

Evolutionary rewiring and reprogramming of bacterial transcription regulation

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Abstract

Rewiring and reprogramming of transcriptional regulation took place during bacterial speciation. The mechanistic alterations among transcription factors, *cis*-regulatory elements and target genes confer bacteria novel ability to adapt to stochastic environmental changes. This process is critical to their survival, especially for bacterial pathogens subjected to accelerated evolution. In the past two decades, the investigators not only completed the sequences of numerous bacterial genomes, but also made great progress in understanding the molecular basis of evolution. Here we briefly reviewed the current knowledge on the mechanistic changes among orthologous, paralogous and xenogenic regulatory circuits, which were caused by genetic recombinations such as gene duplication, horizontal gene transfer, transposable elements and different genetic contexts. We also discussed the potential impact of this area on theoretical and applied studies of microbes.

Keywords: Evo-Devo; Transcription regulation; Rewiring; Reprogramming; Molecular evolution

1. Introduction

In his immortal work “On the Origin of Species, by Means of Natural Selection, or the Preservation of Favored Races in the Struggle for Life”, Chapter V, Charles Darwin summarized that though variation is essential in generation of the morphological innovations either in domestic or in wild creatures, “our ignorance of the laws of variation is profound” (Darwin, 1859). After 150 years, this situation was undoubtedly improved. The development of the modern genetics and genomics has demonstrated that evolution is a two-step process: initially mutations and recombinations in nucleic acid sequences occurred and led to phenotypic changes, and then if these genetic alterations have selective superiority or are subject to genetic drift, they would be eventually fixed in

populations and contributed to the origin of species (Peter and Davidson, 2011). Needless to say, understanding “the law of (genetic) variation” governing phenotypic adaptation of organisms has been and will be one of the central topics in evolutionary biology as well as in genetics.

During the past three decades, the study on molecular basis of evolution has proposed that it is the regulatory genes, rather than the structural genes, that play more important roles during the origin and adaptation of species (Carroll, 2008). Albeit remaining in debate (Hoekstra and Coyne, 2007), the interdisciplinary field of evolution-development (so-called “Evo-Devo”) was initiated under this “regulatory thinking” (Carroll, 2005), aiming at a new synthesis of biology to answer the fundamental question on how adaptive variation is determined by genetic processes (Dean and Thornton, 2007). In this area, the evolution of transcription regulation received more attention because the RNA polymerase (RNAP) directed RNA biosynthesis is essential to all kinds of organisms. Studies have revealed that evolution in transcription regulation can give rise to new phenotypes. Taking advantage from the recent development of high-throughput methodologies, such as ChIP-seq,

Abbreviations: HGT, horizontal gene transfer; RNAP, RNA polymerase; TCSTS, two-component signal transduction system; TE, transposable element; TF, transcription factor; TG, target gene.

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ChIP-chip, massively parallel sequencing and two-dimension gel electrophoresis, a global view of the evolution of transcription regulation has been established, especially in animal and yeast, as being elegantly reviewed elsewhere (Wilson and Odom, 2009; Dowell, 2010; Lind et al., 2010).

Evo-Devo study has received substantial attention in the areas of fungal, animal and plant evolutionary biology, but it was not quite so prosperous in bacteria (Perez and Groisman, 2009b), partially due to that most bacteria do not have the traditional sense of development. However, just as in every breakthrough period of molecular genetics, the study on bacteria also should provide fundamental contribution to the theoretical framework of Evo-Devo. This is due to the simplicity of bacteria as ideal models. First, after 15 years of effort, there are nearly two thousand complete bacterial genomes deposited in the public databases and this number will be dramatically increased in the future by the application of new-generation of sequencing technology. Remarkably, a lot of strains or close-relatives of the same bacterial species were sequenced, providing us an unique opportunity to analyze functional divergence among orthologous and paralogous regulatory cascades. Second, bacterial cells do not have complicated process of development, which simplifies the molecular analysis when using them as model organisms. Third, evolutionary prediction based on the sequence comparison is limited and sometimes misleading, especially for regulatory sequences lacking genetic codes as protein-coding sequences. Because of this, empirical investigation should bring tremendous spirit to the research of Evo-Devo. As for bacteria, genetic manipulation is generally easier than that in plant, animal and fungi, this advantage is meaningful because it can be quickly applied to challenge the hypotheses generated by comparative and large-scale “omic” studies. Finally, the generation times of bacteria are relatively short so that it is feasible to conduct mechanistic study under the strictly-controlled circumstance, which minimizes the possibility drawing to the incomplete conclusion inevitably caused by traditional inductive or comparative methods in evolutionary studies.

This review covered the recent empirical studies about the rewiring and reprogramming of bacterial regulatory circuits, with special reference to molecular mechanisms. Occasionally we compared the research progress to the conclusions generated from animal and fungal studies. We will discuss the current status and the future prospective of this exciting field.

