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IV. CATABOLITE REPRESSION IN OTHER BACTERIA AND IN EUKARYOTES

Life in mucus: sugar metabolism in *Haemophilus influenzae*

L.P. Macfadyen and R.J. Redfield (*)

Department of Zoology, University of British Columbia, Vancouver, B.C., (Canada) V6T 1Z4

Haemophilus influenzae is a small Gram-negative bacterium in the family *Pasteurellaceae*, recently risen to prominence as the first organism whose complete genome has been sequenced (Fleischmann *et al.*, 1995). It is an obligate commensal and opportunistic pathogen in the human respiratory tract and a major causative agent of acute and chronic respiratory tract infections. Close relatives occupy similar niches in other mammals. *H. influenzae* normally colonizes mucosal surfaces, but some invasive serotype b strains can cross epithelia, reaching the bloodstream and ultimately the central nervous system. Until the recent development of an effective vaccine these strains were the major agent of meningitis in infants and small children. These latter are thought to be 'dead-end' infections, largely irrelevant to long-term evolution. However, there have been few studies of

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(*) Corresponding author.

H. influenzae's metabolism, most work having focused either on pathogenesis or on DNA metabolism, especially the efficient natural transformation system.

Bacterial cells have evolved to use very diverse substances as sources of energy and of carbon. In this review, we will concentrate on use of sugars and other carbohydrates, in part because of our interest in the role of sugar uptake in regulating competence for natural transformation and also because very little is known about use of other carbon sources.

Sugar utilization by other bacteria

In enteric bacteria, certain sugars ('PTS sugars') are transported and simultaneously phosphorylated by the phosphoenolpyruvate:carbohydrate phosphotransferase system (PTS). The PTS also controls transcription of genes responsible for transport and catabolism of many other sugars and metabolizable substrates, effectively constructing a sugar preference hierarchy with PTS sugars at the top. This is achieved by regulation of adenylate cyclase activity (and thus cAMP levels) according to PTS sugar availability (for a recent review see Postma *et al.*, 1993). As PTS sugar supplies dwindle, cAMP is synthesized and, in concert with the cAMP receptor protein (CRP), initiates transcriptional activation from promoters preceded by CRP binding sites (Botsford and Harman, 1992). cAMP-dependent and some cAMP-independent sugar operons also have sugar-specific regulation coordinated by specific inducers and regulatory proteins.

H. influenzae is unlikely to have as broad a sugar repertoire as *E. coli* and other enteric bacteria (the closest allies of the *Pasteurellaceae*), both because of limits imposed by its small genome (1.83Mb) and because of the restricted opportunities in its mucosal niche.

Which sugars may be available in the mucosal niche?

Unlike enteric bacteria, which encounter a wide range of carbohydrates in the human gut, *H. influenzae* has no direct contact with the bulk of sugars in the host diet. Furthermore, since *H. influenzae* inhabits an extracellular environment (and indeed, may adhere to respiratory mucus itself rather than to epithelial surfaces (St. Geme, 1994)), it has no direct access to host cell metabolic intermediates. Effectively, only sugars that cross the mucosal cell membrane are available to the mucosal flora. In the absence of definitive studies as to the nature of this mucosal niche, we consider below its likely sugar/carbon-source composition.

The major sugars in the human diet are the monosaccharides glucose and fructose, and the disaccharide sucrose (glucose-fructose). In primitive diets these are largely obtained from fruits and grain and provide between 8% and 21% of dietary energy (Gibney *et al.*, 1995). Dietary lactose (glucose-galactose) intake depends on the consumption of milk products; free galactose may also be contributed by fermented milk products. The second major dietary sugar source is starch, which can provide >70% of dietary energy intake as glucose. Lesser amounts of other sugars may be consumed as cell wall polysaccharides (Southgate, 1995). Because complex carbohydrates and even disaccharides must be hydrolysed to simple sugars before absorption, only the monosaccharides glucose, galactose and fructose enter the circulation from the diet, with glucose predominating. Glycerol also enters the circulation, primarily from breakdown of dietary and stored fats.

