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Research review paper

Bacteriophage T7 transcription system: an enabling tool in synthetic biology

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ABSTRACT

Since its discovery in the 1970s, the T7 RNA polymerase (T7 RNAP) transcription system has been applied extensively as an effective tool in molecular biology because of its robust function in various hosts, including prokaryotic, eukaryotic and cell free systems. Recently, the T7 RNAP transcription system has emerged as a critical component for synthetic biology. The present paper summarizes the advances of the T7 RNAP transcription system in synthetic biology, including the recent progress of T7 RNAP structure and its cognate promoter and terminator and its application in cell free systems, logic gates and orthogonal genetic circuits.

1. Introduction

Synthetic biology is a broad and interdisciplinary filed involving chemistry, physics, mathematics, and biology with the ultimate aims of creating functional parts, devices, circuits, systems and organisms that possess novel, predictable and useful functions of value from catalogued and standardized biological building blocks (Cameron et al., 2014; Cardinale and Arkin, 2012). The interdisciplinary field of synthetic biology also relies engineering principles to design and assemble the standard biological components like machinery constructed for the purposes of improving applications in industrial production or biological research (Osbourn et al., 2012; Wellhausen and Oye, 2008).

Synthetic gene circuits have become invaluable tools for studying the design principles of native gene networks and in facilitating the development of new biotechnologies (Way et al., 2014). Consequently, gene circuits now play a critical role in the synthetic biology research. In the design of gene circuits, RNA polymerase (RNAP) from bacteriophage T7 is one of the most widely used tools. T7 RNAP shows a number of valuable properties including: (i) being a single-subunit enzyme in contrast to multi-subunit bacterial RNAP; (ii) a high processivity; (iii) a high specificity towards the T7 promoter; (iv) an independence from auxiliary transcription factors; (v) an ability to produce very long transcripts; and (vi) termination only by class I and class II termination signals, differing significantly from bacterial transcription termination sites (Kortmann et al., 2015). These properties highlight the potential of developing T7 RNAP as a transcription system that is simple, efficient and sufficiently specific to be used in different organisms. The T7 transcription system has already been shown to be compatible with a variety of hosts, including multiple kinds of prokaryotes and eukaryotes (Table 1), and even cell-free systems. It should be notable that the development of T7 expression system in eukaryotes has lagged far behind that in prokaryotes since eukaryotic mRNA needs to be post-transcriptionally processed and then transported into cytoplasm before translation. Furthermore, most eukaryotes in Table 1 used the DNA virus to transient expression of protein in cytoplasm with T7 system and the protein expression will be lost with the cell division. In the case of protozoa, *Trypanosoma* and *Leishmania* have seen successful construction of T7 expression system as host cell, this ability is attributed to their mRNA trans-splicing mechanism (Wirtz et al., 1994) and this trans-splicing mechanism is seldom happened in other eukaryotic species. In addition, different chemical inducers have been developed and combined with the T7 transcription system to make it more controllable in laboratory research and for industrial production (Table 2). However, despite the fact that T7 transcription system has been widely used for protein expression, its potential value as a synthetic biological tool is based on its unique divisible structure that is much less familiar to scientists. The development of a “plug-and-play” T7 transcription system that is completely independent of host

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Table 1
T7 expression system developed in different hosts.

