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The environment, microbes and bioremediation: microbial activities modulated by the environment

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Abstract

Microorganisms in nature are largely responsible for the biodegradation and removal of toxic and non-toxic chemicals. Many organisms are also known to have specific ecological niches for proliferation and colonization. The nature of the environment dictates to a large extent the biodegradability of synthetic compounds by modulating the evolutionary processes in microorganisms for new degradative genes. Similarly, environmental factors often determine the extent of microbial gene expression by activating or repressing specific gene or sets of genes through a sensory signal transduction process. Understanding how the environment modulates microbial activity is critical for successful bioremediative applications.

Introduction

The environment exerts profound influence on microbial activities. Many microbes are fully active in certain environments but are rendered inactive in others. Bioremediation, which employs biological agents for the detoxification and removal of environmental pollutants, is often carried out in the fields where microbial activities or their products are essential for bioremediation to be effective. If the pollutants are synthetic and new to the environment, the microorganisms may not have the capability to degrade the pollutants since the appropriate biodegradative genes may not have evolved. Even if the genes are present, functional expression of the genes is essential for the appropriate degradative enzymes to be produced. Various factors present in the environment are known to activate or repress gene expression and thereby modulate microbial activities. If bioremediation is to succeed, it is imperative that we learn how the environment controls evolution of new genes in bacteria or the expression of microbial genes. This article deals with an example in each of these two areas.

Environmental modulation of microbial gene evolution

A prominent example of bioremediation often cited in newspapers and scientific magazines is the Exxon Valdez oil spill. The indigenous microorganisms in Prince William Sound were supplied with an oleophilic fertilizer allowing them to proliferate and consume the spilled oil (Pritchard & Costa 1991). In addition, numerous bioremediation studies are being conducted using natural (often indigenous) or adapted microorganisms to degrade and remove a variety of pollutants from the environment (Keeler 1991). Emphasis so far, however, has been on the use of natural microorganisms,

rather than genetically manipulated ones, due to adverse public reaction to, as well as various regulatory constraints on, the release of genetically engineered microorganisms in the environment (Bakst 1991). However, natural mixed cultures are known to degrade pollutants, including crude oil, at slow rates because of interactions among themselves (Friello et al. 1976). Also, since many contaminated sites contain more than one pollutant, it is often difficult for natural microorganisms to degrade, efficiently and simultaneously, a mixture of pollutants. It has been shown (Haugland et al. 1990a) that a single culture with appropriate genetic competence to degrade a mixture of chemicals is much more efficient than a mixture of cultures with equal genetic capability. In addition, indigenous microorganisms, when exposed to a mixture of pollutants, may produce toxic intermediates that do not allow an overall reduction of the toxicity or teratogenicity of the biotreated sample to any significant extent (Mueller et al. 1991). Thus, genetically improved single cultures are considered more efficient than mixed cultures in degrading single or mixed toxic chemicals.

Another important reason for using genetic selection for decontamination of polluted environments is that in many cases natural microorganisms have not evolved the genetic competence to utilize a synthetic compound. For example, the half-life of chlorinated dioxins or a number of polychlorinated compounds is of the order of years, which means that natural microorganisms do not have efficient enzyme systems to break down these compounds. To degrade a newly-made synthetic compound, a microorganism must evolve the appropriate genes encoding enzymes that have high affinities for the target chemical or intermediate products as substrates. This sort of natural evolution may take years, depending on the chemical structure and the solubility of the compound, as well as the nature of the environment. Therefore, genetic selection resulting in an enhancement of the evolutionary process may play a critical role in facilitating the evolution of new biodegradative genes in natural microorganisms for the utilization of such compounds.

How do we enhance the process of natural evolution for biodegradative purposes? To address this question, it is necessary to understand how new genes encoding new types of catabolic enzymes evolve in nature. An interesting example of natural evolution of new catabolic enzymes is the microbial degradation of chlorinated aromatic compounds. Large quantities of these compounds have been synthesized and released into the environment in the form of herbicides and pesticides, or as industrially useful compounds such as PCBs (polychlorinated biphenyls). Because these compounds have many chlorine substituents, natural microorganisms that rapidly degrade the non-chlorinated parent compounds are unable to mineralize the chlorinated ones to any significant extent. However, because of exposure to natural microflora for many years, a number of chlorinated aromatic compounds with fewer chlorine substituents can be degraded by natural microorganisms through the use of newly evolved degradative genes.

