ANNUAL REVIEWS

Annual Review of Microbiology

Evolutionary Origins of Two-Barrel RNA Polymerases and Site-Specific Transcription Initiation

Thomas Fouqueau,* Fabian Blombach,* and Finn Werner

Institute of Structural and Molecular Biology, Division of Biosciences, University College London, London WC1E 6BT, United Kingdom; email: f.blombach@ucl.ac.uk, f.werner@ucl.ac.uk

Annu. Rev. Microbiol. 2017. 71:331–48

First published as a Review in Advance on June 28, 2017

The *Annual Review of Microbiology* is online at micro.annualreviews.org

https://doi.org/10.1146/annurev-micro-091014-104145

*These authors contributed equally to this work.

Keywords

multisubunit RNA polymerases, evolution, LUCA, translation initiation

Abstract

Evolution-related multisubunit RNA polymerases (RNAPs) carry out RNA synthesis in all domains life. Although their catalytic cores and fundamental mechanisms of transcription elongation are conserved, the initiation stage of the transcription cycle differs substantially in bacteria, archaea, and eukaryotes in terms of the requirements for accessory factors and details of the molecular mechanisms. This review focuses on recent insights into the evolution of the transcription apparatus with regard to (*a*) the surprisingly pervasive double-Ψ β-barrel active-site configuration among different nucleic acid polymerase families, (*b*) the origin and phylogenetic distribution of TBP, TFB, and TFE transcription factors, and (*c*) the functional relationship between transcription and translation initiation mechanisms in terms of transcription start site selection and RNA structure.

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INTRODUCTION

Nucleic acid polymerases carry out key functions in DNA replication, repair, and recombination, as well as in RNA transcription. The latter is the first step in gene expression and provides both the templates for protein synthesis (mRNA) and the structural RNAs that form the essential components of the translation machinery (rRNA and tRNA). The two most important superfamilies of nucleic acid polymerases are (*a*) the single-subunit right-handed polymerases encompassing the thumb, finger, and palm motifs and (*b*) the two-barrel polymerases characterized by an active site formed at the interface between two double-Ψ β-barrel (DPBB) motifs. The single-subunit polymerase superfamily includes almost all replicative DNA polymerases; bacteriophage single-subunit RNA polymerases (RNAPs), including mitochondrial RNAP; and reverse transcriptases. As such they are considered the most versatile nucleic acid polymerase family because different members can utilize DNA or RNA templates to synthesize DNA or RNA in any combination. The two-barrel nucleic acid polymerase superfamily comprises multisubunit (ms) RNAPs, which carry out transcription of the cellular genomes of bacteria, archaea, and eukaryotes as well as the chloroplast genome. Recently, some surprising additions have been made to this family that increase its functional breadth not only in terms of template specificity but also with respect to the mechanisms of site-specific transcription initiation.

DNA POLYMERASE D AND Qde-1 CONTAIN DOUBLE-Ψ β-BARRELS

The bulk of a cellular msRNAP is provided by two large catalytic subunits: β′ and β or Rpo1 and Rpo2 in single bacterial or archaeal RNAPs, respectively, or RPB1 and RPB2 in eukaryotic RNAPII. The subunits show striking sequence and structural similarities that are strongest in the active-site microenvironment (21, 32, 51, 86). These include the trigger loop and bridge helix elements, which are essential for the nucleotide translocation cycle (24, 40, 44, 60, 65, 83, 99, 100). The catalytic center is formed at the interface between two six-stranded DPBB domains, the two barrels being DPBB-A and -B. Each catalytic subunit contributes one DPBB

domain (36). The DPBB-A of the largest RNAP subunit (β′, Rpo1, and RPB1 in bacteria, archaea, and eukaryotes, respectively) contributes three invariant aspartic acid residues in the highly conserved NADFDGD motif to the active center, which coordinates the catalytic magnesium-A ion (**Figure 1*a***) (81). The DPBB-B of the second-largest subunit (β, Rpo2, and RPB2) provides two invariant lysine residues involved in substrate binding (22).

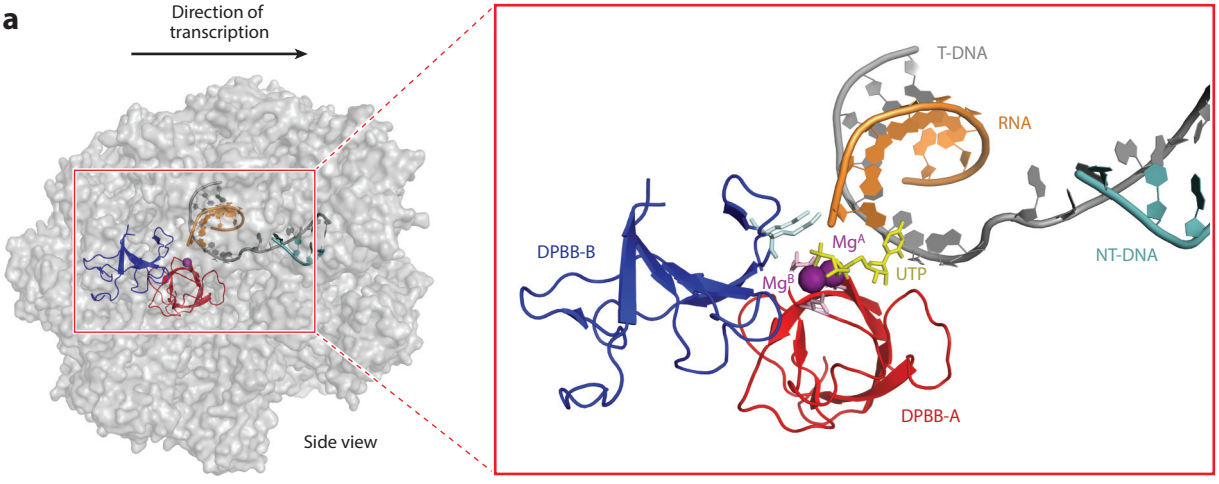
The pervasiveness of the DPBB architecture in msRNAPs is well known and was thought to be restricted to DNA-dependent transcription. Remarkably, the structures of two unorthodox nucleic acid polymerases revealed that this structural framework can also support RNA-dependent RNA synthesis and DNA-dependent DNA synthesis (73, 75). Both the eukaryotic RNA-dependent RNAP Qde-1, which facilitates RNA silencing in the fungus *Neurospora crassa*, and the catalytic subunit DP2 of the replicative archaeal DNA polymerase D (PolD) are two-barrel polymerases (73, 75) (**Figure 1*b***). The DPBB-A-type barrels (named DPBB-2) with catalytic carboxylate residues are located in the C-terminal regions of Qde-1 and PolD DP2. The DPBB-2 domain of Qde-1 contains the consensus DxDGD motif and binds the catalytic Mg^{2+}, whereas the DPBB-2 domain of PolD has only two of the canonical aspartic acid residues (NxDGD), except in species of the phylum of Thaumarchaeota, where the canonical three aspartic acid residues are conserved (e.g., *Nitrosopumilus maritimus* in **Figure 1*c***). The X-ray structure of PolD lacks the catalytic Mg^{2+} ion, but the two aspartic acid residues in DPPB-2 were found essential for PolD activity, consistent with a role in Mg^{2+} coordination (80). According to the paradigm of two-barrel polymerases, the second, a DPBB-B-type barrel named DPBB-1, harbors two canonical lysine residues in both Qde-1 and PolD DP2 (73, 75). Beyond the two DPBB domains, no further structural similarity was detected between the msRNAPs Qde-1 and PolD.