Although circulating sugars may directly pass across epithelia, cells of the respiratory tract also secrete large amounts of sugars as mucus polysaccharides. Mucus cells of submucosal glands and goblet cells of surface epithelium in trachea and bronchi secrete mucus glycoproteins (mucins), which make up 40-60% of the solid phase of respiratory tract mucus. Up to 90% of the mucin mass consists of branched oligosaccharide chains (Boat and Cheng, 1980), and chromatographic analysis has shown that these chains contain five sugars: fucose, galactose, N-acetylglucosamine, N-acetylgalactosamine and sialic acid (N-acetylneuraminic acid, NANA) (Boat *et al.*, 1976). Mucins are consequently a potentially rich source of sugars for mucus inhabitants.

H. influenzae might also scavenge sugars from dead cells, including cells of the host immune system, particularly at sites of infection and inflammation, and from other bacteria with which it shares the mucosal niche. Moreover, and as discussed below, *H. influenzae* may obtain sugars through symbiotic relationships with other mucus-dwelling species.

Sugar utilization by *H. influenzae*

Until recently, our knowledge of *H. influenzae*'s metabolic needs and capabilities came entirely from lab cultures of wild-type cells. These showed that *H. influenzae* requires several amino acids (although different laboratories, working with a variety of strains, report differing requirements (Coulton and Pang, 1983; Butler, 1962)), a pyrimidine or citrul-

line, thiamine, pantothenic acid, hemin and NAD (Kilian, 1976). Phenol red broth assays showed fermentation of glucose, xylose, ribose and galactose (Kilian and Frederiksen, 1981). The reported fermentation of deoxyribose was in error (M. Kilian, pers. commun).

New experimental data, combined with the identification of sugar catabolic operons in the recently sequenced *H. influenzae* genome (Fleischmann *et al.*, 1995), is providing a much more detailed understanding of sugar utilization by this bacterium. (Sequence information can be accessed via the WorldWide Web site of The Institute for Genomic Research [TIGR], address http://www.tigr.org; all *H. influenzae* genes mentioned in this review were first published in the TIGR database unless indicated otherwise). As described in more detail below, *H. influenzae* has a minimal phosphotransferase system (PTS) which transports only fructose. In response to a decrease in fructose availability, this PTS increases intracellular cAMP levels by activating adenylate cyclase. Increased levels of cAMP allow transcriptional activation of genes for uptake and catabolism of ribose, xylose, galactose and fucose (Macfadyen *et al.*, 1996). Each of these pathways is also regulated by availability of substrate. A PTS-mediated rise in intracellular cAMP also regulates development of competence for natural transformation (Macfadyen *et al.*, 1996). Uptake and fermentation of glucose, glycerol and sialic acid are independent of both cAMP and the PTS (Macfadyen *et al.*, 1996). No other sugars are known to be used, although some genes for gluconate and maltose utilization are present (table II).

Specific sugars and regulation

i) Fructose and the PTS

The PTS catalyses phosphotransfer from phosphoenolpyruvate via the "general" transfer proteins HPr and Enzyme I to PTS sugar-specific permeases (Enzymes II) and thence to the transported sugar. In the absence of PTS sugars, PTS proteins remain phosphorylated, and the phosphorylated form of the glucose-specific EIIA regulatory component ($EIIA^{Glc}$) activates adenylate cyclase to produce cAMP (Postma *et al.*, 1993). An *H. influenzae ptsI* mutant (lacking Enzyme I) could not ferment fructose. Because fermentation was not blocked by mutations in *cya* or *crp* and not restored to the *ptsI* mutant by addition of cAMP (table I), we concluded that fructose is a PTS sugar (Macfadyen *et al.*, 1996). This combination of PTS dependence and cAMP independence was not seen for any other tested sugar(*). These findings were confirmed by the discovery of genomic homologues of *E. coli*'s *fruA* and *fruB* genes, specifying the EIIBC fructose permease (Prior and Kornberg, 1988) and a unique fructose-specific

Table I. Sugar fermentation by *H. influenzae* strains.