An example of such an evolutionary process is that of chlorocatechol degradative genes. Catechol is a central intermediate of aromatic degradation (Wheelis & Ornston 1972) and its mode of degradation is illustrated in Fig. 1. Catechol is utilized by a large number of bacteria, including the genus *Pseudomonas*. In *P. putida*, catechol is degraded by a set of chromosomal genes *cat*A, *cat*B, *cat*C etc., leading to the formation of β-ketoadipate. This is finally converted to succinate and other members of the tricarboxylic acid cycle for oxidative metabolism. The enzymes such as pyrocatechase I and cycloisomerase I (muconate lactonizing enzyme I) have high affinity for their substrates, catechol and *cis*, *cis*-muconate, but have little affinity for the chlorinated catechols (Schlomann et al. 1990). Consequently, catechol degrading microorganisms are unable to utilize chlorinated catechols. However, it is possible to isolate from nature various *Pseudomonas* species and *Alcaligenes eutrophus* which are capable of degrading chlorinated benzoic acids, phenoxyacetic acids, and benzenes such as 3-chlorobenzoic acid (3-Cba; Chatterjee et al. 1981), 2,4-dichlorophenoxyacetic acid (2,4-D; Don & Pemberton 1981) and 1,2,4-trichlorobenzene (1,2,4-Tcb; van der Meer et al. 1991a). These compounds are metabolized to their corresponding

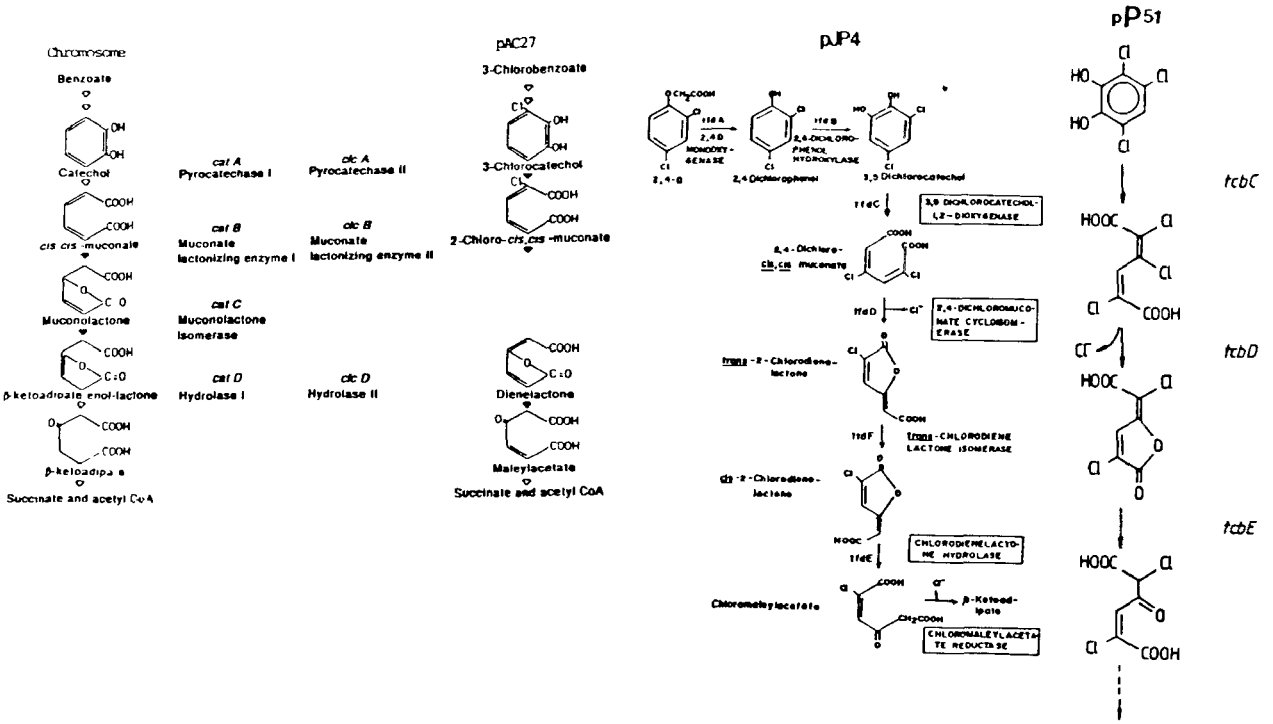

Fig. 1. The degradation pathways of catechol, 3-chlorocatechol, 3,5-dichlorocatechol and 3,4,6-trichlorocatechol encoded by chromosomal genes and the plasmids pAC27, pJP4 and pP51. The genes for each step of the pathway are indicated, as well as the enzymes of the first three pathways. Type II enzymes involved in 3-chlorocatechol and 3,5-dichlorocatechol utilization are also involved in 3,4,6-trichlorocatechol dissimilation.