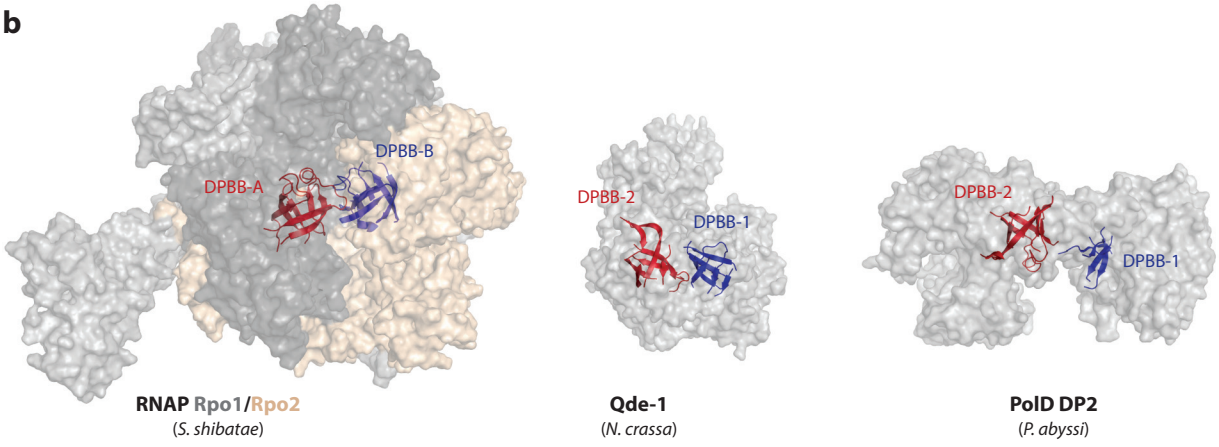
Despite the fact that the catalytic subunits of msRNAPs are highly conserved in all three domains of life, the largest subunit is encoded by two open reading frames of adjacent genes in archaea (Rpo1) and chloroplast plastids (β′) (64, 94). A closer look at RNAP gene organization in archaea reveals even higher levels of complexity. In methanobacteria and halobacteria (both archaea despite the misleading names), the largest (Rpo1) and second-largest (Rpo2) RNAP subunits are split, whereas in Thaumarchaeota and Korarchaeota, Rpo1 is encoded by a single open reading frame (15). These observations suggest that multiple split and fusion events of catalytic core subunits occurred. Interestingly, insertion of the corresponding split sites into the genes encoding the catalytic core subunits of *Escherichia coli* RNAP does result in active enzymes (77). In contrast to msRNAPs, Qde-1 and PolD contain the two DPBB domains within a single polypeptide chain, which could be the result of a fusion of DPBB-encoding genes. The genes encoding the largest and second-largest RNAP subunits are encoded in a polycistronic operon, and this organization is conserved between bacteria and archaea, whether the genes for the two subunits are split or not. In fact, epsilonproteobacteria harbor a single fused catalytic subunit encompassing both β and β′ (50), and the fusion of *rpoB* (encoding β) and *rpoC* (encoding β′) genes in *E. coli* results in functional RNAP in vitro and in vivo (76). The fused single catalytic core subunit encompasses DPBB-A in its C-terminal half and DPBB-B in its N-terminal half in the same order as is found in its counterparts, Qde-1 and PolD. In summary, the two DPBBs at the catalytic heart of msRNAP reveal an intriguing structural and functional conservation across a very broad range of two-barrel polymerases, and the remarkable variation of the arrangement of the genes encoding the DPBB subunits bears witness to several split and fusion events during evolution.

EVOLUTIONARY INSIGHTS FROM VIRAL TWO-BARREL RNAPS

Next to the two catalytic core subunits, all cellular msRNAPs include universally conserved subunits that play important roles for the efficient assembly of the two large catalytic subunits of

msRNAPs (31, 55). These include the α_2 homodimer in bacteria, which is homologous to Rpo3/11 and RPB3/11 in archaea and eukaryotes, respectively, and ω (Rpo/RBP6). The former constitute the RNAP assembly platform that in archaea and eukaryotes also includes the Rpo/RPB10 and Rpo/RPB12 subunits. Owing to the universal nature of assembly platform subunits, it was assumed that they were essential for the correct and stable folding of the catalytic subunits of two-barrel msRNAPs. Recently, Minakhin and coworkers (95) identified and biochemically characterized the





