann and Chamberlin, 1988; Ishihama, 2000, 2010) (Table 1). The sigma factor confers the promoter recognition ability to the core RNAP. The set of promoters recognized by the RNAP holoenzyme (core enzyme-sigma factor complex) is determined by the species of the associated σ factor. Most of the genes expressed in exponentially growing cells of *E. coli* are transcribed by the holoenzyme containing the house-keeping RpoD sigma. The function of alternative σ factors is often linked to specific stresses, such as nitrogen limitation (RpoN), heat shock (RpoH) and nutrient starvation (RpoS). The set of genes for flagella formation and chemotaxis are transcribed by the holoenzyme containing RpoF sigma. Two ECF (extracytoplasmic function) sigma factors, RpoE and FecI, participate in the transcription of a group of genes that are activated in response to envelope stresses. FecI was originally identified as a regulatory gene for the ferric citrate transport system. At the transcription level, an important mechanism of the genome regulation involves the replacement of the RNAP-associated sigma factor (Ishihama, 2010, 2012; Jishage et al., 1996; Maeda et al., 2000). Each of seven sigma factors controls a specific set of target genes and operons, thereby leading to the formation of a unique pattern of genome transcription. This review article summarizes how much the bacterial research community in Japan has contributed to the development of the "sigma paradigm", mainly focusing on the RpoS, RpoH and RpoF sigma factors. It also presents a brief overview of the contributions made towards understanding the second-step modulation of RNAP by more than 300 species of TF.

Stationary-phase Sigma RpoS

The stationary phase is a fixed physiological state affecting the cessation of cell growth. In laboratory cultures, one of the most common stresses leading to entry into the stationary phase is the limited availability of nutrients (Kolter et al., 1993). In *E. coli*, the stationary phase is achieved by triggering a global stress response of gene expression dependent on the alternative sigma factor RpoS (Ishihama, 1999, 2000; Loewen and Hengge-Aronis, 1994). This response allows cells to become more resistant, not only to the initial triggering stress but also to a variety of other stresses. This cross-protection phenomenon is typical of the general stress response system in single-cell bacteria that are directly exposed to a variety of environmental stresses. The *rpoS* gene is not essential for growth under non-stress conditions, but strains carrying mutations affecting *rpoS* activity are extremely sensitive to environmental stresses.

Identification of the stationary phase sigma RpoS

The *rpoS* (renamed from *katF*) gene was originally identified as a positive regulator of the *katE* gene encoding hydroperoxide hydroperoxidase II, the stationary-phase specific enzyme (Loewen and Triggs, 1984). Kan Tanaka and colleagues (The University of Tokyo; Tokyo Institute

Table 1. Sigma factors in *Escherichia coli* K-12.

Sigma	Alternative Name	Size (Mr)	Gene (Map)	Operon	Regulatory functions	Target Promoters (Regulon DB)	Target Promoters (SELEX)*
RpoD	σ^{70}	613 aa (70,263)	*rpoD* (69.12)	*rpsU-dnaG-rpoD*	Growth-related genes	1872	1320
RpoN	σ^{54}	477 aa (53,990)	*rpoN* (71.98)	*lptB-rpoN-hpf-ptsN-rapZ*	Nitrogen-regulated genes	96	793
RpoS	σ^{38}	291 aa (37,972)	*rpoS (katF)* (71.98)	*rpoS*	Stationary-phase genes	164	235
RpoH	σ^{32}	284 aa (32,469)	*rpoH (htpR)* (86.94)	*rpoH*	Heat-shock genes	322	331
RpoF	σ^{28}	230 aa (27,521)	*rpoF (fliA)* (43.11)	*rpoF-fliZ-tcyJ*	Flagellar-chemotaxis genes	144	260
RpoE	σ^{24}	191 aa (21,696)	*rpoE* (58.28)	*rpoE-rseA-rseB-rseC*	Extra-cytoplasmic response genes	518	493
FecI	σ^{19}	173 aa (19,480)	*fecI* (97.33)	*fecI-fecR*	ferric citrate transport genes	1	-
					Sigma, unidentified	693	

*** The number of regulatory target promoters for each sigma factor were estimated from the number of binding sites of RNAP holoenzyme containing each sigma subunit as identified by the genomic SELEX screening system.**

of Technology) made a breakthrough in the identification of the regulatory role of RpoS. They cloned and expressed the *rpoS* gene and identified it as a sigma factor based on the DNA sequence (Tanaka et al., 1993, 1995). Further, they purified the RpoS protein and confirmed its sigma activity *in vitro* using the reconstituted RNAP holoenzyme containing RpoS. Because of the high-level of similarity in both structure and promoter selectivity between RpoD, the major sigma in the growing phase, and the stationary phase RpoS, they proposed that RpoS is the second principal sigma factor that functions in the stationary phase. After an analysis of transcription *in vitro* of a set of *E. coli* promoters, using both RpoD- and RpoS-RNAP holoenzymes, they classified *E. coli* promoters into three groups: promoters recognized only by RpoD; promoters recognized preferentially by RpoS; and the largest group of promoters recognized by both RpoD and RpoS (Tanaka et al., 1995). [Note that the promoter selectivity of these two sigma factors changes in different modes depending on the reaction conditions *in vitro* (see below).] By making chimeric promoters between promoter-35 and -10 signals, the discrimination signal of RpoS recognition was identified to reside within the -10 sequence.

Regulatory roles of RpoS

As in the case of other sigma factors, RpoS interacts with the RNAP core enzyme and modulates its promoter recognition specificity so as to recognize a specific but large set of genes. Two general approaches were employed to define the RpoS regulon: the proteome analysis using two-dimensional gels of whole cell lysates (Cuny et al., 2007) and the transcriptome analysis using ChIP-chip or ChIP-Seq systems (Lacour and Landini, 2004; Pattern et al., 2004; Weber et al., 2005). These studies indicated that RpoS regulates, directly or indirectly, 10% (approximately 500 genes) of the *E. coli* genes, of which only about 140 genes were predicted to be under the direct control of RpoS (Weber et al., 2005). These RpoS-regulon genes are involved in not only cell survival in the stationary phase, but also in cross-protection against various stresses, including nutrient starvation, osmotic stress, acid shock, cold shock, heat shock, and oxidative DNA damage (Ishihama, 1997, 2000; Loewen et al., 1998). RpoS also regulates a number of genes involved in the expression of virulence within host animals, suggesting that RpoS is an excellent potential regulatory candidate for controlling the virulence. Beyond entry into the stationary phase, *E. coli* forms aggregates or biofilms that are morphologically and physiologically distinct from cells of planktonic growth. This requires the coordinated production of an extracellular matrix of polysaccharide polymers and protein fibers that facilitate cell aggregation and adhesion to a solid surface. RpoS is involved in the transcription of a set of genes needed for the production of these extracellular polymers. During prolonged starvation, a certain number of cells are converted into persister cells that are unsusceptible to antibiotics and other bacteriocidal agents. Again, RpoS is needed for the expression of the genes necessary for the establishment of persister cells (Landini, 2009). RpoS is also indicated to be required for transformation into the viable but non-culturable state (VBNC) (Boaretti et al., 2003).

