# CONTROL OF GENE EXPRESSION IN PROKARYOTIC SYSTEMS

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Received 24 January 1974

## 1. Introduction

The problem of how genes are expressed and regulated by environmental conditions, as well as in developing systems, has received considerable attention over the last ten years. The major break through in this area is due, in retrospect, to a convergence of studies, largely initiated in Europe, the aim of which was to analyse the classically known phenomenon of enzyme induction [1–3] as well as the general features of the sexuality in bacteria [4]. In particular, the possibility of constructing partial diploids has permitted the discovery of regulatory genes and of their mode of action. Biochemistry has matched the progress in genetics: the characterization of messenger RNA's [5,6] and of their enzyme forming machinery [7,8] as well as the deciphering of the code [9,10] and of the main translation steps, illustrate some of its major achievements during the same decade. At the present stage, it has become possible to account in precise molecular terms [11] for most facets of gene action and control in prokaryotic systems – some of which with a great luxury of details [12] – and the picture thus obtained provides interesting and provocative models to a similar study in eukaryotes.

Owing to the lack of space and broadness of the theme, we shall however restrict ourselves to situations as they have been analyzed in bacteria and their viruses. Moreover, we shall mostly remain on the biochemical verge of the problem by placing emphasis on transcription control.

## 2. Homeostatic control of gene activity

Enzyme induction [1,2] or repression by the end-terminal products of biosynthetic pathways [13–15] illustrate the homeostatic adjustment of the genetic machinery to environmental changes. In recent years, repressors active on three distinct bacterial [16–18] and one phage [19] operons have been obtained in a high degree of purity. This has enabled one to dissect their mode of interaction with operator targets and inducers. Kinetic studies on repressor–DNA and repressor–inducer complex formation suggests that inducers act by dissociating the repressor–operator complex rather than by causing direct transconformation to free repressor molecules [20]. The repressor molecules, thus far analyzed, are oligomeric, as expected from the allosteric hypothesis. The *lac* repressor is a tetramer [21]. The lambda repressor exists in solution as a monomer, dimer or tetramer in a rapid equilibrium [22,23]. The monomer does not bind DNA but, when associated in a dimer, it binds to the operator. The half life of the repressor–operator fragment complex measured by Riggs et al. [24] is around 10 min, at 0°C.

Recently, the complete structural analysis of the *lac* [25] and lambda operator fragments [26] has been achieved. A common feature is the presence of a two-fold rotational symmetry. The lambda operator contains, however, multiple (around 6) repressor binding sites [27] with different affinity constants. The site of highest affinity in the case of the OL fragment being closest to the N gene. It is assumed that the first dimer subunit binds to this site and that subsequent addition of monomers distal to the highest affinity site 'polymerizes' the repressor along the OL region. The nature of the 'recognition code' is still unknown. Elements of symmetry, as they have been found among the *lac* operator fragments [25] could be an important feature for target selection. By combining genetic analysis and

protein chemistry data, Müller-Hill et al. [16,21 see also 28] have come to the conclusion that the portion of the *lac* repressor that binds the operator is α-amino proximal and includes the first 30 amino acid residues while the inducer recognition part lies between residues 200 and 250 (the subunit includes 347 amino acis residues).

In vivo, gene expression of inducible systems is controlled at the transcription rather than translation level [29–31]. Blockade of *lac* [32,33], *gal* [17], *trp* [34,35] or lambda transcription units by their cognate purified repressors has indeed been observed using in vitro systems including normal or transducing phage DNA templates plus purified RNA polymerase. In vitro repression of the tryptophan operon requires in addition to the product of the tryptophan regulatory gene, the presence of L-tryptophan as a corepressor. Neither tryptophan RNA synthetase nor trp-tRNA appear to be involved [34,35]. RNA polymerase in unable to initiate RNA synthesis on a DNA molecule to which repressor is previously bound [32,33]. However, repressor is also able to block RNA synthesis when the RNA polymerase has *already* formed the initiation complex, so that it seems to have a dual function [36,37]. In two cases (*lac* and *trp*) mapping studies clearly indicate that the operator lies between the promotor region and the genes under its control [38,39]; but while in one case (lambda), the operator is probably not transcribed [40], in the *lac* system it is cotranscribed with the structural genes of the operon [41]. Hence, it is presently difficult to make a choice between the 'fence' hypothesis (RNA polymerase would be blocked during the 'drifting' to the site of chain initiation) and a mechanism implying a more complex alteration of the promotor region (such as one for instance that would render it more compact). This latter model is compatible with Pirotta's findings [23] that the lambda repressor – whose molecular weight is only 40 000 daltons – can protect a DNA of 80 nucleotide base pairs.

