The Periplasmic Galactose Binding Protein of Escherichia coli

This protein is involved in transport as well as in chemotaxis.

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Cell membranes are involved in many biological functions; among them are active transport, excitation, and oxidative phosphorylation. My own interest in membrane functions started with the problem of active transport and the mechanism of energy coupling.

Around 1935, the late August Krogh of Copenhagen lectured on salt regulation by freshwater animals. The ability of the frog to pick up a common physiological electrolyte like sodium chloride was illustrated by the simple fact that when a frog is placed in distilled water it dies within a few hours, whereas in tap water it could survive. In tap water the frog is able to capture and concentrate the traces of salt present there. In distilled water there is nothing to pick up. This phenomenon formed the basis for the studies by Ussing (*1*) on the active sodium transport in frog skin, a process that requires cell respiration.

Active Transport and the Phosphorylation of Sugars

The capture and active transport of glucose, carried out by the epithelium of the intestine, by the kidney cortex tubules or by many tumor cells, presented other examples of considerable interest. In 1933 Lundsgaard had already expressed interest in a theory, proposed by Wilbrandt and Laszt, that a phosphorylation-dephosphorylation cycle might operate in the active transport of glucose occurring in the intestinal and kidney epithelia, processes that are readily inhibited by phlorizin. Both types of epithelia were known to be rich in phosphatases and Lundsgaard showed that these enzymes were also inhibited by phlorizin (*2*).

In the early 1930's it was often taken for granted that phosphatases, which catalyze dephosphorylation, would likewise be the catalysts operating in phosphorylations, a step called "phosphatese" reaction; thus phosphatases were possible catalysts of cyclic reactions. I was much attracted to the Lundsgaard proposal, yet I realized that this type of a cyclic mechanism would not be adequate to account for active transport. In my modified noncyclic model, a vigorous phosphorylation was considered the key step, replacing the inefficient "phosphatese" reaction. A phosphorylation step by an equivalent of one adenosine triphosphate (ATP) was considered the driving force required for the dramatic recapture performance which the epithelial cells of the kidney tubules are able to muster. The existence of a highly effective oxidative phosphorylation mechanism for glucose was indeed disclosed in kidney cortex homogenates in which the phosphatase activity has been subdued by the addition of sodium fluoride (*3*). This type of phosphorylation was shown to depend on the presence of both oxygen and di- or tricarboxylic acids (*3, 4*). When these organic acids were present, much more oxygen was consumed, and this vigorous consumption of oxygen is typical for the Krebs cycle. The dependence on the Krebs cycle is characteristic for the process now called "oxidative phosphorylation." Oxidative phosphorylation was later assigned a role in the translocation of ions through the mitochondrial membrane (*5*). The role of "high energy phosphate" for active transport would obviously have to be of a different nature, depending on the type of substance that was actively transported. In the case of sugars and polyalcohols, a direct phosphorylation, forming the corresponding phosphoric esters of the polyalcohols, seemed a plausible step for the function of active transport.

The first successful demonstration of a phosphorylation mechanism functioning in the transport of hexoses was described in bacteria (Gram-negative as well as Gram-positive organisms) by Roseman and his co-workers (*6, 7*). In this system, the so-called "phosphotransferase" system, the phosphoryl donor is phosphoenolpyruvate (PEP) and not ATP. The subsequent donor is a phosphorylated histidine group in a low molecular protein, HPr. The reaction sequence as described by Roseman and co-workers (*6, 7*) operates as follows:

$$\text{PEP} + \text{HPr} \xleftrightarrow{\text{enzyme I}} \text{pyruvate} + \text{P-HPr} \quad (1)$$

$$\text{P-HPr} + \text{glycosides} \xleftrightarrow[\text{enzymes II}]{\text{(factor III)}} \text{HPr} + \text{glycoside-6-phosphate} \quad (2)$$

The first step is catalyzed by one enzyme. The second step involves several specific enzymes (enzymes II) depending on the nature of the glycosides. The most common glycosides used are α-methylglucosides and β-methylthiogalactoside. The monosaccharides glucose and mannose and the polyalcohol mannitol are also substrates. The phosphoryl acceptor is invariably the 6-hydroxyl of the various substrates. Factor III is a family of sugar specific proteins required for step 2 (*7*). Galactose can serve both as a phosphate acceptor in step 2 (*8*) and as an inducer of the proteins involved in step 2 (*7*).

In contrast to the phosphotransferase system, the lactose or thiogalactoside system (also called "TMGI" permease) of *Escherichia coli* does not seem to operate through a device of phosphorylation of the sugars.

The importance of the PEP system for active transport of sugars in microorganisms was established through the *genetic approach.* Tanaka and Lin (*9*), as well as the Roseman group (*10*), demonstrated that the so-called "car" mutants—that is, mutants unable to grow on a number of carbohydrates (glucose, mannose, mannitol, for instance)—were defective in the enzyme catalyzing reaction 1.

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Active Transport of Sugars without Direct Phosphorylation

Although nature has clearly used the device of phosphorylation of sugars and related compounds in the service of active transport, this device does not appear to be generally used for transport or permease activity; even certain sugars and polyalcohols are captured by other means. Transport systems that operate through a direct active uptake of phosphoric esters, like glycerophosphate or glucose-6-phosphate, have also been described (*11, 12*). Transport of the latter ester is discussed later.

The *E. coli* transport system for lactose and thiogalactosides (*13*) has been examined by Kennedy and his co-workers (*14, 15*) and has resulted in the isolation of the highly lipophilic M protein, the gene product of the Y gene of the lac operon. This gene programs the permease for the transport of lactose and thio-β-galactosides [thiomethylgalactoside (TMG) or thiodigalactoside (TDG)]. Using a modification of the Kepes-Monod model, Kennedy invoked the idea of energy transmission through the membrane protein, ATP eliciting conformational changes of the M protein (*14*). A conformational change of the carrier brought about directly by oxidation reduction has been proposed by Barnes and Kaback (*16*) on the basis of their studies of active transport of amino acids and β-galactosides by bacterial membrane vesicles. Barnes and Kaback found that uptake of lactose and TMG into the membrane vesicles was greatly stimulated by addition of D-lactate as an energy source (*16*).