2. Bacterial transcription regulatory network is highly flexible during evolution

Prokaryotic transcription was roughly divided into several stages: preinitiation (also known as template recognition), initiation, promoter clearance, elongation and termination (van Hijum et al., 2009). Preinitiation takes place when a σ subunit associates with RNAP core enzyme (contains $\alpha_2\beta\beta'\omega$ subunits) to form holoenzyme. In general, the σ subunit enables RNAP to bind to the core element of a promoter (–10 and –35 hexamer regions) upstream of a transcription initiation site. However, in certain promoters, an extended ~20 bp

UP element (a component of bacterial promoters) can also be recognized by the C-terminal domain of RNAP α subunit (α -CTD domain). After the initial binding of RNAP, a transcription “bubble” will be created from the approximate –10 to +2 position of transcription initiation site by isomerization, which helps to form the initial open complex. When the first DNA base is transcribed into RNA, the promoter clearance and escape occur. The slip of RNAP from DNA template generates a set of abortive transcripts, until approximate 23-nt transcripts are formed. Afterward, the RNA transcripts were elongated until transcription termination occurs, either by hairpin structures in DNA (Rho independent termination) or by binding of the Rho-cofactor that dissociates the RNAP from DNA (Rho-dependent termination). During this process, transcription factors (TFs) can activate or repress the synthesis of RNA by binding into different promoter motifs. In addition, nucleoid proteins (such as H-NS, Fis and HU), small ligands, second cellular messengers (such as cAMP, c-di-GMP, cGMP and ppGpp) and small regulatory RNAs, modulate transcription by diverse mechanisms. Detailed description on these topics can be found in other dedicated reviews (Browning and Busby, 2004; van Hijum et al., 2009).

The basic unit of bacterial transcription regulation consists of three components: a TF, *cis*-regulatory elements and the target genes (TGs). These components are extremely flexible in evolution. Even in the close-relatives of Proteobacteria, only 30% of TFs and TGs are conserved. Among them, the global regulators (such as Crp, Lrp, Fnr and PhoP) that used to be regarded as “conserved”, evolved very quickly (Lozada-Chavez et al., 2006). The quick evolutionary properties of bacterial transcription regulation can attribute to the following reasons. First, the *cis*-regulatory sequences are degenerate and short (approximately 5–10 nt). Mutations in the so-called “consensus” regulatory sequences will not only cause quantitative change on the binding affinity, but also lead to complete dissociation or even substitution to rewire novel TFs (Gelfand, 2006). Second, prokaryotic genes are often controlled by a number of *cis*-regulatory sequences, which results in different spatiotemporal expression patterns (van Hijum et al., 2009). Therefore, mutations in *cis*-regulatory sequences usually affect the specific expression pattern, rather than causing lethiferous effect as that in protein-coding regions. Third, bacterial genes are regulated by multiple TFs working cooperatively or competitively. Since different combination of TFs determines the output of gene transcription when bacteria live under diverse ecological niches, TF rewiring caused by mutations may result in establishment of novel combinational regulation. In addition, since transcription is an extraordinary complicated biochemical cascade involving hundreds of biomolecules, any heritable change in these processes promotes its evolutionary flexibility to bacterial adaptation to environments (Alon, 2007; Dowell, 2010).

Significant adaptive changes caused by rewiring and reprogramming of transcriptional regulation have been observed by numerous studies. For example, in the gram-negative bacterium *Vibrio fischeri*, a σ^{54} -dependent response regulator SypG acts as a TF to activate the expression of 18

genes responsible for biosynthesis of exopolysaccharides (Yip et al., 2005). It has been found that its cognate histidine kinase RscS, which can phosphorylate the SypG, is sufficient to alter the host range of *V. fischeri* to colonize with host squid *Euprymna scolopes*. Heterologous expression of RscS fully restored the ability of *V. fischeri* strains which doesn't encode the active *rscS* gene (Mandel et al., 2009), implying that post-translational modification of a TF protein is critical in determining the animal–bacteria mutualism. More familiar examples can be seen in accelerated evolution of pathogenic bacteria which acquire novel virulence-associated genes through horizontal gene transfer (HGT) (Juhás et al., 2009; Boto, 2010; Jackson et al., 2010). As indicated in Enterobacteria, TFs tend to co-transferred with their TGs, which substantially facilitate the signal integration of xenogenic genes into recipient bacterial cells as soon as possible (Price et al., 2008).

3. Genetic recombinations contribute to evolution of transcription circuits

3.1. Duplications create relaxed opportunity for novel functional divergence

Just as Ohno's conceptual book (Ohno, 1970) and numerous subsequent studies have revealed, gene duplication is an abundant resource to create new genes. Since additional copies of a gene are present in the genome, functional constraint and natural selection are relaxed and the duplicated genes obtain opportunities to evolve. Bacteria are haploids so that genome-scale duplications are not as familiar as that in the genomes of diploid, especially plant (mainly by polyploid). However, paralogous genes generated by small-scale duplication are generally identified in bacterial genomes