Using the newly-constructed collection of *E. coli* promoters expressing two-fluorescent reporters, one attached to the test promoter and another to the reference promoter, we performed a systematic quantitative search *in vivo* for *E. coli* promoters that are activated in the stationary phase (Shimada et al., 2004). These *in vivo* data indicated that most of the promoters which are affected in the absence of RpoS, or after over-expression of RpoS, represent those indirectly affected; for instance, due to the increase or decrease of TFs under the test conditions (reviewed in Ishihama, 2010, 2012). Recently, we performed a more direct estimation of the regulatory targets of RpoS by using the genomic SELEX (systematic evolution of ligands by exponential enrichment) screening *in vitro* (Ishihama et al., 2016; Shimada et al., 2005). The total number of constitutive promoters under the direct control of RpoS ranges up to approximately 200 (Table 1) (Shimada et al., 2017).

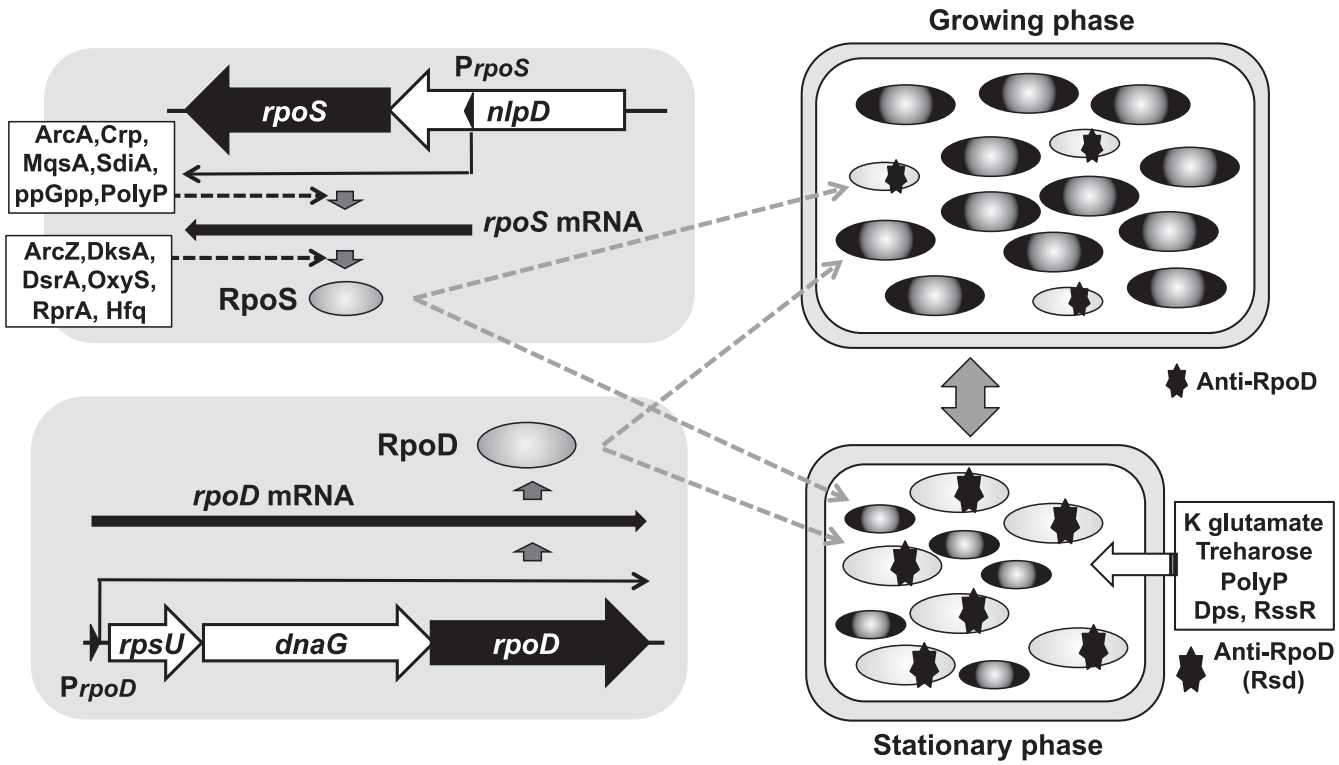

Fig. 2. Growth phase-coupled switching between growth-phase RpoD and stationary-phase RpoS.

E. coli contains two principal sigma factors. RpoD plays a major role in the transcription of growth related genes, while RpoS plays a major role in the transcription of stationary phase-specific genes (Tanaka et al., 1993, 1995). For the switching of sigma from RpoD to RpoS in the stationary phase, several mechanisms are involved: the increase of the functional RpoS level; and the activation of RpoS by intracellular factors or conditions such as glutamate, treharose, *polyP*, Dps, RssR, and DNA super-helicity; and the inhibition of RpoD by anti-sigma Rsd (Jishage and Ishihama, 1998, 1999, 2001).

Level control of RpoS

Since the amount of RNAP core enzyme is limited, i.e., approximately 2,000 molecules per genome, multiple sigma factors compete with each other in binding to the core enzyme (Ishihama, 2010, 2012; Maeda et al., 2000). The selection pressure for RpoS is mainly the competition between two principal sigma factors, i.e., the growth phase-specific RpoD and the stationary phase-specific RpoS, for a limiting number of RNAP core enzyme (Jishage and Ishihama, 1995; Jishage et al., 1996). Therefore, the level of RpoS is carefully controlled, showing a dramatic increase at the onset of the stationary phase, reaching 30% of the level of the house-keeping RpoD. In concert with the regulatory role of the RpoS in the stressful stationary phase, the mechanisms governing the RpoS level is one of the most complex regulatory mechanisms of gene expression. Regulation takes place at the level of transcription, translation, mRNA stability, and protein stability, all being coordinated in response to various stress signals (Hengge-Aronis, 2002).

At the transcription level, a number of stress-response TFs were found to be involved in the regulation of the *rpoS* gene, including ArcA, CRP, Fis, MqsA, SdiA and UvrY (Fig. 2) (Ishihama, 2016). The activity of TCS (two-component system) response regulators ArcA and UvrY are controlled by phosphorylation catalyzed by the respective sensor kinases, anaerobiosis-sensing ArcB and short-chain fatty acid-sensing BarA, respectively (Yamamoto et al., 2005). CRP is activated by cAMP that is produced in the absence of glucose, and regulates as many as more than 350 targets (Shimada et al., 2011). SdiA is the sensor of HSL (homoserine lactone), the quorum-sensing signal AS-1 (Shimada et al., 2014). Since *E. coli* lacks the HSL synthase gene, SdiA is considered to monitor QS signals secreted by other bacteria in the environment. In addition to these protein TFs, the nucleotide effector ppGpp regulates the expression of RpoS (Gentry et al., 1993). The ppGpp alarmone exerts its regulatory function by binding to the RNAP core enzyme at two sites: one at the contact interface between RpoC with RpoZ (Chatterji et al., 1998; Ross et al., 2013), and another at the contact surface between RpoC and DksA (Ross et al., 2016). Thus, ppGpp is a class-IV TF. ppGpp also activates the synthesis of inorganic polyphosphate, poly(P) that accumulates under stress conditions and in the stationary phase (Rao and Kornberg, 1996). Poly(P) directly binds to the RNAP core enzyme (Kusano and Ishihama, 1997b; Ozaki et al., 1992). In the absence of poly(P) synthase, RpoS does not increase upon entry into the stationary phase (Shiba et al., 1997).