Thus, energy needed for active transport might be channeled directly to the specific membrane protein carrier, bringing about a change in its affinity for the ligand.

Permease seems to be involved in active transport as well as in membrane carrier activity. Wong *et al.* (*17*) have found a mutant of the TMGI transport system which showed intact membrane carrier activity (supposedly by way of the permease protein), but which had lost the ability to concentrate TMG inside the cell.

The "lac permease" has been studied mainly with substrate analogs like thio analogs of β-galactosides, especially TMG and TDG. As substrates, these analogs show a relatively low affinity for the transport system, and the concentration gradients achieved are merely of the order of 10 to 100. The affinities of the natural substrates like galactose or galactosides are no higher. However, the existence of high affinity nonphosphorylating transport systems, which are able to accumulate free galactose against very high concentration gradients, has been observed. A study of one of these systems is the main topic of this essay.

High Affinity Galactose Capture Systems

A highly active galactose transport system which operates at very low concentrations of substrate was first described by Horecker *et al.* [(*18*); see also Osborne *et al.* (*19*)] using *E. coli* ML. Since the absence of galactokinase (phenotype K^-) was considered a prerequisite for quantitative studies of galactose permease, only gal K mutants were used. The ability to capture galactose at external concentrations as low as $10^{-6}M$ is characteristic of this system. In some cases the galactose concentration inside the cell was 2000 times higher than that of the medium; moreover, the accumulated free galactose inside the cell was retained after the cells were transferred to a medium that did not contain any galactose. To account for this effective retention, exit mechanisms were studied. It was found that the addition of azide or dinitrophenol abolished the retention of galactose, seemingly activating exit (*19*).

A simple alternative explanation for the retention phenomenon was offered by Rotman (*20*) who focused his attention on the high affinity capture mechanisms and assumed that these could serve as highly effective recapture mechanisms. Recapture of "leaking" endogenous galactose would thus accomplish high retention without any alteration of the rate of exit. Exit is believed to be served by another carrier, different from that of entrance and high affinity recapture. The Rotman recapture mechanism can account for several observations including the phenomenon of endogenous induction of the gal operon, which we have observed in *E. coli* K-12 gal mutants (*21, 22*). This induction will be discussed in a later section.

Terminology for Galactose Transport and Permease Systems

Before the highly effective galactose transport system is described, it is necessary to comment on the terminology of transport systems, especially the galactose transport systems. This terminology requires modifications that should eliminate the present ambiguities. In particular, two points need clarification: (i) a sharper distinction between the steps involved in active transport (permease *sensu stricto*, energy generating systems, and additional factors), and (ii) more emphasis on classification of the transport system according to the specificities and affinities for the substrates. The first point is a very general one; the second point is obviously a more specific one.

With regard to the first point, a distinction has to be made between the use of the name permease ("P") and the name transport. Recently it has been proposed that the specific proteins involved in the first recognition of the substrates to be transported should be classified as permease proteins (*23, 24*).

The present genetic terminology for galactose transport systems is far from clear because of the lack of knowledge of the steps operating in these systems. Thus, a gene involved in the biosynthesis of one of the proteins involved in the galactose capture system is called either "P_{mg}" or "mgl P" (*24, 25*). The name "P" refers to permease and "mg" or "mgl" to the alleged specificity of the permease for β-methylgalactoside permease. At present no one knows for certain whether the "mgl P" gene is really programing for a P, that is, for a permease in the strictest sense. Moreover, the affinity of the P_{mg} system for β-methylgalactoside (called megal) is actually not nearly as high as its affinity for free galactose or for β-glycerogalactoside. The name P_{mg} for this transport system was introduced to differentiate it from another galactose transport system called "P_{gal}" (*25*), because the latter handles only free galactose and not megal.

By present terminology, *E. coli* may have as many as six active transport systems for galactose, some of them designated by symbols which are not rigorous biochemical terms.

1) The phosphotransferase system in which galactose or megal are supposed to be phosphorylated in the 6-hydroxyl position [Roseman (*10*)].

2) The lac system, also called TMGI, described by the Monod School (*13*).

3) A related system in *E. coli* K-12, described by Prestidge and Pardee (*27*) and called TMGII permease (TMGII is temperature sensitive at 37°C).

4) The transport system reported by Rotman *et al.* (*25*), called P_{gal} because it

handles only free galactose and not megal; it may be α-specific (*26*).

5) Rotman's P_{mg} system (*25*), a high affinity active galactose transport system which is specific for galactose and β-galactosides. Megal is not nearly as effective a substrate as galactose and β-glycerogalactoside (*28, 29*). D-Fucose is transported but with low efficiency (*24, 25*).

6) Galactose transport systems linked to L-arabinose transport (*30*).

The picture sketched here may be unnecessarily complicated. For instance, the Roseman system (*10*) may not handle β-galactosides directly (*16*). Also, there is some doubt whether TMGII (*27*) may not be identical with Rotman's "P_{gal}" system since both systems have specificity for melibiose, an α-galactoside (*26*). The "P_{mg}" system, in contrast, is strictly specific for β-galactosides and its binding protein, and as will appear later, shows strict specificity for the β form of free galactose as well as for β-galactosides. When these characteristics are taken into account, it seems worthwhile to impose some renewal of the terminology. Since megal is a much poorer substrate than β-glycerogalactoside or β-galactose we shall replace the term P_{mg} by the term $P_{\beta g}$ in describing the high affinity transport system of galactose and β-galactosides.

The $P_{\beta g}$ transport system shows not only an extraordinarily high affinity for its substrates (especially glucose and galactose), but it also plays an essential role in a number of interesting cell physiological phenomena.