(Craven and Neidle, 2007; Sandegren and Andersson, 2009). It was estimated that the frequency of gene duplication ranges from 10^{-2} to 10^{-4} in a bacterial population (Andersson and Hughes, 2009). If the natural selection is absent, duplications are unstable and typically disappear after a few generations of growth. But selection can fix duplications with adaptive advantage and then generate substantial genomic variations. For example, Martinez-Nunez et al. (2010) estimated that there are 477 and 483 groups of paralogous genes in the genomes of *Escherichia coli* and *Bacillus subtilis*, respectively. Gevers et al. (2004) analyzed the pan-genomes of 106 bacteria and summarized that the proportion of paralogs in these genomes ranged from 7% (*Rickettsia conorii*) to 41% (*Streptomyces coelicolor*). Among them, duplication of TFs is familiar in different gene categories. In the genome of phytopathogenic bacterium *Xanthomonas campestris* pv. *campestris*, which encodes 54 response regulators of two-component signal transduction systems (TCSTSs), 13 paralogous groups are identified among the total of 28 response regulators with putative TF activity (Qian et al., 2008). Very recently, Soo et al. (2011) artificially duplicated and overexpressed each gene of *E. coli* and then challenged the recombinants by exposing them to toxins and antibiotics. Promotion in bacterial growth was identified and duplications of a group of TFs, such as MarA, YcgZ and CpdA, were found to confer toxin resistance. They estimated that duplication and overexpression of a randomly chosen *E. coli* protein promoted bacterial resistance with a probability of 0.4% (Soo et al., 2011). It is obvious that TF duplication may add adaptive potential of bacteria, and investigation of the functional divergence among these regulatory paralogs will be one of the interesting topics in the post-genomic era.

As shown in Fig. 1, although there is a remarkable tendency that the duplicated TF gene accumulates deleterious mutations

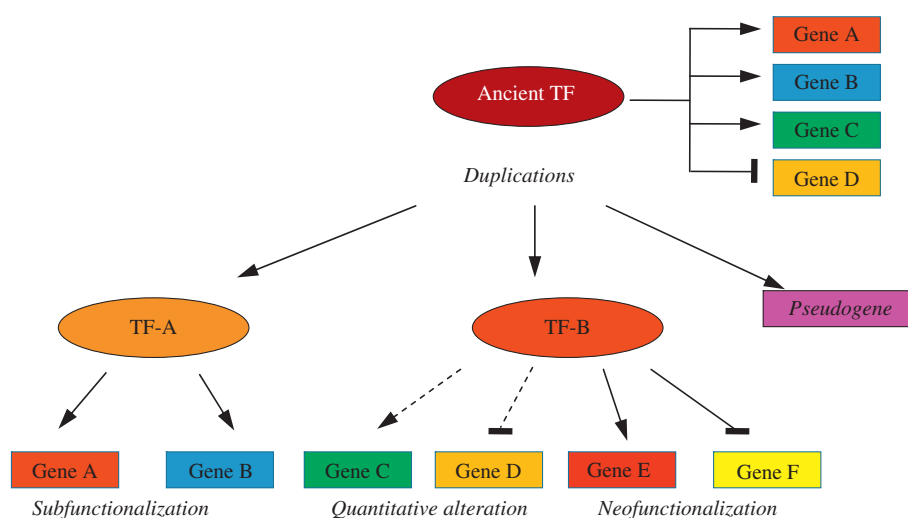


Fig. 1. Influence of gene duplication on the transcriptional circuits. The ancient transcription factor (TF) initially regulates the expressions of gene A, B, C and D. After duplication, the progeny paralog TF-A reserves partially regulatory function to control the transcriptions of gene A and B (subfunctionalization). The paralog TF-B not only modulates the expression of gene C and D, but also evolves novel ability to regulate the expression of gene E and F (neofunctionalization). In addition, duplication also creates a paralogous gene that accumulates point mutations and turns to be a pseudogene. TFs are marked as ellipses with different colors, and genes are indicated by rectangles. The arrowheads indicate the activation of gene expression, and T-shaped lines represent the repression of gene expression. The dotted lines indicate quantitative change in TF binding affinity. The solid lines indicate evolutionary or regulatory processes.

and turns to be a pseudogene, it may adopt partial functions from the ancestral gene (subfunctionalization) or even gain novel functions (neofunctionalization). Initially, the paralogous TF recognizes the same DNA motif as the parental regulator, but it can be differentiated soon to interact with new DNA-binding sites of the other genes, and thus become a novel regulator responding to alternative environment (Zhang, 2003). In *Salmonella enterica* serovar Typhimurium, two MerR-family TFs, CueR and GolS, are unambiguous paralogs that origin from gene duplication of a common ancestor. Both proteins contain N-terminal DNA-binding helix-turn-helix domains and highly diverse C-terminal regions, and share a similarity of 42% in amino acid composition (Checa et al., 2007). GolS responds to golden salt stress (Au) whereas CueR prefers to Cu, Ag and Au induction. Although highly specific regulation was observed between GolS and its target gene *gesABC* (Pontel et al., 2007), the binding sites of most of their target genes (such as *golB*, *copA*, *golTS* and *cuiD*) are remarkably similar so that GolS and CueR can cross recognize and activate the transcription of these genes (Perez Audero et al., 2010). However, each TF showed higher affinity for its own operators than for that of the other one. For example, by using resonant mirror biosensor technique, Perez Audero et al. (2010) estimated that the dissociation equilibrium constant (K_D) between GolS and its own operator *P_{golB}* is 8.7×10^{-9} mol/L, nearly 150 times higher than 5.7×10^{-7} mol/L between GolS and *P_{copA}* which is the innate operator of CueR. Therefore, GolS and CueR represent an intermediate stage that duplicated TFs evolve to have more specific regulatory function by rewiring novel *cis*-regulatory sequences. In addition, it strongly indicated that quantitative change in TF-DNA binding affinity also has adaptive potential during the duplication of TFs.