chlorocatechols, *viz.* 3-chlorocatechol, 3,5-dichlorocatechol, and 3,4,6-trichlorocatechol, which are further degraded via chloromuconates and dienelactones (Reineke & Knackmuss 1988). Thus, the pathways of catechol and chlorocatechol degradation are quite similar, as shown in Fig. 1, where *cat*A, *clc*A, *tfd*C, and *tcb*C encode type I or type II 1,2-dioxygenases acting on catechol or chlorinated catechols, *cat*B, *clc*B, *tfd*D, and *tcb*D encode cycloisomerases acting on *cis*, *cis*-muconate or its chlorinated derivatives, and *cat*D, *clc*D, *tfd*E, and *tcb*E encode hydrolases having affinity for enol-lactone or dienelactones. Although the pathways are similar, the evolved enzymes active on chlorinated catechols or their intermediate metabolites have altered substrate specificities. For example, the *cat*A gene product (type I dioxygenase) has little activity towards chlorinated catechols, while the *clc*A gene product (type II dioxygenase) has high activity towards 3-chlorocatechol, with lower activity towards 3,5-dichlorocatechol. In contrast, the *tcb*C gene product preferentially acts on 3,4-dichlorocatechol, whereas the *tfd*C product has a higher affinity for 3,5-dichlorocatechol (Schlomann et al. 1990; van der Meer et al. 1991a). In addition, similar substrate specificity differences among the enzymes acting on chlorinated and non-chlorinated compounds exist in subsequent steps of the pathway.

How do microorganisms evolve new degradative genes that encode enzymes with new substrate specificities? It is interesting to note that the catechol degradative (*cat*) genes are chromosomal while the chlorocatechol degradative (*clc*, *tfd*, and *tcb*) genes are all plasmid-borne. Recruitment of genes in nature is facilitated by the transmission of plasmids to other natural microorganisms to allow degradation of chlorinated compounds, as shown by mesocosm studies in the presence of chlorobenzoates (Fulthorpe & Wyndham 1991). Since chlorobenzoate, chlorophenoxyacetate, and chlorobenzene degradation initially requires oxygenases to generate

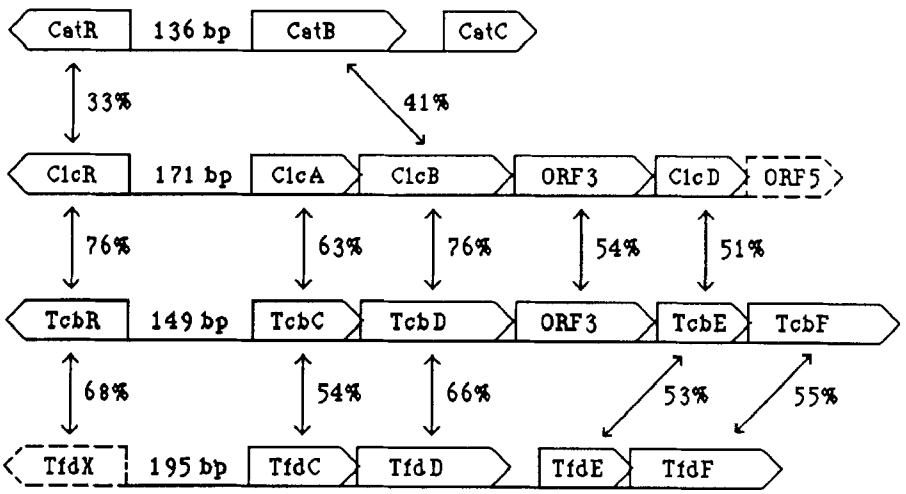

Fig. 2. The organizational similarity between the catechol and chlorocatechol degradative operons as shown by their gene products. Each of the operons contains a divergently transcribed regulatory gene with 100 to 200 base pairs between the ATG of the first gene in the operon and the ATG of the divergently transcribed regulatory gene. TfdX is the translated gene product of part of an open reading frame with amino acid identity to TcbR and other regulatory proteins, but its exact function has not been delineated. The extent of amino acid identities among the gene products is shown by arrows (the amino acid identity of TcbR to ClcR is only over a portion of ClcR, not the whole protein). In addition, other identities between the gene products are: CatR and TcbR, 31%; ClcA and TfdC, 59%; CatB and TcbD, 44%; CatB and TfdD, 42%; ClcB and TdfD, 63%; ClcD and TfdE, 54%. Also, ORF5 whose function is unknown, shows about 47% identity to TfdF in its N-terminal region.

chlorocatechol, it is interesting to note that the genes for oxygenases are often recruited by transposable elements on a plasmid either as separate gene clusters flanked by IS elements or as a composite transposon (van der Meer et al. 1991b; Nakatsu et al. 1991). A dehalogenase gene involved in dechlorinating chlorinated aliphatic compounds has been reported to be present on a mobile genetic element capable of recombining at high frequencies with plasmid and chromosomal DNA (Thomas et al. 1992). Molecular cloning, DNA sequencing, as well as purification and amino acid sequence determination of some of the catechol and chlorocatechol degradative enzymes, have provided considerable insight into the mode of evolution of the chlorocatechol degradative genes.

