Review

DNA as a Double-Coding Device for Information Conversion and Organization of Self-Referential Unity

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DNA as a Double-Coding Device for Information Conversion and Organization of Self-Referential Unity

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Abstract: Living systems are capable on the one hand, of eliciting a coordinated response to changing environment (aka adaptation) and on the other hand, they are capable of reproducing themselves. Notably, adaptation to environmental change requires the monitoring of the surroundings, while reproduction requires monitoring oneself. These two tasks appear separate and making use of different sources of information. Yet both the process of adaptation as well as that of reproduction are inextricably coupled to alterations of genomic DNA expression, while a cell behaves as an indivisible unity in which apparently independent processes and mechanisms are both integrated and coordinated. We argue that at the most basic level, this integration is enabled by the unique property of the DNA to act as a double coding device harboring two logically distinct types of information. We review biological systems of different complexity and infer that inter-conversion of these two distinct types of DNA information represents a fundamental self-referential device underlying both systemic integration and coordinated adaptive response.

Keywords: self-referential system; DNA information; supercoiling; gradients; nucleoprotein complexes; inter-conversion of distinct information types

1. Introduction

The distinctive organizational hallmark of living systems is their ability to self-reproduce. For that matter, living systems are regarded as self-referential systems implying the capacity to monitor oneself, that is, to perpetually assess their status quo [1,2]. At the same time, living systems are capable of monitoring their surroundings and eliciting a functionally coordinated adaptive response to environmental change (Figure 1). The capacity of 'monitoring oneself' assumes that the system divides itself, as it were, into two parts, that which monitors and that which is monitored. Furthermore, monitoring oneself and monitoring environment appear as separate tasks utilizing different sources of information. In multicellular eukaryotes relative independence of the information used to control cellular reproduction and functional specialization is apparent in separation of the processes of proliferation and differentiation [3] observed in most cells, especially during development.

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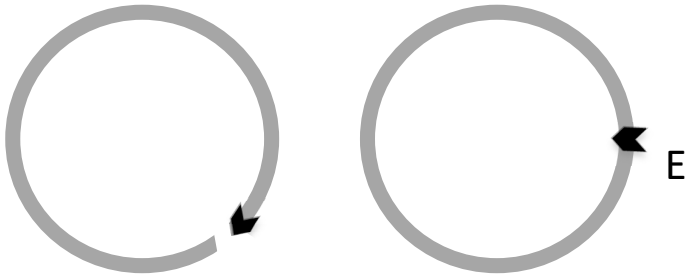

Figure 1. The self-referential organisation of living system is represented by an arrow, which closes on itself (left panel). The self-pointing arrow is a symbol for the condition in which the system divides itself into that which monitors and that which is monitored [1]. The system thus constitutes an isolated, operationally closed circuit. The environment (the space outside of the circle circumference) is marked as 'E' (right panel). The environmental impact on the system is indicated by black arrow crossing the circle circumference from outside to inside. Unless this environmental impact is deteriorating, the operational closure of the system is retained.

In unicellular organisms such as bacteria, alteration of e.g. the cell motility in response to environmental signals (chemotaxis) and the process of cell division are also regulated independently. For example, control of bacterial cell density (quorum sensing) is executed by autocrine/paracrine signaling pathways involving autoinducer molecules [4], whereas bacterial chemotaxis is induced by environmental factors including e.g., those produced by plants [5–7]. In *Caulobacter* system the cell differentiation can be decoupled from DNA replication [8] and the processes of cell division and differentiation are distributed between the two morphologically and developmentally distinct daughter cells [9]. However, both in eukaryotes and prokaryotes the information underpinning the apparently independent processes of reproduction and adaptation is encoded in the very same DNA genome being reflected in, and largely governed by, the genetic control mechanisms. Furthermore, even if the cellular reproduction and the adaptive environmental response may utilize different information sources and independent regulation mechanisms, it is obvious that the intrinsic organization of a cell endows it with the capacity to behave as a whole, indivisible unity in which apparently independent processes and mechanisms are both coordinated and integrated [3,7,10]. Importantly, a coordinated switch in gene expression during transition between bacterial motile and biofilm lifestyles appears to involve a change in chromosome structure [11]. Also the switching between alternative gene expression programs both during the growth cycle and in response to various stress impacts involves coordinated alterations of DNA topology, coherently modulating the gene expression in extended chromosomal domains [12,13]. So then, assuming that both the genetic expression and the structural dynamics of the genomic DNA polymer are intimately involved in this integration process, the central question to address is the nature of the coordinating device.

2. DNA Is a Source of Two Distinct Types of Information

The still largely underappreciated characteristic of the double helical DNA polymer is that it is a source of two logically distinct types of information. One is the well-known linear genetic code, which is discontinuous (digital) being embodied in discrete triplets of base pairs – the codons (Figure 2). The other source of information stored in the DNA is of continuous (analogue) nature, being embodied in juxtaposition of distinct base steps, which partly overlap [14–16].

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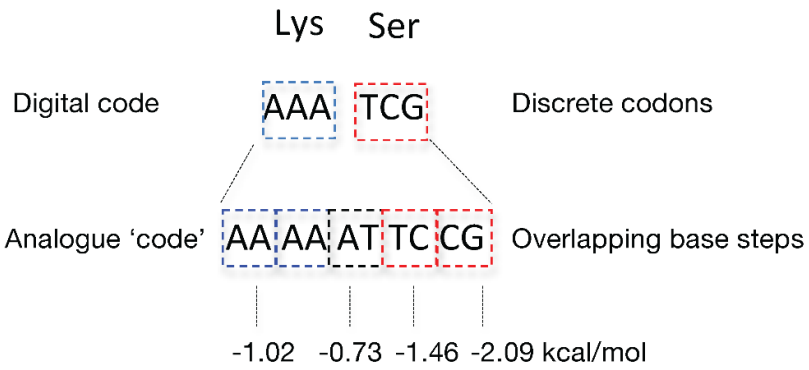

Figure 2. Relation between the digital genetic code and the analog information stored in overlapping base steps of the DNA. Two deliberately chosen triplets coding e.g. for lysine and serine are indicated as 'digital code'. Indicated below as 'analogue code' are the five consecutive overlapping base steps harbored in these two codons. The free stacking/melting energies of base steps [14] are indicated underneath (see also Table 1).

Table 1. Free stacking/melting energy.

Base steps	kcal/mol
AA/TT	-1.02
AT/AT	-0.73
TA/TA	-0.6
CA/TG	-1.38
GT/AC	-1.43
CT/AG	-1.16
GA/CT	-1.46
CG/CG	-2.09
GC/GC	-2.28
GG/CC	-1.77

Since in contrast to the codons, the base steps are overlapping (that is, each first base of any base step is the second base of a previous step and each second base of any base step is the first base of a following step), it is exactly this latter feature, which confers the character of continuity to the DNA analogue 'code'. Importantly, various base steps are characterized by distinct stacking/melting energy levels (Table 1) and also, can adopt different preferential conformations [14–18] (Figure 3).