Endogenous Induction of the Gal Operon and the Gene for $P_{\beta g}$

At this point it may be appropriate to make a few remarks about the "gal operon" and the physiological role of the enzymes programed by it. The three genes for galactose metabolism form a cluster (*31*) that can be transmitted through a prophage λdg (*32*) or by an episome F-gal (*33, 34*). The enzymes programed by the three genes were identified with the three enzymes of the uridine diphosphogalactose (UDPGal) pathway; this pathway is also the predominant one in the galactose metabolism of man (*35, 36*). The enzymes are galactokinase (K), galactose-1-phosphate uridyltransferase (Gal-1-P-uridyltransferase) (T), and UDPGal 4-epimerase (E). The programing and coordinate regulation of the biosynthesis of these enzymes (*21, 22*) has been compared with that of the lac operon, and the gene cluster was therefore called the gal operon (*33*). Unlike the lac operon, the gal operon is never completely repressed; hence, relatively high levels (*36*) of the enzymes appear in the uninduced state. Induction brings about increases in enzyme levels about tenfold higher than those of the uninduced state. Induction can be elicited by the addition to the growth medium of galactose (*36*) or D-fucose (*37*). Buttin (*38*) also found a specific regulator gene R_{gal} for the gal operon.

With regard to the programing and control of the transport system, the structural gene for $P_{\beta g}$ is located in the vicinity of the histidine region (*20*). It is not subject to regulation by the R_{gal} gene (*25*). Yet, several mutations in the gal operon affecting its regulation also seem to exert an effect on the regulation of the $P_{\beta g}$ system (*25, 28*); moreover, D-fucose as well as galactose can act as inducer for $P_{\beta g}$ synthesis (*25, 28*). Coordinate endogenous induction of βg P gene and of the gal operon was encountered in the following way.

It was first observed that *E. coli* K-12 mutants of the phenotype $K^-T^+E^+$ show a derepression of the gal operon; that is, the remaining active enzymes T and E are synthesized at a rate corresponding to that of full induction (*21, 39*). A study of the nature of this type of "constitutivity" pointed to an endogenous induction which acted through a peculiar metabolic "cul de sac" by which UDPGal released free galactose in the cell without the galactose being reutilized (*22, 28, 40*). The lack of galactokinase activity is indeed one of the main prerequisites for this induction; the other main prerequisite is a rapid formation of UDPGal from endogenous UDPG by the action of epimerase (*22*).

A closer analysis disclosed furthermore, that the presence of a high affinity galactose recapture system, such as the $P_{\beta g}$ system, secured the conditions for a full-fledged endogenous induction (*28, 40, 41*). In addition, there seems to be a peculiar "mutuality" attached to this regulation; the endogenous inducer, which is accumulated, further induces the $P_{\beta g}$ system (*28*). Since the synthetic growth medium used was freed of even traces of galactose, the inducer stemmed from a cellular source; the cellular precursor was shown to be UDPGal (*22, 28*).

Studies of the $P_{\beta g}$ system and especially of its regulation requires special precautions owing to its high affinity for free galactose as follows.

Basal growth medium: Synthetic media with succinate or glycerol as carbon sources, and ammonia as nitrogen source, are recommended. Commercial nutrient broth contains sufficient traces of galactose to elicit a simulated endogenous induction in $P_{\beta g}$ strains which are also K^-E^- or K^-UDPG$^-$ (UDPG synthetase–defective) (*22*).

The state of the gal operon: The presence of gal K and gal E mutations must be taken into consideration (see later).

Concentrations and permease substrates: Galactose as well as β-glycerogalactoside (labeled with ^{14}C) must be used in concentrations below $10^{-6}M$. This low concentration is imperative, especially if galactose is used as substrate, otherwise activity due to other galactose permeases becomes too high. The use of β-glycerogalactoside requires Z^-Y^- strains for proper analysis, since this galactoside is also substrate for β-galactosidase and is transported by the lac system.

If these precautions are disregarded the evaluation of many experiments is apt to be more difficult. Thus, previous arguments for or against the involvement of endogenously produced galactose in the gal K type of induction (*21, 42, 43*) failed to take into account the action of the high affinity capture system which makes it mandatory to use well-defined synthetic media when studying this type of induction. In his thesis, Wu has demonstrated that strains of the phenotype "$P_{\beta g}^+$," K^-, UDPG$^-$, show no induction of T and E provided that they are grown on ammonia mineral succinate medium (*40*); replacement of synthetic medium by commercial nutrient broth as growth medium, however, brings about full induction in this strain [(*22*); see also table 9 in (*40*)]. Apparently many brands of nutrient broth contain galactose or a related inducer that can be greatly concentrated by the $P_{\beta g}$ system and thus simulate endogenous induction (*40*). Since the regulation of additional membrane functions, including galactose chemotaxis (see later) may be governed by the same mechanisms, it is vitally important to distinguish between a genuine endogenous induction and an induction brought about by the "scavenging" of impurities in the medium, especially when studying the K^-E^- or K^-, UDPG$^-$ double mutants.

Table 1. Relation between intracellular and extracellular galactose concentrations for various K mutants of *E. coli* K-12 (*28*).

Strain	Phenotype	Gal operon regulation *	Galactose concentration	
			Intracellular ($10^{-4}M$)	Extracellular ($10^{-6}M$)
W3092c	$K^-P^+_{\beta g}$	Internally induced	2.47	0.45
W3092i	$K^-P^-_{\beta g}$	Inducible	0.82	3.5
CN 30.2	K-UDPGPP-	Inducible	0.26	0.1
316E-, 1–4	K^-E^-	Inducible	0.1	0.1
W3092c rev	$K^+P^+_{\beta g}$	Inducible	0.26	0.1
54	$K^-P^+_{\beta g}$	Internally induced	1.95	0.27
C3-3	F'Gal K^-/K^-	Internally induced	3.50	

* The status of the gal operon regulation was determined as described (*22*).

From Fig. 1A and Table 1, it may be seen that the endogenous induction is dependent on epimerase activity (E^+), converting UDPG to UDPGal. Apparently, a fraction of intracellular UDPGal is converted to free galactose. If this free galactose cannot be phosphorylated because of the K^- state and is unable effectively to escape from the cell, because of the presence of a highly effective and fully induced recapture mechanism (for example, $P_{\beta g}{+}{+}$), then the galactose liberated from UDPGal accumulates inside the cell.