Translation of *rpoS* mRNA is also under the complex regulatory network, including a number of regulatory sRNA such as ArcZ, DksA, DsrA, OxyA, and RprA, and Hfq, the master regulator of translation (Hengge-Aronis, 2002; Loewen et al., 1998). The RNA-binding Hfq protein is an RNA chaperone. It was first identified as the *E. coli* factor, referred to as the host factor for the Qβ phage, that is employed for the replication of the RNA genome of the bacteriophage Qβ (Kajitani and Ishihama, 1991). In *E. coli*, Hfq stimulates the translation of a set of mRNA including *rpoS* mRNA, by modulating the mRNA second-

ary structure. The increase in the RpoS level in the stationary phase results in part from a substantial increase in its stability (Hengge-Aronis, 2002). RpoS is unstable in the exponential phase but it is relatively stable in the stationary phase. The instability of RpoS in the exponential growing phase is due to the activity of the ClpXP protease (Yanning and Gottesman, 1998). The adaptor protein RssB plays a regulatory role in this degradation pathway. Phosphorylated RssB directly interacts with RpoS and delivers it to the ClpXP protease complex. An anti-adaptor protein IraP interacts with RssB and interferes with the RssB-mediated degradation of RpoS (Bougdour et al., 2006).

Activity control of RpoS; Influence of intracellular conditions

Many genes are transcribed *in vitro* by both RpoD and RpoS holoenzymes (Tanaka et al., 1995). Accordingly, the promoter sequences recognized by these sigma factors overlap, to certain levels, and two sigma proteins share a set of common amino acid sequences. Tanaka and colleagues proposed that both RpoD and RpoS are the principal sigma factors that provide the RNAP core enzyme with the recognition properties of promoters associated with most of the growth-related genes in *E. coli* (Tanaka et al., 1993, 1995). A switch must take place in the utilization of two principal sigma factors from RpoD in the growing phase to RpoS in the stationary phase. Several mechanisms are involved in this switching.

We suspected the possibility that the regulatory functions of RpoD and RpoS might be controlled in different ways depending on the intracellular conditions. In fact, the transcription activity of the RpoD holoenzyme decreases at high concentrations of salt, but the RpoS holoenzyme exhibited an opposite response (Ding et al., 1995). The activity of the RpoS holoenzyme increased concomitantly with the increase in potassium glutamate concentration up to 200 mM. In contrast, the activity of the RpoD holoenzyme decreased concomitantly with the increase in glutamate concentration. Tanaka and colleagues found that the C-terminal proximal segment of RpoS is needed for retaining the sigma activity at high concentrations of glutamate (Ohnuma et al., 2000). Selective activation of the RpoS holoenzyme was also observed in the presence of trehalose that increased in stationary-phase cells (Kusano and Ishihama, 1997a). Not only does the difference in the cytoplasmic conditions between exponentially growing cells and stationary phase cells, but also the difference in the configuration of genome DNA, influences the selective utilization of RpoD and RpoS. Upon entry to the stationary phase, DNA super-helicity decreases, but the RpoS RNAP favors a template DNA with low super-helicity (Kusano et al., 1996). This series of research was an objective lesson to learn that the *in vitro* system for the analysis of biological reactions, such as transcription, should be set up so as to mimic the *in vivo* conditions, and, with regard to this aspect, no single standard condition exists for *in vitro* reactions.

Rsd: Anti-sigma factor against RpoD

From the stationary-phase cell lysates, we isolated an inactive form of RpoD, which was associated with a novel protein, designated as Rsd (*r*egulator of *s*igma *D*) (Jishage and Ishihama, 1998). Rsd was found to sequester RpoD and displace it from the RNAP core enzyme, thereby generating free core enzyme acceptable to RpoS or other sigma factors. We consequently designated it as an anti-sigma factor for RpoD. Rsd binds to region 4 of RpoD, the recognition surface of the promoter-35 signal (Jishage and Ishihama, 2001). The Rsd level increases upon entry into the stationary phase, and, concomitantly, the activity increases for RpoS-dependent promoters, while the activity decreases for RpoD-dependent promoters (Jishage and Ishihama, 1999). The discovery of the anti-sigma factor Rsd created a new window towards understanding the switch of the promoter selectivity of RNAP through the replacement of bound sigma factors, including DnaKJ as anti-RpoH, FlgM as anti-RpoF, and RseA as anti-RpoE (Hughes and Mathee, 1998; Ishihama, 2016).

Coupling with modulation of the translation machinery

During the transition from exponential growth to the stationary phase, the core machinery of translation—the ribosome—is also modulated in parallel with the modulation of the transcription apparatus. Akira Wada and colleagues (Kyoto University; Osaka Medical College) performed the fine mapping of ribosome-associated proteins by the RFHR (radical-free highly reducing) system of 2D gel electrophoresis, and identified two major players of ribosome inactivation: the ribosome modulation factor (RMF) that converts the functional 70S ribosomes into inactive 100S dimers (Wada, 1998; Wada et al., 1990), and the ribosome hibernation promoting factor Hpf that stabilizes the 100S ribosome dimers (Maki et al., 2000). Upon transfer of the stationary-phase cells into non-stress conditions, the 100S ribosomes immediately dissociate into the translationally active 70S ribosomes by releasing RMF. The cell cycle including the structural and functional inter-conversion of ribosomes is referred to as the hibernation (Yoshida and Wada, 2014).

Polymorphism of RpoS

Polymorphisms including the loss of function of RpoS have been identified in many laboratory strains of *E. coli* K-12 W3110 donated from the major *E. coli* laboratories in Japan (Jishage and Ishihama, 1997). Following this, frequent mutations have been identified for the *rpoS* gene. Given its role in adaptation under stressful conditions, it is natural that *rpoS* mutations are common because RpoS mutations arise readily during prolonged incubation and nutrient starvation (King et al., 2004). Accordingly, the involvement of RpoS has been proposed for the induction of GASP (growth advantage in the stationary phase) (Zambrano et al., 1993) and SPANC (self-preservation and nutritional competence) (King and Ferenci, 2005) phenotypes, leading to an enhancement of high-level mutations for survival under starved conditions.

Heat-shock Sigma RpoH

When *E. coli* cells are exposed to higher temperatures, a set of heat-shock proteins (HSPs) are markedly and transiently induced, which are widely conserved in a wide