New facets of negative controlling mechanisms have been unravelled, very recently, by the discovery of a particular class of operons involved in the biosynthesis of amino acids. In the 'histidine system' from *Salmonella*, for instance, it was found that the first enzyme of the pathway not only displays sensitivity to feedback inhibition by histidine, but also functions as a corepressor for the operon as a whole, histidyl-tRNA being a corepressor. This has been shown both in vivo [42] and in vitro [43]. A somewhat similar situation has been encountered in the isoleucine biosynthesizing system [44] as well as in the *hut* operon [45]. The difference between these situations and the one exhibited by the *trp* operon which is described above needs to be emphasized. But, the fact that the same molecule can function as an allosteric enzyme in a metabolic pathway and as a gene controlling element of the same pathway is certainly of great interest from an evolutionary standpoint.

Detailed analysis of arabinose gene regulation in *E. coli* [46,47] has led one to conclude that alternative situations, in which gene expression is controlled *positively* rather than *negatively* can also be encountered. In the *ara* system, a regulatory gene, *ara C*, codes for a protein which functions both as a repressor (in the absence of L-arabinose), or as a positive internal inducer in its presence. The 'activated' C protein triggers expression of the arabinose system by interacting with a genetic locus called initiator, or 'I', distinct from the promotor, or 'P' site, [47]. In vitro synthesis of ribulokinase (*ara B*) directed by DNA from phage φ80 carrying the *ara* operon, in a system prepared from a ribulokinase deletion mutant demonstrates the requirement of L-arabinose, cAMP [48,49] as well as of the *ara C* protein [50]. Positive control of gene action has proven to be very wide spread in bacteria and phage systems. A situation closely resembling the arabinose type of control has been unravelled in the rhamnose [51] and in the maltose [52,53] systems. The mode of action of *mal T*, a maltose regulatory gene equivalent to *ara C* gene has been studied in detail. Mutations in *mal T* can cause a pleiotropic defect which totally curtails the cell's ability to synthesize the *mal* permease, amylomaltase and phosphorylase, as well as to adsorb lambda phage. Existence of an initiator gene could also be established. An interesting feature distinguishes however the maltose from the arabinose controlling system. This is the fact that the *mal T* product does not function as a repressor in the absence of the metabolic inducer. Thus, regulation in the *mal* system is purely positive, while regulation in the *ara* system is partly negative and partly positive.

A break towards a better understanding of 'catabolite repression' [54,55] at a molecular level was the

observation that cyclic 3′, 5′-AMP (cAMP) can counteract the classically known 'glucose effect' [56,57, 57b] in vivo, a result also bridging certain concepts about homeostasis in bacteria and higher organisms [58].

Pleiotropic mutations that totally hinder expression of most cell inducible systems can either be corrected by exogenous cAMP (adenyl cyclase defect) or not. Mutants of this latter class were found to lack a protein factor required for 'turning on' the lac operon since a protein synthesizing system from a factor defective mutant did not support the synthesis of β-galactosidase, unless fortified by purified extracts containing the factor [59,60]. The protein factor is usually referred to as CAP (for catabolite gene activator protein) or CRP (for cAMP receptor protein, since it is found to bind cAMP with a relatively high affinity constant $K_a = 10^6$ liters/mole). A considerable body of evidence indicates that CRP – a protein of 40 000 daltons which contains two subunits – acts by changing the DNA conformation near the promoter region [61,62] so as to permit formation of a correct initiation complex. From a series of genetic studies, it can be concluded that the promoter region from catabolite sensitive operons would contain two adjacent 'sub-sites': one for RNA polymerase binding proper, the other for CRP binding. This model predicts that CRP should be stimulated by cAMP to bind to *lac* DNA in vitro and this was found to be so [63]. However, conditions restricting its binding to the *lac* promoter region have not yet been found [63], a result suggesting that CRP action in vivo might perhaps involve another factor. Accordingly, studies on *lac* and *ara* expression in adenylcyclase and *crp* mutants have revealed the existence of a factor (coded for by a so called 'alt' gene) which can mutate to provide a substitute for the CRP–cAMP system [64]. Unpublished data from our laboratory also suggest that $H_1$, a low molecular weight DNA-binding protein discovered by Jacquet et al. [65] (very similar in properties to the D factor from Ghosh and Echols [66]) could also be involved at this stage. It has been observed, for instance, that the integrity of the CRP binding site, near the promoter region, is an absolute requisite for $H_1$-dependent stimulation of *lac* DNA transcription in an in vitro system.