The cellular threshold concentration of galactose for induction of the galactose operon in gal K strains is 1 to $2\times 10^{-4}M$ (Table 1). A galactose concentration of $0.8\times 10^{-4}M$, found inside cells that are "recapture defectives" (that is, lacking the $P_{\beta g}$ system), is below the threshold for induction (*28*). However, the relatively high levels of total free galactose in the $_{\beta g}$P, gal K mutants, as compared with a gal fermenter (such as the W3092 revertant) are noteworthy. The lowest cellular galactose concentrations were found in gal E mutants that are unable to convert UDPG to UDPGal, indicating that the cellular galactose stems from UDPGal. Strains that are able to phosphorylate or metabolize galactose also show galactose concentrations that are far below the threshold level for induction of the gal operon. From Table 1 it also appears that a mutant with the phenotype $P_{\beta g}{-}K^-E^+$, unable to recapture the endogenously produced free galactose (hydrolyzed from its own UDPGal), should be able to act as a "feeder of inducer" for strains with inducible $P_{\beta g}$ and gal operon (test strains), if grown together. A responsive test strain should have the following phenotype: K^-E^- and a $P_{\beta g}$ system which at the start of the mixed culture is uninduced. The test strain does not generate galactose endogenously because of its inability to form UDPGal from UDPG. On the other hand, traces of endogenous galactose leaking out from the "recaptureless" feeder strain can be effectively captured and concentrated by the uninduced responsive test strain during the mixed growth experiment (Fig. 1B). After one generation time of mixed growth, the $P_{\beta g}+$ strain showed indeed a typical induction of its $P_{\beta g}$ (*28*). Such an induction did not develop if feeder and test strains were merely mixed for a few minutes [see (*28*) and Fig. 1B]. Neither did a mixed growth experiment of two test strains (that is, phenotype $P_{\beta g}+$, K^-E^-) elicit any induction of the active $P_{\beta g}$ since no endogenous galactose was generated in either of these strains. As would be expected, addition of exogenous galactose to the growth medium of either of the test strains elicited, after one generation time, an induction of $P_{\beta g}$. Conversely, if the feeder strain was grown in a medium containing galactose, no induction of $P_{\beta g}$ was elicited for the reason that this strain has no $P_{\beta g}$ (*28*).

In the feeding experiment, the strain in which the $P_{\beta g}$ system is induced by capturing "inducer" from the feeder

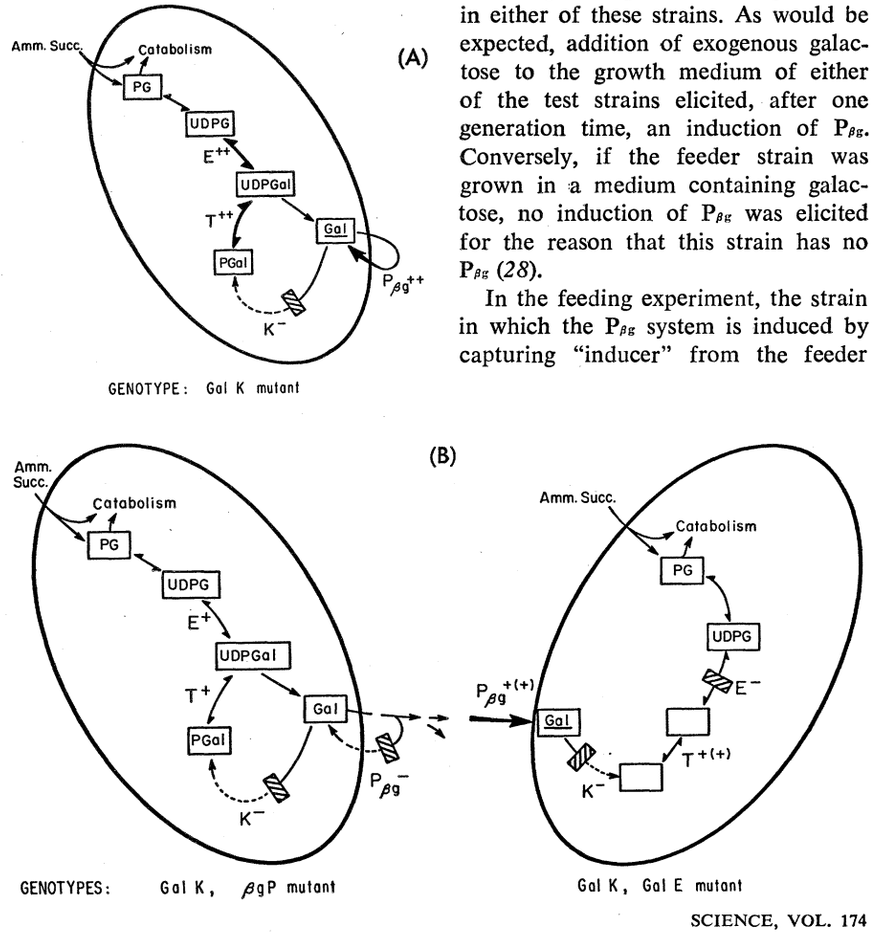

Fig. 1. (A) Scheme of endogenous induction of the gal operon through endogenous release of galactose which remains nonphosphorylated and is retrieved completely by a recapture mechanism. Special symbols and abbreviations as follows: Amm. succ., ammonium succinate; PG, α-glucose-1-phosphate; Gal and *Gal* for endogenous levels of galactose which are, respectively, below and above the threshold for induction of the gal operon. The symbols +, +(+), and ++ designate enzyme (or permease) levels corresponding to no induction, beginning induction, and full induction, respectively. Pathways are indicated by ↔ (reversible) or → (irreversible). The hatched symbol signifies blocked pathway corresponding to the defect in galactokinase, "K^-." (B) Scheme of a feeding experiment by which one strain generates the inducer galactose, but because of its lack of a capture or recapture system it loses its inducer (gal levels below the threshold for induction of the gal operon). In a mixed growth culture with a K^-E^- mutant which is unable to make UDPGal and endogenous galactose but has a capture mechanism, induction of the E^- strain ensues during the mixed growth. Galactose "fed" from the "donor" strain to the E^- strain cannot be used as a nutrient by the K^-E^- strain but because of the K^- state the galactose captured accumulates to level *"Gal"* meaning that the threshold for induction is reached. Hence biosynthesis of the enzyme T of the gal operon as well as $P_{\beta g}$ are being induced, that is, $T^{+(+)}$, $P_{\beta g}{}^{+(+)}$.

strain apparently responds to intracellular concentrations of galactose of the order of $10^{-4}M$ or somewhat lower. It may be recalled that the cellular threshold for the gal operon is between 1 to $2 \times 10^{-4}M$ galactose. The threshold for the regulation of some of the high-affinity transport mechanisms (and as described later for the galactose binding protein) is probably even lower.