It should be noted that the host strains harboring the three plasmids pAC27, pJP4, and pP51 containing the *clc*ABD, *tfd*CDEF and *tcb*CDEF gene clusters (Fig. 1) were isolated in different continents *viz.* the United States, Australia, and Europe. These plasmids appear to be different with respect to their host range, *inc* property, and substrate profiles. If these three plasmids evolved independently in the natural microflora in response to the release of chlorinated substrates into the environment, then we can ask if the genes of the pathway will show any similarity to one another as well as to any presumptive ancestral genes, such as the *cat* genes. Indeed, the organization of the *cat*BC operon which is positively regulated by *cat*R regulatory gene, shows similarity to that of the *clc*ABD operon and its regulatory gene *clc*R (Rothmel et al. 1991a). In addition, both operons are similar in their organization (Fig. 2) to the *tcb*CDEF operon and its regulator gene *tcb*R (van der Meer et al. 1991c). In the above cases, the positive regulatory genes are transcribed divergently from the operons they control in such a way that the promoters overlap (Rothmel et al. 1991a; van der Meer et al. 1991c; Rothmel et al. 1991b). In contrast, the *tfd*CDEF operon is controlled by a negative regulatory gene *tfd*R, which acts as an activator in the presence of inducers such as 2,4-D, 2,4-dichlorophenol, and 4-chlorocatechol; also *tfd*R maps several kilobases upstream of the *tfd*CDEF operon (Kaphammer et al. 1990). However, just upstream of *tfd*C, there is a divergently transcribed open

reading frame (*tfd*X, Fig. 2) which shows similarity to the other regulatory genes (Perkins et al. 1988). In addition, CatR shows significant amino acid sequence homology with the other two regulatory proteins ClcR and TfdX (Rothmel et al. 1991a), while TcbR shows significant homology with parts of CatR, ClcR, and TfdX (van der Meer et al. 1991c). Similarly, the structural genes of the four operons *cat*BC, *clc*ABD, *tfd*CDEF, and *tcb*CDEF exhibit extensive homology with one another (Fig. 2). The chromosomally encoded CatA shows a good deal of homology to ClcA (Neidle et al. 1988; Frantz et al. 1987) while ClcA shows 50 to 60% homology (Fig. 2) with TcbC and TfdC (van der Meer et al. 1991a; Perkins et al. 1990; Ghosal & You 1988). Similarly CatB shows 40 to 50% homology with ClcB, TfdD, and TcbD (Aldrich et al. 1987; Frantz & Chakrabarty 1987; van der Meer et al. 1991a; Perkins et al. 1990; Ghosal & You 1988), while ClcD shows about 50% homology with TfdE and TcbE (van der Meer et al. 1991a; Perkins et al. 1990). Finally, about 50% homology exists between TfdF and TcbF and between TfdF and the N-terminal portion of a putative open reading frame at the end of the *clc*ABD operon.

In addition to sequence identity among the evolved genes, there is striking organizational similarity in the *clc*, *tfd*, and *tcb* operons (Fig. 2). For example, not only are the genes organized in the same order as the steps of the pathway but there is a 4 base pair overlap (ATGA) between the stop codon of *clc*A and the start codon of *clc*B (Frantz & Chakrabarty 1987). This 4 base pair overlap also exists between the stop codons of *tfd*C and *tcb*C and the start codons of *tfd*D and *tcb*D (Perkins et al. 1990; van der Meer et al. 1991c). Similarly, this 4 base pair overlap can be found between the stuctural genes of *clc*D and ORF5, *tcb*E and *tcb*F and *tfd*E and *tfd*F. Even more interesting is the presence of an additional open reading frame, called ORF3, between the *clc*B and *clc*D genes and also between the *tcb*D and *tcb*E genes. These ORF3 regions have no known function in chlorocatechol degradation but are present in both operons with more than 50% homology between the two (van der Meer et al. 1991c). Thus, the evolved gene clusters exhibit a great deal of organizational and sequence similarity to each other and to the chromosomal *cat* genes that allow degradation of nonchlorinated catechol.

We have so far discussed the evolution of genes that took place over a long period of time in response to the release of chlorinated compounds in the environment. In nature, these compounds are often present with other biodegradable lignocellulosic materials, and therefore, the urgency to recruit and evolve new biodegradative genes is absent. We have described another system (Rothmel et al. 1991a) in which microorganisms from dump sites were subjected to strong selection in a chemostat for the utilization of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). This recalcitrant compound was supplied to the microorganisms as the only major source of carbon and energy. Thus, the evolution of a degradative pathway for the utilization of 2,4,5-T was necessary for the survival of the microorganisms in the chemostat. It is interesting to note that 2,4,5-T degradative (*tft*) genes which evolved in a strain of *Pseudomonas cepacia* AC1100, showed little DNA homology to other members of the genus *Pseudomonas* (Tomasek et al. 1989; Haugland et al. 1991). A transposable element termed IS931 (Haugland et al. 1990b) present near the *tft* genes with a large number of copies on the genome of *P. cepacia* AC1100 also did not show any hybridization with genomic digests from a large number of pseudomonads (Tomasek et al. 1989). Recently, a cluster of *tft* genes has been sequenced and DNA (and protein) databank searches revealed homologies with genes encoding glutathione reductases, glutathione transferases, and (chloro) catechol 1,2-dioxygenases (Fig. 3). Glutathione transferases are often associated with xenobiotic detoxification in a variety of organisms including eukaryotes and have not been reported in pseudomonads. Thus, it appears that a stressed environment such as the chemostat with a single recalcitrant carbon source favors gene recruitment from any available source. Therefore, environmental conditions to a large extent dictate the mode of evolution of new degradative genes.