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msRNAP DPBB-A		Qde-1 DPBB-2		PolD DP2 DPBB-2	
Sulfolobus shibatae (Rpo1)	NADFDGD	*Neurospora crassa*	GGDYDGD	*Pyrococcus abyssi*	RRNCDGD
Homo sapiens (RPB1)	NADFDGD	*Ceraceosorus bombacis*	GGDYDGD	*Haloferax volcanii*	RRNCDGD
Homo sapiens (A190)	NADFDGD	*Arabidopsis thaliana*	GSDLDGD	*Methanococcus maripaludis*	RRNCDGD
Homo sapiens (C160)	NADFDGD	*Nicotiana tabacum*	GSDLDGD	*Korarchaeum cryptofilum*	RRNVDGD
Escherichia coli (β′)	NADFDGD	*Caenorhabditis elegans*	GSDLDGD	*Nitrosopumilus maritimus*	RRDADGD

first two-barrel msRNAP encoded by a giant bacteriophage. ϕKZ nonvirion RNAP (nvRNAP) is related to the msRNAP of the host it infects and is likely the result of a horizontal transfer of the genes encoding the two catalytic subunits. Interestingly, ϕKZ nvRNAP does not include any classical assembly platform subunits. However, ϕKZ nvRNAP harbors gp68, a subunit without any similarity to other proteins except for its homologs in related giant bacteriophages, which may play a role in nvRNAP assembly. Similarly, several msRNAPs from different eukaryotic virus families appear to lack assembly platform subunits. Insect baculoviruses encode nvRNAPs composed of only four subunits: The two largest subunits (LEF-8 and -9) share sequence similarity with the DPBB-A and -B of msRNAPs, and two additional subunits (LEF-4 and p47) have no sequence homology with any known msRNAP subunits (28, 71). Thus, these nvRNAPs lack distinguishable assembly subunits, which altogether demonstrates that these are not required for the efficient assembly of DPBB msRNAPs per se. Detailed structural and functional analysis of the nvRNAPs and its kindred is sure to reveal many surprises in the coming years.

THE ORIGINS OF THE BARRELS IN THE RNA WORLD?

Although msRNAPs chiefly function as DNA-dependent RNAPs, they can utilize RNA templates in some special cases, in vitro and in vivo. For example, human RNAPII facilitates replication of the hepatitis virus D genome by RNA template–dependent RNA synthesis (19, 67, 88). X-ray structures of yeast RNAPII with RNA scaffold templates show that it can accommodate an RNA duplex in a similar manner to the RNA-DNA hybrid in DNA-dependent msRNAPs. These results highlight the potentially ancient RNA-dependent activity of msRNAPs (53) and are in line with the idea of an RNA-protein world preceding the modern era of cells employing DNA as genetic material. The common ancestor of extant msRNAPs likely evolved from a primordial RNA-dependent two-barrel RNAP that consisted primarily of the DPBB motifs. In msRNAPs, the DPBB may later have contributed to its adaption so as to utilize double-stranded DNA as templates. It is thought that the primordial RNA-dependent RNAP appeared at the RNA world era and was a self-replicating RNA ribozyme (**Figure 2**). If that were the case, the processivity and fidelity of the primal ribozyme must have been sufficiently high for self-replication, something that has not been achieved yet with synthetic ribozymes in vitro (38). Following the emergence of templated protein synthesis, binding of an RNA-binding proteinaceous cofactor containing a DPBB domain to the catalytic core of the RNAP ribozyme may have increased its stability, processivity, and fidelity—all critical factors for efficient and faithful transcription. Given that

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Figure 1

Evolution of the catalytic core of two-barrel polymerases. (*a*) Structure of the conserved catalytic core of two-barrel multisubunit RNAPs (msRNAPs). Three conserved aspartic acid residues of double-Ψ double-β barrel (DPBB)-A (stick representations in *light pink*) are coordinating the catalytic magnesium ion (Mg^A). The two conserved lysine residues of DPBB-B are shown as stick representations (*light blue*). The catalytic center is occupied by UTP in complex with a second magnesium ion (Mg^B). The schematic is based on the structure of *Saccharomyces cerevisiae* RNAPII [Protein Data Bank identification (PDB ID): 2NVZ]. (*b*) Structural overview of the conserved catalytic core of two-barrel nucleic acid msRNAPs: RNA-dependent Qde-1 and DNA polymerase D (PolD). (*c*) Multiple sequence alignments of conserved catalytic motifs of (*left*) DNA-dependent RNAPs from *Sulfolobus shibatae* Rpo1 (ACL36488.1), *Homo sapiens* RPB1 (RNAPII: CAA45125.1), *H. sapiens* A190 (RNAPII: AA126304.1), *H. sapiens* C160 (RNAPIII: AAH41089.1), and *Escherichia coli* β′ (AIX65985.1); (*middle*) RNA-dependent RNAPs from *Neurospora crassa* (EAA29811.1), *Ceraceosorus bombacis* (CEH11733.1), *Arabidopsis thaliana* (AEE29226.1), *Nicotiana tabacum* (CAA09697.1), and *Caenorhabditis elegans* (CAA88315.2); and (*right*) PolD subunit DP2 from *Pyrococcus abyssi* (CAB49044.1), *Haloferax volcanii* (CAG38138.1), *Methanococcus maripaludis* (CAF29582.1), *Korarchaeum cryptofilum* (ACB08273.1), and *Nitrosopumilus maritimus* (ABX 13690.1). The conserved motifs are highlighted in pink. Catalytic aspartic acid residues are in boldface. Abbreviations: NT-DNA, nontemplate DNA; T-DNA, template DNA.