It is evident, then, that galactose even in very low concentrations acts as an efficient inducer of $P_{\beta g}$ as well as of the gal operon (*22, 28*).

Selective Aspects of Endogenous Induction

Endogenous induction as observed in gal K mutants is readily lost by mutations giving rise to $P_{\beta g}$ defects. It might not be surprising if there were a selection against this type of endogenous induction. After all, a 10- to 15-fold increase in the rate of synthesis of two of the enzymes programed by the gal operon (T and E) as well as of the $P_{\beta g}$ systems seems sheer waste since the galactokinase step is missing in these strains.

The $P_{\beta g}+ K^-$ strains may not release as much intracellular galactose as the corresponding $P_{\beta g}$ defective strains that are able to sustain surprisingly high internal and external galactose levels (see last column of Table 1). It would not be unreasonable to expect that free galactose inside the cell might exert a feedback inhibition either of UDPGal formation of UDPGal splitting (spilling of more galactose) or of the recapture system, before the threshold level for induction was reached. Even if a negative feedback is elicited in the $P_{\beta g}+ K^-$ strain, it is not sufficently effective to prevent the idle endogenous induction.

Additional Regulatory Control of βg P Genes

Although the $P_{\beta g}$ system seems to follow a coordinate regulation with the enzymes of the gal operon as seen in endogenous induction or in the exogenous induction by galactose or D-fucose, this feature does not account for the entire regulatory control of this transport system.

It has, for instance, been observed that certain Hfr derivatives, PL-2 and PL-2-7, K^+E^- and K^-E^- phenotypes, respectively, show derepressed $P_{\beta g}$ activity, although these strains are completely devoid of epimerase activity (*44*). This type of derepression, reminiscent of an "operator constitutive," may be characteristic of certain HfrH derivatives [for characterization of Hfr and HfrH, see (*35*)].

Moreover, levels of $P_{\beta g}$ (whether noninduced, induced, or constitutive) tend to decrease if all the enzymes of the gal operon are induced or constitutive (*45*). The basis for this type of opposing regulation is under further study. It should be added that the same investigators have identified a specific regulator gene for the "$P_{\beta g}$" system (*45*). This gene is called mgl R, or βg R, and is located far away from the gal operon; yet it is distinctly different from the gal R (*45*). Hence, although the gal operon as well as the gene for $P_{\beta g}$ are both induced by galactose, the two gene loci respond to different regulator genes.

Induction and Repression of Transport via the Membrane

Our observations have led us to subscribe to Rotman's recapture theory. I am thus prompted to raise the question of whether the endogenous induction of the gal operon and the $P_{\beta g}$ transport system occurs truly from within or whether it occurs from without, elicited by the recapture of the galactose through the membrane.

In fact, Heppel and Dietz [see (*12*)] found that induction of the glucose-6-phosphate transport system is specifically elicited from the exogenous glucose-6-phosphate. Any glucose-6-phosphate accumulated inside the cell, as seen in certain mutants, is unable to elicit induction. This situation would be reminiscent of the observations on arginine as corepressor of its own pathway. Endogenously accumulated arginine is inactive as a corepressor; only arginine transported through the membrane acts as a corepressor (*46*). Apparently, the mixing of the two types of arginine is a relatively slow process (*47*). Perhaps the endogenous induction of the gal operon reflects the pattern of control from without, through a recapture. Then, the existence of a genuine endogenous induction could be disputed. However, observations on the endogenous induction of the operon for histidine catabolism by urocanic acid (*43*) argues in favor of an endogenous induction. Moreover, as we shall see in the next section, the regulation of the biosynthesis of the periplasmic galactose binding protein presents a striking example of a genuine endogenous induction.

Periplasmic Galactose Binding Protein

Substrate recognition by specific binding of ligands to be transported must be the first event in active transport as well as in facilitated flow and counterflow as catalyzed by permeases. Techniques for release of membrane proteins, so-called periplasmic proteins with specific binding activities, were successfully initiated about 5 years ago (*48*). Anraku and Heppel (*49*) developed a technique by which *E. coli* cells suspended in sucrose ($0.5M$) and subjected to $10^{-4}M$ ethylenediaminetetraacetate (EDTA) for 10 minutes and subsequently shocked with cold water release some of the periplasmic binding proteins, among them a protein that binds galactose.

Perhaps the use of the word "periplasmic" first needs a few comments. Whether these proteins in the intact cells are located on the membrane or between the membrane and the cell wall is an open question (*12*). The phenomenon was perhaps first brought into focus by the observations of Malamy and Horecker (*50*), who found that alkaline phosphatase is one of the enzymes released readily from *E. coli* when cells are converted to spheroplasts by lysozyme and EDTA. In this essay we shall use the term "periplasmic" solely as an operational term.

Anraku (*51, 52*) has given a biochemical description of a galactose binding protein whose molecular weight (M.W.) is approximately 36,000. This protein is insensitive to sulfhydryl reagents (*51*). According to Anraku each unit of M.W. 36,000 binds one molecule of galactose. In order to obtain saturation it was necessary to use galactose concentrations of about $10^{-5}M$. The dissociation constant for galactose was reported to be approximately $10^{-6}M$. Since no other galactose derivatives such as β-glycerogalactoside or β-methylgalactoside were tested for binding and no highly defective transport mutants were available at that time, it remained indeterminate whether Anraku's periplasmic galactose binding protein belonged to the TMG systems, the P_{gal} system, or the $P_{\beta g}$ system.

In our laboratory Winfried Boos initiated a genetically oriented study of the

Table 2. Summary of results of joint studies on transport, binding protein, and chemotaxis (*29, 59*).