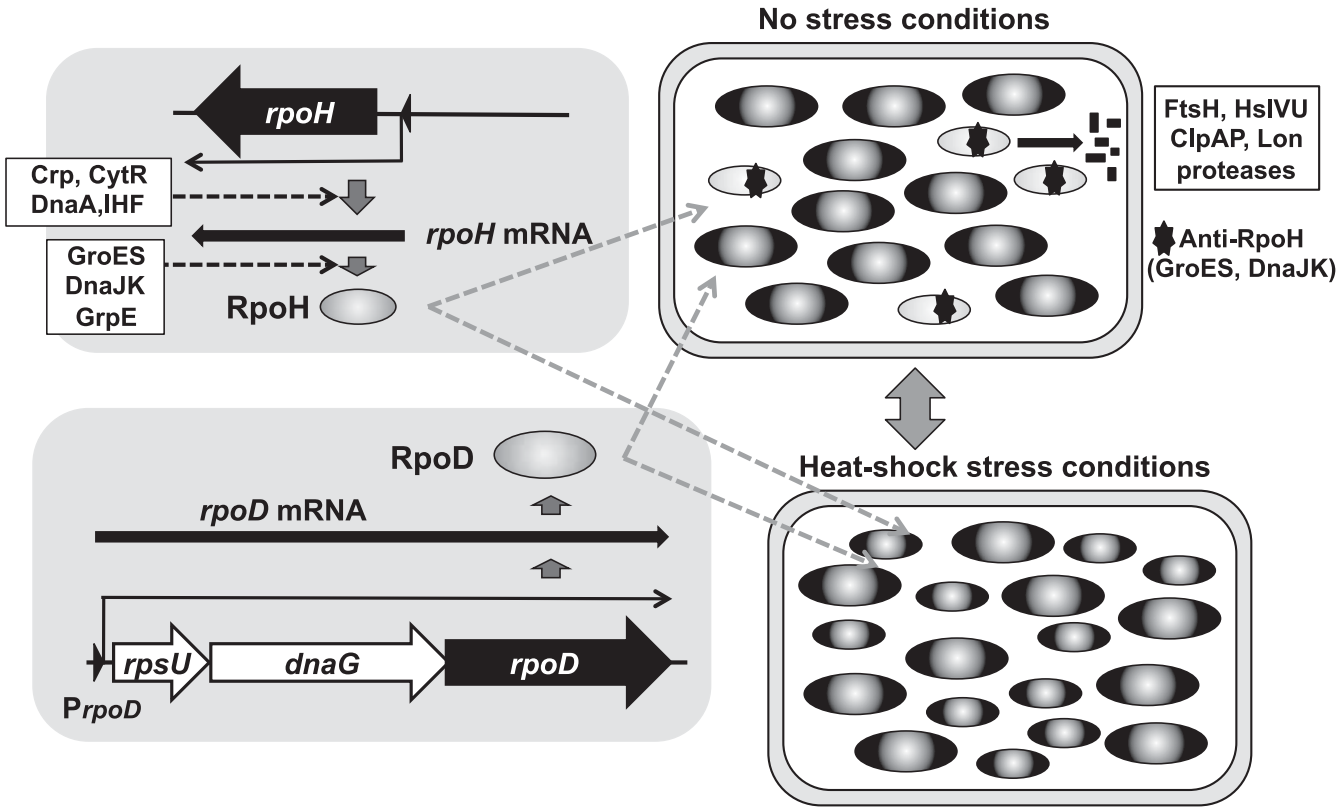

Fig. 3. Role of RpoH sigma in heat-shock response.

Upon exposure of *E. coli* to heat shock, RpoH sigma is induced, which is involved in the transcription activation of a set of heat-shock genes (Yura, 1993). Under non-stress conditions, or during the adaptation of heat-shocked cells to normal growth, heat-shock proteins GloES and DnaJK bind to RpoH, thereby leading to its degradation by heat-induced proteases ClpAB and Lon (Gamer et al., 1996).

variety of bacteria. The laboratories of Takashi Yura (Kyoto University), and Fred Neidhardt in the US, made a pioneering contribution in the discovery of heat-shock response in *E. coli* (Herendeen et al., 1979; Yamamori et al., 1978). Heat-shock induced proteins (HSPs) play major roles in controlling the structure and function of various proteins, including protein folding, assembly, transport, repair and degradation during normal growth, as well as under stress conditions (Georgopoulos et al., 1994; Hendrick and Hartl, 1993). The heat-shock response is thus a cellular protective system for the maintenance of protein homeostasis. The initial event of HSP synthesis is the transcriptional activation of a set of heat-shock response genes as mediated by the heat-shock transcription factor HtpR (Neidhardt and VanBogelen, 1981; Yamamori and Yura, 1982).

Identification of heat-shock sigma RpoH

The discovery of heat-shock regulation in *E. coli* originated when a heat-shock negative mutant, which lacked the *htpR* gene, was isolated (Cooper and Ruettinger, 1975). Upon shifting cells of *E. coli* from 30° to 42°C, a set of more than 30 heat-shock genes are transiently induced, as first detected by proteome analysis (Yamamori et al., 1978). The induction of HSPs takes place immediately after temperature up-shift, reaching a maximum after only a few minutes and, thereafter, decreasing down to the pre-shift level during the adaptation to the post-shift culture conditions (Yamamori and Yura, 1980). This finding led to an open window to view a new paradigm of bacterial response to not only heat shock, but also to other varieties of environmental stress. Induction of HSPs depends on the *htpR* gene, because such induction does not take place in an *htpR*-deleted mutant. In concert with this finding, the level of HSP production correlates with the level of HtpR protein (Yamamori and Yura, 1982). Based on the sequence, the *htpR* gene product was identified as a minor sigma factor that was then renamed RpoH (Cowing et al., 1985; Landick et al., 1984). Among the six alternative factors, RpoH (renamed HtpR) was the first minor sigma factor that was discovered in *E. coli*. Transcription initiation of the HSP genes is regulated largely by RpoH (Grossman et al., 1984; Yura, 1993).

Regulatory role of RpoH sigma

RpoH is specifically required for expression of the genes encoding a set of HSPs as identified by proteome (Lemaux et al., 1978; Yamamori et al., 1978) and also by transcriptome analyses (Chuang and Blattner, 1993) (Fig. 3). Genome-wide transcription profiling of the regulatory targets of RpoH was identified under the moderate induction of a plasmid-borne *rpoH* gene under defined, steady-state growth conditions (Zhao et al., 2005). The set of HSPs include the GroEL (HSP60) and DnaK (HSP70) chaperones and the Lon and Clp proteases. A total of 126 genes were identified to be influenced in the absence, or in the over-expression, of RpoH (Guisbert et al., 2007), which are organized in 85 operons. Besides the expression of

HSPs, the RpoH regulon includes the genes for a response to other environmental insults, such as ethanol, alkaline pH, and hyperosmotic shock. The genes for proteolysis and cell division are also under the control of RpoH. It is, however, dispensable at low temperatures.

The set of RpoH-regulon genes thus identified *in vivo*, however, vary depending on the culture conditions, because transcription of the majority of RpoH-regulon genes is influenced by not only RpoH alone, but also a number of collaborating factors (Ishihama, 2010, 2012, 2016). Using the Genomic SELEX screening, we have identified a total of 72 constitutive promoters that are recognized *in vitro* by RpoH alone in the absence of other regulatory proteins (Table 1) (Shimada et al., 2017). Using these RpoH-regulon genes, the consensus sequence of TNtCNCcCTTGAA (-35) and CCCCATtTa (-10) was proposed for the RpoH promoter (Cowing et al., 1985).

Level control of RpoH sigma

The heat-shock induction of HSPs depends on the amount of RpoH (Yamamori and Yura, 1982). The level of RpoH sigma plays an active and dynamic role in regulating HSP gene expression under a variety of conditions. The intracellular level of RpoH is negligible under the steady-state of cell growth (Jishage et al., 1996; Maeda et al., 2000). Following a temperature shift from 30° to 42°C, the synthesis of RpoH increases markedly leading to its rapid accumulation (Fig. 3). The *rpoH* gene is associated with at least four promoters: three (P1, P4 and P5) are transcribed by RpoD RNAP (Fujita and Ishihama, 1987), whereas one (P3) is transcribed by RpoE (Erickson and Gross, 1989). The complex promoter organization allows fine tuning of the transcription level in response to environmental conditions. The promoter of the *rpoH* gene is also under the control of a group of transcription factors, such as CRP, CytR, DnaA, that collaborate with RNAP for controlling the expression of RpoH (Ishihama, 2016).