Control of ribosomal RNA synthesis in bacteria has been subject to extensive studies since as early as 1956 [67–69] when it was found that amino acid starvation in *E. coli* auxotrophs can cause cessation of RNA synthesis. This response, which is termed amino acid control or 'stringent control', is a negative control function exercised by the product of the RC gene; mutations at this locus which are recessive [70] allow the cell to accumulate RNA even if some amino acids are absent or cannot be activated [71]. During the normal RC response, RNA synthesis is interrupted at the stage of initiation [72] and there is a preferential reduction in the rate of rRNA synthesis relative to the average rates of all other RNA species [73–75]. A break in the mechanism of the stringent control came after the discovery that a new guanine nucleotide (first designated as 'magic spot I' or MS-I) was an early and specific feature of the $RC^+$ response to amino acid deprivation [76]. MS-I was isolated and identified as guanosine-5′-diphosphate 2′ or 3′ diphosphate, or ppGpp [77]. Relaxed mutants fail to accumulate ppGpp during amino acid starvation [78]. Synthesis of this nucleotide results from an ATP-dependent conversion of GDP. This may possibly represent an idling translational reaction occurring on the messenger–ribosome complex, which would be 'signalled' by the presence of uncharged tRNA [79]. ppGpp biosynthesis by in vitro ribosomal systems requires, in addition to ATP, GDP, messenger and transfer RNA's, some factors found in the ribosomal 'wash'. Factor(s) from 'relaxed' ribosomes exhibit(s) greater thermosensitivity than that (or those) derived from 'stringent' ribosomes. How ppGpp does actually control rRNA synthesis is not fully understood: ppGpp feed back inhibits the activities of the first enzymes of the pathway leading to GTP and ATP. Studies on the behaviour of guanine auxotrophs suggest that the rate of accumulation of stable species of RNA is especially sensitive to the intracellular level of GTP [80,81]. But, a more provocative model was proposed by Travers et al. [82]. These authors reported on the existence of $\psi_r$, a positive controlling element, specifically acting on the production of rRNA during in vitro transcription of *E. coli* DNA. Quite interestingly, the $\psi_r$-dependent stimulation effect was abolished by ppGpp. Unfortunately, the model appears controversial, other reports indicating that in vitro ribosomal transcription is only weakly influenced by $\psi_r$ [83]. According to more recent investigations however, $\psi_r$ would be equivalent to the

Tu–Ts elongation factor system [84,84b], the activity of which is highly sensitive to ppGpp. A 'polymerase Tu–Ts complex' (sedimenting at 16 S) has been found in crude bacterial extracts. It would be responsible for in vitro transcription of *E. coli* DNA to ribosomal RNA [85] and ppGpp (or GDP) would strongly inhibit this reaction. If these findings can be substantiated, a very subtle intrication would turn out to exist between translation and ribosomal RNA synthesis thanks to the intermediary role of elongation factors. Another important parameter has to be taken into consideration to account for the transcription of ribosomal genes: this is the 'conformational state' of the promoter. In particular, promoters could exist in 'open' and 'closed' forms [86] and the ribosomal promoters would be more often in the closed configuration (at 37°C) relative to promoters for other structural genes. Until more becomes known about the incidence of these parameters, the exact interrelationship between ppGpp, $\psi_r$ and the ribosomal transcription machinery will not be definitively established.