Strains	$P_{\beta g}$ transport	Galactose binding protein		Gal chemotaxis (Adler test)*	Reference
		Gal binding	Cross reaction		
W3092c	+	+	+	+	(*28, 29, 59*)
W3092i	−	+	+	+	(*28, 29, 59*)
W4345†	−	−	−	−	(*24, 29, 59*)
AW520‡	−		−	−	(*59*)
AW521‡	+		+	+	(*59*)

* Tested by Adler and co-workers according to their recent assay method (*57*). † Strain obtained from Dr. Boris Rotman. ‡ Strain obtained from Dr. Julius Adler.

galactose binding protein. He first showed that this protein is genetically related to the $P_{\beta g}$ transport system. The binding specificities and affinities of the galactose binding protein are similar to those of the $P_{\beta g}$ system, in that they both show the highest affinities for glucose, galactose, and β-glycerogalactoside (*29*). A subsequent investigation of a βg P mutant from Rotman's collection showed that this mutant did not produce any detectable amounts of the galactose binding protein (*29*). Moreover, the gene locus for the defect in permease activity is closely linked to if not identical with a gene locus necessary for the expression of the binding protein (*53*); βg P mutants isolated after mutagenesis of a wild type strain also showed defects in the expression of the galactose binding protein (*53*). But matters are more complex in that strains highly defective in galactose transport may, nevertheless, show a high production of galactose binding protein (*29*). An analogous situation had already been encountered by Pardee and co-workers, who surveyed mutants defective in sulfate transport (*54*). A survey of *E. coli* strains for galactose binding protein showed that all the transport positive strains investigated invariably produced galactose binding protein (*53*), whereas the transport negative strains could be divided into two types, one which still produces the binding protein and another that has lost the capability to synthesize it (*29, 53*).

Attempts to find membrane recognition sites on the galactose binding protein which can restore genuine galactose transport in EDTA-shocked cells have so far not given reproducible results and, at best, have shown only a modest restoration (*52, 55*).

Both the demonstration of structural $P_{\beta g}$ mutants, such as temperature-sensitive mutants and affinity mutants, and the development of reproducible methods for the restoration of transport in shocked cells (or in the bacterial membrane vesicles (*16*)—with the use of binder proteins from wild type—would be desirable tools in any attempt to establish a correlation between galactose binding protein and the transport systems.

Galactose Binding Protein and Galactose Chemotaxis

About 3 years ago I tried to correlate the presence or absence of the galactose binding protein with another membrane function, *specific galactose chemotaxis*, as described by Adler and co-workers (*56, 57*). The characteristics of chemotaxis are only partly understood; yet it is considered one of the most primitive neurobiological reactions known. In this article, only the few aspects pertinent to the discussion

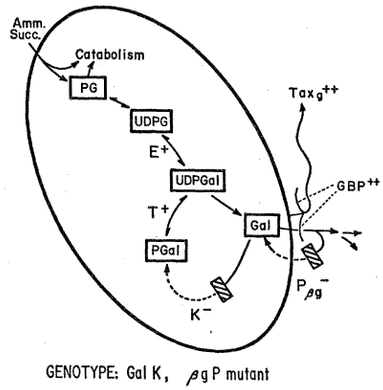

Fig. 2. Scheme of induction from within (endogenous induction) of two "ektobiological" systems located on the outside of the membrane, the galactose binding protein (GBP^{++}) and galactose chemotaxis (Tax_g^{++}) $P_{\beta g}^-$ means in this case that the capture (and recapture) system is defective because a mutation renders the affinity of GBP for galactose abnormally low. The galactose binding protein levels were, therefore, determined by its cross reactivity; GBP^{++} signifies that the levels were high after growth in the presence as well as in the absence of the exogenous inducer, D-fucose. The gal operon remained uninduced (T^+E^+) in this strain, which is unable to recapture its endogenous galactose.

of the galactose binding protein are mentioned.

Chemotaxis is the movement of organisms toward or away from a chemical. It was first described by Engelmann, Pfeiffer, and other biologists more than 70 years ago. They suspected that the type of chemotaxis observed in bacteria is an avoidance reaction, or a swimming away from particularly low concentrations of an attractant when the organisms are placed in a gradient between high and low concentrations of the test chemical. It is in general assumed that unlike the "topotaxis" found in higher organisms, chemotaxis in bacteria, avoiding low (threshold) concentrations of a chemical, is a "phobotaxis" [see Adler's review of 1966 (*58*)].

Adler and co-workers showed that chemotaxis of *E. coli* to specific hydrophilic amino acids (*56*) or to specific sugars (*56, 57*) is not only independent of the function of the corresponding metabolic pathways but also of permeases.

The independence of gal chemotaxis with respect to the presence or absence of active transport resembles significantly the independence of production of galactose binding protein to that of the presence or absence of β-galactoside transport (*29*). It seemed plausible, therefore, that galactose binding protein may represent the first step in the recognition of galactose, serving the two membrane functions, galactose chemotaxis and the $P_{\beta g}$ transport system.

This idea was tested by Adler with two types of β-galactoside transport–defective mutants: one which showed plenty of galactose binding protein (our strain W3092i) and another strain (Rotman's W4345) which showed no trace of it (*29*). These two types of transport defective strains were also gal K mutants. A third gal K mutant, which was transport positive (our W3092c), was tested together with the defective strains (*59*). The results are summarized in Table 2. The only discernible correlation is between galactose binding protein and gal chemotaxis.

Moreover, the substrate affinities in gal chemotaxis show the characteristics of those of the $P_{\beta g}$ transport system as well as those of galactose binding protein (*59*) and differ from those of gal binding systems belonging to other transport systems (*60, 61*). Table 3 summarizes quantitative data on affinities by the sensitive inhibition test by

Hazelbauer and Adler (*62*). The affinities of galactose binding protein toward various sugars are strikingly parallel with those of chemotaxis.