The increase in the RpoH level under heat-shock conditions is also attributable, at least in part, to enhanced translation and stabilization of both *rpoH* mRNA and RpoH protein (Grossman et al., 1984; Yura, 1993). Translational enhancement of RpoH synthesis is mediated by heat-shock induced partial melting of the mRNA secondary structure formed within the 5′-coding sequence of *rpoH* (Morita et al., 1999; Nagai et al., 1991). Under a steady-state of cell growth, RpoH is metabolically unstable (half-life of about 1 min) through proteolytic cleavage of chaperone-associated RpoH by membrane-bound ATP-dependent protease FtsH (Herman et al., 1995; Tomoyasu et al., 1995) and several cytosolic proteases, such as ClpAP, ClpQY, and Lon (Kanemori et al., 1997). The proteolysis of RpoH is prevented through sequestering of heat-shock induced chaperones such as DnaK-DnaJ-GrpE and GroEL-GroES from RpoH to other heat-shock induced abnormal proteins (Gamer et al., 1992; Tilly et al., 1983). Together, these observations indicate that the increased level of RpoH appears to be subject to a complex regulatory system, including the enhancement of both transcription and translation and the stabilization of both the *rpoH* mRNA and the RpoH protein.

Activity control of RpoH

The control of sigma activity by an anti-sigma factor was established, for the first time, for the major sigma RpoD by Rsd (Jishage and Ishihama, 1998, 1999). Using purified RpoH protein, a set of HSPs were found to associate with RpoH, including DnaK, DnaJ, and GrpE (Gamer et al., 1996). The activity of RpoH is directly controlled by the DnaK-DnaJ-GrpE chaperone system (Fig. 3). These HSPs work synergistically to interfere with the activity of RpoH. DnaK and DnaJ compete with RNAP core enzyme for stable binding to RpoH (Gamer et al., 1996; Liberek and Georgopoulos, 1993). Such an apparent sequestration of RpoH from core RNAP could presumably render RpoH a substrate for one or more proteases. The binding of chaperones leads to the degradation of unused RpoH by FtsH protease (Blaszczak et al., 1999). With respect to the transcriptional regulation, the role of HSPs is to prevent the function of RpoH as an anti-sigma factor by interfering with its association with RNAP and, moreover, by permitting the efficient degradation of RpoH by proteases. The set of HSPs inhibit the activity of RpoH by interfering with its association with RNAP core enzyme, forming feedback regulatory circuits. Since these HSPs with chaperone functions associate with a variety of proteins, it is, however, difficult, in practice, to discriminate between anti-sigma and chaperone functions. For instance, a considerable amount of GroE and DnaK is also associated with purified RNAP containing RpoD sigma (Ishihama, 2016; Ishihama et al., 1983).

Flagellar-chemotaxis Sigma RpoF

The bacterial flagellum is a complex organelle consisting of three distinctive structural parts: the basal body, the hook, and the filament (Macnab, 1992). The research group of Tetsuo Iino (The University of Tokyo) and Kazuhiko Kutsukake (Hiroshima University; Okayama University) devoted their efforts to identify the genetic control system of flagella formation using *Salmonella typhimurium* as a model bacterium. Consequently, it became clear that the synthesis and function of the flagellar and chemotaxis system required the expression of more than 50 genes, which are divided between at least 17 operons which constitute the large, coordinately regulated flagellar regulon (Kutsukake et al., 1988; Macnab, 1992). The transcription of the flagellar-regulon genes forms a highly organized cascade, together forming a coordinated hierarchy of the flagellar assembly (Kutsukake and Iino, 1994; Kutsukake et al., 1990; Macnab, 1992). Within the regulon, the operons are divided into three temporally regulated transcriptional classes: class-I (early), class-II (middle), and class-III (late) (Kutsukake et al., 1990). The class-1 (early) consists of a single operon including two genes, *flhD* and *flhC*, each encoding the transcription factors FlhD and FlhC, respectively, which together form a complex, $FlhD_2$-$FlhC_2$ or $FlhD_4$-$FlhC_2$, which activates the transcription of a set of class-2 (middle) genes, including both the *rpoF* sigma gene (renamed *fliA*) and the *flgM* gene encoding the anti-RpoF factor (Macnab, 1992).

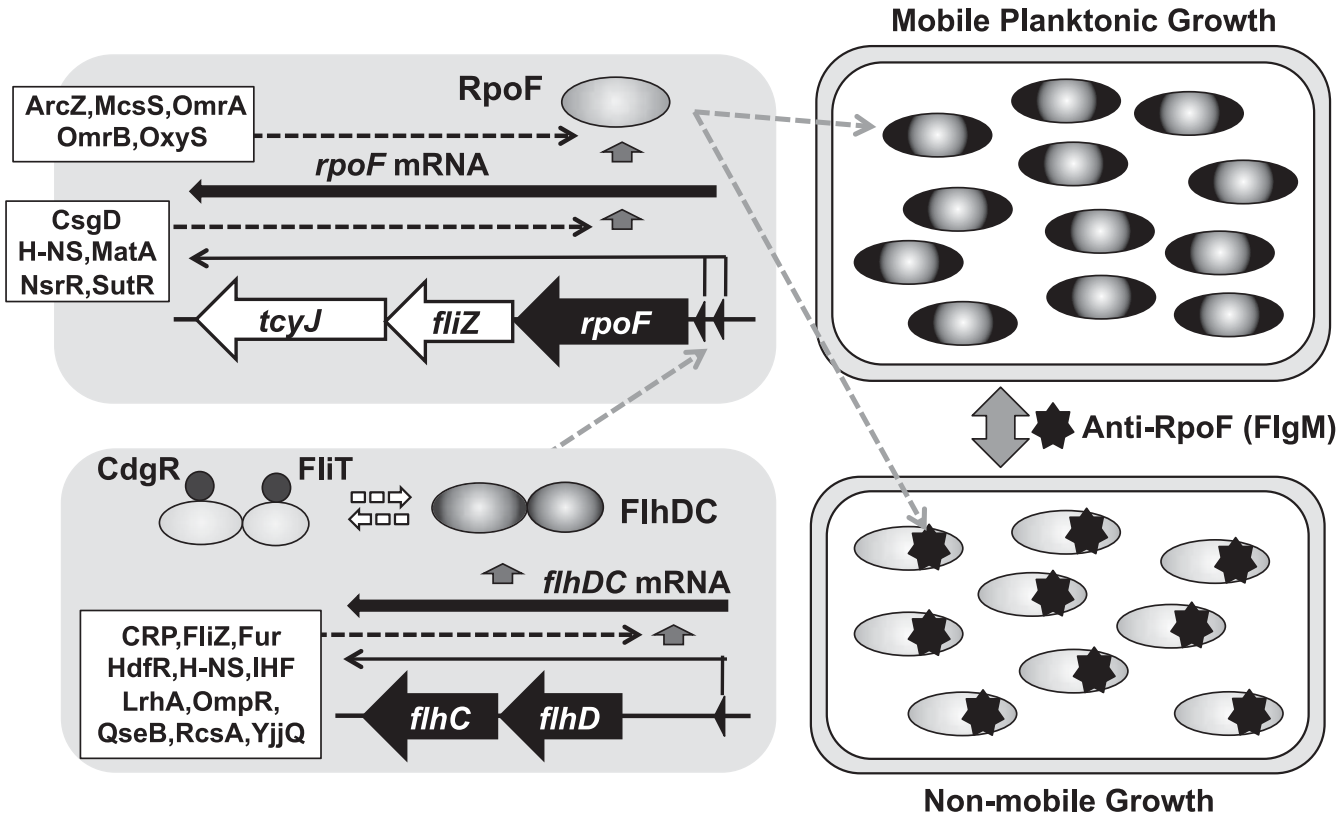

Fig. 4. Role of RpoF sigma in flagellar formation and chemotaxis.