Sugar	KW20 cAMP⁻	RR745 cAMP⁻	RR745 cAMP⁺	RR668 cAMP⁻	RR668 cAMP⁺
fructose	+	–	–	+	+
fucose	+	+	+	–	+
xylose	+	+	+	–	+
ribose	+	–	+	–	+
galactose	+	–	+	+/–	+
glycerol	+	+	+	+	+
sialic acid	+	+	+	+	+
glucose	+	+	+	+	+

Overnight culture (50 µl) was inoculated into 2-ml phenol red assay broth (PRB) (Difco) supplemented with 10 mg/ml hemin, 2 µg/ml NAD, 10% brain heart infusion (Difco) and 0.5% sugar. Results were scored after at least 8-h growth in rolled, loosely capped tubes. KW20=wild-type *H. influenzae* Rd (Alexander and Leidy, 1951); RR745=KW20 *ptsI*::mini-Tn*10kan* (Macfadyen *et al.*, 1996); RR668=KW20 *cya*::mini-Tn*10kan* (Dorocicz *et al.*, 1993).

(*) The following sugars were negative in a phenol red broth assay of *H. influenzae*: deoxyribose, maltose, mannose, arabinose, sucrose, glucose-6-phosphate, sorbitol, mannitol, inositol, erythritol, galactosamine, glucosamine, mannosamine, N-acetylgalactosamine, N-acetylglucosamine, gluconic acid, glucoronic acid, methyl-α-glucoside, methyl-β-glucoside.

diphosphoryl transfer protein that combines EIIA and HPr activities (J. Reizer *et al.*, 1996). Subsequently, *H. influenzae* membranes have been shown to contain an active fructose permease (Macfadyen *et al.*, 1996).

This fructose-specific PTS regulates intracellular cAMP concentrations, which in turn control fermentation of several other sugars as well as development of natural competence (see below). These findings suggest that fructose is at the top of *H. influenzae*'s sugar preference hierarchy. This is unexpected because fructose is much less abundant than glucose in body fluids (0.14 mg/dl versus ~93 mg/dl, respectively, in serum (Watanabe *et al.*, 1989)). If glucose is the most common sugar in *H. influenzae*'s environment, why does it ignore glucose and employ a fructose-specific PTS to sense carbon source availability and regulate uptake and catabolism of other carbon sources?

Firstly, it may be significant that fructose is a central intermediate of *H. influenzae* sugar catabolism (fig. 1). In terms of the number of catabolic steps required to feed a sugar into glycolysis, fructose is the most efficiently used monosaccharide. Secondly, glucose is efficiently and preferentially used by host cells, and it is possible that little free glucose actually reaches the mucosal niche; competing with the human host for glucose may not be a useful strategy. Finally, fructose differs from glucose levels in that they vary significantly between different locations in the human body. For example, cerebrospinal fluid fructose levels (~4 mg/dl) are 30-fold higher than serum levels (~0.14 mg/dl) (Watanabe *et al.*, 1989). Fructose levels in the human host may well be a more sensitive indicator of exploitable niches.

H. influenzae joins species of *Rhodobacter*, *Pseudomonas*, *Streptomyces* and *Listeria* (Saier, 1977; Romano and Saier, 1992; Mitchell *et al.*, 1993; Titgemeyer *et al.*, 1995) in having a fructose-specific PTS. Saier *et al.* (1985) have suggested that the primordial PTS was fructose-specific. However, although *H. influenzae* lacks a PTS glucose permease, it retains homologues of HPr and $EIIA^{Glc}$ (the latter required by *E. coli* for both glucose uptake and adenylate cyclase activation). In the absence of phylogenetic information, it may be more parsimonious to postulate that the glucose permease has been lost.