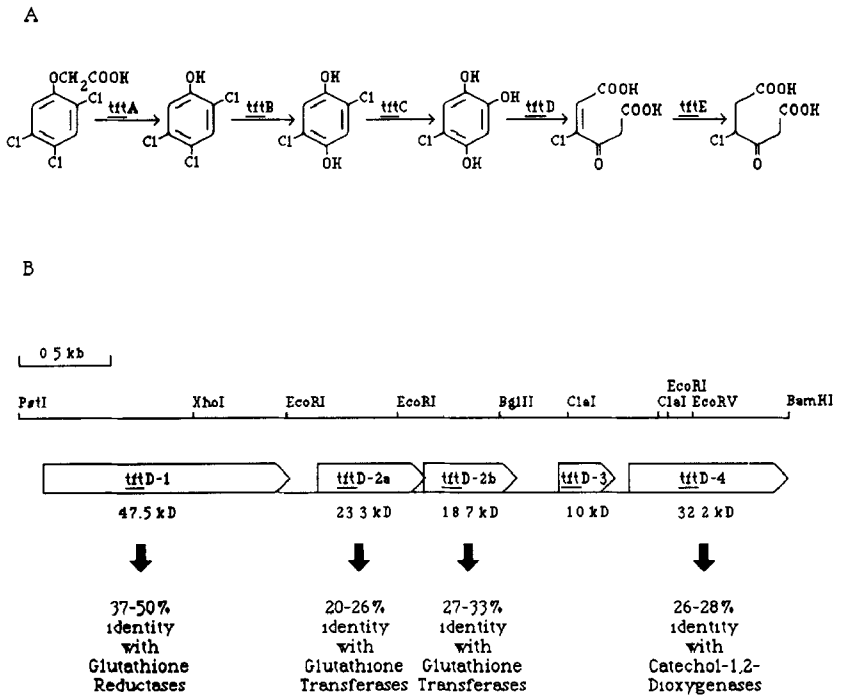

Fig. 3. (A) A tentative pathway of 2,4,5-T degradation and (B) DNA sequence analysis of a 4.2 kb *Pst*I-*Bam*H1 fragment which complements a 2,4,5-T negative mutant. The *tft*D gene cluster is believed to encode a chlorohydroquinone oxygenase complex. This cluster revealed the presence of five potential open reading frames, *tft*D-1, *tft*D-2a, *tft*D-2b, *tft*D-3 and *tft*D-4. The molecular mass of each polypeptide encoded by these genes is indicated. Four of the polypeptides, excluding the gene product of *tft*D-2b, were confirmed by expression of these genes from the T7-promoter in *E. coli*. In addition, amino acid comparison searches of the protein databanks found significant homologies with the gene products of *tft*D-1, *tft*D-2a/b, and *tft*D-4 and enzymes such as glutathione reductase, glutathione transferase, and catechol 1,2-dioxygenase respectively.

Environmental modulation of microbial gene expression

In the preceding section, we discussed how the environment modulates the recruitment and evolution of degradative pathway genes in bacteria. The environment also plays a major role in regulating the expression of specific genes in microorganisms, such that the habitat of some microorganisms is dictated by the environment they live in. An example of how the ecological niche of a bacterium is influenced by the environment is the proliferation of mucoid cells of *P. aeruginosa* in the lungs of cystic fibrosis (CF) patients. Normally, *P. aeruginosa* cells are nonmucoid and do not produce appreciable amounts of extracellular or capsular polysaccharide. The *P. aeruginosa* cells are, however, known to infect the lungs of CF patients, where they become heavily mucoid on continued proliferation in this environment due to production of an exopolysaccharide termed alginic acid (May et al. 1991). Alginate is a polymer of D-mannuronic and L-guluronic acids (Fig. 4A) which is a strong gelling agent. Alginate is believed to encapsulate *P. aeruginosa* cells to help the infecting bacteria adhere to the epithelial cells of the CF lung tissues, thereby giving rise to microcolonies on the tissue surfaces. The strong gel formation by the alginate capsule is also beneficial to the infecting *P. aeruginosa* cells since the alginate layer is believed to protect the cells from antibiotic therapy and the body's immune system. Therefore, mucoid, alginate-producing *P. aeruginosa* cells proliferate extensively in the CF lung, causing serious problems for the patients due to production of other virulence factors such as elastase, various proteases, phospholipase C, exotoxin A, etc (May et al. 1991) that cause extensive damage to the lung.