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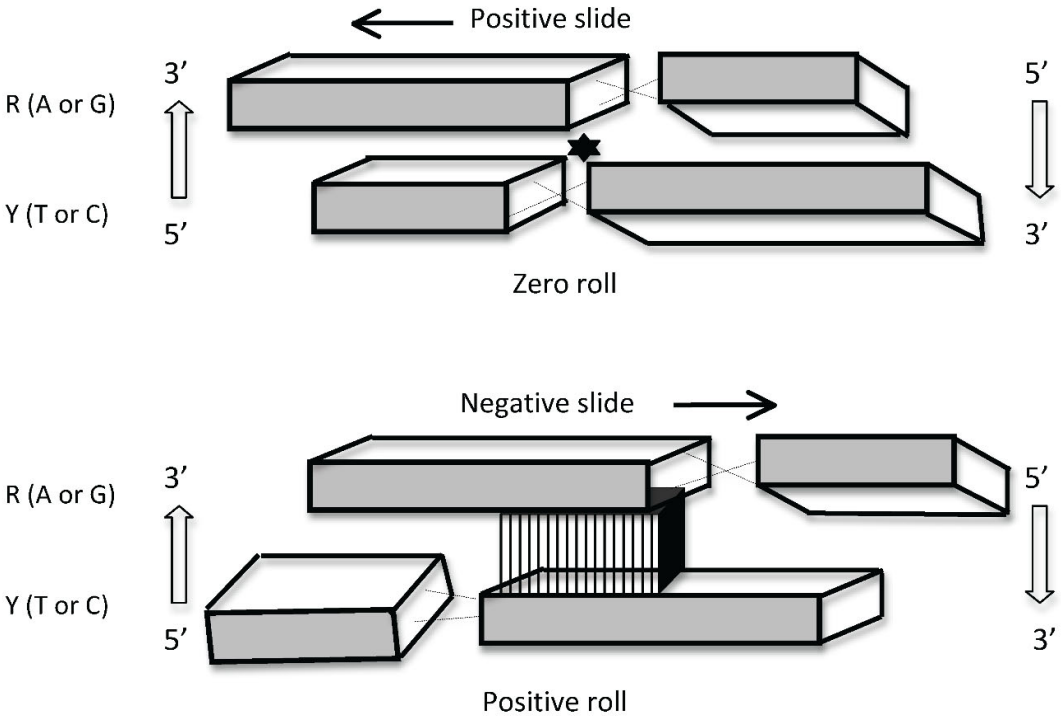

Figure 3. Pyrimidine-purine (YR) base steps are flexible and can adopt various configurations. Purine and pyrimidine bases respectively are indicated by large and small rectangles. The asterisk in upper panel indicates the potential steric clash between large purine bases, which is avoided by positive slide of the base pairs. In lower panel this base step has a negative slide and positive roll, and this alternative configuration is stabilized by increased cross-chain stacking interaction between the large purine bases (vertically striated region between the large rectangles). The minor groove side of the bases is shaded (after [18]).

The contiguous base steps favoring various local conformations can determine the 3D configuration and average trajectory of the DNA [15,16,18,19]. Importantly, the base stacking and accordingly, the conformation of DNA base steps can be modulated by environmental conditions inducing alterations of DNA twist eventually affecting the DNA helical repeat and the torque accommodated by the double helix. The configuration of DNA depends both on sequence organization and average superhelical density [20–22] as well as on the size of the affected topological domain [23].

In the bacterium *E. coli* the DNA superhelical density varies as a function of cellular energy charge, which depends on, and changes with, the environmental conditions. The level of negative DNA superhelicity varies as a function of ATP/ADP ratio, primarily because ATP is utilized by DNA gyrase, an enzyme introducing negative supercoils into the DNA [24–27]. Both the ATP/ADP ratio as well as gyrase activity increase on nutritional shift-up, e.g., when the starved bacterial cells are inoculated in fresh growth medium. However, at this stage there is also a more direct effect on DNA topology (namely on DNA twist) mediated by changing ionic composition [28–33]. In other words, changing environmental conditions altering the DNA superhelicity eventually stabilize distinct DNA structures depending on sequence organisaton and size of the affected domain. Ultimately, the sensing of available metabolic energy and ionic composition by DNA would select the configuration and topology optimally adapted to given environmental impact. In turn, alterations of DNA configuration are relevant to gene expression, as the DNA binding ligands, architectural proteins and enzymes (e.g. the transcription and replication machinery) show preferences for particular DNA topology [34–44]. The DNA binding proteins can in turn stabilize different DNA deformations such

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as bending, over- or under-twisting, wrapping, looping, bridging as well as can constrain DNA supercoils [45–49]. All these effects are pertinent, as the various assembled nucleoprotein complexes modulate the genetic expression. Furthermore, on translocation along the DNA template, the transcription and replication machineries directionally modulate the DNA superhelicity, inducing positive supercoils ahead and negative supercoils in their wake [50]. Diffusion of these induced free supercoils can distinctly affect the activity of neighboring genes in genome [51–53]. In addition, this topological differentiation of DNA on opposite sides of the moving DNA translocases has a potential to spatially organize the binding of regulatory proteins recognizing distinct DNA supercoil structures [23].

Thus in principle, coordination of different genetic programs (e.g. those governing self-reproduction and those for adaptive response) could be achieved simply by arranging the genomic DNA analogue information in such a way as to couple the emergence of distinct 3D DNA structures and particular DNA topology to different internal and external impacts on the one hand, and on the other hand, to employ these distinct structures for selective and coordinated readout of the digital (genetic) code optimizing the expression of traits apt for coping with given demands. The expression of different genetic programs in response to both environmental and internal signals would be then integrated and coordinated by variation of a single, continuous tunable parameter sensitive to both internal alterations and environmental change. Conceivably, the superhelicity of DNA serving as an interface between the external and internal milieu, is the most plausible contender for the role of pivotal variable adjusting the dynamics of DNA analogue information (i.e. the genomic DNA configuration) and the pattern of gene expression in response to both internal and external signals. Indeed, in bacteria the induction of distinctly different patterns of gene transcription coupled to activation of disparate genetic functions have been observed in response to directional modulation of DNA superhelical density by environmental stress or topoisomerase poisons and inhibitors as well as in response to topoisomerase gene mutations [12,13,54–59].

In bacteria, the role of DNA topology in coordinating the genetic adaptive response with various environmental cues is well documented [13,60,61]. Also in experimental evolution studies, modulation of global regulatory networks and DNA topology were identified as main internal factors subject to the process of selection [62,63]. During the bacterial growth cycle, the successive stages of cell reproduction (aka exponential growth phase) and maintenance (aka stationary phase) are long known to be associated with distinct – respectively high and low – negative superhelical densities of the DNA [64]. Notably, the spatial separation of relatively G/C-rich and relatively A/T-rich sequences, organized respectively around the *oriC* and *ter* poles of the *E. coli* chromosome, allows for temporal separation of gene expression at the two chromosomal poles due to growth phase-dependent changes of superhelicity. Indeed, the chromosomal *oriC* pole is not only G/C-rich relatively to the *ter* pole but is also enriched for gyrase binding sites [54,65,66]. On nutritional shift-up, the increase of negative superhelicity at this chromosomal pole is reinforced by production of negative supercoils trailing both the translocating replisomes and the trains of RNA polymerase σ70 holoenzyme molecules transcribing the numerous strong ribosomal RNA operons, all of which are directionally oriented from *oriC* towards the terminus of chromosomal replication [65]. The vegetative σ70 RNA polymerase and the stationary phase σS holoenzymes respectively prefer highly supercoiled and relaxed DNA templates and accordingly, are activated in succession during the growth cycle [34,36,67,68]. The frequency distributions of σ70 and σS binding sites form correspondingly decreasing and increasing spatial gradients along the chromosomal *oriC-ter* axis [66]. Thus the *oriC* pole (the Ori macrodomain and the flanking left and right non-structured domains) of the *E. coli* chromosome is enriched for σ70 binding sites and transcribed by σ70 RNA polymerase both earlier and more actively than the *ter* pole enriched for σS binding sites, giving rise to early gene products underpinning fast growth and replication [12,66,69]. Furthermore, the anabolic and catabolic genes are respectively enriched at the *oriC* and *ter* poles of the *E. coli* chromosome [70]. As a result, anabolic pathways are activated early during the reproduction stage under conditions of high negative superhelicity and catabolic pathways are activated later under conditions of low negative superhelicity characteristic of the maintenance stage [12,56]. Thus during the bacterial growth cycle, the temporal separation of