Since duplicated TFs developed novel recognition abilities and maintained substantial similarity in their structures, they may rewire complicated functional interactions during controlling transcription. In *E. coli*, the TF NusG and its paralog RfaH regulate the transcriptional pausing and termination, and constitute an intriguing model in studying the functional divergence of duplicated genes (Bailey et al., 1997). NusG is an essential protein and a global regulator that is involved in transcription of nearly all genes of *E. coli*. However, its paralog, RfaH, is a non-essential and a highly specialized regulator that participates in the expression of a limited number of HGT-acquired genes containing *ops* DNA motif (Artsimovitch and Landick, 2000). Since RfaH homolog is absent in archae, it is evident that NusG and RfaH duplication might occur after eubacteria and archae differentiation (Belogurov et al., 2009). Interestingly, functional roles of NusG and RfaH are opposite. Although both NusG and RfaH bind to RNAP, NusG has additional ability to bind to Rho factor and facilitates its recruitment to the termination complex. As a result, NusG increases Rho-dependent termination. In contrast, RfaH doesn't have Rho-binding affinity and the presence of RfaH even modestly reduces Rho-dependent termination *in vivo* and *in vitro* (Cardinale et al., 2008). Protein structures of NusG and RfaH have been

dissected. They are similar in their N-terminal domains (NTD) which bind to RNAP but quite different in their C-terminal domains (CTD): NusG contains a β -barrel CTD that binds to Rho factor, whereas RfaH has an α -helical hairpin and loses Rho-binding activity (Belogurov et al., 2007). Interestingly, Belogurov et al. (2009) revealed that RfaH can compete with NusG for effects on Rho-dependent termination by stably binding to RNAP. Because Rho-dependent termination assistant by NusG is a major mechanism to silence foreign DNA obtained through phage or horizontal gene transfer (Cardinale et al., 2008), the role of RfaH in cells is supposed to exclude NusG from the transcription elongation complex, thereby blocking NusG-assistant Rho-dependent RNA release (Belogurov et al., 2009) and promote the transcription of HGT-acquired virulence factors. Therefore, duplication and subsequent divergence of NusG and RfaH not only changed a global regulator into a specific regulator, but also created a functionally-opposite TF that modulates transcription termination competitively.

3.2. Horizontal gene transfer and the fate of acquired regulatory circuit

As mentioned above, HGT (also known as lateral gene transfer) is one of the most important genetic processes that determine the dynamics of bacterial genomes. By obtaining foreign DNA *via* transformation, conjugation and transduction, bacteria evolve very quickly to adapt to imaginable ecological niches (Ochman et al., 2000). Although estimation of how many genes were obtained from HGT in bacterial genomes depended on the various methods to discriminate acquired genes, the percentages are impressive: for example, Koonin et al. (2001) calculated that 1.6%–32.6% of bacterial genes may be obtained by HGT. Consequently, investigation on the function of HGT-acquired genes is a central topic in microbiology, especially considering that HGT contributes to the emergence of novel pathogenic bacteria of human beings.

However, the integration of HGT genes into pre-existing signal networks is not a royal road. This evolutionary process consists of at least three episodes: i) destroying or silencing of HGT genes by recipient bacterial cells; ii) counteracting the silencing effect and iii) rewiring and reprogramming of the regulatory cascade to accommodate these foreign genes (Fig. 2). At the first stage, the majority of the foreign DNA will be destroyed by restriction-modification systems or silenced by bacterial “innate immunity systems”, such as H-NS or CRISPR-mediated gene silencing (Lucchini et al., 2006; Hale et al., 2009). With the helper proteins such as Hha and YdgT, the histone-like nucleoid-structuring protein H-NS prefers to bind AT-rich DNAs that usually origins from HGT and silences gene expression by blocking the recruitment of RNAP in transcription (Paytubi et al., 2004). To counteract negative effect from gene silencing, bacteriophages, pathogenicity islands and transposable element encode H-NS antagonists. In *S. enterica*, it was found that when the functional H-NS is present, a TF SlyA could simultaneously occupy the promoter region of the HGT-acquired gene *pagC* together with