It should be pointed out that the CF lung environment is different from a normal lung environment in that the CF disease is characterized by a defective chloride channel which leads to an accumulation of abnormal fluids in the CF lung. Such a genetic defect allows accumulation of high levels of electrolytes such as NaCl and a highly dehydrated, sticky mucus in the respiratory tissues. The CF lung is, therefore, another example of a stressed environment similar to the chemostat with 2,4,5-T as the only major carbon source, as described previously. When mucoid *P. aeruginosa* cells isolated from the CF lung are cultured in the laboratory in a rich medium, they tend to revert back to non-mucoidy (i.e. no longer produce alginate). This suggests that there are specific genetic mechanisms that allow the *P. aeruginosa* cells to switch on the alginate genes in the stressed CF lung (to help survive in a dehydrated, salty environment), but to turn the switch off when the environment is favorable. In addition, although *P. aeruginosa* is known to cause other infections in burn and eye or urinary-tract, they are seldom mucoid. This suggests that mucoidy due to alginate production is primarily a characteristic of growth in the CF lung. Only mucoid *P. aeruginosa* cells appear to use the CF lung as their primary habitat.

How do *P. aeruginosa* cells sense the CF lung environment when they happen to end up in the lung, and how do they transduce the signals to trigger the activation of the alginate genes? In nature, microorganisms are known to scan the environment, resulting in the expression of a specific gene or sets of genes. Such environmental sensory signal transduction is well known for gene expression involving chemotaxis, nitrogen assimilation, medium osmolarity, virulence, etc (Bourret et al. 1991; Stock et al. 1990). Sensory signal transduction often employs a so-called two-component system; a sensor, usually a trans-membrane protein, scans the environment and in the presence of appropriate signals, undergoes a conformational change, which triggers autophosphorylation. The phosphate is then transferred from the sensor kinase to a cytoplasmic regulator protein, which in its phosphorylated form activates the promoter of a gene or set of genes by functionally binding the promoter region (Stock et al. 1990; Bourett et al. 1991). If the kinase is a soluble protein, it often needs additional transducer proteins that can sense external signals by a membrane spanning domain and interact with the kinase to trigger phosphorylation.

To understand how the alginate genes, which are normally silent in *P. aeruginosa*, are specifically expressed in the CF lung environment, we have delineated the alginate biosynthetic pathway (Fig. 4B) and the organization of the alginate biosynthetic (*alg*) genes (Fig. 4C). The mannuronate units of alginate are synthesized from fructose 6-phosphate *via* formation of mannose 6-phosphate, mannose 1-phosphate, GDP-mannose and GDP-mannuronic acid (Fig. 4B). Four enzymatic activities *viz.* PMI (Phosphomannose isomerase), PMM (Phosphomannomutase), GMP (GDP-mannose pyrophosphorylase) and GMD (GDP-mannose dehydrogenase) are involved in this conversion. Two of the enzymes, PMI and GMP, are encoded by a single gene *alg*A, while PMM is encoded by *alg*C and GMD by *alg*D (Fig. 4B). Another gene, *alg*E, believed to be involved in alginate polymerization (Chu et al. 1991) is present downstream of *alg*D. All these genes which are clustered at 34-min region of the chromosome (except *alg*C), have been completely sequenced and their gene products hyperproduced from the *tac* promoter (Shinabarger et al. 1991; Zielinski et al. 1991; Roychoudhury et al. 1989; Roychoudhury et al. 1992a). The functions of other genes present downstream of the *alg*D gene such as *alg*8, *alg*44, *alg*60 etc, are not known although they are believed to be involved in alginate polymerization or excretion. The gene *alg*G, downstream of *alg*E (Fig. 4C), is known to be required for insertion of guluronate residues in alginate (Chitnis & Ohman 1990). Very little is known about the detailed steps involving epimerization, acetylation or excretion of alginate.