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anabolic (reproductive) and catabolic (maintenance) gene expression 'subprograms' is achieved by strategic spatial organization of the DNA analogue information (such as the *oriC-ter* gradients of the DNA thermodynamic stability and relative frequencies of the gyrase, σ70 and σS binding sites) coordinated with asymmetric enrichment of anabolic and catabolic genes around the chromosomal poles (Figure 4).

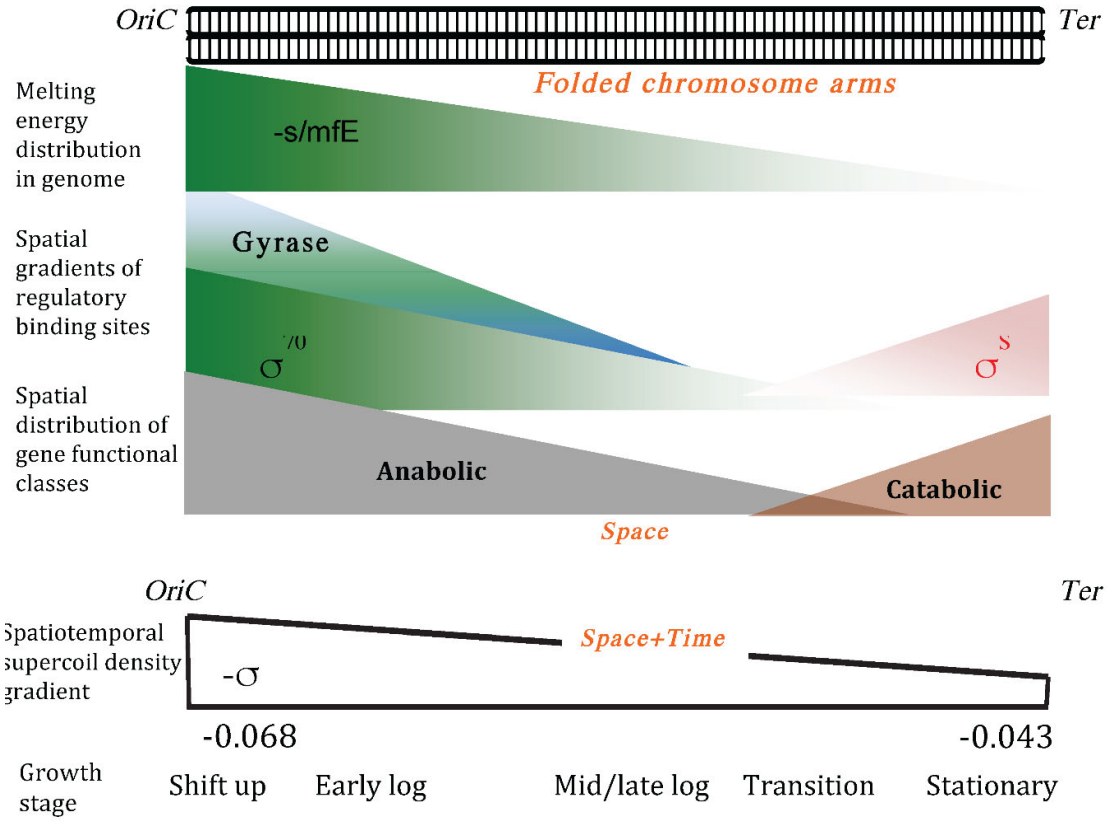

Figure 4. Organisation of the gene expression program during the *E. coli* growth cycle. The circular bacterial chromosome is indicated on the top folded, with two arms aligned along the *oriC-ter* axis. Indicated below are the spatial gradients of DNA average negative stacking/melting free energy (approx. the G/C content), relative frequencies of gyrase binding sites, of the σ70 and σS binding sites and the spatial organisation of anabolic and catabolic genes along the *oriC- ter* axis. Shown underneath is the spatiotemporal gradient of negative superhelical density (-σ), which changes both temporally with growth phase (from ~ -0.068 on shift-up to ~ -0.043 in stationary phase) as well as forms a spatial gradient along the *oriC-ter* axis of the chromosome [44,66]. This spatiotemporal gradient of superhelical density is proposed to coordinate the sequential expression of the anabolic and catabolic genes during the bacterial growth cycle [66,71].

In addition to the enrichment of anabolic and catabolic genes around opposite chromosomal poles, also the order of cognate regulatory genes along the *oriC-ter* axis is correlated with their successive expression during the growth cycle [66]. The genes of reproduction stage regulators are located in the vicinity of the *oriC* pole, whereas the genes of maintenance function regulators are positioned closer to the *ter* pole of the chromosome (Figure 5).

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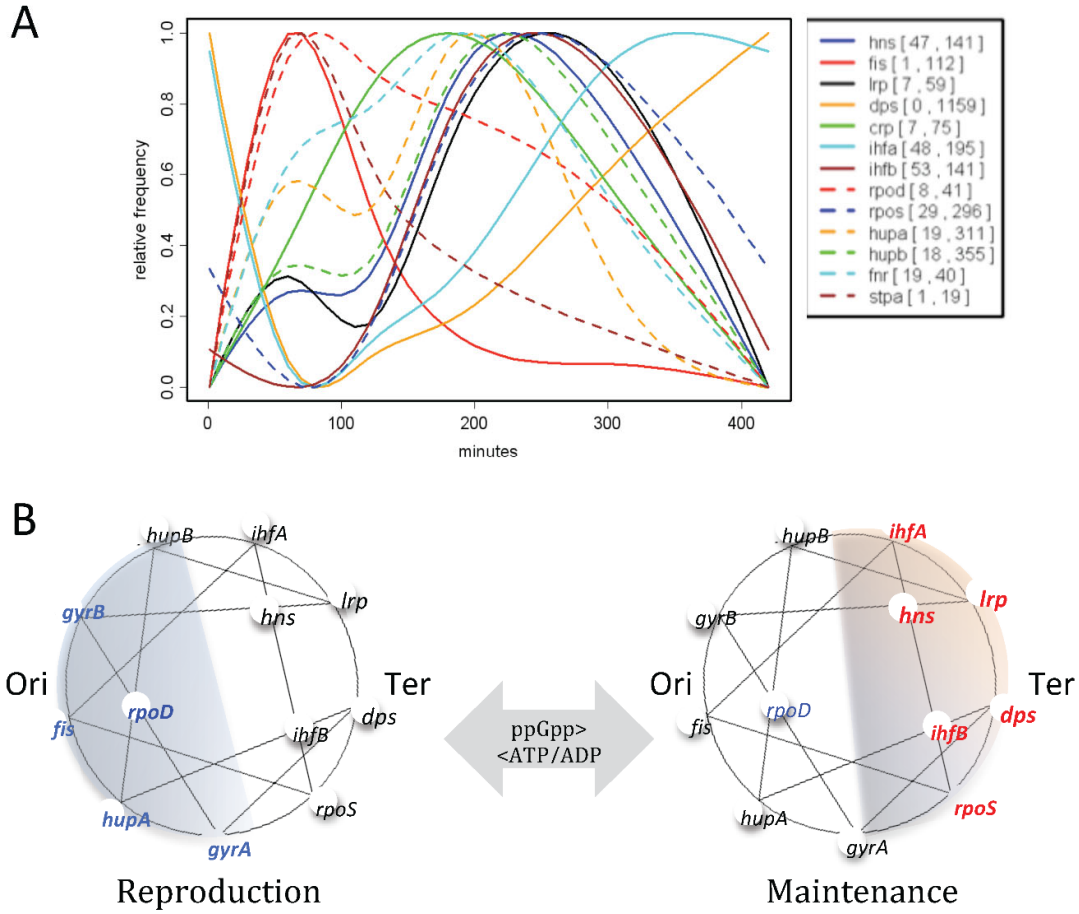