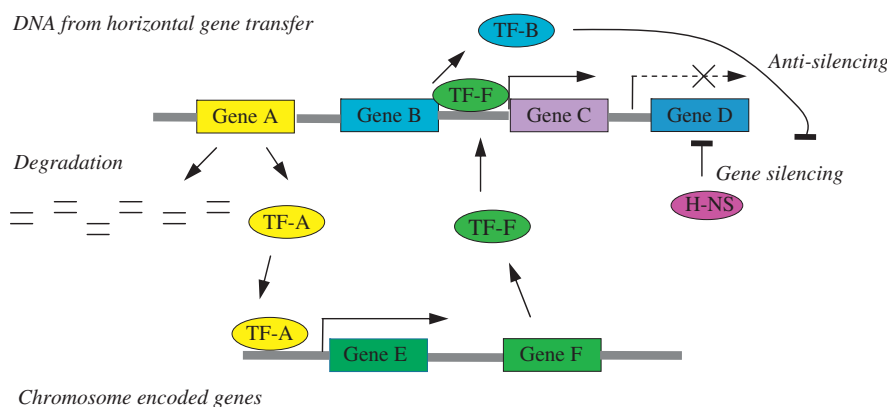


Fig. 2. Rewiring and reprogramming of transcription regulations during horizontal gene transfer. Once a DNA fragment is transferred to a recipient bacterial cell, the fragment is either degraded by endogenous restriction system in bacterium or silenced by H-NS-like protein-mediated repression (e.g., gene D). However, TFs transcribed from the xenogenic fragment (e.g., TF-B) may conquer the gene silencing process by targeting silencing complex. Afterward, xenogenic TFs can regulate the expression of their own target gene in the DNA fragment. In addition, novel regulatory circuits may be established during evolution between xenogenic TFs and chromosome encoded genes (e.g., TF-A and gene E), or between chromosome encoded TFs and xenogenic genes (e.g., TF-F and gene F). The denotation is the same as those in Fig. 1.

H-NS and promote *pagC* transcription. However, in the *hns* mutant, SlyA was dispensable to *pagC* transcription. In addition, SlyA was unable to increase *pagC* expression *in vitro* by itself. These results suggested that the major function of SlyA is to overcome the negative effect of H-NS, possibly by changing the DNA-H-NS complex to recruit other TFs, rather than by a competitive substitution mode (Perez et al., 2008). Besides counteracting with the gene silencing system by regulating its activity, Lind et al. (2010) recently revealed that duplication of acquired genes to amplify their expression level is also a beneficent way to overcome sub-optimal expression level of HGT genes.

After the successful escape from degradation and gene silencing, the last stage of signal integration of HGT genes is rewiring or reprogramming of the pre-existing signal networks (Fig. 2). The factors which determine the rewiring events are critical in this stage, but the answer may be quite simple: in an animal Evo-Devo study that artificially transferred the human 21 chromosome into a mouse Down syndrome model (lacking murine 21 chromosome), the authors concluded that it is the regulatory DNA sequence, rather than any other species-specific factor, is the single most important determinant to rewire gene transcription (Wilson et al., 2008). This is also true in prokaryotes. For example, In the *S. enterica*, the function of HGT-acquired pathogenicity islands-1 and -2 (SPI-1 and SPI-2) was well documented (Fass and Groisman, 2009). Among them, SPI-2 contains a two-component signal transduction system SsrA-SsrB that not only regulates all SPI-2 functional gene clusters, but also modulates genes encoded by other genomic regions, most of the latter are also potential HGT-acquired genes (such as *srfH*, *J*, *K*, *G*). Genome-wide ChIP-chip analysis showed that a degenerate, 18 bp palindrome with a conserved 7-4-7 DNA motif, is the minimal *cis*-regulatory sequence to bind with SsrB (Tomljenovic-Berube et al., 2010). This flexible sequence determines the SsrB-mediated regulation during *Salmonella*–animal interaction. However, additional regulatory sequence was evolved during host-pathogen co-evolution. Because

another bacterial species, *S. bongori*, doesn't have SPI-2, comparison between these two species provides informative clues on signal integration of HGT genes. Osborne et al. (2009) identified a virulence-associate gene *srfN* that is regulated by SsrA-SsrB in *S. enterica*. The TF SsrB directly controls the transcription of *srfN* by binding into a specific DNA region 654 bp upstream of the transcription initiation site. In *S. bongori*, which lacks SsrA-SsrB, this DNA-binding site is absent, albeit it contains an *srfN* ortholog. To establish the regulatory relationship between SsrA-SsrB and *srfN*, co-transferring the SsrA-SsrB and this specific DNA-binding site into *S. bongori* is indispensable. Phylogenetic analysis revealed that this specific regulatory sequence exists only in *Salmonella* subspecies infecting warm-blooded animals, suggesting that rewiring of *cis*-regulatory DNA sequence and acquired TFs is an essential mechanism of adaptive evolution of bacteria (Osborne et al., 2009). Moreover, it seems that SsrB-SsrA has been well integrated into signal network of *Salmonella* since its expression is under the control of ancestral two-component signal transduction systems, including EnvZ-OmpR and PhoQ-PhoP. Among them, the phosphorylated OmpR binds to the promoter region of *ssrA* and *ssrB* (Feng et al., 2003). PhoP can bind to the promoter region of *ssrB* to modulate its transcription when *Salmonella* lived in macrophages. However, it is noticeable that PhoP doesn't interact with the promoter of *ssrA*, but controls its protein level by a post-transcriptional manner that is associated with its 5'-UTR sequence (Bijlsma and Groisman, 2005).