The gene *alg*C, encoding PMM, is not part of the cluster at 34-min but has been shown to map (Shortridge et al. 1991) on a 60-kb *Dra*I fragment at 10-min region near three *alg* regulatory genes *alg*R1, *alg*R2 and *alg*R3 (Fig. 4C). The nucleotide sequence of the *alg*R1 gene demonstrates that it is a member of the two-component response regulator

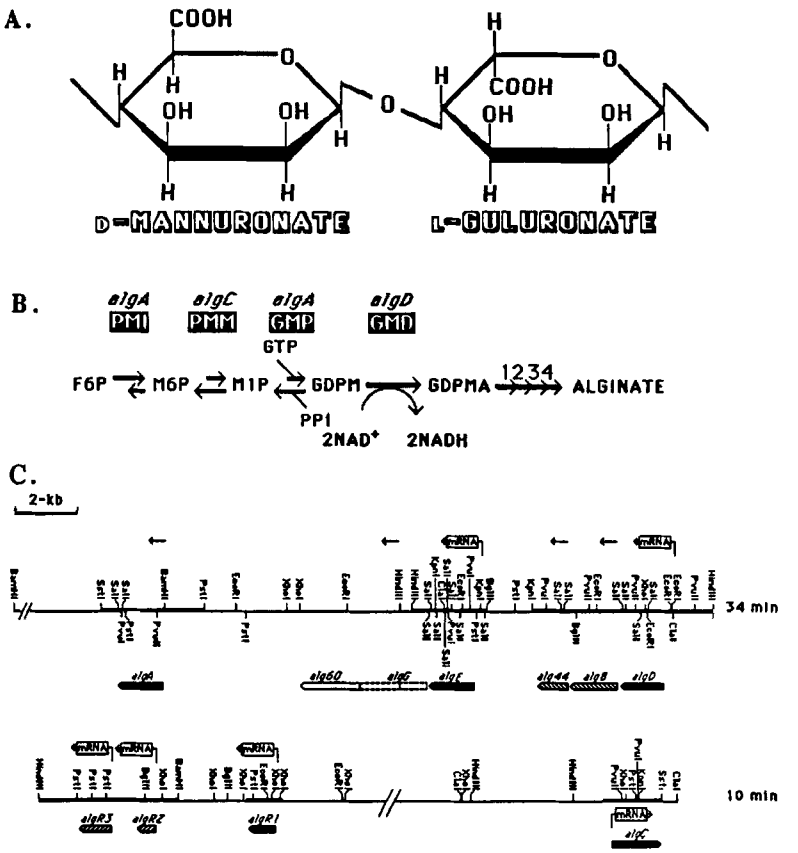

Fig. 4. (A). Chemical structure of alginate. (B). Alginate biosynthesis pathway. Arrows (1, 2, 3, 4) indicate the undefined steps of polymerization, acetylation, export, and epimerization. The gene encoding each enzyme is indicated above the enzyme name. Equilibria for the alginate reactions are known for PMI, GMP, and GMD and are indicated by the relative sizes of the arrows for each direction of the reaction. F6P, fructose 6-phosphate; M6P, mannose 6-phosphate; M1P, mannose 1-phosphate; GDPM, GDP-mannose; GDPMA, GDP-mannuronic acid. (C). Organization of alginate genes. The locations of the two gene clusters on the *P. aeruginosa* chromosome are indicated in minutes. Restriction enzyme sites are indicated by vertical lines: (——) Sequenced DNA); (——) unsequenced regions; (⟶) direction of transcription; (⌐mRNA¬) denotes the direction of transcription and transcriptional start; (◀) protein encoded by the indicated gene and confirmed by N-terminal amino acid sequencing; (◁) protein-coding region predicted from DNA sequence analysis of the indicated gene; (◁) protein encoded by the indicated gene for which the exact coding region has not been determined.

proteins which in their phosphorylated form, are involved in binding and activating or repressing specific promoters. Indeed, AlgR1 has been shown to be required for the activation of both the *alg*D (May et al. 1991) and the *alg*C (Zielinski et al. 1991) promoters. AlgR1 has been purified and shown to bind two far upstream regions centered at -382 and -458 of the *alg*D promoter (Kato & Chakrabarty 1991). The binding of AlgR1 at such far upstream sites is believed to lead to looping out of the intervening region (Fig. 5) so that AlgR1 molecules bound at -382 and -458 regions can make contact with the RNA polymerase bound at the GG-N10-GC promoter region (at -21 to -33 of the *alg*D transcription initiation site). The bending of the DNA is believed to be facilitated by proteins such as AlgR3. AlgR3 is a basic histone-type of protein homologous to sea-urchin histone H1 (Kato et al. 1990). The looping of the *alg*D promoter region is also facilitated by a putative analogue of the *Escherichia coli* cyclic AMP receptor protein (CRP), which is known to be a DNA-bending protein involved in gene regulation (DeVault et al. 1991). It was previously demonstrated that AlgR2 is required for alginate synthesis and for maximal activation of the *alg*D promoter by AlgR1 (Kato et al.