Type	Host	Reference
Eukaryoter	*Autographa californica*	(van Poelwijk et al., 1995)
	Human Hela cells	(Fuerst et al., 1987)
	Human hepatocellular carcinoma cell line (FLC4)	(Aoki et al.,1998)
	Human embryonic kidney (HEK-293 cells)	(Chou et al., 2010; Ghaderi et al., 2014)
	Leishmania chagasi	(Yao et al., 2007)
	Leishmania mexicana	(Ishemgulova et al., 2016; Kraeva et al., 2014)
	Leishmania tarentolase	(Kovtun et al., 2010; Kushnir et al., 2005)
	Mouse cells (L(A9) cells)	(Elroy-Stein and Moss, 1990)
	Pichia pastoris	(Hobl et al., 2013)
	Saccharomyces cerevisiae	(Benton et al., 1990)
	Trypanosoma brucei	(Kelly et al., 2007; Poon et al., 2012; Wirtz et al., 1998)
	Trypanosoma cruzi	(Alonso et al., 2014)
	Tobacco (*Nicotiana tabacum*)	(Emadpour et al., 2015; McBride et al., 1994; Terpe, 2006; Sheen and White, 2018)
	Zebrafish (*Danio rerio*)	(Verri et al., 1997)
R	*Agrobacterium tumefaciens*	(Kang et al., 2007; Zhu et al., 2003)
	Bacillus megaterium	(Gamer et al., 2009)
	Bacillus subtilis	(Chen et al., 2010; Conrad et al., 1996; Troeschel et al., 2012)
	Burkholderia cepacia	(Jia et al., 2010)
	Chromobacterium violaceum	(Kang et al., 2007)
	Corynebacterium acetoacidophilum	(Equbal et al., 2013)
	Corynebacterium glutamicum	(Kortmann et al., 2015)
	Escherichia coli	(Chao et al., 2002a, 2002b; Choi et al., 2010; Chou et al., 2010; Kang et al., 2007; Troeschel et al., 2012)
	Erwinia carotovora/ amylovora	(Gaudriault et al., 1997; Kang et al., 2007)
	Lactococcus lactis	(Wells et al., 1993a, 1993b)
	Lactobacillus plantarum	(Heiss et al., 2016)
	Pseudomonas sp. ATCC19151	(Davison et al., 1989)
	Pseudomonas aeruginosa	(Brunschwig and Darzins, 1992; Kang et al., 2007; Schweizer, 2001)
	Pseudomonas putida	(Herrero et al., 1993; Troeschel et al., 2012)
	Rhodobacter capsulatus	(Katzke et al., 2010)
	Ralstonia eutropha	(Barnard et al., 2004)
	Salmonella choleraesuis	(Kang et al., 2007)
	Salmonella enterica	(Lee et al., 2006b)
	Streptomyces lividans	(Lussier et al., 2010)
	Synechocystis sp.	(Camsund and Lindblad, 2014)

transcription components is challenging. The traditional T7 system always requires the T7 polymerase gene to be integrated into host genome and transcribed by host RNA polymerase to avoid the lethality issue of over transcription. This review primarily focuses on summarizing recent advances of T7 RNAP in synthetic biology to provide a guide to address such questions. The structure of T7 RNAP is introduced, as well as how it can be divided into multiple functional parts, and the unique promoter/terminator components of T7 system is also briefly summarized. The special properties of T7 components, which have led to the development of a T7 logic gate for a synthetic biological circuit, are then introduced. Lastly, current advances in developing an orthogonal T7 transcription system are summarized, followed by a prospective of how the combination of the T7 transcription system and synthetic biology can be used in cell free systems, which have the potential to become the cleanest and easiest *in vitro* biosynthesis system for future applications.

2. Advances in T7 RNAP structure

The continued advancement of synthetic biology calls for the construction of larger and more complex synthetic gene circuits, which in turn necessitates the development of additional parts and component libraries with which to build these circuits. Phage derived T7 RNAP is an important molecular tool for the study of synthetic gene circuits. Original full-length T7 RNAP can often cause cellular stress and result in mutations that affect the functionality of the underlying gene circuit (Shis and Bennett, 2014). Therefore, in recent years researchers have generated split proteins, based on the full sequence of T7 RNAP, to reduce lethality to the host (Schaerli et al., 2014; Segall-Shapiro et al., 2014). A synthetic gene circuit, driven entirely by fragmented T7 RNAP prepared using a split intein can interact more stably in gene circuits, further increasing the availability of these components (Schaerli et al., 2014; Segall-Shapiro et al., 2014).

Protein function is determined by protein structure, and, thus, understanding the structure of T7 RNAP is the key to the splitting of T7 RNAP. Several researchers have published a comprehensive review of relationship between the structure-function relationship of T7 RNAP (Tunitskaya and Kochetkov, 2002; Sousa and Mukherjee, 2003; Borkotoky and Murali, 2018). The present review describes the T7 RNAP structure mainly on the basis of Sousa and Mukherjee (2003), in which T7 RNAP functional domain is comprised of residues ~313–448, ~532–738, and ~770–838, while residues ~449–531 and ~739–770 are inserted between these three domains(Fig. 1).

The three T7 RNAP subdomains have been referred to as the "thumb," "palm," and "fingers." The "thumb" subdomain is comprised of residues ~330–410, and forms a long helical projection on one side of the template-binding cleft. The "palm" subdomain is comprised of residues ~386–448, ~532–540, and ~788–838, and contains most of the catalytically critical residues. Residues ~541–737 and ~771–778 make up the "fingers" subdomain. The thumb, fingers, and palm subdomains together form the polymerase domain. T7 RNAP also contains four additional structural elements known as "accessory" modules. These are the N-terminal domain (residues 1–310), the extra four-helix bundle (residues 449–531), the promoter recognition loop (739–770) and the C-terminal loop (839–883) (Sousa and Mukherjee, 2003).