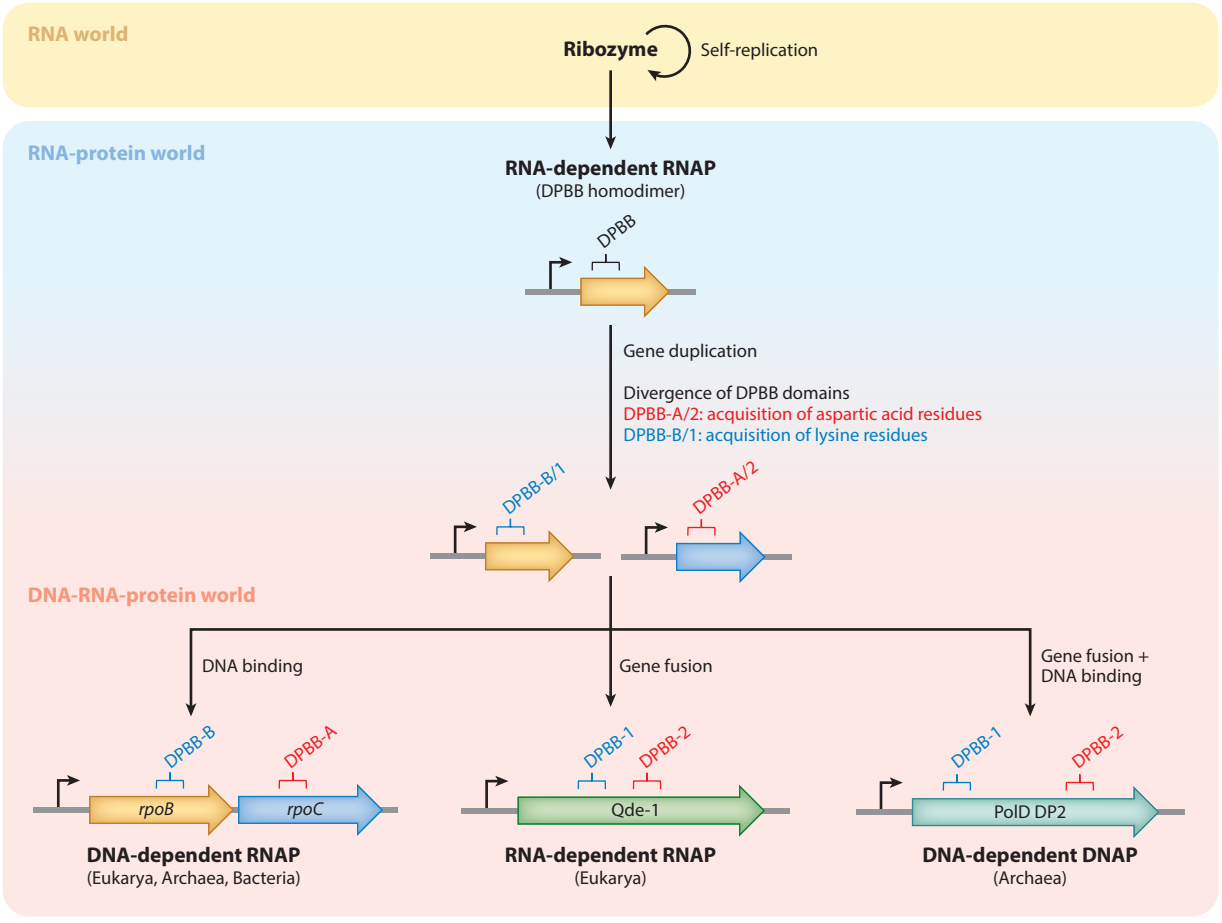

Figure 2

Hypothetical schematic for the evolution of two-barrel nucleic acid polymerases. The primordial RNAP was a self-replicating, RNA-dependent ribozyme that emerged in the RNA era (*yellow*). In the RNA-protein era (*blue*), the ribozyme was invaded by a cofactor containing a double-Ψ double-β barrel (DPBB) domain, forming a ribonucleoprotein complex. The ultimate ancestor of two-barrel polymerases functioned as a homodimer enzyme. Duplication followed by divergent evolution resulted in acquisition of aspartic acid residues by DPBB-A/2, and acquisition of lysine residues by DPBB-B/1. In the modern DNA-RNA-protein era (*pink*), the template specificity changed from RNA to DNA for multisubunit–RNA polymerase (msRNAP) and DNA polymerase D (PolD). In Qde-1 and PolD, the two DPBB domains are in a single polypeptide (DPBB-1 at the N terminus and DPBB-2 at the C terminus), suggesting a fusion of DPBB-encoding genes. msRNAP genes containing the DPBB domains are encoded in a polycistronic operon, and the organization is conserved between bacteria and archaea.

the two DPBB domains of two-barrel polymerases are related, the ancestor of those enzymes most likely functioned as a homodimer. Duplication followed by divergent evolution resulted in functional specialization of the two DPBB domains: acquisition of metal chelating aspartates by DPBB-A/2 and acquisition of basic residues by DPBB-B/1 (**Figure 2**). Crucially, the template specificity changed from RNA to DNA, and in an unexplained fashion catalysis was usurped by the DPBB proteins, and the now obsolete ancestral RNA was lost. RNAP evolved by increasing its bulk through the acquisition of modules/domains into the DPBB-containing large subunits and by increasing its subunit repertoire via accretion of additional, reversible associated factors

around the conserved core (37, 89). The ultraminimal active site of two-barrel msRNAPs appears to be composed of the two DPBB domains, the switch 2 element that interacts with the template DNA strand, and the secondary channel that allows for entrance of the nucleotide substrates (71). The reduced subunit repertoire of the different viral msRNAPs described above supports the idea that primordial msRNAPs were mainly composed of the two catalytic core subunits.

THE SEARCH FOR THE EVOLUTIONARY ORIGINS OF THE GENERAL TRANSCRIPTION FACTORS

In contrast to single-subunit RNAPs, such as bacteriophage T7 RNAPs, which are able to initiate transcription without additional factors, all cellular msRNAPs strictly rely on basal transcription factors. Basal transcription factors facilitate promoter recognition, local melting of DNA, and template strand loading into the RNAP active site to form the open complex (OC) ready for transcription initiation. Bacterial RNAPs rely chiefly on a single σ^{70}-related transcription factor, whereas archaeal transcription initiation involves three basal transcription factors, TBP, TFB, and TFE (29, 66, 92). TFE appears to have evolved originally as a heterodimeric factor with α and β subunits, but many archaea retained only the α subunit (11). In eukaryotes TBP- and TFB-related factors are required for transcription initiation by the three canonical nuclear RNAPs. Similar to the case of the archaea, the combination of TBP and TFIIB is necessary and sufficient for site-specific transcription initiation of eukaryotic RNAPII in vitro on strong promoters using a negatively supercoiled DNA template topology (29, 61, 66). Archaeal TFE has counterparts in the RNAPII and III transcription machineries (TFIIE and the RNAPIII subcomplex C82/34, respectively) that appear to carry out similar functional roles in OC formation, with additional functions in the recruitment of basal transcription factors or RNAP itself (11, 17, 33, 58, 63, 92). All other eukaryotic basal transcription initiation factors appear to be specific to this domain of life.

None of the three archaeo-eukaryotic basal transcription factors has a clear homologous counterpart in bacteria; nevertheless, some intriguing clues about their evolutionary origin are emerging. (*a*) TBP is a highly symmetrical, saddle-shaped protein that consists of two β-sheet domains called TBP domains. Interestingly, individual TBP domains that are ubiquitous in all three domains of life are present in some bacterial nucleases (RNaseH III) and DNA glycosylases, which demonstrates that the ancestry of the TBP domain predates the last universal common ancestor (LUCA) (14) (**Figure 3*a***). (*b*) The C-terminal core domain of TFB/TFIIB includes two multihelix bundle–type helix-turn-helix (HTH) motifs that are distantly related on the structural level to the three-helix HTH motifs present in bacterial σ^{70} (1, 34) (**Figure 3*b***). The internal symmetry of the TFB/TFIIB core domain dictates that the evolutionary ancestor must have contained a single multihelix-type HTH motif. (*c*) The two subunits of TFE/TFIIE contain winged HTH motifs, structural motifs abundant in all three domains of life. Sequence analysis of the winged HTH motifs from both TFE subunits suggest that they share a common ancestry with transcription regulators of the MarR family (1, 10, 12) (**Figure 3*c***).