ii) cAMP-dependent sugars: galactose, ribose, xylose, fucose

H. influenzae has homologues of genes for the high affinity *mgl* galactose uptake system (Rotman *et al.*, 1968; Harayama *et al.*, 1983), but not for the *galP* low affinity system (Macpherson *et al.*, 1983). These are adjacent to the *galTK* genes, whose products carry out galactose catabolism (Shapiro and Adhya, 1969). A putative CRP site lies upstream of the first gene, *mglB* (Macfadyen *et al.*, 1996), which encodes the periplasmic galactose binding protein. Galactose may also enter the bacterial cell via one or more cAMP-independent glucose permeases (Henderson, 1990), which could explain the intermediate levels of fermentation of this sugar by *cya* mutants (table I). *H. influenzae* has homologues of the inducible high affinity *E. coli* ribose (Anderson and Cooper, 1970) and xylose (Rosenfeld *et al.*, 1984) uptake systems, as well as a full complement of catabolic genes. As in *E. coli*, fermentation is blocked by mutations in *cya* and is restored by added cAMP. CRP sites have been identified upstream of *rbsD*, the first gene of the *rbs* operon, and of *xylH*, the xylose permease gene (Macfadyen *et al.*, 1996). Lastly, *H. influenzae* contains a complete set of fucose uptake and catabolism genes (Chakrabarti *et al.*, 1984), with a potential CRP site upstream of *fucP*, the fucose permease gene (Macfadyen *et al.*, 1996). Associated with the genes for uptake and metabolism of each sugar is a gene for a sugar-specific repressor or activator, which links gene expression to presence of the appropriate substrate.

These data suggest that the *H. influenzae* PTS, like its enteric homologues, controls transcription of cAMP-dependent sugar operons. Catabolism of ribose, galactose, xylose and fucose is dependent on cAMP, requires a functional PTS EI (Macfadyen *et al.*, 1996), and is repressed by fructose (Macfadyen *et al.*, 1996). We suggest that these sugars are present at low levels in *H. influenzae*'s mucosal environment (supported by the presence of only high affinity uptake systems for each); most of the time it is energetically wasteful for *H. influenzae* to transcribe these genes and ferment these sugars. These operons will only be switched on as preferred sugars run out and rarer sugars become more desirable.

iii) cAMP-independent sugars and carbohydrates: glycerol, glucose, sialic acid

Although *H. influenzae* lacks the PTS glucose permease, it has been shown to take up glucose, phosphorylate it via an ATP-dependent kinase (Macfadyen *et al.*, 1996) and feed it into central metabolism (fig. 1). As in *E. coli*, the *mgl* system for galactose uptake may also transport glucose (Death and Ferenci, 1993), and indeed, some inhibition of galactose uptake by glucose has been observed in *H. influenzae* (Macfadyen *et al.*, 1996). However, the cAMP independence of glucose fermentation argues for additional as yet unidentified

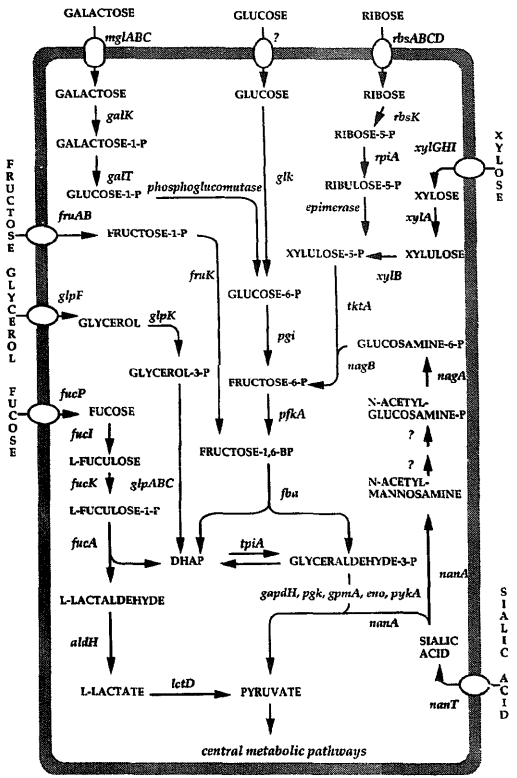

Fig. 1. *H. influenzae* sugar metabolism.

uptake mechanisms, as has been postulated for *E. coli* (Death and Ferenci, 1993).