The split version of T7 RNAP, is commonly referred to as nicked RNA polymerase and was originally discovered during its purification (Davanloo et al., 1984). Ikeda and Richardson reported that T7 RNA polymerase was cleaved by a protease at a single site between amino acids 172 (lysine) and 173 (arginine), located in the H-loop domain (Ikeda and Richardson, 1987). After cleavage, if the two fragments from the nicked T7 RNAP remain associated, the complex retains T7 RNAP activity, but with a 3.5-fold reduction in specific activity (Ikeda and Richardson, 1987). If the two fragments of T7 RNAP dissociate, their ability to catalyze RNA synthesis is lost. Further studies demonstrated that the C-terminal fragment of nicked T7 RNAP (amino acids 173–880) could bind T7 promoter on its own but was unable to synthesize full-length mRNA. The addition of the N-terminal domain (amino acids 1–172) was able to rescue the C-terminal fragment's ability to transcribe mRNA (Muller et al., 1988). Recently, Segall-Shapiro and colleagues showed that T7 RNAP could be divided into three fragments. T7 RNAP is split initially between amino acids 601 and 602, forming 'core fragment(amino acids 1-601)' and 'σ fragment(amino acids 602-880)'. The DNA-binding loop is included in a 'σ fragment' and the 'core fragment' possesses the catalytic activity for RNA synthesis. Furthermore, the 'core fragment' is split at residue 67 to form the '*α* core fragment' and '*β* core fragment'. The activity of T7 RNAP requires all three of these subunits to be co-expressed (Segall-Shapiro et al., 2014). Schaerli and coworkers divided T7 RNA polymerase into two expression domains between amino acids 514 and 515 and fused each with a split intein, N-terminal Npu intein (102 amino acids) from *dnaE* in *Nostoc punctiforme* and the C-terminal Ssp intein (36 amino acids) from *dnaE* in *Synechocystic* sp. *strain* PCC6803. When both domains are co-expressed, the split intein mediates protein trans-splicing yielding a full-length T7 RNA polymerase, which can transcribe genes by binding the T7 promoter (Schaerli et al., 2014).

Table 2
Inducers developed for T7 expression system.

Host	Inducer	Operon	Reference
Agrobacterium tumefaciens	IPTG/Lactose	*lac*	(Kang et al., 2007)
Bacillus megaterium	Xylose	*xyl*	(Gamer et al., 2009)
Bacillus subtilis	Xylose	*xyl*	(Conrad et al., 1996; Troeschel et al., 2012)
	Tetracycline	*tet*	(Kushwaha and Salis, 2015)
Corynebacterium glutamicum	IPTG/Lactose	*lac*	(Kortmann et al., 2015)
Chromobacterium violaceum	IPTG/Lactose	*lac*	(Kang et al., 2007)
Escherichia coli	Blue light	*ara*	(Baumschlager et al., 2017)
	Choline	*bet*	(Ike et al., 2015)
	Cumate	*cym and cmt*	(Choi et al., 2010)
	Galactose	*gal*	(Menzella and Gramajo, 2004)
	Heat	λP_L	(Chao et al., 2002b; Gupta et al., 1999)
	IPTG/Lactose	*lac*	(Kang et al., 2007; Xu et al., 2014)
	L-arabinose	*ara*	(Chao et al., 2002a; Wang et al., 2011; Wycuff and Matthews, 2000; Zei et al., 2005)
	Light, IPTG/Lactose	*lac*	(Binder et al., 2014; Chou et al., 2010)
	Malonyl-CoA	*fapO/fapR*	(Xu et al., 2014)
	Propionate	*prp*	(Lee and Keasling, 2006a)
	Rhamnose	*rha*	(Giacalone et al., 2006)
	Sucrose/sorbitol, NaCl	*proUp*	(Bhandari and Gowrishankar, 1997; Pal et al., 2001)
	Tetracycline	*tet*	(Kushwaha and Salis, 2015)
Erwinia carotovora	IPTG/Lactose	*lac*	(Kang et al., 2007)
Human embryonic kidney (HEK293T) cells	Light(caged T7 RNAP)	*lac*	(Chou et al., 2010)
Lactobacillus plantarum	IPTG/Lactose	*lac*	(Heiss et al., 2016)
Lactococcus lactis	IPTG/Lactose	*lac*	(Wells et al., 1993a, 1993b)
Leishmania mexicana	Tetracycline	*tet*	(Kraeva et al., 2014)
Pseudomonas aeruginosa	IPTG/Lactose	*lac*	(Kang et al., 2007)
Pseudomonas phaseolicola	Rhamnose	*rha*	(Pagratis and Revel, 1993)
Pseudomonas putida	Xylose	*xyl*	(Troeschel et al., 2012)
	Tetracycline	*tet*	(Kushwaha and Salis, 2015)
Ralstonia eutropha	Phosphate	*PhaP*	(Barnard et al., 2004)
Rhodobacter capsulatus	Fructose	*fru*	(Katzke et al., 2010)
Salmonella choleraesuis	IPTG/Lactose	*lac*	(Kang et al., 2007)
Salmonella enterica	Propionate	*prp*	(Lee and Keasling, 2006b)
	Arabinose	*ara*	(McKinney et al., 2002)
Streptomyces lividans	Thiostrepton	*tip*	(Lussier et al., 2010)
Tobacco	Ethanol	*phb*	(Lössl et al., 2005)
Trypanosoma cruzi	Tetracycline	*tet*	(Darocha et al., 2004)