ARE BACTERIAL SIGMA AND ARCHAEO-EUKARYOTIC TFB/TFIIB FACTORS RELATED?

It has been proposed by Iyer and Burton that bacterial σ^{70} and archaeal and eukaryotic TFB/TFIIB share ancestry, based on the fact that the C-terminal HTH motifs in both cases are involved in promoter recognition at similar positions relative to the transcription start sites (TSSs) (18, 34). Their binding mode follows the canonical manner in which DNA binds HTH motifs, i.e., intercalating α-helix 3 into the major groove of the DNA template. Recent high-resolution structures

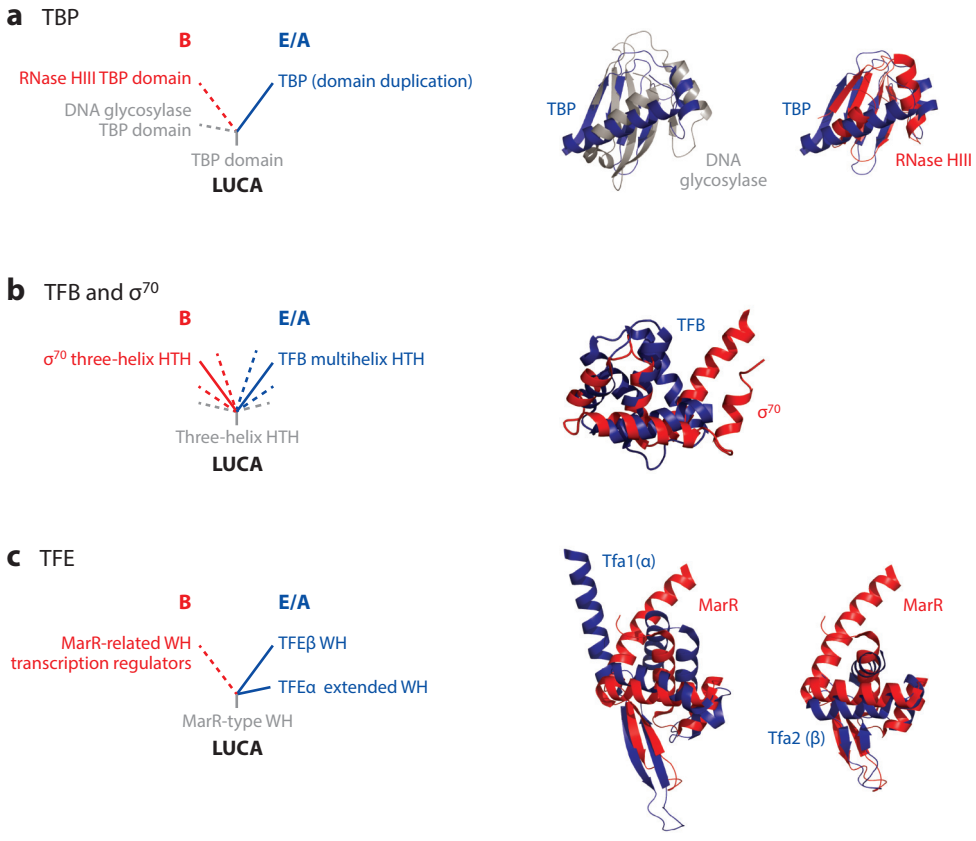

Figure 3

Evolutionary origins of basal transcription factors. Schematic phylogenetic relationships of structural motifs present in bacterial (B, *red branches*) and eukaryotic/archaeal (E/A, *blue branches*) basal transcription factors, based on References 1, 10, and 14. Structurally related proteins with different functions are indicated by dotted lines. Gray branches indicate presence of the respective structural motifs in universally conserved proteins. (*a*) Proteins containing TBP domains were already present in the last universal common ancestor (LUCA), but the domain duplication observed in TBP is unique to this protein. (*b*) In TFB/TFIIB, two multihelix bundle–type helix-turn-helix (HTH) motifs are found that are probably derived from basic three-helix HTH motifs (1). Three-helix HTH motifs are found in bacteria-specific and archaea-eukaryotic lineages as well as in several universally conserved proteins (1). The bacteria-specific proteins with basic three-helix HTH motifs include σ^{70}. (*c*) MarR-related winged HTH (WH) motifs are abundant in bacterial transcription regulators and are distantly related to the WH motifs present in the two subunits of archaeal TFE (TFEα and TFEβ) and eukaryotic TFIIE (termed Tfa1 and Tfa2 in yeast). The overlay of the structural motifs is based on the following Protein Data Bank (PDB) entries: *Sulfolobus acidocaldarius* TBP (PDB ID: 1MP9), *Escherichia coli* DNA glycosylase II (1MPG), *Geobacillus stearothermophilus* RNase HIII (2D0A), *Saccharomyces cerevisiae* Tfa1 and Tfa2 (5FYW), *E. coli* MarR (1JGS), *Pyrococcus woesei* TFB (1D3U), and *E. coli* σ^{70} (4YG2).

of bacterial and eukaryotic closed and open complexes have provided us with a more detailed picture of transcription initiation of σ^{70}- and TBP/TFB-related transcription machineries (5, 30, 63, 102). But even with this additional structural insight, it appears the homology between σ^{70} and TFB is limited to the canonical HTH recognition of promoter DNA.