RpoF sigma plays a major role in the transcription of a set of genes for flagellar formation of chemotaxis. The motility of *E. coli* is controlled by monitoring the environmental conditions. Accordingly, the level and activity of RpoF are controlled by various regulatory proteins at various stages including transcription, translation, and mRNA and protein stability. The master regulator FlhDC complex plays a major role in the transcription of the *rpoF* gene. The function of the FlhDC complex is controlled by two regulators, CdgR and FliT, each interacting with FlhD and FlhC, respectively (Wada et al., 2011a, b). Likewise, the function of RpoF sigma is controlled by anti-sigma FlgM (Kutsukake, 1994; Macnub, 1992).

FlhDC: The master regulator of flagellar formation

The *flhD* and *flhC* genes of the class-1 operon encode the master regulator of flagellar formation. The *flhDC* complex is required for the expression of all the remaining flagellar operons. The class-2 operons are transcribed by RpoD RNAP in the presence of the FlhDC complex. The promoter of the *flhDC* operon is under the control of RpoD, RpoF and RpoN (Dong et al., 2011) and a number of TFs, such as CRP and OmpR (Fig. 4) (Yanagihara et al., 1999). Using the PS (promoter-specific)-TF screening system, we have identified the participation of more than 10 TFs in the transcriptional regulation of the *flhDC* promoter (Ishihama, A. and Ogasawara, H., in preparation), including Crp, FliZ, Fur, IHF, LrhA, MatA, OmpR and RcsAB. The expression of FlhDC is also under several regulatory sRNAs, such as ArcZ, McaS, OmrAB and OxyS (Mika and Hengge, 2013).

In *Salmonella*, the function of the FlhDC complex is controlled by a negative regulator CdgR (renamed YdiV), an EAL domain protein, which is expressed under low-nutrient conditions and binds to the FlhD subunit, leading to interference with its binding to the class-2 promoters. Thus, CdgR is considered to be an anti-FlhDC factor that plays a role in the nutritional control of the flagella regulon (Fig. 4) (Wada et al., 2011a). Although *E. coli* and *Salmonella* have a similar flagellar regulatory system, the response of flagellar synthesis to the nutrient conditions is different in the two systems. Under low-nutrient conditions, the flagellar synthesis is inhibited in *Salmonella* but enhanced in *E. coli*. The *cdgR* gene is transcribed efficiently in *E. coli*, but the intracellular level of CdgR is low due to its inefficient translation (Wada et al., 2012). As well as CdgR, FliT acts as an anti-FlhDC factor which binds to the FlhDC complex through an interaction with the FlhC subunit, and inhibits its binding to the class-2 promoter (Fig. 4) (Yamamoto and Kutsukake, 2006). The nutritional control of flagellar formation in *Salmonella* also takes place through the regulation of *cgdR* transcription by a negative regulator FliZ, which binds to the promoter of the *cdgR* operon (Wada et al., 2011b).

Identification of flagella-chemotaxis sigma RpoF

The genes specifying chemotaxis, motility, and flagellar function in *E. coli* are coordinately regulated, and form a large and complex regulon. In the case of *B. subtilis*, a novel sigma factor of RNAP of an approximately 28 kDa molecular mass was identified, which provides the core enzyme with the recognition specificity of flagellar and chemotaxis genes in *Bacillus subtilis* (Gilman and Chamberlin, 1983). Likewise, a minor form of *E. coli* RNAP was identified, which specifically transcribes several *E. coli* chemotaxis and flagellar genes *in vitro* and *in vivo* (Arnosti and Chamberlin, 1989). The promoter selectivity of this RNAP was identified to be associated with a polypeptide of an approximately 28 kDa molecular mass, which restores the specific when added to the core RNAP,

leading to the proposal of a novel sigma factor, referred to as σ^{28} or RpoF. Hence, the *E. coli* RpoF holoenzyme appears to be analogous to the *B. subtilis* RpoF RNAP.

Through genetic studies, the Iino-Kutsukake group identified that the *S. typhimurium fliA* gene product of 239 amino acid residues in length includes the conserved sequence of a protein homologous to that of σ^{28}; namely, a flagellar specific sigma factor of *B. subtilis* (Ohnishi et al., 1990). The *fliA* gene product purified from an overproducing strain activated the *in vitro* synthesis of flagellin, the *fliC* gene product, indicating that the FliA protein functions as an alternative sigma factor specific for *S. typhimurium* flagellar operons, and FliA was then renamed to RpoF. Using the reconstituted *E. coli* RNAP holoenzyme from the purified RpoF and sigma-free core enzyme, we confirmed its sigma function in an *in vitro* transcription system (Kundu et al., 1997).

Regulatory roles of RpoF

The regulatory target of RpoF in *Salmonella* was identified to include a set of genes that were classified into the class-3 operons of the flagella regulon (Ide et al., 1999; Kutsukake et al., 1990; Ohnishi et al., 1990). More than 30 genes have been proposed to carry promoters that are under the control of RpoF sigma, including a set of the structural genes for flagella formation, and the chemotaxis genes encoding sensor of environmental signals affecting the motility control (Arnosti and Chamberlin, 1989; Ohnishi et al., 1990).

Using a combination of ChIP-chip, ChIP-seq and RNA-seq systems, a more comprehensive screening was recently performed for the identification of the regulatory targets of RpoF sigma in *E. coli* (Fitzgerald et al., 2014). A total of 52 RpoF-binding sites were identified *in vivo* on the genome of exponentially growing *E. coli* K-12 MG1655 cells in a rich LB medium, with a considerable level of overlapping with the hitherto identified target genes of the RpoF regulon. After Genomic SELEX screening of the binding sites *in vitro* of the RNAP RpoF holoenzyme alone, we also identified more than 100 binding sites *in vitro* in the absence of other supporting regulatory proteins (Table 1) (Shimada et al., 2017).

The binding sites of RNAP and TF identified *in vivo* using ChIP-chip, ChIP-seq and RNA-seq systems do not represent the whole set of their binding sites because: i) their binding to regulatory sites is often interfered with by other DNA-binding proteins; and ii) their binding to potential regulatory targets depend on the simultaneous presence of supporting factors.

Level control of RpoF

The total cellular levels of seven sigma factors exceed that of the RNAP core enzyme (Ishihama, 2000; Jishage et al., 1996), suggesting the competition between sigma factors for binding to the limiting amount of RNAP (Ishihama, 2000, 2010). Under steady-state growth of *E. coli* K-12 W3110, the level of RpoF is about half the level of major sigma RpoD, but decreases upon sudden exposure to heat shock (Jishage and Ishihama, 1995). Transcription of the *rpoF* operon is regulated by three sigma factors, RpoD, RpoS and RpoF itself. The positive control by RpoN is mediated through the flagella regulator FlhDC complex (Dong et al., 2011). Transcription of *rpoF* is under the control of multiple TFs, including CsgD (the master regulator of biofilm formation) and CpxR (an envelope stress response regulator) (Dudin et al., 2014). During the transition from exponential growth to the stationary phase, *E. coli* changes from a motile-planktonic to an adhesive-sedentary biofilm. CsgD activates a set of genes for an extracellular polysaccharide matrix, but represses both the *fliDC* and *rpoF* genes encoding the master regulators of flagellar formation and chemotaxis (Ishihama, 2010, 2012). On the other hand, the FlhDC complex represses transcription of the *csgD* gene, indicating the cross-regulation of two pathways of cell behavior, biofilm formation and planktonic growth, by the respective master regulators.