3. Promoter and terminator of T7 transcription system

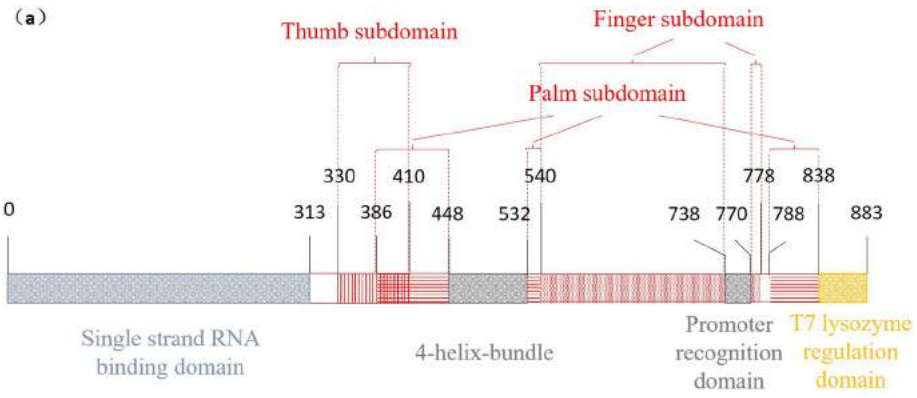
Reliance on the host transcription system for the construction of circuit elements, such as logic gates, is difficult to implement for obtaining predictable and independent circuits (Iyer et al., 2013). T7 RNA polymerase can orthogonally and specifically recognize its cognate promoter and does not require co-factors to activate transcription. This makes T7 RNAP an important foundation for synthetic biological circuitry in both *in vivo* and *in vitro* applications (Iyer et al., 2013; Meyer et al., 2015). Residues 739–770 are the promoter recognition loop in T7 RNAP and allow T7 RNAP to specifically contact the promoter (Cheetham et al., 1999). Mutations to these critical parts of the promoter usually result in a substantial loss in promoter recognition (Meyer et al., 2015). Attempts to create T7 RNAP mutants, capable of strong and specific recognition of more divergent promoters, generally rely on directed evolution (Chelliserrykattil et al., 2001; Esvelt et al., 2011), phylogenetic part mining, or domain grafting (Meyer et al., 2015). In recent years, some researchers have used compartmentalized partnered replication (CPR) (Abil et al., 2017) and systematic evolution of ligands by exponential enrichment (SELEX) (Keefe and Cload, 2008) to create T7 RNAP mutants and orthogonal T7 RNAP promoter pairs, improving the utility of T7 RNAP for use in complex transcriptional circuitry.

In addition to T7 RNAP and T7 promoter, T7 terminator has also aroused researcher interests over the past few decades with efforts aimed at improving its termination efficiency. Two distinct types of terminators, class I and class II terminators, have been described for T7 RNA polymerase (Macdonald et al., 1994). Class I terminators have a structure similar to the rho-independent intrinsic terminators of *E. coli* RNAP (Macdonald et al., 1994). Class II terminators do not appear to possess any distinctive secondary structure, and are comprised of the sequence ATCTGTTTTCTTGC with further analysis showing that the critical element of class II terminators are the ATCTGTT sequence, which must be invariant for function (Lyakhov et al., 1998).

Although the class I terminator is the most widely-used terminating element, its inefficiency of termination and large size (100 bp) is problematic for multigene construction and expression (Du et al., 2009). Class II terminator has been engineered to express multigenes *in vitro* and *in vivo* to overcome this problem. Du et al. (2012) concluded that, the transcription termination of T7 RNAP could be made more efficient by substituting a standard, single, class I terminator with adjacent copies of the class II terminator (Du et al., 2012, 2009). Thirteen different terminators were designed *in vitro*, improving the termination efficiency of T7 terminator and leading to an increase in termination efficiency from 80% to 99% (Mairhofer et al., 2015). Kesik-Brodacka and coworkers took a different approach, utilizing the inefficiency of T7 terminator or the read-through mechanism of T7 RNAP to improve the stability of plasmids. Their work suggests that the terminators are not only used as the signal of the end of transcription processes, but also a “bandpass filter”, providing a new perspective for efficient design of genetic circuits, in which the terminator's function is one of the regulating elements (Kesik-Brodacka et al., 2012).