3.3. Transposable elements represent an evolutionary power to regulatory innovation

In bacteria, transposable elements (TEs) include insertion (IS) elements (usually 0.7–2 kb), transposons (>2 kb), retrons (transposition *via* an RNA intermediate), mobile introns and small repetitive sequences that like the eukaryotic MITEs (Miniature Inverted Repeat Transposable Elements). In prokaryotes or in eukaryotes, genomic analyses indicated that

these mobile elements play a creative role in rewiring of gene expression regulation, rather than acting as a destructive factor. For example, Jordan et al. (2003) estimated that nearly 25% of experimentally studied human promoters have transposable element-derived sequences. Feschotte (2008) proposed a model that TE insertion can affect gene transcription and translation by diverse mechanisms, including i) disruption of a promoter; ii) addition of new *cis*-elements; iii) generation of antisense RNA; iv) formation of heterochromatin region; v) affect 3'-UTR and post-transcription regulation; vi) formation of a binding site for small RNAs; vii) interference with RNA splicing (Fig. 3). Not all of these models are experimentally confirmed in prokaryotes, therefore, future experimental investigation shall confirm and add more mechanisms to the impact of TEs on innovation of regulatory networks.

The adaptive and functional significance of transposable elements inserted into *cis*-regulatory regions is that they may bring novel control inputs to an existing cascade. For example, Luque et al. (2006) identified an IS element *ISTosp1* that inserted into the glutamyl-tRNA synthetase gene *gltX* of a cyanobacterium *Tolypothrix* sp. PCC 7601. This insertion resulted in co-transcription of *ISTosp1* and *gltX* and created a NtcA TF binding site. Although there lacks furthermore genetic evidence, compared with the reference species *Nostoc* sp. PCC 7120 which doesn't contain the insertion, the transcription of *gltX* of *Tolypothrix* sp. PCC 7601 was up-regulated when the bacterium was subjected to different nitrogen sources, indicating that *ISTosp1* insertion may direct nitrogen-regulated transcription to *gltX*. Similar studies, of which gene transcription is activated by TE insertion, were observed in other genes or operons (Petersen et al., 2002; Sun and Dennis, 2009). Different from these studies, Zhang and

Saier (2009a) found that in *E. coli* the activation of *glpFK* operon (contains a glycerol facilitator gene and a glycerol kinase gene) by an IS5 insertion is intriguing. IS5 insertion destroyed the pre-existing repression derived from negative, CRP-cAMP dependent TF GlpR. Meanwhile, it substantially increased the transcription level of *glpFK*, even in the absence of the induction factor, glycerol. The 117-bp of the IS5 3' end is enough to increase the *glpFK* transcription. Further analysis revealed that this region contains an integration host factor (IHF, bacterial proteins that bind to specific DNAs to introduce DNAs sharp bend) binding site and A-tracts. Both the IHF protein and native promoter of *glpFK* are indispensable to the activation, and genetic analyses indicated that the IHF binding resulted in the permanent DNA bend and an additive effect on *glpFK* transcription (Zhang and Saier, 2009a). Meanwhile, they also found that IS5 insertion caused the directed mutation by generating the elevated mutation rate of glycerol utilization mutants (Zhang and Saier, 2009b). Consequently, these studies indicated that there are many mechanisms of adaptive gene activation by TE insertion, which need to be investigated.

In recent years, it has been recognized that TE insertion can cause even more complicated results in the rewiring and reprogramming of transcription in animal genomes (Fig. 3). But evidences from bacterial studies are lacking. For example, homologous TE inserted into *cis*-regulatory region of different genes may connect unrelated genes into a signal network, if TE contains regulatory motifs that bind to the same TF. This rewiring model, initially proposed by Britten and Davidson (1969), has been experimentally confirmed in determining sex determination of medaka fish (Herpin et al., 2010) and transcription circuit of human embryonic stem cells (Kunarso et al., 2010). Another untouched but interesting area is that TE