Figure 5. Switch between the reproduction and maintenance programs in *E. coli*. (**A**). Growth phase-dependent expression of the NAP and sigma factor genes (data from [136]). The different expression curves were normalized to [0;1] to compare them in one plot. Minimum and maximum values are indicated in brackets in the legend. Abscissa—time in minutes after inoculation of cells in fresh growth medium. The *Escherichia coli* CSH50 overnight (16 h) cultures were inoculated at an initial OD600 of 0.1 in rich double yeast-tryptone (dYT) medium and grown in a fermenter under constant pH 7.4 and high aeration (5 L air per min) at 37 ◦C for 7 h (420min). Samples for RNA-seq were taken at 1, 2, 3, 5 and 7 h after inoculation. The different curves were normalized to [0;1] to compare them in one plot. **B.** The circular chromosomes are depicted with Ori and Ter poles indicated. The reproduction regulatory genes are indicated in blue (left panel), the maintenance program regulatory genes are indicated in red (right panel). The original position of these genes on the circular chromosome is approximated. Note that the reproduction and the maintenance regulators are located around the opposite poles of the chromosome. Colored areas indicate the putative spatiotemporal concentration gradients of regulators. Connecting lines indicate the crosstalk between regulatory genes [72]. The 'alarmone' ppGpp produced on shortage of nutritional resources acts as a switch from reproduction to maintenance program. Conversely, high ATP/ADP ratio (established e.g. on nutritional shift-up) favors the commencement of reproduction program.

Therefore, also these regulators are expressed sequentially, first because as already mentioned, on the commencement of growth the *oriC* pole is activated earlier than the *ter* pole and second, because the iterative rounds of chromosomal replication initiation at *oriC* increase the copy numbers of early regulatory genes located in its vicinity relative to that of the genes of maintenance regulators located closer to the chromosomal replication terminus [12,66,73–75]. Most important among these sequentially expressed regulators, besides the DNA topoisomerases and RNA polymerase sigma

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factors, are the highly abundant nucleoid-associated proteins (NAPs), which potentially form spatiotemporal gradients (Figure 5) interacting with cognate binding sites spatially organized in the genome [66,76–80]. Importantly, the abundant NAPs bind DNA with different affinities depending on its 3D structure, are capable of constraining supercoils and stabilizing topological domains and thus of partitioning and storing the superhelical energy [81–83] which can be used to do work, e.g. to separate the DNA strands and facilitate transcription initiation.

Similar organizational logic applies to the operation of the aerobic/anaerobic switch during the bacterial growth. The *atp* operon responsible for ATP production under aerobic growth conditions is located in close vicinity of *oriC* while the *fnr* gene, encoding the major DNA binding regulator of anaerobic growth, is located in the vicinity of *ter*. Accordingly, the *arcA* and *arcB* genes encoding the two-component system responsible for gene regulation under conditions of microaerobiosis are located in-between *atp* and *fnr* (Figure 6A). Thus again, these genes are spatially ordered in the genome according to their sequential requirement during growth while the expression of aerobic and anaerobic gene groups appears to be correlated with the gradual alteration of oxygen partial pressure (Figure 6B). As with nutritional shift-up, the topoisomerase activities are involved in the regulation of DNA supercoiling during aerobic-anaerobic transitions in *E. coli* [84], whereby the growth under high oxygen conditions is correlated with high negative superhelicity of plasmid DNA [85].