H. influenzae has the *glpF* gene, which in *E. coli* facilitates diffusion of glycerol across the membrane. *glpT*, a transporter for glycerol-3-phosphate, is also present. Moreover, a complete glycerol operon (Lin, 1987) allows glycerol to be fed directly into the glycolytic pathway (fig. 1).

H. influenzae ferments free sialic acid (N-acetylneuraminic acid, NANA). A sialic acid permease

was overlooked in the original gene assignments, but the hypothetical HI1104 protein has been found to have significant homology to the recently sequenced *E. coli* sialic acid permease, *nanT* (Casari *et al.*, 1995; Martinez *et al.*, 1995). N-acetylneuraminate lyase (*nanA*) is present and would yield pyruvate and N-acetylmannosamine; the latter is probably phosphorylated and converted to N-acetylglucosamine-phosphate by an as yet unidentified epimerase (epimerases with this activity have been purified from *Aerobacter cloacae*, *E. coli* and *Clostridium perfringens* (Warren, 1972), but not yet sequenced). N-acetylglucosamine phosphate can then be further metabolized to fructose-6-phosphate (Warren, 1972) by sequential action of a deacetylase (*nagA*-encoded) and a deaminase (*nagB*-encoded), or incorporated into the bacterial cell wall as required. (It should be noted that *H. influenzae* synthesizes sialylated lipo-oligosaccharide endogenously (Mandrell and Apicella, 1993), and the lyase activity may function to control intracellular sialic acid levels, since build-up of sialic acid has been shown to be toxic in *E. coli* (Vimr and Troy, 1985)).

Sialic acid is abundant in the human body (60-900 mg/ml in serum), but less than 0.05% of this is the free sugar (Waters *et al.*, 1992), the bulk being conjugated to glycoproteins. Mucus is a particularly rich source; sialic acid and fucose always occupy terminal (unsubstituted) positions in mucin oligosaccharides and together represent 30-35% of mucin sugar content (Wolf *et al.*, 1980). It is thus somewhat surprising that *H. influenzae* appears to lack a gene for the neuraminidase required to cleave sialic acid from oligosaccharides (although one early study reported neuraminidase activity (Mueller and Hinz, 1977)). One possibility is that it competes for free extracellular sialic acids produced by neuraminidase-secreting organisms with which it shares its niche, as has been suggested for *E. coli* (Vimr and Troy, 1985). For example, the neuraminidase-secreting *Pseudomonas aeruginosa* is likely to be part of the normal flora of the human upper respiratory tract (Taylor *et al.*, 1990) and is commonly found alongside *H. influenzae* in chronic respiratory infections. Alternatively, *H. influenzae* may secrete sialic acid lyase and take up the resulting N-acetylmannosamine and pyruvate (E. Vimr, pers. comm.).

Glucose, glycerol and sialic acid — all of which feed directly into central metabolic pathways — may be available in fluctuating levels depending on diet and the presence of other mucosal microbes. The *glpR*-encoded repressor protein allows expression of the *glp* operon only when induced by glycerol or glycerol-3-phosphate. Expression of the *E. coli* sialic acid operon is dependent on the presence of sialic acid (Martinez *et al.*, 1995), although this has not yet been shown for *H. influenzae*. cAMP-independent (but inducer-dependent) regulation may allow these carbon sources to be used on a 'take when available' basis, without reference to fructose levels. However, constitutive expression of sialic acid genes may be crucial to *H. influenzae* if it uses these same genes to synthesize sialylated LOS as protection against the host immune response (Mandrell and Apicella, 1993).

iv) Genes for using maltodextrins and gluconate

Although *H. influenzae* does not ferment maltose (Macfadyen *et al.*, 1996), it has a gene for amylomaltase (*malQ*), which breaks down amylopectin and dextrins to glucose. The *mal*-encoded proteins that facilitate the crossing of the inner membrane by dextrins (Lin, 1987) are absent. Digestion of starch by amylases begins in the mouth, and produces dextrins. While *H. influenzae* does not inhabit the mouth, some of its close relatives do (Kilian and Schiott, 1975), so this amylomaltase gene may be an evolutionary relic. Similarly, *H. influenzae* has a partial gluconate operon, as well as a number of genes encoding enzymes of hexonate catabolism (Lin, 1987). The organism may still take up gluconate via GntP, but we have found that it does not ferment this sugar (Macfadyen *et al.*, 1996).