## 3. Control mechanisms operating during expression of genetic programs

### 3.1. *Sporulation*

Considerable work has been devoted over the last 10–15 years to the control of gene expression during the phenomenon of sporulation in *Bacilli* (for review see: [87,88]). Great hope for a molecular approach to this problem was formerly placed on Losick and Sonenshein's observation [89,90] dealing with changes in the template specificity of the transcription machinery in early sporulating cells. This change was revealed in vivo by the cessation of multiplication of phage $\phi$e if cells are infected at the onset of the stationary phase. Its in vitro counterpart was the loss by the RNA polymerase from sporulating cells of the ability to transcribe $\phi$e DNA, that same DNA being readily used as template by the vegetative enzyme. Other modifications have also been noted: the RNA polymerase from sporulating cells does not react on SPI and T4 DNAs efficienly as does the 'vegetative polymerase', the reverse being observed with T7 DNA [91]. Studies involving *B. subtilis* DNA as template indicate that RNA polymerase from wild type sporulating cells synthesizes little rRNA in vitro as opposed to the polymerase from stationary phase cells [92]. These changes are paralleled by drastic modifications of the polymerase molecule itself, for the $\beta$ subunit from the sporulating enzyme is appreciably shorter in size than the corresponding subunit from the 'vegetative' polymerase [93,94]. That changes in template specificity and $\beta$ subunit modification coincide with the onset of sporulation is substantiated by use of appropriate mutants: for instance, a rifampicin-resistant mutant, which fails to sporulate, known as rfr10 [95] alters the RNA polymerase in such a way that it retains vegetative template specificity during stationary phase and continues synthesizing ribosomal subunits and rRNA, after the end of the logarithmic phase. Yet, this beautiful scheme is at least partly controversial. According to more recent findings [96] the $\beta$ subunit conversion would be no longer observable when RNA polymerase *extraction* is carried out sufficiently rapidly under conditions blocking the proteolytic activity which, as is known, greatly increases at this phase [97]. An endoprotease recently isolated by Millet et al. [98] was reported to carry out in vitro the modification of the $\beta$ subunit when incubated in the presence of *B. subtilis* RNA polymerase [99].

### 3.2. *Phage development*

It is well known [100,100b,101] that T4-phage DNA transcription by the host polymerase is *restricted** to genes coding for the 'very early' or 'immediate early' [102–104] species and this also applies to lambda DNA [105,106]. 'Immediate early' sequences are found at some later stages in molecules at least twice as large, this process of covalent extension, being probably under viral control [107]. More significant are the modifications of the transcription machinery accompanying the switch from 'early' to 'late' gene function. This involves: a conversion of the phage DNA template from a 'resting' into a 'replicative' state [108,109], numerous changes of the RNA poly-

* See, however, recent data by L. Gold's group [166–168] which reveal the existence of a '2nd mode' of transcription (sensitive to rifampicin and eliminated by mutation t 61). This prereplicative transcription probably gives rise to what had been formerly described as 'quasi late' species.

merase core (under control of genes 33 and 55) as well as the synthesis of new sigma factors [110,111]. Modifications of the core consist of: i) the covalent addition of ADP [112] to one of the two $\alpha$ subunits; ii) a structural change in $\beta'$ giving rise to a net increase of its negative charge [113]; and iii) the appearance of low molecular weight subunits (so called $\omega$-like peptides) [114,115].

Other mechanisms are apparently involved to 'turn on' late functions in T7 and T3 phages. In T7, immediate early transcription is initiated by the *E. coli* enzyme at a unique promoter site situated at or near an extremity of the phage chromosome [116,117]. In vitro, a $2.4 \times 10^6$ daltons species is formed, which resolves into five discrete species in the presence of the termination factor Rho [118,119]. Similar species also appear in vivo but a post transcriptional modification has been proposed to account for their production since RNAase III from uninfected cells can cleave the $2.4 \times 10^6$ species into just those five main species which occur in vivo after infection by a late defective T7 phage [120]. Processing of the polycistronic message would be required for translation [121]. Among the immediate early sequences transcribed by the host enzymes, one corresponding to gene 1 codes for a new polymerase; this phage specific transcriptase is monomeric and can copy late genes in the absence of additional sigma factor [122,123]. Its template specificity is restricted to T7 and T3 DNA templates. Preliminary evidence suggests that the late genes are transcribed in 3–4 polycistronic units [118,124]. Gene control during T3 development also involves a similar mechanism [125].

Perhaps the best documented model to date, as far as the temporal control of gene expression is concerned, is that of the lambda bacteriophage, a small virus ($3.2 \times 10^7$ daltons) whose genetics is remarkably well known after the pioneering studies of Jacob and Wollman [126], Campbell [127] and others. Contrary to T4, where the genetic origin of the various messenger classes is actually unknown, lambda offers a situation of choice since most transcription units could be topographically related with the genes located on the 'control', 'early' and 'late' regions of the chromosome. Such an achievement was made possible by convergent technical approaches such as hybridization – using DNA's from deletion mutants as well as separated strands or halves [128–130,130b] – or the EM analysis of artificial heteroduplexes [131]. Aside from a classical repression mechanism involving the CI gene product, whose physico-chemical properties have been analysed in details by Ptashne and his colleagues [19,22] and which blocks transcription from the prophage genome at two operators sites (VI–V3 and V2), lambda gene expression harbours a great luxury of positive controlling devices which can only be summarized at this stage.