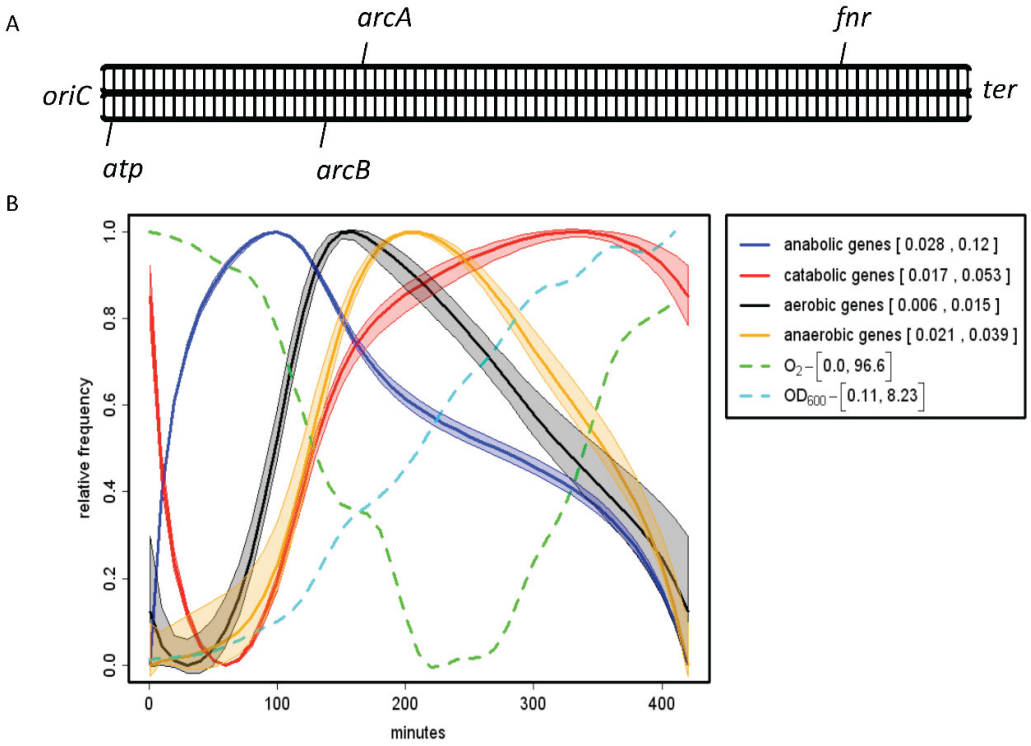

Figure 6. Chromosomal order of regulators and temporal pattern of regulated gene expression. **A.** Spatial ordering of aerobic/anaerobic growth regulatory genes on the *E. coli* chromosome along the *oriC–ter* axis. Genes on the clockwise (right) replichore are indicated on the upper bar, and genes on anti-clockwise (left) replichore are indicated on the lower bar. The *atp* operon encodes ATP synthase. *arcA*/*arcB* encode a two-component system active under microaerobic conditions [86,87]. ArcA also represses *rpoS* [88]. *fnr* has a dominant role under more strictly anaerobic conditions [86]. **B**. Temporal dynamics of expression of various gene classes (data from [136]). The *Escherichia coli* CSH50 overnight (16 h) cultures were inoculated at an initial OD600 of 0.1 in rich double yeast-tryptone (dYT) medium and grown in a fermenter under constant pH 7.4 and high aeration (5L air per min) at 37 °C for 7 h. Samples for RNA-seq were taken at 1, 2, 3, 5 and 7 h after inoculation. The different curves were normalized to [0;1] to compare them in one plot. The envelopes of the curves indicate the standard

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deviation at 10% random remapping of the expression patterns to genes. Minimum and maximum values are indicated in brackets in the legend. Expression values (anabolic, catabolic, aerobic, anaerobic) in brackets are normalized to the expression of all genes. The optical density and partial oxygen pressure are indicated respectively, by the dashed, light blue and green lines. Note the correlation between the maximal expression of anaerobic genes and minimal partial oxygen pressure.

3. Coupling of Logically Distinct Types of Information

The pivotal question is as to how the coupling of DNA analogue information (i.e. the spatial distribution of DNA torsional energy, which is a continuous variable) with the digital information (i.e. selective expression of unique genes manifesting a discontinuous pattern) is accomplished in the genome? More compellingly, how do the DNA analogue information and digital code communicate with each other? In bacteria, it has been shown that variation in G/C content of the promoter sequence context [55,89] as well as the peculiar sequence organization of the different promoter elements such as e.g. the deviation of the -35 hexamer from the consensus sequence, as well as the G/C-richness and/or extension of the discriminator sequence and the length of the spacer between the -10 and -35 hexamers, confer the ability to distinctly respond to alterations of DNA superhelical density [90–95]. Also the sequences located upstream of the core promoter and characterized by anisotropic bending modulate the response to DNA superhelicity [91,96–98]. Thus a simple way to produce a coordinated transcriptional response to changes of supercoiling would be to put all the functionally relevant genes under the control of promoters with similar sequence organisation and indeed, that is the case for many stringently regulated genes (that is, the genes down-regulated by the alarmone ppGpp; see below) including the stable RNA (transfer and ribosomal RNA) operons [99]. However, several studies identified supercoiling-dependent, spatially extending coherent gene expression patterns in the bacterial genome, which cannot be readily expounded by the promoter sequence similarity scenario [12,13,54,80,82,100]. Various explanations have been proposed for the organization of such extended topological domains including coherent domains of gene expression (aka CODOs) [12,13,71,101] but the issue remains controversial [71,102–104]. Importantly, the CODOs were found to harbor distinct genetic functions [12,13,71] consistent with spatial coupling of the DNA analog information and the digital code in genome.

4. Switching between Alternative Gene Expression Programs

In bacteria, global alterations of gene expression can be induced not only by alterations of DNA superhelicity but also by small intracellular effectors, such as the nucleotide guanosine tetraphosphate (ppGpp). In metazoan cells the role of ppGpp is less clear [105] while in bacteria it serves as an alarmone, reprograming the cell physiology by interacting directly with the transcription and translation machinery [106,107]. However, ppGpp also interferes with replication initiation by modulating the DNA topology at *oriC* [108]. For that matter, ppGpp, the production of which is sharply induced on shortage of nutritional resources, appears to act as a switch curtailing cell reproduction and promoting the establishment of maintenance program.

This ppGpp-dependent switch in gene expression occurs on exhaustion of nutritional resources at later stage of growth. At this stage ribosome production and gyrase activity subside, whereas sharply increased ppGpp concentration facilitates the compositional change of the transcription machinery, e.g. partial substitution of σ70 by stationary phase σS factor in the RNAP holoenzyme and for that matter, ppGpp also switches the supercoiling preferences of polymerase. While RNA polymerase is a direct target of ppGpp, the ppGpp sensitivity of the σ70 holoenzyme *in vitro* can be attenuated by increased DNA superhelicity [109]. In addition, ppGpp appears to stabilise the so-called 'tight' conformer of the σ70 holoenzyme at the expense of the 'ratcheted' conformer favoring supercoiled DNA (Malcolm Buckle, GM and AT, manuscript in preparation). Also the composition of the abundant NAPs changes at this stage such that overall, the intracellular milieu and the bacterial chromatin composition facilitate the transcription of more relaxed and relatively A/T rich DNA around the terminus of replication (the Ter macrodomain), which is enriched for genes involved in maintenance functions. The ppGpp-facilitated growth phase-dependent substitution of σ70 by the

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stationary phase ⊚S factor in the RNAP holoenzyme is associated with switching between the reproduction and maintenance programs and thus, resembles the genetic switch between the alternative growth pathways of temperate bacterial phages such as phage ⊚. The ⊚ switch is also sensitive to both the cell density and metabolic state [110,111] as well as to supercoiling level of the DNA [112,113].

In these two systems, despite the huge difference in complexity, there is a notable organizational similarity manifest in the conversion of distinct information types occurring during the establishment of both the bacterial switch between the reproduction and maintenance programs and the ⊚⊚phage switch between the lytic and lysogenic pathways. In the latter case the system is much simpler and ultimately, the switch boils down to competition between two DNA binding transcriptional regulators (the Cro and CI repressors) for binding specific operator sites in the ⊚⊚regulatory region. However, in both systems first the information of continuous (analogue) type is produced and then converted into information of discontinuous (digital) type.

Table 2. Estimated number and concentrations of the most abundant nucleoid-associated proteins in *E. coli* [121]. Approximate numbers for RNA polymerase and *lac* repressor are shown for comparison.

Protein	**Exponential phase**		**Early stationary phase**	
	No/cell	Concn (μM)	No/cell	Concn (μM)
Dps	8000	7	120000	100
FIS	60000	50	not detectable	
H-NS	20000	17	15000	13
HU	55000	45	25000	20
IHF	10000	8	50000	41
StpA	25000	28	15000	17
Totals		155		191
LacI(LacR)	10			
RNAP	4000-6000			

In *E. coli* system as mentioned above, analogue information is manifest in the *oriC-ter* skew of DNA binding site frequencies for DNA gyrase, ⊚70 and ⊚S, interacting with the changing ratio of the RNAP ⊚70 and ⊚S holoenzymes, the spatiotemporal gradient of chromosomal superhelical density and the temporal concentration gradients evident in various growth phase-dependent combinations of the NAPs (Table 2). These DNA architectural proteins form distinct spatiotemporal patterns of regulatory nucleoprotein complexes in genome [69,114–116]. The NAPs compete for stabilization of alternative supercoil structures (Figure 7) but can also cooperate depending on DNA sequence organisation [47,117]. The mutations of NAP genes alter both the gene expression patterns and DNA topology, consistent with the notion that the NAPs coordinate the growth phase-dependent chromosome structure and function [11,13,56,65,118–120].