Genetic information about use of other carbon sources

a) Amino acids

A search of the TIGR database reveals that *H. influenzae* has transporters/permeases for arginine, branched chain amino acids, glycine, glutamine, leucine, serine, tyrosine, alanine, glutamate, tryptophan and proline, as well as peptides, dipeptides and oligopeptides. Potential sources of amino acids in the mucosal niche are unknown, but respiratory tract mucus is known to contain proteases secreted by leukocytes (Boat and Cheng, 1980). *H. influenzae* and other microbial inhabitants may also secrete proteases. We suggest that protein degradation could supply both amino acids and peptides as carbon (and nitrogen) sources for *H. influenzae*.

b) DNA

E. coli and *S. typhimurium* can use exogenous nucleotides as nucleic acid precursors. Since the

cytoplasmic membrane is impermeable to nucleotides, these must be dephosphorylated to nucleosides by periplasmic enzymes before uptake (Munch-Petersen, 1983). *H. influenzae* has at least one periplasmic 5'-nucleotidase, UshA, as well as the *nupC*-encoded nucleoside transport system (Casari *et al.*, 1995), which in *E. coli* transports ribonucleosides and deoxyribonucleosides (except those of guanine and hypoxanthine). In *E. coli*, synthesis of the *nupC* system is regulated both by the *cytR* repressor and by cAMP-CRP. *H. influenzae* has no apparent CytR homologue, and we have been unable to identify a putative CRP site upstream of *nupC* (Macfadyen and Redfield, unpublished observations). Thus, *nupC* may be constitutively expressed in this organism, which makes energetic sense when one considers the DNA-richness of the mucosal environment (see below), and the estimate that 80% of the energy involved in DNA synthesis is required for biosynthesis of nucleotides (Stouthamer, 1979).

A search of the TIGR database shows that *H. influenzae* lacks DeoB, which converts (deoxy)-ribose-1-phosphate to (deoxy)ribose-5-phosphate. Nonetheless, all other genes of the metabolic pathway that catabolizes nucleosides to glycolytic intermediates are present (table II). It is possible that a divergent or novel enzyme fulfills the DeoB role in *H. influenzae*. In the absence of experimental data, it is not clear whether this organism can utilize scavenged nucleosides as carbon or nitrogen sources as well as nucleic acid precursors.

The presence of homologues of nucleotide scavenging pathway genes (table II) implies that *H. influenzae* scavenges DNA degradation products from lung mucus. However, most of the nucleotides in mucus are not free but polymerized in DNA, as sialic acid is in mucins, and *H. influenzae* does not secrete any nucleases. It may nevertheless exploit this resource by using its natural competence system to take up DNA for intracellular degradation. Natural competence and transformation have generally been assumed to have a genetic function, producing cells with recombinant genotypes which may be better adapted to a changing environment. However, the advantages of this are weak and unpredictable, compared to the substantial immediate benefit provided by the DNA's nucleotides (Redfield, 1993).

H. influenzae may encounter DNA at concentrations of up to 300 μg/ml in healthy respiratory tract mucus (Matthews *et al.*, 1963). This DNA is almost entirely human in origin, although microbial DNA content has not been quantitatively assessed. Moreover, the DNA content of sputum has been shown to increase with the degree of infection in respiratory disease, especially cystic fibrosis (Lethem *et al.*, 1990).