ANALOGOUS INITIATION MECHANISMS IN THE THREE DOMAINS OF LIFE

Despite the lack of robust homology between the basal transcription factors, there are several features shared between the bacterial and archaeal/eukaryotic transcription initiation machineries. First, the extent of the DNA bubble formed during OC formation is nearly identical in the archaeal and the bacterial OCs, based on both permanganate footprinting assays (7, 11, 57, 74) and recent high-resolution structures of the bacterial OC (102). In bacteria, the border of the DNA bubble is marked by the −10 promoter element, an AT-rich promoter element, while archaeal promoters generally show a preference for A/T around position −10 without constituting a sequence motif, as found in proper promoter elements facilitating specific interaction with basal transcription factors (12). Second, transcription initiation universally prefers purine residues as initial nucleotides preceded by a pyridine in the corresponding −1 position on the nontemplate strand, as revealed by RNA-seq TSS mapping data from bacteria and archaea (4, 20, 42, 93). *E. coli* in vitro transcription experiments using a library of randomized sequences confirmed the YR nucleotide preference at position −1/+1 (87). The same preference appears to be echoed in the consensus of human and *Drosophila* initiator promoter elements (39). Third, TF(II)B and σ^{70} both facilitate transcription initiation by stabilizing the template strand through the TF(II)B B-reader element and σ^{70} region 3.2, respectively (3, 5, 48, 72, 102). And lastly, σ^{70} and TFE/TFIIE both prevent the universally conserved transcription elongation factor NusG/Spt5 and its bacterial paralog RfaH from associating with the RNAP during the initiation stage (9, 27, 54, 78). It is important to stress that a shared feature between the bacterial and archaeal transcription initiation machineries does not necessarily mean that the feature is evolutionarily conserved. Rather, the YR preference at position −1/+1 appears to reflect the ability for a template strand purine at position −1 to stabilize the incoming +1 nucleotide via base-stacking interactions, and this feature might be common to all types of RNAPs. This was identified not only in the initially transcribing complex of an msRNAP from *Thermus thermophilus* (6) but also in the evolutionarily unrelated single-subunit bacteriophage N4 RNAP (25).

The apparent absence of universally conserved basal transcription factors facilitating transcription initiation is in contrast to the universal conservation of the transcription elongation factor NusG/Spt5 (90). This prompted us to speculate (*a*) that the regulation of elongation preceded the regulation of initiation in the primordial transcription system of LUCA—the elongation-first hypothesis—and (*b*) that initiation may not have been start-site-specific prior to the emergence of dedicated initiation factors (91). It remains impossible to infer whether the basal transcription machinery of LUCA contained TFB/TBP-like or σ^{70}-like factors, a combination of both, or none (91). Nevertheless, it is worth considering the possible scenarios in the context of other basal transcription machineries in extant life-forms and their viruses. The focus on TFB/TFIIB and σ^{70} blends out the real complexity of the different transcription initiation pathways that evolved in cellular life as well as in the virosphere. In fact, a third, phylogenetically unrelated basal transcription factor evolved in bacteria: σ^{54}. Although σ^{54} and σ^{70} are composed of multiple domains with similar functions, these domains are not homologous (96). It is generally thought that an evolutionary advantage of σ^{54} may lie in tighter gene regulation, as σ^{54}-mediated transcription initiation is fully dependent on the ATPase activity of bacterial enhancer binding proteins. σ^{54} and σ^{70} are able to regulate transcription of the same genes by using alternative promoters with different TSSs (16). The patchy but phylogenetically broad distribution of σ^{54} suggests that two different types of basal transcription factors that coevolved with their own sets of transcriptional regulators have coexisted in bacteria since the early stages of bacterial evolution.

Within the boundaries of cellular life, the strict separation of TBP/TFB- and σ^{70}-based transcription systems in archaea and bacteria, respectively, was recently challenged by the discovery of genes encoding σ^{70} homologs in several novel archaeal species by single-cell genomics (69). Phylogenetic analysis of these genes suggests that they are derived from horizontal gene transfer from bacteria. Even though the genome sequences of these archaeal species are still incomplete, it appears that all of these species also possess the canonical archaeal basal transcription factors (69). Whether the archaeal σ factors actually play a role in transcription in these species remains to be functionally verified.

CLUES FROM UNORTHODOX RNAPS FROM BACTERIOPHAGES AND EUKARYOTIC VIRUSES

The ability of msRNAPs to evolve an alternative basal support machinery, unrelated to TFB and σ factors, was recently highlighted by the biochemical characterization of transcription initiation by ϕKZ nvRNAP (95). ϕKZ nvRNAP appears to be required for transcription from late promoters in the bacteriophage genome. The full context of promoter elements directing transcription initiation is not yet fully understood, but a TATG motif stretching from −3 to +1 relative to the TSS is essential. Transcription initiation of ϕKZ nvRNAP is not dependent on additional basal transcription factors; however, it is possible that the gp68 subunit plays a role in transcription initiation in vivo.

The discovery of giant viruses belonging to the proposed order Megavirales may bring yet more surprises about the evolution of msRNAPs in the virosphere and their mechanisms of transcription initiation. Megaviridae and Poxviridae are double-stranded DNA viruses that encode msRNAPs related to eukaryotic RNAPII (45, 56, 79, 98), and in some cases divergent homologs of the basal transcription factors TBP and TFIIB (35, 97). The African swine fever virus (ASFV) is an extremely potent pathogen that causes hemorrhagic fever in domesticated pigs. ASFV genomes encode seven polypeptides that are related to RNAPII subunits, including the two large DPBB-containing catalytic subunits, and a fusion protein containing the two RPB3 and RPB11 assembly platform subunits. Perhaps most surprising, although ASFV encodes a protein that is distantly related to TFIIB, no TBP homologs could be identified (70). Extracts prepared from ASF viroids are transcription competent (49), and since ASFV is propagated in two very different host environments (wild pigs, such as warthogs and bushpigs, and Argasidae ticks), it is likely that the viral genome indeed encodes all of the components required for transcription without the need to co-opt factors from the host cell. Only a few ASFV promoters have been partially characterized, none of them including classical RNAPII-like promoter elements such as BRE or TATA motifs (i.e., binding sites for TFIIB and TBP) at a meaningful distance from the mapped TSSs (70).

Despite the increasing volume of information regarding the molecular mechanisms of transcription initiation in bacteria, archaea, and eukaryotes, the lack of extended homology between TFB/TFIIB and σ^{70} makes it challenging if not impossible to draw persuasive conclusions about the nature of basal transcription factors in LUCA. Meanwhile, an increasing amount of genomic and biochemical data from microbial dark matter, bacteriophages, and eukaryotic viruses draw a more complex picture, with alternative modes of transcription initiation and interdomain gene transfer of both RNAP subunits and basal transcription factors. The example of σ^{54} and σ^{70} as two basal transcription factors coexisting in the same organism and coregulating transcription might suggest that TFB/TBP and σ^{70} coevolved in LUCA from independent origins, rather than both factors evolving from the same proto–transcription factor present in LUCA before their structural and functional divergence in bacteria and archaea/eukaryotes (12).