4. T7 transcription system as tool in a logic gate construction

The binary number system (0 and 1) is used extensively by modern computers and computer-based devices. The logical operation of this binary system is performed based on the Boolean function or logic gates. From the view of the biologist, biological molecules can potentially be built up into bio-computers and the logic operations of a



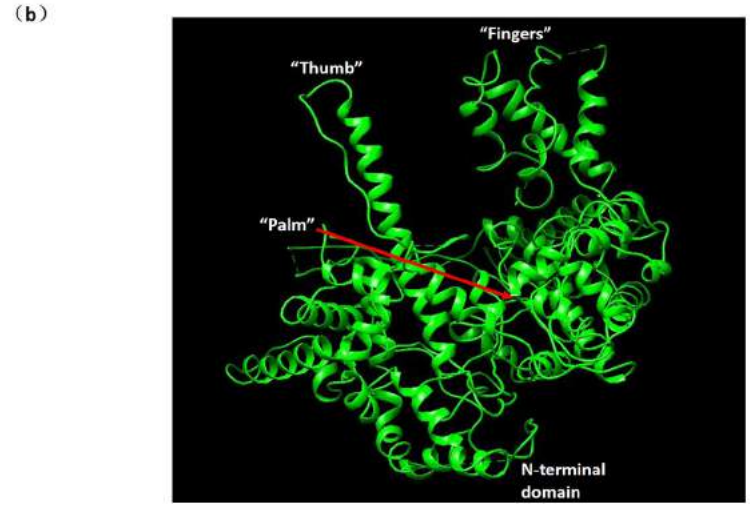


Fig. 1. Structure of T7 RNA Polymerase (domain structure (a) and 3-D structure (b)). T7 RNA polymerase can be divided into one catalytic domain (aa 313–448, aa 532–738 and aa 770–838) and four "accessory" domains, including a single strand RNA binding domain (aa 0–312), a promoter recognition domain (aa 739–769), a T7 lysozyme regulation domain (aa 839–883) and a 4-helix-bundle (aa 449–531) of unknown function. The catalytic domain can be further divided into three subdomains, including a thumb (aa 330–410), a palm (aa 386–448, aa 532–540 and aa 788–838) and a finger (aa 541–737 and aa 771–778). Furthermore, research has demonstrated that T7 RNA polymerase can be divided into two parts at either aa 172 or aa 601, but still remain its complete function if the two parts exist together.

computer can be performed by the substitution of a computer's electronic components with biological molecules, constructing a logic gate through the regulation of biological molecules (Ausländer et al., 2012; Katz and Privman, 2010). Based on this idea, a variety of biological molecules such as nucleic acids (DNA or RNA) (Seelig et al., 2006; Willner et al., 2008), enzymes (Ikeda et al., 2014; Katz and Privman, 2010; Strack et al., 2008) and others small molecules (Park et al., 2010; Sivan and Lotan, 1999), have been used to construct logic gates. The design and construction of organisms with logic gates is the objective of this synthetic biology research.

In synthetic biology, logic gates are constructed based on a principle similar to those used to design electronic circuits (Simpson et al., 2004). The output of the gene circuit is directed partially by promoter activity. However, the difficulty in constructing a biological logic gate is finding an orthogonal transcription system. Over the past few decades, the method for generating biological logic gates has often been at the transcriptional level, and has been carried out by splitting T7 RNAP (Shis et al., 2014). Until now, T7 RNAP has been effectively applied in the construction of transcriptional 'AND' logic gates. Research has demonstrated that T7 RNAP can be divided into two or more fragments, and these split fragments can be transcribed under the control of different inducers. Only when fragments can be assembled into a complete and active T7 RNAP can the target gene be transcribed and translated into a protein. Based on this concept, logic gates can be constructed to regulate the expression of target genes on the basis of input inducers (Shis et al., 2014).