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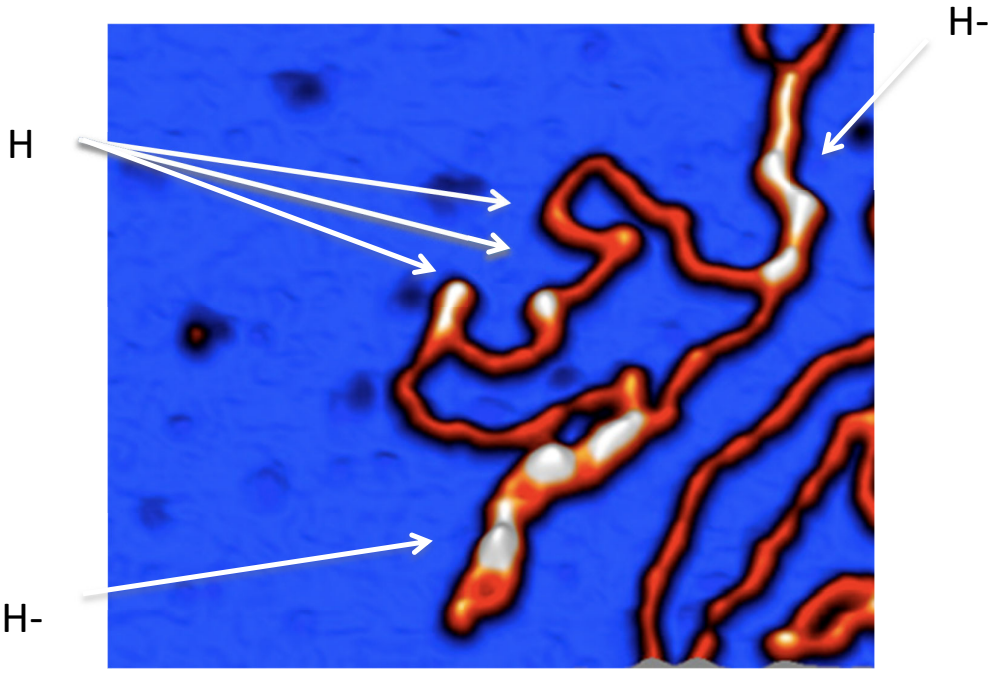

Figure 7. Competition between the bacterial NAPs, HU and H-NS, which stabilise alternative supercoil structures on binding DNA. Distinct supercoil structures stabilized by HU and H-NS are indicated by white arrows. HU stabilizes more open toroidal coils, whereas H-NS stabilizes tightly interwound, stiff plectonemic DNA structures (AFM image, courtesy Sebastian Maurer).

In the case of bacteriophage ◎ the analogue information is manifest as continual bidirectional extension of transcription initiated from the divergent pR and pL promoters located in the ◎ control region, producing on extension distinct sets of regulatory proteins, including those involved in sensing physiological conditions (e.g. CII) and eventually, by modulating the CI/Cro repressor ratio, favoring either the lytic or lysogenic pathway (Figure 8). Continual transcription is both contingent on, and also results in, formation of a spatiotemporal pattern of regulatory nucleoprotein complexes.

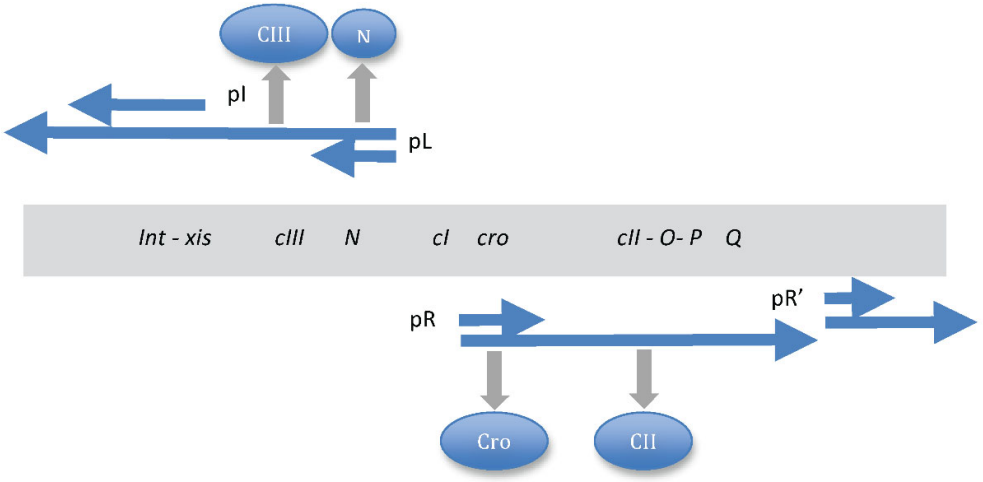

Figure 8. Regulation of lysis-lysogeny decision in λ phage development. Gene and early transcription map of λ is shown (simplified). Genes are indicated in the shaded rectangle. The early transcripts produced from pL and pR promoters are shown as blue arrows. The immediate early gene (short

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arrows) products are N and Cro. On extension of the transcripts known as 'delayed early transcription' (long arrows underneath) the CIII and CII proteins are produced. The N and Cro proteins support lytic development, whereas CIII and CII proteins support lysogeny. Note that synthesis of transcripts of different lengths (i.e. generation of analogue information) results in production of distinct sets of specific proteins (digital information). O and P are DNA replication genes involved in lytic growth. Q protein turns on the late genes for production of phage tails and heads. *cI, cII, cIII* and *int* genes are involved in the establishment of lysogeny. The *xis* gene (together with *int*) is involved in excision of the integrated prophage. Cro and CI are repressor proteins competing for binding at the operator sites in the ◎◎regulatory region. Critical for the active production of CI repressor is the CII protein, the stability of which in turn is sensitive to physiological conditions.

In *E. coli* the switch between the reproduction and maintenance stages is primarily dictated by the energy status, which in turn depends on environmental conditions. Notwithstanding the difficulty of considering phage an organism, it not only can reproduce itself (albeit hijacking the cellular components and machinery) but also responds to environmental conditions. For example, the ◎ phage may prefer lysogenic to lytic growth under conditions of starvation, perhaps since starving cells cannot provide components supporting efficient lytic growth [110]. Starving bacterial cells produce low amounts of proteases, which at high concentrations observed in rich medium destroy the phage CII protein required for activation of the phage *cI* and *int* genes essential for establishing lysogeny. The CI repressor produced under conditions of high CII activity inhibits transcription from the divergent pR and pL promoters in the ◎ regulatory region and thus turns off the expression of all the phage genes except that of its own. However, if CII is rapidly degraded no CI repressor is synthesized, the Cro repressor occupies the ◎ regulatory region instead of CI and lytic growth ensues. So while the phage senses the energy status of the cell, this latter is ultimately translated into specific nucleoprotein complexes competing for binding at the ◎ regulatory region and acting as a switch between alternative developmental pathways. Furthermore, regulation of the lysogenic/lytic switch by CI repressor appears sensitive to DNA supercoiling [112,113], as is also the RNAP ◎70/◎S holoenzyme switch in *E. coli* [36]. A similar relationship of DNA topology-dependent competitive binding at the overlapping DNA sites has been observed between the early and late NAPs, respectively FIS and Lrp, involved in the control of type 1 fimbrial genetic switch in *E. coli* [122].