THE CONNECTION BETWEEN TRANSCRIPTION INITIATION AND TRANSLATION INITIATION

The functional and structural diversity of basal transcription initiation mechanisms in cellular life make it difficult to draw conclusions on the nature of the basal transcription machinery in LUCA. However, some of its functional properties can be deduced. To this end, it is worth considering the products of transcription, coding and noncoding RNA (ncRNA), in regard to their specific requirements of TSS selection. All three domains of life share two conserved translation initiation factors: IF1 and IF2 in Bacteria, and aeIF-1a and aeIF5B in Archaea and Eukarya. Their conserved role is thought to be guiding the aminoacylated initiator tRNA to the P site (8). Additional nonhomologous translation initiation factors are present in Archaea and Bacteria, and the two primary domains especially diverged in the selection of the aminoacylated initiator tRNA (8).

Two conserved modes of translation initiation can be distinguished in bacteria and archaea: 70S ribosome initiation on leaderless mRNA and initiation starting with binding of the 30S ribosomal subunit to a ribosomal binding site (RBS) present in mRNAs with a 5′-UTR (untranslated region) as well as in downstream cistrons of polycistronic mRNAs (8). In bacteria, translation initiation from leaderless mRNA can occur in a factor-independent manner (85). The molecular basis of leaderless translation initiation in archaea is not yet understood. RBS-dependent translation initiation generally requires the aid of initiation factors. Based on their broad occurrence across the two prokaryotic domains of life, it is highly likely that both leaderless and RBS-dependent translation initiation mechanisms were operating in LUCA (59, 101). It has been argued, however, that leaderless translation initiation is more ancient (8). This is based on the fact that leaderless mRNAs can be utilized in all three domains of life: more generally in Archaea and Bacteria (20, 93, 101), but also in the protozoan *Giardia lamblia* (23) as well as in a rabbit reticulocyte in vitro translation system (26). Since leaderless translation initiation requires that the TSS and the start codon overlap, the universal preference of msRNAPs to initiate transcription with guanine nucleotides and the choice of ATG/GTG as start codons could be functionally linked.

RBS-dependent and leaderless translation initiation have distinct advantages in terms of gene regulation. RBS-dependent translation is thought to aid the coordinated expression of genes organized in operons (**Figure 4*a***) (101). This is of critical importance especially for larger hetero-oligomeric complexes such as ribosomes and msRNAPs themselves. Indeed, in organisms that preferably use leaderless mRNAs, such as *Mycobacterium* and the archaeon *Sulfolobus*, 5′-UTRs are still retained in the mRNAs of ribosomal protein-encoding genes (20, 93). The operon encoding the two catalytic subunits of RNAP is a rare example of gene organization being conserved between bacteria and archaea, testifying to the importance of operons and RBS-dependent translation initiation in the coordinated expression of components of large hetero-oligomeric complexes. In both primary domains, transcription and translation are physically coupled, and RBS-dependent translation initiation might facilitate the coordination between the two processes. Indeed, recent NET-seq (native elongating transcript sequencing) data from *E. coli* and *Bacillus subtilis* RNAPs show that they tend to pause at translation start sites, possibly ensuring maintenance of coupling (52). RBS-dependent translation initiation also allows for multiple promoters/TSSs to be used to regulate transcription of a gene (**Figure 4*a***). Lastly, RBS-dependent translation from 5′-UTRs containing mRNAs can be regulated by small RNAs either by blocking access to the RBS or by enabling access to it through changes in secondary structure (82). On the other hand, leaderless mRNAs are thought to allow for tighter regulation, preventing gene expression from spurious transcription or read-through from transcriptional units placed upstream in sense orientation (**Figure 4*b***) (13, 101).

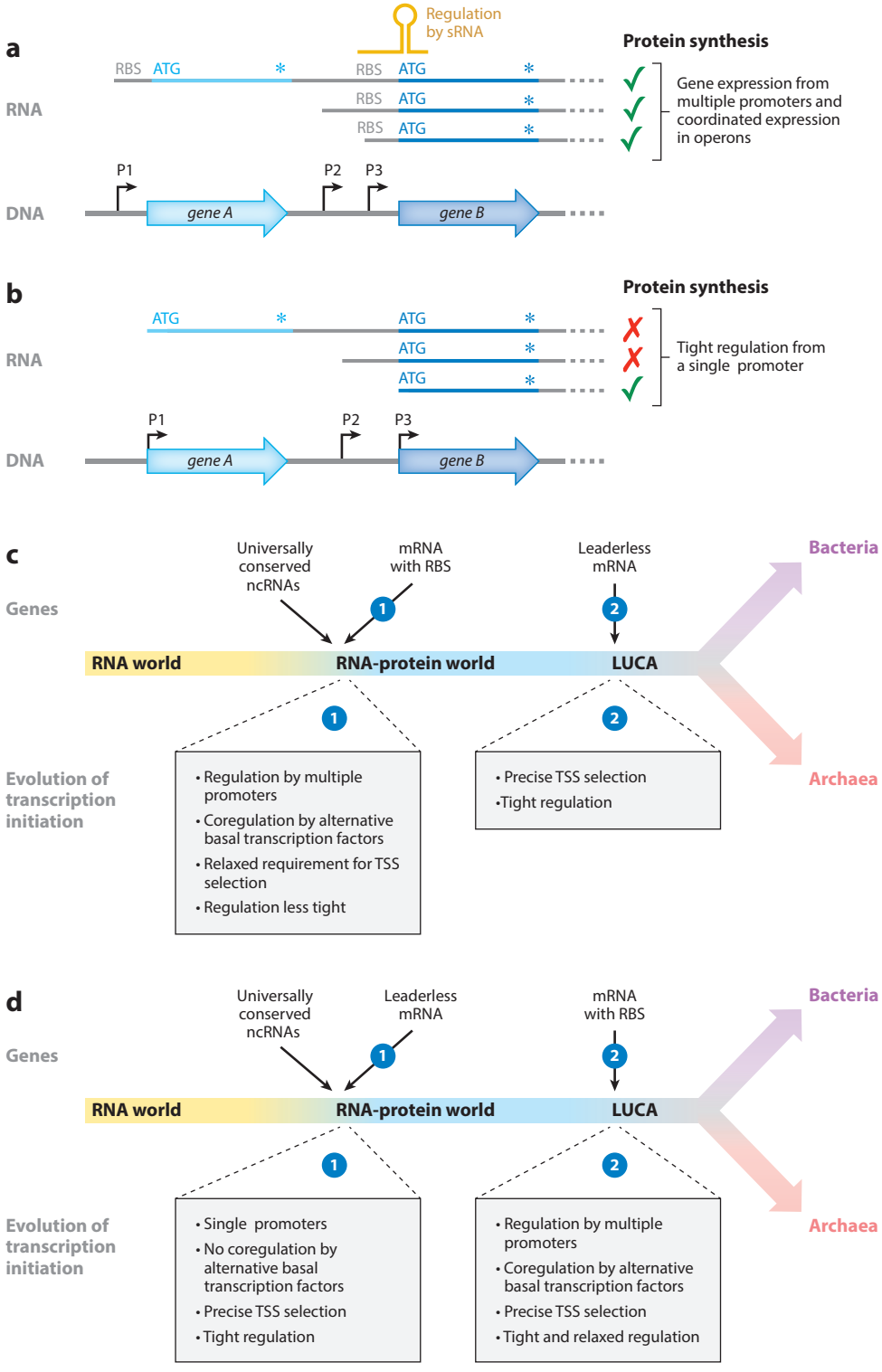
a
Regulation by sRNA
Protein synthesis
RBS ATG * RBS ATG *
RBS ATG *
RBS ATG *
RNA
Gene expression from multiple promoters and coordinated expression in operons
P1 P2 P3
DNA
gene A
gene B
b
Protein synthesis
ATG * ATG *
ATG *
ATG *
RNA
Tight regulation from a single promoter
P1 P2 P3
DNA
gene A
gene B
c
Universally conserved ncRNAs
mRNA with RBS
Leaderless mRNA
Bacteria
Genes
1
2
RNA world
RNA-protein world
LUCA
1
2
Evolution of transcription initiation
• Regulation by multiple promoters
• Coregulation by alternative basal transcription factors
• Relaxed requirement for TSS selection
• Regulation less tight
• Precise TSS selection
•Tight regulation
Archaea
d
Universally conserved ncRNAs
Leaderless mRNA
mRNA with RBS
Bacteria
Genes
1
2
RNA world
RNA-protein world
LUCA
1
2
Evolution of transcription initiation
• Single promoters
• No coregulation by alternative basal transcription factors
• Precise TSS selection
• Tight regulation
• Regulation by multiple promoters
• Coregulation by alternative basal transcription factors
• Precise TSS selection
• Tight and relaxed regulation
Archaea