Activity control of RpoF: Anti-RpoF sigma factor FlgM

A negative regulatory gene, *flgM*, which is responsible for the coupling of the expression of class-3 operons to a flagellar assembly, encodes an anti-sigma factor that binds to RpoF and prevents its association with an RNAP core enzyme (Kutsukake et al., 1990; Ohnishi et al., 1992). The purified FIgM repressed transcription from the *fliC* promoter, one that is activated by the sigma factor RpoF. No DNA-binding activity was detected in FIgM. Chemical cross-linking experiments showed that the purified FIgM bound to RpoF and disturbed its ability to form a complex with the RNAP core enzyme. These results indicate that FIgM is a novel type of negative regulator that probably inactivates the flagellum-specific sigma factor through direct interaction, i.e., it is an anti-sigma factor (Fig. 4).

The sequential expression of flagellar operons is coupled to the assembly process of flagellar structures. This coupling is achieved by the fact that FlgM is exported out of the cell through the flagellar structures that are formed by the functions of the class 1 and 2 genes. FlgM is excreted from the cells into the culture medium through the hook-basal body of flagellar. FlgM is a sensor of the assembly state of a flagellar structure, leading to couple the flagellar gene expression to flagellar assembly (Kutsukake, 1994). FlgM has a dual function: it can bind to RpoF and is capable of being exported through a flagellar structure. FlgM is the anti-sigma factor for RpoF sigma; as in the case of Rsd, the anti-RpoD sigma for the major sigma RpoD (Jishage and Ishihama, 1998, 1999). The C-terminal portion of FlgM could inhibit the RpoF-dependent transcription of the class 3 genes while its N-terminal domain is necessary for its export through the flagella (Iyoda and Kutsukake, 1995). On the other hand, the C-terminal region of RpoF contains the FlgM-binding domain (Kutsukake et al., 1994).

Structure-function Relationship of RNAP

Because of its important role in the transcription of genetic information, a number of experts in protein structures, including those from Japan devoted much effort, albeit without success, to solve the structure of RNAP following its discovery. The structure was first determined by NMR for the C-terminal domain of *E. coli* RNAP (Jeon

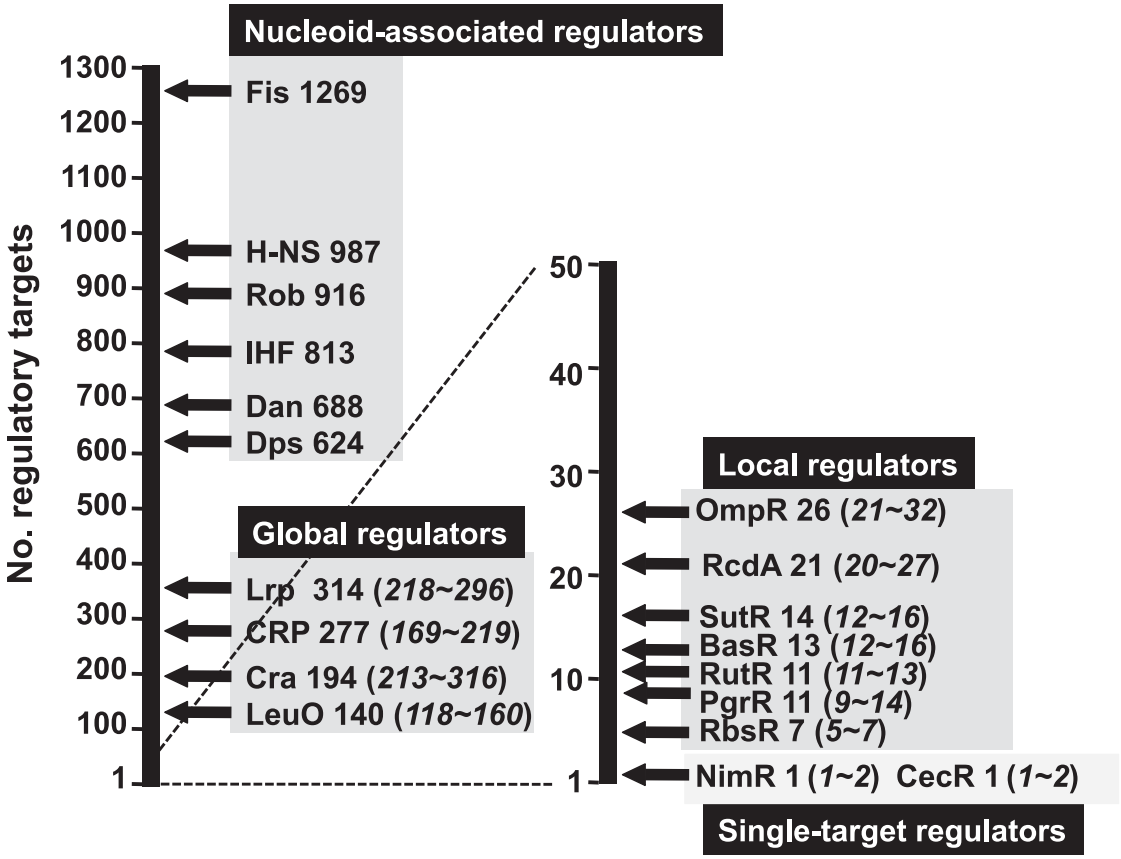

Fig. 5. Classification of the transcription factors based on the number of regulatory targets.

E. coli K-12 contains a total of approximately 300 transcription factors (TFs). Using the genomic SELEX screening *in vitro*, the binding sites on its genome were identified for more than 200 TFs (Ishihama et al., 2016). Based on the information of TF-binding sites (shown after each TF), the regulatory target promoters' genes and operons were identified or predicted for these TFs (shown in parentheses). Based on the number of regulatory targets, TFs are classified into nucleoid-associated regulators (targets, 600~1200), global regulators (targets, 100~300), local regulators (targets, 10~30), and single-target regulators (targets, 1~2). Some representative TFs from each group are shown. In the case of nucleoid-associated TFs, only the total number of binding sites on the entire genome is indicated.

et al., 1995), which plays a key role in the interaction with class-I TFs and promoter UP elements (Ishihama, 1992, 1993). The success of solving the entire structure of bacterial RNAP was achieved with the use of the core enzyme from the thermophilic bacterium *Thermus aquatics* (Zhang et al., 1999). The structure-function relationship of RNAP has long been analyzed using *E. coli* RNAP. In 2013, Murakami finally succeeded in solving the crystal structure of *E. coli* RNAP holoenzyme containing RpoD sigma (Murakami, 2013). [Note that the cover figure includes this crystal structure of *E. coli* RNAP.] This pioneering research made a breakthrough toward understanding the function and regulation of RNAP on a structural basis (for instance, see Murakami, 2015).