'Establishment' of the lysogenic state involves prior activation of lambda 'immunity' region, a process requiring early synthesis of two phage specified proteins, CII and CIII [132,133,133b], presumably acting like other positive controlling elements on a promoter situated between *cro* and CII [134,134b]. As the repressor begins to form and reaches a certain level inside the infected cell, it 'shuts-off' CII and CIII genes. Yet, expression of the CI gene is now ensured by a new control mechanism which resides in the fact that it is the repressor *itself* which *activates* the transcription of its own messenger [128,135,136]. This 'maintenance-control' mechanism termed 'autogenous regulation' by Goldberger [137] apparently involves a promoter distinct from the previous CII, CIII target [134]*. Aside from this rather complex mechanism which permits the *establishment* and *maintenance* of the lysogenic state, the synthesis of the repressor can also be controlled negatively at the transcription level by gene *cro* (or *tof*) [138]. Control of lambda gene expression during the transition from the lysogenic to the lytic cycle, following prophage induction, can be visualized as a 'cascade' mechanism. Three major steps, at least, are triggered in an ordered fashion:

Step 1 – Repressor inactivation permits copying of the immediate early genes ('*N*' and '*cro*') from the $P_L$ and $P_R$ promoters respectively [130b, 139,140]. Propagation of the host polymerase along the phage chromosome is stopped at the end portion of these genes due to the existence of ($\rho$ dependent) termination signals [141].

Step 2 – The N product formed at step 1, is now acting as an 'antitermination' factor, counteracting Rho action and allowing for 'downstream' propagation of the RNA polymerase molecules beyond the ter-

* A somewhat similar situation has been observed for the control of T4 gene 43 product [169].

mination barriers [142–144]; this causes expression of distal genes of 'excision' and 'replication' function as well as of the regulatory gene *Q*.
Step 3 – During circularization of the excised prophage DNA, a new promoter for late gene transcription is formed [145]. The *Q* gene product appearing at step 2 activates synthesis of messenger RNA for late genes (maturation functions). Thus, gene expression during phage formation involves at least two sequential actions from positive controlling elements, one at the termination step (N) and the other (Q) at the initiation step of transcription.

Recent studies have been oriented towards a better characterization of the N factor, a short lived protein which, according to the behaviour of certain bacterial mutants, (*groN*), is operating at the level of the host polymerase [146] rather than of the template. Supporting its action as an antiterminating agent, it has been found that messenger from lambda $N^+$ prophage are very long molecules which include, in their 5′ proximal region, the messenger sequence from lambda $N^-$ prophages [147]. Using lambda DNA-directed lysozyme synthesis as an indirect assay system, Greenblatt [148] was able for the first time, to demonstrate the in vitro activation of purified phage DNA transcription by an N containing extract.

## 4. Conclusion

Refinement of biochemical technology over the last decade, has permitted the demonstration of the validity of the classical operon model at a molecular level. But, many of the former views about regulatory proteins or the genetic elements of regulatory circuits have proven to be over-simplified. This short survey, although very incomplete, shows for example, the degree of complexity that repressor molecules can display, which can, in some instances, harbour defined enzymic functions or punctuation signals and genetic targets such as promoters or operators, whose structural redundancy was unexpected. Considerable effort has been devoted to the characterization of positive controlling mechanisms involved in the ordered expression of genetically programmed systems. The notion that a gene transcribed first in a temporal sequence of a developing organism can activate transcription of other battery of genes intervening later in development has been largely substantiated.

Moreover, it is also becoming increasingly evident that many parameters other than those involving incidence of regulatory proteins on defined genetic targets have to be taken into consideration. For instance, the physico-chemical state of the DNA template – namely its circularity and superhelicity – can be of cardinal importance for the fidelity of control (see studies by Echols et al. [149] on lambda DNA). Recent work by Worcel [150,151] indicates that *E. coli* chromosomes harbour a very high degree of organization. DNA binding proteins ($H_1$, $H_2$, D, etc...) which could unwind or cover specific portions of the chromosome could represent primitive substitutes for the nuclear proteins in eukaryotes.

We have not discussed in this review the possibility of controls at a translational level although very clear-cut situations of this nature have been described both in homeostatic [152] or in developing systems [153,154]. Not only is transcription tightly coupled with the attachment of ribosomes to messenger RNA [155–158,158b], a situation which is specific of prokaryotes, but a large body of information points towards the 'modulation' of messenger recognition mechanisms involving processing of messenger RNA, or changes in the specificity of ribosomes [159] and initiation factors [160–165]. There is no doubt that the problematics of gene expression and its control will present even more unexpected facets as work with eukaryotic systems will progress.

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