Ptashne [110] suggested that the regulatory sequences initiated at the ◎ control region essentially generate a 'cascade' along each pathway, sequentially turning *on* and *off* groups of genes. In this cascade, one regulatory protein turns *on* or *off* a block of genes, which includes another regulatory gene the product of which in turn regulates another block of genes and so on. Here, the regulatory cascade is established by protein binding only at a few DNA sites on the phage genome. This type of regulation is made possible due to peculiar spatial organization of functionally related genes in genome, as they are grouped together and also transcribed in the same direction. Thus while emphasizing the role of cascades, this mode of regulation also implicates the spatial gene organization and the directional extension of transcription – properties, considered here as belonging to analogue (continuous) information type – by contrast to the 'cascade control' which essentially turns the genes *on* or *off* and therefore, provides purely digital information.

Despite the difference in complexity and details, in both the bacterial and phage systems there is a discernible common organisational design: initial utilization of analogue information (spatial *oriC-ter* gradients of DNA binding sites interacting with temporal gradients of regulatory proteins in the former, and gradually extending transcription starting from the divergent pR and pL promoters in the latter) and its subsequent conversion into digital information (the differing nucleoprotein complexes producing specific gene expression patterns sustaining either reproduction or maintenance in the former and, the distinct sets of regulatory proteins underpinning either the lytic or lysogenic pathway in the latter).

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5. Analogue/Digital Information Conversion Operates as a Regulatory Device in Living Systems of Diverse Structural Complexity

Given the similarity of underlying regulatory design in the bacterial and phage systems the pertinent question is, whether this mode of information conversion obtains also in more complex multicellular organisms. Indeed, over three decades ago Ptashne [110] drew parallels between the processes of gene regulation in phage ◎◎◎and higher organisms, in particular the process of *Drosophila* embryogenesis, where formation of the pattern of stripes expressing the segmentation gene even-skipped (*eve*) depends on sequential turning *on* and *off* of transcriptional regulators, a form of cascade control similar to that of the ◎◎life cycle. Actually, during *Drosophila* embryogenesis, notwithstanding the role of digital *on* or *off* type 'cascade control', the importance of analogue information in the pattern formation is most conspicuous.

During *Drosophila* embryonic development the maternal gene messages are strategically deposited at opposite – anterior and posterior - poles of the embryo, such that the translated proteins diffuse from the poles forming spatial concentration gradients along the anterior-posterior axis (Figure 9). These overlapping concentration gradients lead to spatially determined, locally fixed ratios of transcriptional regulators and thus establish boundaries of target gene expression. Spatially determined threshold concentrations of transcriptional regulators lead to sequential activation of the various segmentation genes eventually producing a distinct pattern of seven stripes expressing the even-skipped pair-rule gene, which is essential for segmentation of the embryo [123,124]. So here we have a clear case of the conversion of analogue information (continuous protein concentration gradients) into digital information (specific pattern of seven discrete *eve* stripes). This conversion of protein concentration gradients into particular pattern of stripes is enabled by existence of seven distinct enhancers of the *eve* gene (one for each stripe), each of which binds different combinations of regulatory proteins depending on their spatially determined threshold concentrations established along the anterior-posterior axis. Thus seven distinct enhancers binding different combinations of regulatory proteins generate alternative nucleoprotein complexes independently activating *eve* expression - albeit in a spatially defined manner – and producing the specific pattern of seven stripes.

At this stage of development (syncytial blastoderm) the *Drosophila* embryo is not yet cellularized and contains about 1500 nuclei evenly distributed underneath the membrane, while each stripe extends over 6 nuclei on average [110]. The *Drosophila* genome is about 180Mb in size, so a single row of nuclei expressing even-skipped gene would contain about 1Gb of DNA. In contrast, the *E. coli* genome is 4.6Mb in size and that of phage ◎ is about 48.5Kb. The *Drosophila* embryo is (longitudinally) about 500 times the size of *E. coli* cell. Thus, concerning the spatial extension of implicated gradients, there is a difference in orders of magnitude. Furthermore, in the case of phage ◎◎and *E. coli,* the gradients (directionally elongating transcripts in the former and the putative sigma factor and NAP gradients in the latter) extend over single genomes (albeit differing in size by two orders of magnitude), whereas in the case of *Drosophila* the protein concentration gradients extend over more than a thousand of spatially arranged genomes (nuclei) in the embryo. Finally, in the phage and bacterial genomes the regulatory proteins have a relatively easy access to DNA binding sites, whereas the binding of cognate regulatory sites is obstructed in *Drosophila* nuclei by tight packaging of the DNA in chromatin. Furthermore, in phage◎◎◎the spatial extension of genomic transcription produces distinct sets of proteins that are put to work in temporal succession. Also the temporal gradients of NAPs and sigma factors do not coexist in a single bacterial cell but are successively established in the progeny. In contrast, the opposite concentration gradients of Bicoid and Caudal extending from the poles and responsible for the formation of the anterior and posterior structures coexist in a single *Drosophila* embryo.

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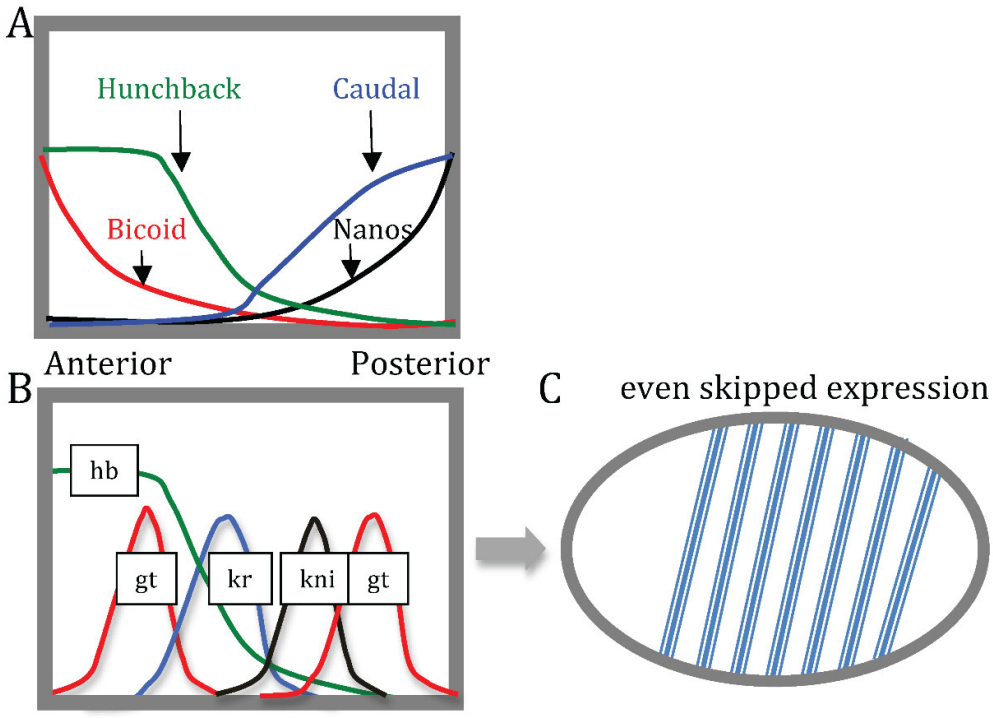