Transcription Factors Involved in the Second-Step Modulation of RNAP

The promoter selectivity of RNAP is further modulated in the second step after interaction with a total of approximately 300 TFs (Ishihama, 2010, 2012, 2016; Ishihama et al., 2016). Most of these TFs are DNA-binding proteins that bind to DNA and interact with DNA-bound RNAP for expression of their regulatory functions. Based on the binding subunits, we classified these TFs into four groups: α-contact class-I; σ-contact class-II; β-contact class-III; and β'-contact class-IV (Ishihama, 1992, 1993) (see Fig. 1). The identification of regulation targets for all these TFs is one of the important subjects for understanding the genome regulation within a single cell as a whole. Since the DNA-binding affinity of this group of regulators is low, but important for an effective interaction with RNAP and the quick replacement of RNAP-bound TFs. The regulation targets of *E. coli* TFs have been analyzed *in vivo* using the modern techniques of transcriptome and proteome, and the data are assembled in databases such as EcoCyc and RegulonDB, which contain varieties of data with different levels of accuracy, including a number of theoretical predictions based on the small number of experimentally identified results. More serious problems originating from the *in vivo* experiments are the competition or collaboration of test TFs with a total of 400~500 DNA-binding proteins in binding to the DNA targets. Using innovative computational systems and databases constructed from a number of publications describing gene expression and regulation in *E. coli*, the modeling research of networks and regulation is prevalent. A number of regulation models of genome transcription have been proposed using such mixed data collections. However, the basis of these models should be carefully examined, mainly because the data sets used include certain levels of erroneous predictions, which arise from the use of varieties of

E. coli strains with different genetic backgrounds, the use of varieties of experimental systems, and theoretical predictions without experimental confirmation.

Genomic SELEX search for the regulatory targets of 300 TFs

To overcome these problems, we have performed a systematic search for the binding sites of all 300 TFs on the *E. coli* K-12 genome by using the improved system of genomic SELEX (systematic evolution of ligands by exponential enrichment) system (Ishihama et al., 2016; Shimada et al., 2005). The regulatory targets for these TFs can be predicted once their binding sites are identified on the *E. coli* genome. To date, we have purified a total of about 270 TFs from a single, and the same, *E. coli* K-12 W3110 type-A strain containing the complete set of seven sigma factors, and subjected to SELEX screening for about 200 TFs. The comprehensive list of the regulation targets and regulatory functions for these TFs together provides a novel paradigm of the genome regulation. For instance, TFs can be classified into three groups based on a hierarchy of TF networks, from the single-target regulators, the local regulators, the global regulators, and the nucleoid-associated regulators (Fig. 5). Most of *E. coli* TFs belong to the local regulators, but the global regulators such as Cra, CRP, LeuO, and Lrp, regulate hundreds of the genes. Most of the nucleoid proteins are bifunctional proteins, playing both structural roles and regulatory roles (Ishihama, 1999, 2016; Ishihama et al., 2016). These nucleoid-associated TFs are involved in the regulation of about 1,000 genes where these proteins bind (Fig. 5). Details of the genomic SELEX screening of both sigma factors and TFs are summarized in the newly-constructed TEC database (www.shigen.nig.ac.jp/ecoli/tec/).

Prediction of the Genome Regulation

Once the regulatory target promoters, genes and operons are identified for all seven sigma subunits by using the genomic SELEX screening system, the level of transcription of their targets can be predicted if we know: 1) the intracellular concentrations of sigma factors; and 2) the binding affinity of each sigma factor to the RNAP core enzyme. So far, we have identified the intracellular concentrations of all seven sigma factors (Jishage and Ishihama, 1995; Jishage et al., 1996) and their binding affinity to the RNAP core enzyme (Maeda et al., 2000). Likewise, the pattern of genome transcription could be predicted once the list of regulatory targets for all TFs are established by using both genomic SELEX screening and PS-TF screening. For this ultimate purpose, we have already determined the intracellular concentration of more than 200 TFs in growing *E. coli* K-12 W3110 cells by a quantitative immunoblot analysis (Ishihama et al., 2014). The intracellular levels of nucleoid-associated proteins have also been determined for *E. coli* cells at various growth phases and growth conditions (Ishihama, 2009; Talukder et al., 1999), and the recognition specificity and affinity of DNA-binding have also been determined for all these proteins (Ishihama, 2009; Talukder and Ishihama, 1999).

Resources for Research of the Genome Regulation

The complete genome sequence of *E. coli* K-12 W3110 has been determined as a result of collaborative research involving more than 10 laboratories in Japan (Hayashi et al., 2006). Comprehensive experimental resources, such as the complete ORF clone library (ASKA library) (Kitagawa et al., 2005) and the complete collection of deletion mutants (Keio collection) (Baba et al., 2006), are fundamental tools for the elucidation of genome regulation. Both ASKA and Keio resources were constructed by Hirotada Mori (Nara Institute of Science and Technology) and his colleagues, and their quality control is constantly maintained. Details are described in GenoBase (http://ecoli.naist.jp/GB/). Both ASKA and the Keio collection are deposited into the *E. coli* strain stock center, which is organized and maintained by Hironori Niki (National Institute of Genetics, Mishima) under the support of the NBRC (National BioResource Project) (Yamazaki et al., 2009). The collection of wild-type and mutant *E. coli* strains is listed in the PEC database (https://shigen.nig.ac.jp/ecoli/strain) which is maintained, together with the TEC database, by Yukiko Yamazaki and Shoko Kuwamoto (National Institute of Genetics, Mishima).

To perform the SELEX screening of regulatory targets of all sigma factors and TFs, we have constructed the collection of expression plasmids for all these regulatory proteins (Ishihama et al., 2016). Using the sigma and TF proteins purified for SELEX, we have also constructed a collection of antibodies, which were used for the quantitative immunoblot analysis of the intracellular concentrations of all the regulatory proteins (Ishihama et al., 2014; Jishage and Ishihama, 1995; Jishage et al., 1996). For the quantitative measurement of promoter activity in *E. coli* K-12, we have constructed the two-fluorescent reporter vectors for more than 1,000 promoters from *E. coli* K-12, and measured the promoter activity recognized by RpoS RNAP at various growth phases from exponential growth to the stationary phase (Shimada et al., 2004). A systematic screening of promoters, genes and operons recognized *in vitro* by each sigma factor and each TF from *E. coli* K-12 is being carried out using the improved genomic SELEX system (Shimada et al., 2005) and PS-TF screening system (Shimada et al., 2014). The first-group results are published in Ishihama et al. (2016) and deposited into the TEC (Transcription Profile of *Escherichia coli*) database (www.shigen.nig.ac.jp/ecoli/tec/). For a complete understanding of the genome regulation of a single model organism *E. coli* K-12, these experimental resources of considerable value have been used extensively in a wide range of research, including that described in this report.

Concluding Remarks

Japan has been one of the leading countries in the field of the molecular genetics of bacteria, and, in particular, in *E. coli* research. As described in this review, scientists in Japan have made substantial contributions to constructing a complete view of genome regulation within a single, and the same, bacterial strain *E. coli* K-12. This genome-wide research depends on the development of a va-

riety of resources, such as the Keio collection of the single-gene knock-out *E. coli* mutants and the ASKA library of single-gene expression vectors, as well as the innovation of experimental systems such as the genomic SELEX, and the PS-TF, screening systems. The success achieved has resulted from the fact that scientists in Japan have been able to concentrate on a single research subject, and to make this their life work. The long-term continuation of the same line of research has been generally supported by accumulated research activities. In addition, the unique funding system for group research in Japan has provided scientists with the opportunity to organize genome-wide research. At present, however, the government in this country forces scientists to delve into the fascinating world of cutting-edge applied science for the development of new biotechnology. Nevertheless, we should continue our goal of achieving further advances in the building of a complete view of genome regulation in bacteria as a research model in modern fundamental bioscience.

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