We have found that the fructose-sensing PTS of *H. influenzae* regulates competence development via activation of adenylate cyclase and increases intracellular levels of cAMP, thus linking DNA uptake to lack of the preferred sugar. Both *cya* and *crp* mutations prevent competence development (Dorocicz *et al.*, 1993; Chandler, 1992). We have shown that a *ptsI* mutant is 250-fold less competent than the wild type on transfer to a competence-inducing medium (lacking sugars and nucleotide precursors) (Macfadyen *et al.*, 1996). Full competence is restored to this mutant by addition of exogenous cAMP to the medium. These findings suggest that the primary benefit of competence is acquisition of nucleotides, and we propose the following model: cells preferentially obtain nucleosides by uptake. If these are insufficient, availability of energy resources will determine whether nucleotides are synthesized *de novo*, or whether cells become competent in order to take up any free DNA in the environment.

Conclusions

In the past year, two new sources of information about *H. influenzae* metabolism have become available, and together these are providing us with a partial understanding of the nature of the mucosal niche and the intrahost survival tactics of this organism. Firstly, experimental data have given us some insight into the genetic capabilities of *H. influenzae* and have indicated some sugar sources that may be available to it. Ongoing genome analysis and gene identification have supported experimental findings and will direct further experimentation. To date, there is little information available on actual sugar composition of respiratory tract mucus.

Clearly, *H. influenzae* has maintained genes which allow it to exploit the major sugars likely to be available in the mucosal niche: glucose, fructose, galactose and glycerol. Moreover, it retains the ability to exploit some less common monosaccharides, probably obtained from mucins. Our current synthesis of metabolic information highlights *H. influenzae*'s efficiency as a scavenger in an apparently novel niche, rich in DNA and oligosaccharide subunits as well as more conventional nutrients. This information has both genetic and medical implications.

Table II. *H. influenzae* genes for uptake and catabolism of carbon sources.

H. influenzae no.	Gene	Product
Fructose		
0446	*fruA*	fructose-permease IIBC component
0447	*fruK*	1-phosphofructokinase
0448	*fruB*	fructose-permease IIA/FPr component
Galactose		
0819	*galK*	galactokinase
0820	*galT*	galactose-1-phosphate uridylyltransferase
0821	*galS*	*gal* operon repressor
0822	*mglB*	D-galactose-binding protein
0823	*mglA*	methylgalactoside permease ATP-binding protein
0824	*mglC*	mglC protein
Ribose		
0501	*rbsD*	high-affinity ribose transport protein
0502	*rbsA*	high-affinity ribose transport protein
0503	*rbsC*	high-affinity ribose transport protein
0504	*rbsB*	periplasmic ribose-binding protein
0505	*rbsK*	ribokinase
0506	*rbsR*	*rbs* repressor
0464	*rpiA*	ribose phosphate isomerase (Casari *et al.*, 1995)
Xylose		
1106	*xylR*	xylose operon regulatory protein
1109	*xylH*	xylose transport permease protein
1110	*xylG*	D-xylose transport ATP-binding protein
1111	*xylI*	D-xylose-binding periplasmic protein
1112	*xylA*	xylose isomerase
1113	*xylB*	xylulose kinase
1023	*tktA*	transketolase
Fucose		
0610	*fucP*	L-fucose permease
0611	*fucA*	fuculose-1-phosphate aldolase
0612	*fucU*	fucose operon protein
0613	*fucK*	fucokinase
0614	*fucI*	L-fucose isomerase
0615	*fucR*	L-fucose operon activator
0499	*aldH*	aldehyde dehydrogenase
Glucose		
0144	*glk*	glucose kinase *(Streptomyces coelicor)*
Sialic acid		
0140	*nagA*	N-acetylglucosamine-6-phosphate deacetylase
0141	*nagB*	glucosamine-6-phosphate deaminase
0142	*nanA*	N-acetylneuraminate lyase
1104	*nanT*	sialic acid permease (Casari *et al.*, 1995)
Glycerol		
0683	*glpC*	glycerol-3-phosphate dehydrogenase, subunit C
0684	*glpB*	glycerol-3-phosphate dehydrogenase, subunit B
0685	*glpA*	glycerol-3-phosphate dehydrogenase, subunit A
0686	*glpT*	glycerol-3-phosphate transporter
0690	*glpF*	glycerol uptake facilitator
0691	*glpK*	glycerolkinase
1009	*glpR*	glycerol-3-phosphate regulon repressor