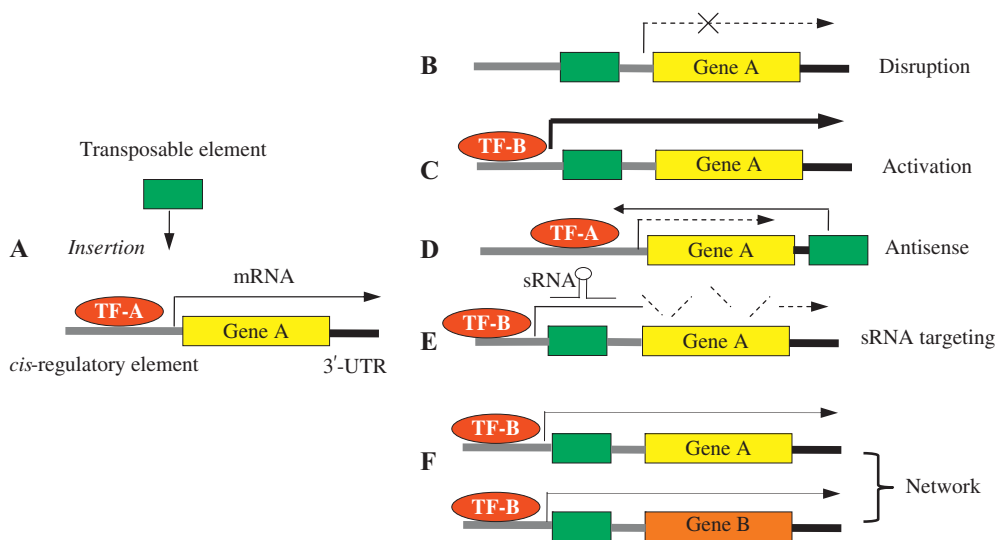


Fig. 3. Rewiring and reprogramming of transcription regulations during transposable element (TE) insertion. **A:** the general mechanism of the gene A transcription that is activated by TF-A. **B:** the insertion of TE destroys the interaction between TF-A and *cis*-regulatory element of gene A, which resulted in transcription termination. **C:** the insertion of TE creates an opportunity to recruit a novel TF-B which subsequently activates the transcription of gene A under an alternative condition. **D:** the TE insertion activates an antisense transcription, which represses the expression of gene A. **E:** the sequence of TE acts as the target of small RNAs and leads to degradation of gene A mRNA or represses its expression by post-transcription manner. **F:** TE inserted into previously unrelated genes (A and B) and rewired them into a network since the TE sequences act as *cis*-regulatory elements that are controlled by a TF. The denotation is the same as those in Fig. 1.

potentially provides target sites for small RNAs regulation. As antisense regulatory RNAs, either encoded in *trans* or in *cis*, have recently been found to be a genome-wide phenomenon in bacterial genomes (Toledo-Arana et al., 2009; Sharma et al., 2010), future studies will be seen to focus on this topic.

3.4. Genetic context determines the architecture and complexity of transcription regulatory circuit

Although single-cell bacteria are considered one of the simplest organisms on the earth, they must be the most variable cell-types since each bacterial species/strain adapts to a specific ecological niche. To respond to various outside stimuli, orthologous TFs and regulatory circuits co-evolved to cope with natural selection imposed by environmental challenges. As being repetitively verified by functional genomic studies (Rodionov et al., 2002; Rhodius et al., 2006; Cooper et al., 2008), orthologous TFs usually contain distinct regulatory functions, i.e., drive the expression of different set of genes, even if they are highly conserved in primary sequences. This is usually caused by gain/loss of target genes, or development of novel recognition specificity between TFs and *cis*-regulatory sequences. For example, although *Salmonella* and *Yersinia* share a conserved PhoQ-PhoP, a two-component signal transduction system controlling virulence factor expression by sensing Mg^{2+} concentration and antibiotic peptides (Groisman and Mouslim, 2006), substitution of PhoQ-PhoP of *Salmonella* by the orthologs from *Yersinia* resulted in virulence attenuation. By ChIP-chip and tiling microarray analyses, it was estimated that about 30% of the PhoP targeted genes experienced transcriptional rewiring events (Perez et al., 2009). Similar to the result from the pan-genomic comparison, PhoP regulon also contains “core” and “accessory” portions. Further mechanic study revealed that the TF PhoP of *Yersinia* can bind to the promoter of *Salmonella* virulence gene *mgfA* and *ugtL*, but it failed to recruit RNAP to the *ugtL* promoter and cannot drive its transcription. The promoter of *Salmonella ugtL* represents a group of α -CTD dependent, *cis*-regulatory sequences of which the PhoP binding boxes located further upstream from the -10 region. The orientation of PhoP binding boxes is opposite between *Salmonella* and *Yersinia* in such promoters, indicating that PhoP of *Salmonella* and *Yersinia* evolved species-specific mechanism in recognizing the architecture of this kind of promoters (Perez and Groisman, 2009a).

In studying the evolution of animal body plan, it was found that there is no major change in signaling network after Cambrian Explosion that occurred 500 million years ago. This implies that after basic regulatory cascade being established, it remains relatively conserved and more adaptive change in regulation to specify the basic cascade (Peter and Davidson, 2011). This kind of conservation also took place in bacteria, as being systematically studied in regulatory network of sporulation in endospore-forming bacteria, such as *B. subtilis* (de Hoon et al., 2010). Consequently, novel TFs introduced into an ancient, established regulatory cascade, which was named as “intercalary evolution”, was proposed to explain the