Schaerli and coworkers made a split T7 RNAP intein for the construction of transcriptional 'AND' logic gate. They nicked T7 RNAP into C-terminal polymerase domain (1–514) and N-terminal polymerase domain (514–884) and fused split inteins (N-terminal Npu intein, NpuN; C-terminal Ssp intein, SscP) into two split domains leading to an auto-catalytic excision of the inteins resulting in full-length T7 RNAP (Schaerli et al., 2014). In their paper, the C-terminal polymerase domain is regulated by P_{BAD}, which is induced by arabinose, and the N-terminal polymerase domain is regulated by P_{tac}, which is induced by IPTG. Their results showed that there was no fluorescence when cells were induced by arabinose or IPTG alone, but fluorescence appeared only when cells were induced by both arabinose and IPTG. This demonstrates that it is possible to construct an 'AND' logic gate using the recombined T7 RNAP formed by two split intein linked T7 RNAP subunits to catalyze T7 promoter transcription just like the native T7 RNAP (Fig. 2a). However, a single logic gate is insufficient to meet demands of the range of activities for promoters, thus, a library of synthetic transcriptional 'AND' logic gates were constructed using split T7 RNA polymerase by Shis and Bennett (2013). In this study, the DNA recognition domain, within the C-terminal of T7 RNAP for T7 promoter specificity, was mutated and transformed into five different strains to create 25 mutants. A library of transcriptional 'AND' gates was built using different T7 RNAP/ T7 promoter pairs in an inducible green fluorescent protein (GFP) expression system (Fig. 2b) (Shis and Bennett, 2013). This library enables the construction of larger and more complex gene circuits. In addition to the two split fragments of T7 RNAP, it can be further split into three parts ('α fragment', 'β core fragment' and 'σ fragment') (Segall-Shapiro et al., 2014), with which Segall-Shapiro and coworkers created a more complex and adjustable 'double AND' system. In this system the core fragment was used as a T7 RNAP 'resource allocator', and two 'AND' logic circuits and different T7 RNAP/promoter pairs were introduced into the system to compete with each other as a

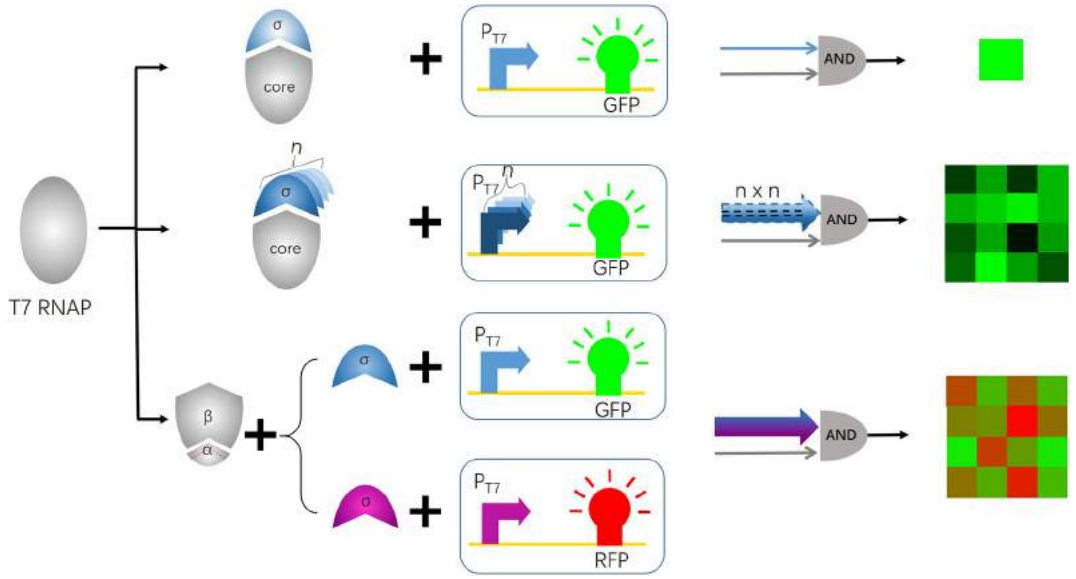


Fig. 2. Construction and regulation of 'AND' logic gate using T7 system. (a) By splitting T7RNP into a core-part and a σ-part, an 'AND' logic gate is built where only when the two parts are co-expressed can there be a functional T7 RNAP and hence an output of a downstream green fluorescent signal. (b) By combining different σ-part and T7 promoter mutants, the 'AND' logic gate described in Fig. 2a can be improved to generate a downstream green fluorescent signal of different brightness. (c) In this model the core-part is split into a α-part used as a switch of the logic gate (which can be further split if needed) and a β-part. By using two pairs of "σ-part-T7 promoter-reporter gene" and by setting the expression of β-part at a constantly low level, the 'AND' logic gate described in Fig. 2b can be further modified to generate a tunable bicolor (green fluorescence and red fluorescence of different ratio) downstream signal. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

'resource allocator'. Using this system, a library of green color, resulting from GFP expression, and red color, resulting from red fluorescent protein (RFP) expression were mixed in different proportions (Fig. 2c). Furthermore, split T7 RNAP ('core' and 'alpha') binding different σ factors can create additional in-put signal regulatory modularity (Shis et al., 2014).