The regulation of gal chemotaxis (*56*) as compared with that of production of the binding protein (*46*) may indicate a coordinate regulation. As far as production of the binder is concerned, full induction or close to full induction seems to occur even in strains with a highly defective $P_{\beta g}$ system (*29, 63*). Yet, one of these strains (W3092i) is not able to retain sufficient galactose to induce the gal operon (*28*). Apparently induction of galactose binding protein is elicited at levels lower than those required for inducing the gal operon. More important, the absence of recapture in the $P_{\beta g}$ strains indicates that induction of the binding protein does not require recapture of endogenous galactose; hence, this induction represents, as was mentioned in the previous section, a genuine endogenous induction (induction from within). Interestingly enough, gal chemotaxis can also be induced by this type of endogenous induction (*64*). Figure 2 is meant to illustrate an induction from within of two systems either belonging to the membrane or located even farther out.

Direct attempts to restore gal chemotaxis in shocked cells by addition of crude preparations of galactose binding protein have given promising results (*62*). Moreover, a newly found gal chemotaxis mutant has been isolated that shows chemotaxis only if the galactose concentrations are raised at least 30-fold over those used in the responsive strains. From this low affinity "structural" mutant, Hazelbauer and Adler (*62*) have collected a preparation that binds galactose only at high concentrations and can restore chemotaxis to shocked cells only if the galactose concentrations are correspondingly high.

The mechanism of the restoration, as well as the nature of the so-called low affinity chemotaxis mutant, can best be explored by purification and high resolution methods. The following questions arise.

1) What are the physical-chemical properties of the normal galactose binding protein? Does the binding of galactose to the binding protein elicit any conformational changes which may be of importance in transport or taxis systems?

2) What are the properties of the galactose binding protein from the low affinity mutant?

Specific Conformational Changes of Galactose Binding Protein

The biochemical basis for membrane functions like galactose chemotaxis or galactose transport can perhaps best be explored by an intensive macromolecular and physicochemical study of the normal high affinity galactose binding proteins. Quantitative binding tests [Boos (*65, 66*)] indicate that the binding protein may have two types of "affinity conformations." One type seen only at very low galactose concentrations showed an apparent dissociation constant as low as $10^{-7}M$. Another form, the prevailing one in the presence of galactose, had a dissociation constant for galactose of about $10^{-5}M$. A Scatchard plot reveals that this form binds two molecules of galactose per one monomer (*66*). The possibility of the existence of negative cooperativity brought about by the binding of galactose ought to be explored by various techniques. This may be possible since galactose exerts a number of other effects on its binding protein.

Analytical polyacrylamide electrophoresis on purified galactose binding protein disclosed also the existence of two forms of the protein with a difference in migration rate. Under detergent condition (sodium dodecyl sulfate polyacrylamide electrophoresis), only one band is seen corresponding to a M.W. of 35,000 (*65, 66*). However, under nondetergent conditions, it has been possible by prior treatment of the polyacrylamide gel with low concentrations of the specific substrates to increase the electrophoretic mobility of the binding protein. This increase of electrophoretic mobility is brought about by treating the gel with high affinity substrates such as glucose, galactose, or β-glycerogalactoside. α-Methylglucoside and TMG are ineffective (*66*). A change in the charge of the binding protein brought about by the binding of a specific yet uncharged ligand indicates a change in the conformation of the protein. This type of conformational change may also be manifest by an increase in the fluorescence of some of the tryptophans of the binding protein when galactose or β-glycerogalactoside is added. The galactose-induced increase of the tryptophan fluorescence is also accompanied by a blue shift in the emission spectrum (*66*). The emission change elicited by addition of glucose is smaller and does not show a blue shift (*66*) (Table 4).

It appears from the same study that glucose as well as galactose is able to "induce" these changes in concentrations as low as $10^{-7}M$ to $10^{-6}M$ and β-glycerogalactoside is able to elicit the changes at concentrations almost as low ($10^{-6}M$). At concentrations higher than $10^{-4}M$ a less specific and smaller rise in tryptophan emission is seen by addition of megal, TMG, and other thiogalactosides. The rapid fluorescence technique permits the investigator to perform binding studies in an independent and rapid way. It was, for instance, found that the binding protein emission response is instantaneous if equilibrated solutions of galactose are used in which the β component is predominant. However, if α-galactose is added the emission response shows a marked lag period (*66*); the slow spontaneous mutarotation is undoubtedly responsible for the lag period. This observation underlines once more that the galactose binding protein, as part of $P_{\beta g}$, is decidedly specific for β anomers.

It is of considerable interest to compare the properties of galactose binding protein with a purified protein from one of Adler's chemotaxis mutants which

Table 3. Specificity of the galactose chemoreceptor and the galactose binding protein as determined by inhibition studies. The data is adapted from (*62*). Inhibition is measured by determining how many bacteria enter a capillary tube containing an attractant when inhibitor, that is, a second attractant, is present in both the bacterial suspension and the capillary. The effectiveness of the inhibitor is determined by varying the concentration of inhibitor at a constant concentration of attractant, and the result is represented as the concentration which inhibits the accumulation of bacteria by 50 percent (*62*).

Item	Concentration (μM) required for 50 percent inhibition		
	Taxis toward 5 μM galactose	Binding of 5 μM galactose	Ratio taxis/ binding
D-Glucose	0.005	1.0	200
D-Galactose	0.036	7.0	190
1-D-Glycerol-β-D-galactoside	0.15	25	170
D-Fucose	6.2	1,100	180
β-Megal	30	3,500	120
L-Arabinose	95	17,000	180
D-Xylose	120	18,000	150

Table 4. Some biochemical characteristics of *E. coli* strains which are gal chemotatic positives or defectives (structural chemotaxis mutants).

E. coli K-12 strains	$P_{\beta g}$ per 10^9 cells (nmole/min)	Tax_g*	Galactose binding protein			
					Gal induced changes	
			Gal binding	Cross reaction	Electrophoretic migration	Tryptophan fluorescence
W3092cy⁻	1.2	+	+	+	+ Increase	+ Increase
AW550	0.01	[∓]†	ND‡	+	ND	ND
EH3039	< 0.01	−	ND	+	ND	[∓] Increase§

* Tax_g is abbreviation for chemotaxis for galactose. † Nondetectable chemotaxis at $10^{-6}M$ galactose, detectable at $10^{-4}M$. ‡ Nondetectable. § Detectable increase in fluorescence at $10^{-3}M$ galactose.

seems to be an affinity mutant requiring about 50-fold higher concentrations of galactose to elicit chemotaxis. The purified protein from the affinity mutant is precipitated by antibody directed against purified galactose binding protein (plus cross reactivity), and it can therefore be characterized as a product of a structural mutation in the gene for the binding protein (*63, 67*). The mutant protein migrates slightly faster than normal galactose binding protein in the analytical polyacrylamide electrophoresis. However, it fails to show any detectable change in migration rate in the presence of galactose (that is, if the gel contains galactose, up to $10^{-3}M$) (*63, 67*). The changed character of the cross-reacting protein is summarized in Table 4.