H. influenzae no.	Gene	Product
Nucleoside uptake		
0206	*ushA*	5' nucleotidase
1116	*nupC*	nucleoside uptake system C (Casari *et al.*, 1995)
Nucleoside scavenging		
0349	*adk*	adenylate kinase
1230	*apt*	adenine phosphoribosyl transferase
0674	*gpt*	xanthine guanine phosphoribosyl transferase
1743	*gmk*	5' guanylate kinase
1219	*cmk*	cytidylate kinase
1646	*cmk*	cytidylate kinase
0529	*tdk*	thymidine kinase
0132	*udk*	uridine kinase
0905	*thyA*	thymidylate synthase
0954	*dut*	deoxyuridine triosephosphatase
Incomplete regulons or pathways		
Maltose		
1108	*malY*	*mal* inducer biosynthesis blocker
1356	*malQ*	amylomaltase
1611	*sfsA*	maltose metabolism regulator
Hexonates		
0049	*kdgK*	2-keto-3-deoxy-D-gluconate kinase *(Erwinia chrysanthemi)*
0054	*uxuR*	*uxu* operon regulator
0055	*uxuA*	D-mannonate hydrolase
Nucleoside catabolism		
0280	*udp*	uridine phosphorylase
0518	*deoD*	purine nucleoside phosphorylase
0519	*deoC*	deoxyribose aldolase *(Mycoplasma homini)*
1350	*cda*	cytidine deaminase

All unreferenced gene assignments are taken from the TIGR *H. influenzae* genome database (Fleischmann *et al.*, 1995). All identifications are to *E. coli* homologues unless otherwise noted.

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Catabolite repression control in the *Pseudomonads*

D.N. Collier, P.W. Hager and P.V. Phibbs, Jr.

Department of Microbiology and Immunology,
East Carolina University School of Medicine, Greenville NC 27858 (USA)

The growth response of microorganisms presented with two carbon/energy sources is frequently biphasic (diauxic), reflecting the sequential, rather than simultaneous, utilization of the carbon/energy sources. During the first exponential phase of growth, when the preferred carbon source is being utilized, expression of genes encoding the catabolic enzymes required for assimilation of the second carbon source is repressed, despite the continued presence of the second substrate in the media. This so-called catabolite repression control (CRC) is abrogated as the preferred carbon source approaches depletion. Cell growth may lag (slow or cease) until the catabolic machinery required to assimilate the second carbon source is fully established. At this point a second distinct logarithmic growth phase is exhibited.

Established mechanisms of catabolite repression control in bacteria

For Gram-negative enteric bacteria such as *Escherichia coli*, glucose is the preferred carbon source. In these organisms, transport of glucose by the phospho*enol*pyruvate (PEP)-dependent transport system (PTS) drives a protein phosphorylation cascade in which a phosphate is sequentially transferred from PEP to enzyme I to HPr to enzyme IIA^{glc} and ultimately to glucose. Because the activity of adenylate cyclase is stimulated by phosphorylated IIA^{glc}, and certain permeases are inhibited by unphosphorylated IIA^{glc}, the dephosphorylation of IIA^{glc} that results from glucose transport (1) lowers adenylate cyclase activity, thereby reducing cyclic AMP (cAMP) levels, and (2) inactivates transport systems for certain solutes (so-called inducer exclusion). Hence, glucose will effect CRC of any operon whose transcription requires cAMP-activated catabolite activator protein [CAP (or CRP)] and/or an intracellular inducer whose transport has been blocked (for a recent review, see Saier, 1993).

Cis-acting catabolite responsive elements (CRE), similar in sequence to *lacO* and *galO*, which are capable of imparting CRC on heterologous genes in *Bacillus subtilis*, have been identified in numerous genes in Gram-positive organisms. The catabolite control protein (CcpA), which is required for imposing CRC on many CRE-containing genes, is a member of the LacI family of helix-turn-helix transcription regulators. It is possible, though not yet conclusively demonstrated, that the presence or

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