Figure 9. Generation of anterior-posterior pattern of even skipped (*eve* pair-rule gene) expression initiated by gradients of the *Drosophila* maternal effect genes. **A.** The Bicoid protein gradient extends from anterior to posterior while the Nanos protein gradient extends from posterior to anterior. Nanos inhibits the translation of the *hunchback* message (in the posterior), while Bicoid prevents the translation of the *caudal* message (in the anterior). This inhibition results in opposing Caudal and Hunchback gradients. (**B**) Spatial distribution of Bicoid-responsive segmentation (gap) gene expression such as giant (gt), krueppel (kr) and knirps (kni). The gap gene products mutually repress each other's expression, resulting in spatially defined domains of gap gene expression along the anterior-posterior axis of the embryo, which ultimately define the pattern of even skipped (a pair-rule gene) expression in seven stripes (**C**). Each stripe has an independent transcriptional control system consisting of different constellations of transcription factors (specific combinations of activators) interacting with seven different enhancer sequences all of which can activate the even skipped gene expression.

Notwithstanding the abovementioned differences, there are also remarkable similarities between these systems. During *Drosophila* embryonic development as well as during the *E. coli* growth cycle the transcriptional and metabolic programs appear tightly correlated [56,125,126]. Regulation of the activity of DNA topoisomerases is associated with both the *E. coli* growth cycle and the *Drosophila* embryogenesis [27,127]. Also, the *Drosophila* development time, the phase transition during *E. coli* growth cycle and the ⊚ phage lytic/lysogeny decision, all respond to nutritional supply [110,128,129]. However, the most important similarity between the systems is the phenomenon of conversion of continuous data (analogue information) into discrete data (digital information). First, in all three systems there is a directional dispersion of analogue information (gradients of proteins in *Drosophila* and *E. coli* and continually elongating transcripts in phage ⊚) from spatially localized sources (anterior and posterior poles in *Drosophila*, proximities of the chromosomal *oriC* and *ter* poles in *E. coli* and divergent pR and pL promoters in regulatory region of phage ⊚). Second, in all three systems the conversion of analogue into digital information is manifest in formation of distinct nucleoprotein complexes involving DNA binding proteins interacting with DNA sites spatially organized in corresponding genomes, or in the embryo. In the latter case, this interaction is facilitated and fine-tuned by ATP-driven chromatin remodelers [130]. Finally and more generally, assuming

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that the initial gradients possess higher entropy than the ensuing discrete patterns of DNA-protein interactions, we may also assume an energy-driven decrease of entropy associated with pattern making in all three systems.

Thus, despite the substantial differences in size, complexity and structural detail between these three living systems (although the phage system can barely qualify as such), in all cases we have a spatiotemporally organized gene regulation program. The temporally organized regulatory cascades alone cannot provide for the unity of living system - a cascade has a beginning and an end – yet it does not necessarily close onto itself while as mentioned above, from systems-theoretical perspective, the living system constitutes a self-referential circuit. What is assumed here is the closure of the system onto itself and this organisation of unity implicates spatial coordinates [131–133]. A relevant example of integration of cell division and differentiation by coordinating the temporal gene expression and spatial organization of gene products and protein gradients has been provided in studies of the *Caulobacter crescentus* system [9,134,135]. All the three systems discussed here have similar organization embodied in the conversion of two distinct information types manifesting a coordinated unity (Figure 10). On this view, a phage acquires the properties of a living system primarily by tapping into its intrinsic organization, namely, appropriating the device of analogue/digital information conversion.

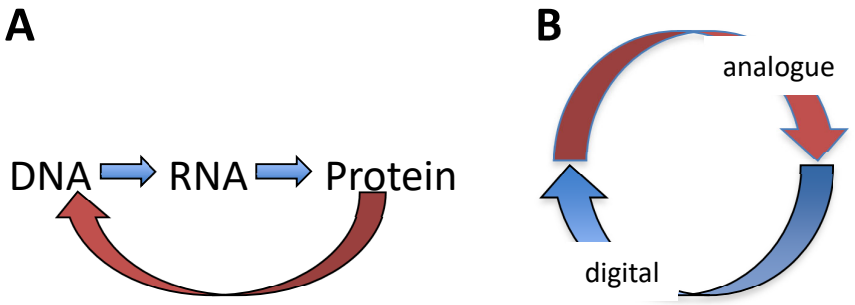

Figure 10. Organization of information flow and inter-conversion in the living system. **A**. Depicted is the central dogma of molecular biology: digital genetic information flows only in one direction, from DNA, to RNA, to protein (blue arrows). However the DNA analogue information is recognized directly by the DNA binding proteins stabilizing various 3D conformations of the DNA (curved brown arrow) and thus modulating the content of digital information. **B**. Systems-theoretical model for inter-conversion of DNA information. The terms 'analogue' and 'digital' refer to two distinct information types stored in the genomic DNA polymer. DNA analogue information implies both static (sequence organization viz. arrangement of base steps) and dynamic parameters (DNA 3D configuration and superhelicity). Digital information implies differential gene expression patterns and the gene interaction networks (including regulatory cascades) emerging thereof. Analogue information provides an integrative sensory interface for both internal and external signals as well as a regulatory context for digital code expression, whereas the latter provides information for reproduction and maintenance of the former. Together, the inter-converting DNA 'codes' form a coordinated self-referential circuit responding to both the internal and external signals as an indivisible unity.

6. Conclusions

The central dogma of molecular biology is stating that genetic information flows only in one direction, from DNA, to RNA, to protein, or from RNA directly to protein. This theory highlighting the unidirectional flow of genetic information does not consider the DNA analogue information and the crosstalk between the DNA and DNA binding proteins – essentially a feedback (Figure 10A, brown curved arrow). It thus cannot account for the main organizational hallmark of the living system manifesting a self-referential circuit. We argue here that this latter organizational feature is inherent in the structure of the DNA double helix, representing a basic device for interconverting

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information. This conversion of information is made possible by existence of two logically different – digital and analogue – information types stored in the DNA. While the digital genetic information encodes all the DNA binding proteins and enzymes, the DNA appears to 'read itself' via DNA-protein interactions. These interactions are informative as they lead to modulation of gene expression according to nascent external and/or internal signals. The coordinated DNA 'self-readout' mediated by DNA binding proteins is based on a strategic spatial organization of regulatory DNA binding sites and regulated genes in genome (or spatial organization of nuclei in case of *Drosophila* embryo). This spatial organization in turn is determinative for successive formation of distinct regulatory nucleoprotein complexes and the emergence of temporal regulatory 'cascades.' Thus the DNA genome can generate spatiotemporally coordinated patterns of activated and repressed genes in response to both internal and external signals. And so then, the genomic DNA acting as a double-coding device, represents the integrative interface where 'that which monitors and that which is monitored', meet to generate a coordinated unity (Figure 10B) responding to both the internal and external signals as an indivisible whole.

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