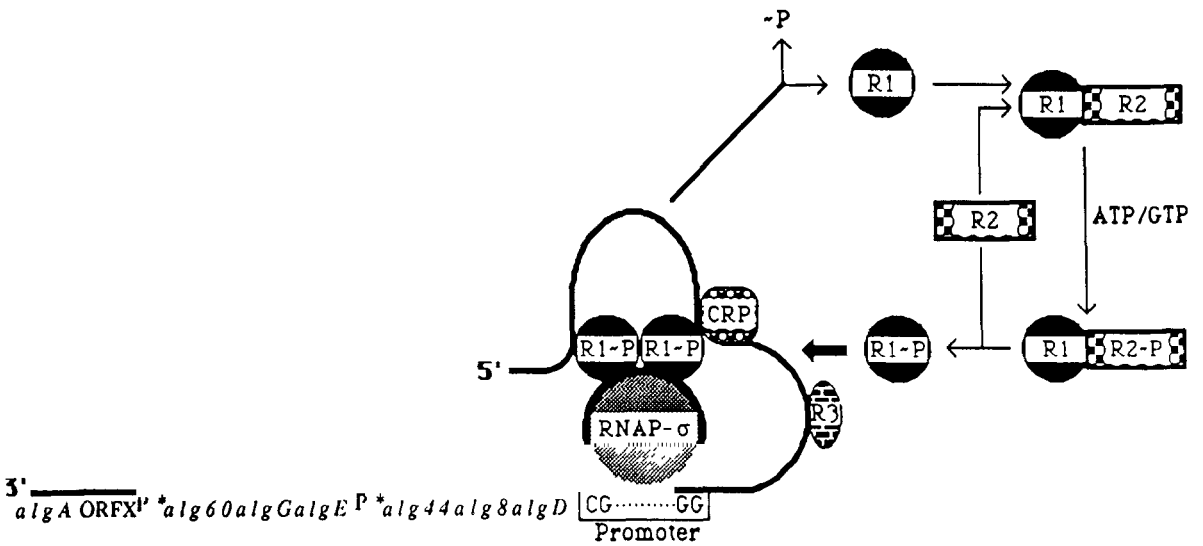

Fig. 5. The pathway for AlgR1 phosphorylation and the DNA looping model for *alg*D promoter activation. AlgR1 and AlgR2 have been shown to occur in a 80 kDa complex where AlgR2 undergoes autophosphorylation in presence of either ATP or GTP and subsequently transfers the phosphate to AlgR1. AlgR1-P functionally binds at the two binding sites centered at -382 and -458 of the *alg*D upstream region and allows bending of the intervening DNA (aided by CRP and AlgR3) to interact with the RNA polymerase at the promoter region. While this allows transcription to be initiated, it is not clear if the entire 16 kb segment down-stream of the *alg*D gene is transcribed as a unit. The location of AlgR3 is arbitrary while CRP site is known to be centered at -362 near one of the AlgR1 binding sites.

1989). It has been subsequently shown that AlgR2 is a protein kinase capable of autophosphorylation in the presence of either ATP or GTP (Roychoudhury et al. 1992b). AlgR2 can then transfer the phosphate to AlgR1 and allow binding of the phosphorylated AlgR1 to the upstream region of the *alg*D promoter for functional initiation of transcription (Roychoudhury et al. 1992c). Since the *alg*D promoter is activated under conditions of high osmolarity due to the presence of high NaCl concentrations in the growth medium (Berry et al. 1989) or under conditions of membrane perturbation induced by ethanol, a known dehydrating agent (DeVault et al. 1990) and since such conditions are reminiscent of the environment present in the CF lung, it appears that specific environmental factors unique to this diseased tissue trigger autophosphorylation of AlgR2. AlgR2 allows phosphorylation of AlgR1 which can then initiate efficient transcription from the *alg*D and the *alg*C promoters, resulting in alginate synthesis. Since AlgR2 is a soluble protein without a membrane spanning region, it is not clear how the osmolarity or membrane perturbation induced signals are transmitted to AlgR2, nor is it completely clear as to how many genes downstream of the *alg*D promoter (other than *alg*D) are under the control of this environmentally regulated promoter. It is interesting to note that expression of other pathogenesis-related genes such as neuraminidase, which appears to be involved in the adherence of *P. aeruginosa* to eukaryotic cell surfaces, might be modulated by high osmolarity and require AlgR1 as an activator (Cacalano et al. 1992).

In conclusion, environmental signal transduction is an important feature in enabling microorganisms to scan their environment and take appropriate measures to establish themselves in a particular ecological niche. Since the CF lung is a highly stressed environment, it is interesting to note that *P. aeruginosa* uses an efficient signal transduction system to turn on the alginate genes for production of the alginate capsule. This capsule not only protects the infecting cells from dehydration or high osmolarity in the CF lung, but also from the body's defense mechanisms or antibiotic treatments, al-

lowing them to colonize the host tissue. If bioremediation is to succeed, the remediating microorganisms must not only evolve and express the degradative genes, but must also establish themselves in the environment where they are to be used. Understanding how microorganisms establish themselves in certain ecological niches as in biofilms on plastic or metal surfaces and human or animal tissues, as well as in soil or sediments (Costerton et al. 1987), is an important aspect of developing successful bioremediation processes.

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