Gal Chemotaxis, Galactose Binding Protein, and the Membrane

I am inclined toward the view that galactose binding protein as well as the chemotaxis system are part of the membrane. Admittedly, the binding protein might be located between the membrane and the cell wall, and chemotaxis might be an exclusive "ektobiological" phenomenon. Let us assume, however, that the membrane is also involved. Defects of the high affinity galactose system can obviously be ascribed to defects of many types.

1) Total lack of galactose binding protein due to a nonsense mutation in the gene programing the binding protein. This also obliterates gal chemotaxis. The strain W4345 (Table 2) exemplifies this case.

2) Structural change of galactose binding protein exemplified by the so-called affinity mutant.

3) Normal high affinity galactose binding protein with fully active gal chemotaxis but lack of active galactose transport due to a defect or an uncoupling of the energy generating systems of the membrane (phosphorylation, electron transport system, chemiosmotic mechanisms).

4) Uncoupling due to an alteration of the sites of the membrane involved in transport by which galactose binding protein remains active as a galactose capture system, but is only able to couple with the chemotaxis system.

Restoration experiments of chemotaxis of shocked cells seem already promising (*62*) and may pave the way for interesting cross experiments with various strains of shocked cells in assays for the binding proteins which may have lost one or the other type of recognition site. However, restoration techniques of transport systems need to be developed.

It should be recalled that other periplasmic binding proteins (sulfate binder, leucine binder, L-arabinose binder) are known in *E. coli* (*12*), yet these proteins seem not to be involved in any type of chemotaxis, although these binders are presumably functioning as part of the corresponding transport systems. None of them cross react with the galactose binding protein. It may be one of the freaks of evolution that the galactose binding protein of the $P_{\beta g}$ system became a common denominator for two membrane functions, gal transport as well as gal chemotaxis. Perhaps the intestine of the infant being regularly supplied with lactose as the only carbohydrate source would be the optimum milieu for the enrichment of mutants that have developed chemotaxis to galactose and glucose.

A number of intriguing problems arise from the joint biochemical and cell physiological studies of galactose transport and chemotaxis. Among the most important biochemical observations one might list the fact that galactose binding protein is a monomer with two binding sites and that binding elicits a conformational change. This conformational change affects the charge of the binding protein, the microenvironment of some of the tryptophan residues, and seemingly decreases the binding affinity. If the galactose-induced conformational change presents an example of what Koshland has termed negative cooperativity (*68*) it would be of special interest, since galactose binding protein is supposed to be the first link in active galactose transport, and most models invoke conformational changes; negative cooperativity would certainly be an additional feature of importance to deal with when discussing transport.

With regard to chemotaxis, the responses to doses and gradients of attractants have been compared with other sensory responses (*56, 69*). Since the purified galactose binding protein appears to be a monomer with two sites, it is conceivable that it transmits signals to the chemotaxis system only if one site is occupied and the other free. Hence the fraction of a population of monomers which is in the so-called "semi-filled" state might determine the intensity of the tactic response.

Summary

A specific high affinity galactose transport system called $P_{\beta g}$ can be induced by trace amounts of galactose in the medium by virtue of its own ability to capture and accumulate galactose. The transport system is coregulated with the production of a high affinity periplasmic galactose binding protein, which constitutes but one part of the transport system. Some transport negative mutants still remain producers of this binding protein.

A close correlation exists between production of the active binding protein and the presence of galactose chemotaxis. The hypothesis, that this binding protein is a common element of the specific galactose transport system, $P_{\beta g}$, and of galactose chemotaxis is supported by observations on structural mutants, being defective in galactose binding protein as well as showing a lack of galactose chemotaxis. The binding protein is a monomer with two binding sites for galactose. Binding of one or two of the galactose molecules elicits specific conformational changes of the galactose binding protein (lowered affinity for galactose, increase of charges of the protein, increased fluorescence of tryptophan residues). The importance of these features for transport and for chemotaxis is discussed (*70*).

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70. The work by Dr. W. Boos was supported by grants from NIH, NSF, and the Wellcome Trust to the biochemical research department, Massachusetts General Hospital. I thank reviewers for many helpful suggestions. Drs. Winfried Boos, Max Delbrück, and Leon Heppel made detailed comments that encouraged me to make extensive revisions and clarifications of the text. Drs. Julius Adler and Winfried Boos kindly gave me permission to quote recent, partly unpublished, material from their work.

Physics Department Ratings: Another Evaluation

Institutional ratings of physics departments by faculty can be predicted by using data in the public domain.

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Evaluation procedures in graduate education are mentioned briefly by authorities on higher education (*1*). Cartter's study (*2*) occupies a prominent place among the sparse comments usually devoted to this topic. Although the Cartter report has received its share of methodological criticism from various persons, mostly those who did not win, place, or show in the ratings, it has not been the subject of serious investigation. Roose and Andersen (*3*) have conducted a survey similar to but more comprehensive than Cartter's. Although scholars rated programs in their respective fields on a numerical scale, these ratings were not published. As might be expected, public comment was divided (*4*).

This study demonstrates that the institutional ratings of physics departments appearing in the Cartter study can be predicted by the use of simple data that are readily available to the general public.

Method

Graham (*5*) has provided aspiring graduate students with some objective data concerning graduate programs. From this volume, the following variables were arbitrarily selected for each graduate program in physics that was rated in the Cartter study: (i) the number of areas of specialization within a department, (ii) the number of faculty, (iii) the number of Ph.D.'s awarded between 1960 and 1964, (iv) the number of full-time students, (v) the number of

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