Anderson and co-workers described the use of two inducible promoters to control the expression of *gfp* and constructed a 'AND' logic gate. Their 'AND' logic gate has two components, one component was translated as a T7 RNAP, containing two amber stop codons (UAG), the second component was transcribed as Ser-tRNA when a nonsense suppressor *supD* was transcribed. When these two components are both induced, an active T7 RNA polymerase is synthesized, which in turn activates a T7 promoter to express *gfp* resulting in a green color (Anderson et al., 2007). This 'AND' logic gate was integrated with natural promoters and implemented for use in mammalian cells.

Until now, most logic gates based on the T7 RNAP system are 'AND' gates. Several years ago, a 'NOT' gate based on the T7 system was built that relied on a RNA aptamer against T7 RNA polymerase (Kim et al., 2014; Ohuchi et al., 2012). In 2017, a multiple 'NOT' gate was constructed in *E. coli* by the combination of T7 RNAP allocator and CI and PhlF repressor, and this was used to develop the red/green/blue color vision system (Fernandez-Rodriguez et al., 2017).

5. T7 transcription system in the construction of genetic circuits with orthogonality

Orthogonality is one of the focuses in synthetic biology. When these engineered gene circuits are embedded into cells to perform new functions, it is expected that the circuits take full advantage of host's resources and will not be limited by the host's native regulatory networks. The property of orthogonality can avoid unnecessary crosstalk between the embed elements in gene circuits and the genetic elements of the host, guaranteeing the universal application of these genetic elements in different hosts. T7 RNAP and its cognate promoter shows a very high specificity and the speed of RNA synthesis by T7 RNAP is 5-fold faster than that by *E. coli* RNAP (Chamberlin and Ring, 1973; Golomb and Chamberlin, 1974). Consequently, the T7 transcription system represents an ideal tool for the construction of an orthogonal system.

Although the T7 transcription system has been applied successfully in various species (Table 1), to date all these extensively used T7 transcription systems are host-dependent, such as pET plasmid series. The T7 RNAP needs to be integrated into the host genome, and only T7 promoter is placed in the plasmid, due to lethality caused by the powerful transcribing capability of T7 RNAP. Previous researchers have investigated the placement of T7 RNAP together with T7 promoter in a single plasmid or the construction of host-independent T7 transcription system in single plasmid (Dubendorff and Studier, 1991; Studier et al., 1991), but all of these have resulted in the death of the host cell. This host dependency limits the universal and convenient application of T7 transcription system as an orthogonal element. Recently, Wang and colleagues have developed a novel host-independent T7 transcription system that integrates T7 RNAP and T7 promoter into a single plasmid with antisense RNA used to reduce the amount of T7 RNAP. Compared to the BL21 (DE3) host, this host-independent T7 transcription system can efficiently express proteins in non-DE3 *Escherichia coli* strains (JM109) and a wild-type *Sinorhizobium* strain TH572. Furthermore, the same laboratory has described the construction of a fine regulating transcription system of T7 RNAP in single plasmid by combining antisense RNA technology, CAP site deletion, terminator substitution and ribosome-binding site (RBS) design, demonstrating the effective expression of proteins in extensive hosts, including five different Gram-negative strains and one Gram-positive strain (Liang et al., 2018). Kushwaha and coworkers investigated another method to maintain the expression of T7 RNAP at non-toxic levels, by using integrated positive and negative feedback loops to tune T7 RNAP expression levels. After construction and characterization of the 50 variants of this integrated system, the resulting engineered system showed autonomous self-regulation that could function in diverse Gram-positive and Gram-negative bacteria (Kushwaha and Salis, 2015). Thus far, orthogonality has not only been discussed as it relates to a transcriptional orthogonal system, in recent years, translation orthogonal systems have also undergone rapid development in gene circuit construction and synthetic biology