Figure 4

Interdependency of transcription and translation initiation: (*a*) The consequences of a 5′-UTR (untranslated region) containing messenger RNAs (mRNAs) and (*b*) leaderless mRNAs on the regulation and coordination of gene expression. (*a*) Whereas ribosomal binding site (RBS)-dependent translation initiation allows for protein expression from mRNAs synthesized from different transcription start sites (TSSs), (*b*) leaderless translation initiation requires transcription to initiate from a single TSS. The order of evolution of translation initiation on leaderless mRNA and RBS-dependent translation initiation has major implications for the evolution of transcription initiation with regard to TSS selection and regulatory mechanisms. (*c*) An early evolution of RBS-dependent translation initiation ❶ would allow for gene expression to be directed from multiple promoters with a relaxed requirement for precise TSS selection. It would also facilitate the coordinated expression of genes, such as those coding for the components of hetero-oligomeric complexes. The evolution of translation initiation from leaderless RNAs ❷ would add a mechanism that enables tight gene regulation and minimization of gene expression arising from spurious transcription read-through. (*d*) A late appearance of RBS-dependent translation initiation ❷ with translation initiation exclusively from leaderless mRNAs at early stages of evolution of cellular life ❶ would require the early evolution of basal transcription factors enabling precise TSS selection and the use of single promoters. Abbreviations: LUCA, last universal common ancestor; ncRNA, noncoding RNA.

Structural features of ncRNA genes may provide additional clues to these questions. There are four types of universally conserved ncRNA genes/operons: tRNA, rRNA operons, 4.5S RNA (the RNA component of the signal recognition particle), and the RNA component of RNase P. The majority of these universally conserved ncRNA genes undergo 5′-end processing: The 16S rRNA gene is the first gene in the rRNA operon. The 5′ end of mature 16S rRNA is generated via the combined action of several RNases in bacteria (2). RNase P is required for 5′-end processing of tRNA as well as 4.5S RNA (41, 62, 84). The requirements for the maturation of the RNA components of RNase P itself are less clear, but it has been reported that the productive transcription of M1 RNA, the RNA component of RNase P in *E. coli*, is driven from a proximal promoter that does not require 5′-end processing (46). Most of the universally conserved ncRNAs require 5′-end processing, and this requirement could reflect the functional properties of the early transcription initiation machinery. However, it should be mentioned that 5′-end processing is also required for many domain-specific ncRNAs, such as transfer-messenger RNA and 6S RNA, which evolved later in the bacterial domain (43, 47, 84). On the other hand, it has been shown that the universal requirement for 5′-end processing of tRNA by RNase P is not essential for life and has been overcome by transcription initiation at the proper 5′ end in the archaeon *Nanoarchaeum equitans* (68). Independent of the 5′-end processing requirements for these universally conserved RNAs, it can be inferred that the arguably oldest genes probably have a relaxed requirement for TSS selection, allowing for multiple promoters/TSSs to be utilized.

CONCLUSION

The discovery of viral msRNAPs with a reduced subunit repertoire and basal transcription factor requirement and the discovery of two-barrel DNA polymerases have advanced our understanding of the evolution of msRNAPs and the crucial role of the DPBB domains. Viral msRNAPs have evolved divergent catalytic subunit assembly pathways and mechanisms for site-specific transcription initiation that may provide clues to the evolution of transcription in cellular life. Transcription is the first step in gene expression and protein synthesis. The mechanisms of transcription initiation and TSS selection thereby directly affect the mechanism of translation initiation and vice versa. We argue that RBS-dependent translation initiation (and 5′-end processing of ncRNAs)

might have contributed to an environment conducive to the evolution of alternative basal transcription factors, such as σ^{70} and TBP/TFB in the same organism (**Figure 4*c***). An alternative model, with precise selection of a single TSS coupled to leaderless translation initiation, would impose several restrictions on the organism in terms of the regulation of gene expression and the ability to evolve alternative basal transcription factors (**Figure 4*d***). For these reasons, we consider the most likely scenarios to be the early appearance of RBS-dependent translation initiation and the parallel evolution of multiple basal transcription initiation factors.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This work was supported by Wellcome Trust Investigator Award WT096553MA to F.W.

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