evolution of complex systems such as animal eyes (Gehring and Ikeo, 1999). A recent example is found in eukaryotic yeast. Booth et al. (2010) revealed that in dairy yeast *Kluyveromyces lactis*, a transcriptional repressor Rme1 was intercalated into the ancient mating control system, which is represented by bakers' yeast *Saccharomyces cerevisiae*. This intercalation not only added a new hierarchical layer of regulation, but also confers *K. lactis* the ability to react to starvation stress, which is absent in *S. cerevisiae* (Booth et al., 2010). In fact, similar evolutionary mode has long been identified in bacterial regulatory networks. In enterobacteria, proteins encoded by *pbgP* operon and *ugd* are responsible for covalent modification of lipopolysaccharide (LPS) with 4-amino-4-deoxy-L-arabinose. These genes are controlled by TCSTSs PhoP-PhoQ and PmrA-PmrB, the latter can sense Fe^{3+} (Gunn et al., 1998). In *Yersinia pestis*, both TFs PhoP and PmrA bind *pbgP* promoter and activate its transcription (Winfield et al., 2005). However, in *S. enterica*, PhoP doesn't directly modulate *pbgP* expression. It controls a connector protein PmrD that can stabilize the active form of phosphorylated PmrA by a post-translational manner, and it is PmrA that solely control *pbgP* expression in *S. enterica* (Winfield and Groisman, 2004). The genome of *Y. pestis* didn't encode PmrD. Comparing with direct control, intercalation of the connector PmrD in *S. enterica* prolonged the persistent state of active PmrA after the disappearance of inducing condition, which helps the bacterium express virulence factors continuously in fluctuating environments within its host (Kato et al., 2007; Perez and Groisman, 2009b).

Although previous investigations of bacterial transcription rewiring were mainly focused on the relationship between TFs and *cis*-regulatory sequences, evolution of other factors affecting transcription process, such as cellular secondary messengers and regulatory RNAs, will take more attention in future study since they are critical participants in transcription regulation. A good example is the functional roles of small RNAs (sRNAs) in regulating quorum-sensing of close-relative *Vibrio harveyi* and *Vibrio cholera*. *V. harveyi* encodes five Qrr sRNAs whose transcriptions are activated by the phosphorylated TF LuxO under low-cell density state. These five Qrr sRNAs destabilize the mRNA of the downstream TF LuxR and repress its expression by an additive manner, i.e., deletion of any single *qrr* gene causes phenotypic change in quorum-sensing. In *V. cholera*, although most of the essential components modulating quorum-sensing are nearly identical to *V. harveyi*, it only encodes four Qrr sRNAs (lacking Qrr-5). Furthermore, these four sRNAs repress the expression of HapR (LuxR analog) in a redundant manner, that is, any Qrr sRNA is sufficient to target *hapR* mRNA so that simultaneous inactivating of the four sRNAs is required to prevent the quorum-sensing process (Tu and Bassler, 2007). Two negative feedback loops responsible for this regulation have been identified: LuxO negatively autoregulates its transcription by a phosphorylation-independent mode; and Qrr sRNAs repress LuxO translation by targeting its mRNA. The two feedback loops reduce LuxO protein level and disruption of any of them resulted in increased level of phosphorylated LuxO and

expression of downstream quorum-sensing regulated genes (Ng and Bassler, 2009; Tu et al., 2010). Consequently, these results indicated that bacterial regulatory circuits were subtly shaped and reprogrammed by natural selection to accurately control their gene products, which are critical in maintaining cellular homeostasis in coping with stochastic environmental changes.

4. Conclusion

The study on bacterial “Evo-Devo”, currently being focused on the rewiring and reprogramming of bacterial transcription circuits during adaptive evolution and speciation, is on its golden age now. From the point of evolutionary biology, comparative genomics has prompted large numbers of hypothesis explaining the biology of bacteria, which can only be verified and developed by experimental analysis. This represents a remarkable paradigm shift from the “modern synthesis” of evolutionary biology, which was established from 1920’s and took a mathematical-oriented methodology to trace the dynamics of gene frequency in populations. Accordingly, fundamental questions are just opening in this area, including: i) what are the mechanic differences of regulatory cascades that are important in bacterial adaptation and speciation? ii) deciphering the biochemical and structural nature of recognizing specificity between regulators (such as TFs and sRNAs) and their targets, and how do genetic context and natural selection affect these specificity? iii) does quantitative change in gene expression contribute to the eventually qualitative alteration, as Darwinian Theory proposed? iv) how did regulatory circuits balance the functional conservation and evolutionary flexibility? v) how did the regulators and their targets be co-optioned and re-adjusted to deal with environmental changes? The answer of these questions, with a story of prokaryotic version, will give insights into the molecular basis of evolution.

Likewise, from the point of molecular biology, the introduction of evolutionary methods is also important in post-genomic era. The investigation of the gene expression in what time, at what cellular location, in how much quantities and with what speed is more and more attractive. As briefly reviewed above, the evolutionary method, which compares the subtle functional differences among orthologous, paralogous and xenogenic circuits, provides us invaluable clues for extensive genetic study because the natural selection has completed the most meaningful “genetic recombination” during evolution. Moreover, tracing back the origin of the current regulatory circuits helped us to understand the reason for this form, rather than any other alternatives, to be the most suited and being fixed in bacterial population. For example, why in some case is the α -CTD region, rather than the σ factors of RNAP, responsible for recognizing promoter sequences? Why do bacteria employ antisense RNAs, rather than proteins, to repress and regulate translation of certain target genes? In this respect, the study of bacterial Evo-Devo is also fundamental, to elucidate the evolutionary basis of molecular regulation.

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