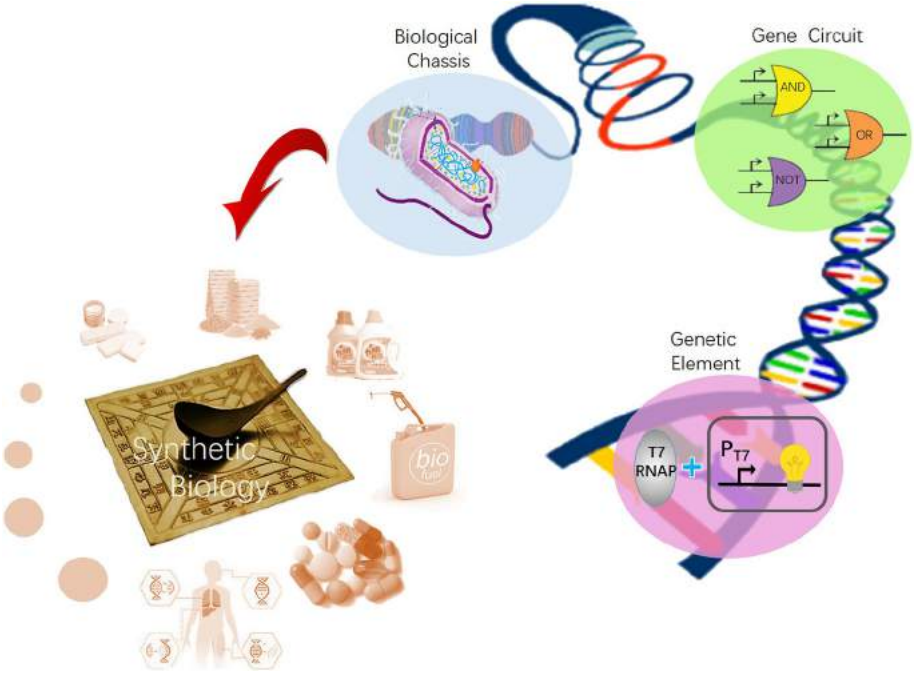


Fig. 3. Perspective of implanting of T7 system into synthetic biology and industrial manufacture. As a transplantable genetic element of high efficiency, specificity, orthogonality and controllability, T7 transcription system, in combination with gene circuit such as logic gates, will create powerful synthetic biology tools in the future to add, modify or control metabolic pathways in different biological chassis, and therefore can help optimize and maximize the production of various kinds of biological products.

(Rackham and Chin, 2005a, 2005b). Moreover, orthogonal transcription-translation networks have been created as the transcription–translation feed-forward loops for detailed studies on cellular decision making processes (An and Chin, 2009), in which the T7 system has been used for the construction of transcription circuit.

6. T7 transcription system in cell-free system

Recently, a cell-free protein synthesis system (CFPS) has been demonstrated as a powerful alternative to classical *in vivo* biosynthesis. CFPS is particularly useful for generating proteins that are: 1. toxic to the host cell; 2. rapidly degraded by intracellular proteases; 3. easily form inclusion bodies; and 4. incorporated with modified or isotope-labeled amino acids. Most importantly, CFPS also saves time and effort in the transformation of target genes into matched host cells by directly adding template DNA, either plasmid or linearized DNA generated by PCR, into a simplified and controllable mixture of *in vitro* biomachinery. Due to its high speed and specificity, the T7 system has been extensively used in CFPS for synthetic biology. The highest protein yield reported using T7 CFPS is 2.3 mg/ml (Caschera and Noireaux, 2014). The synthesis of different types of macromolecules, such as immunoglobins (Cai et al., 2015) and membrane proteins (Elbaz et al., 2004), and even complexes, such as *E. coli* ribosome (Jewett et al., 2014) and RNA virus (Kobayashi et al., 2012), has achieved great success using this powerful *in vitro* system. Since the T7 system has few regulatory components, efforts have been made to introduce synthetic circuits into T7 CFPS. For example, Karig et al. (2012) constructed a negative feedback system by inserting a tetR and a tetO sequences downstream T7 promoter, and Iyer and coworkers (2013) built an 'IMPLY' logic gate circuit using Teto and LacO and verified its function in T7 CFPS. Despite difficulties to scale-up CFPS for industrial manufacture, its convenience and high efficiency still make it a powerful tool for research uses, and there is no doubt that the incorporation of T7 system together with synthetic circuits will further broaden the application of CFPS in the future.

7. Conclusion and perspectives

Since the T7 transcription system functions robustly in a variety of hosts, prokaryotic, eukaryotic and cell free systems, the T7 transcription system has become a tractable and effective tool in synthetic biology. With a transplantable genetic element of high efficiency, specificity, orthogonality and controllability, T7 transcription system will facilitate future fundamental biological research. The T7 transcription system, in combination with gene circuits such as logic gates, will undoubtedly be used to create powerful synthetic biology tools in the future. Such tools will add, modify, and control metabolic pathways in different biological chassis, and help optimize and maximize the production of a variety of fine and bulk chemicals through synthetic biology (Fig. 3). Recently, the several studies combined the T7 transcription system with CRISPR-Cas9 (Cress et al., 2016; McCutcheon et al., 2017) and optogenetics (Baumschlager et al., 2017), improving its effectiveness and broadening its applications. Zhao et al. (2017) mined the T7 RNAP analogues in the database and applied a resultant analogue in non-model strains *Halomonas sp* TD01, paving a new way for the study on T7 transcription system in industrial microorganisms. In the near future, it is envisioned that biological organisms might be assembled as the same way as one assembles a machine but instead with the aid of T7 transcription system and other synthetic biology tools.

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