J. Mol. Microbiol. Biotechnol. (2001) 3(3): 355-359.

Routes for Fructose Utilization by *Escherichia coli*

Hans L. Kornberg*

Department of Biology, Boston University, 5 Cummington Street, Boston, MA 02215, USA

Abstract

There are three main routes for the utilization of fructose by *Escherichia coli.* One (Route A) predominates in the growth of wild-type strains. It involves the functioning of the phospho-enolpyruvate:glycose phosphotransferase system (PTS) and a fructose operon, mapping at min. 48.7, containing genes for a membrane-spanning protein (*fruA*), a 1-phosphofructose kinase (*fruK*) and a diphosphoryl transfer protein (*fruB*), under negative regulation by a *fruR* gene mapping at min. 1.9. A second route (Route B) also involves the PTS and membrane-spanning proteins that recognize a variety of sugars possessing the 3,4,5-D-*arabino* -hexose configuration but with primary specificity for mannose (*manXYZ*), mannitol (*mtlA*) and glucitol (*gutA*) and which, if over-produced, can transport also fructose. A third route (Route C), functioning in mutants devoid of Routes A and B, does not involve the PTS: fructose diffuses into the cell via an isoform (PtsG-F) of the major glucose permease of the PTS and is then phosphorylated by ATP and a manno(fructo)kinase (Mak$^+$) specified by a normally cryptic 1032 bp ORF (*yajF*) of hitherto unknown function (Mak-o), mapping at min. 8.8 and corresponding to a peptide of 344 amino acids. Conversion of the Mak-o to the Mak$^+$ phenotype involves an A24D mutation in a putative regulatory region.

Introduction

In his tragically curtailed but brilliant scientific career, Jonathan Reizer was among the first to use genomics to explore the cryptic treasures of microbial chromosomes. By doing so, he revealed the existence of many DNA sequences that, though normally not expressed, might serve as genes potentially able to open up novel metabolic routes, which might thus act also as the engines of evolutionary change.

The utilization of fructose as sole carbon source for the growth of *Escherichia coli* beautifully illustrates this metabolic flexibility: several permeases can admit (or can be made to admit), albeit with various degrees of readiness, fructose as well as their normal sugar substrate. Moreover, the conversion of external fructose to fructose 1,6-*bis* phosphate inside the cell - the crossroads of paths for cellular biosyntheses and for the generation of energy - is achieved by a variety of mechanisms. And (and this most relevantly demonstrates Jonathan's thesis) when other normally used metabolic pathways have been closed, a novel route can be opened up by bringing into play genes that, up to then, had not appeared to be involved in fructose utilization. It is with these thoughts in mind that this review is dedicated *in piam memoriam* Jonathan Reizer.

There are three main modes of utilization of fructose. In the first (Route A, Figure 1), fructose is taken up via the membrane-spanning protein FruA and concomitantly phosphorylated to 1-phosphofructose, this latter process being effected by the transfer of a phosphoryl moiety from phosphoenolpyruvate (PEP) to the hexose, by the concerted action of two cytoplasmic proteins: Enzyme I of the phosphoenolpyruvate:phosphotransferase system (PTS) and a membrane-associated diphosphoryl transfer protein (DTP). The 1-phosphofructose thus formed is further phosphorylated by ATP and 1-phosphofructokinase (FruK) to the 1,6-*bis* phosphate ester.

In the second mode of fructose utilization (Route B, Figure 1), fructose enters the cells via membrane-spanning proteins that have a general ability to recognize sugars possessing the 3,4,5-D-*arabino* -hexose configuration; these include the permeases for mannose (Man XYZ), glucitol (SrlA) and mannitol (MtlA). In each of these instances, fructose has to be supplied in relatively high concentrations (>2 mM) for it to support growth at 37^{o}C with doubling times of less than 4 h, and in each case it is 6-fructophosphate and not the 1-phospho ester that is formed. Moreover, the phosphate is transferred to the hexose via the general cytoplasmic components of the PTS and the DTP appears not to be involved.

In the third mode of fructose utilization (Route C, Figure 1), fructose enters the cell by facilitated diffusion, using an isoform of the glucose transporter PtsG. Since this mode of entry does not involve the PTS, the free fructose has to be phosphorylated by ATP and a manno(fructo)kinase (Mak$^+$) that is normally present in only trace activity (Mak-o). Growth on fructose by *E.coli* devoid of the capacity to use either Route A or Route B thus necessitates the occurrence of at least two mutational events: the selection of the isoform PtsG-F, which differs from the form PtsG-I generally regarded as the "wild type" in having undergone a V12F mutation and which has thereby acquired the ability to translocate fructose as well as glucose, and the conversion of Mak-o to high activity (Mak$^+$). This latter change brings into play an ORF (*yajF*) of previously unknown function.

Route A

There are a number of features that distinguish Route A from other pathways capable of transforming external fructose to intacellular fructose 1,6-*bis* phosphate.

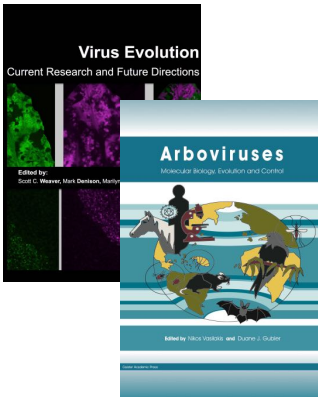
In the first place, the phosphorylated product that appears in the cell is fructose esterified at the 1-position;

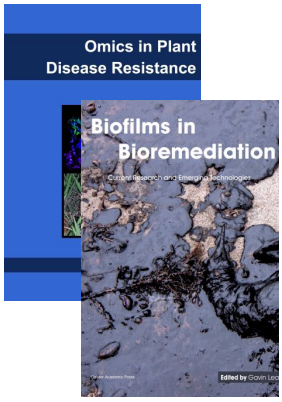
*For correspondence. Email hlk@bu.edu; Tel. (617) 358-1774; Fax. (617) 353-5084.

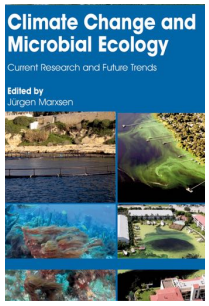
Further Reading

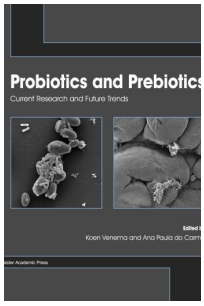
Caister Academic Press is a leading academic publisher of advanced texts in microbiology, molecular biology and medical research. Full details of all our publications at **caister.com**

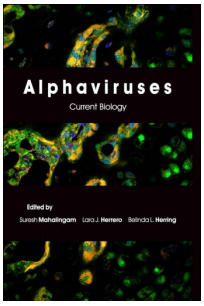
- **MALDI-TOF Mass Spectrometry in Microbiology**
  Edited by: M Kostrzewa, S Schubert (2016)
  www.caister.com/malditof
- ***Aspergillus* and *Penicillium* in the Post-genomic Era**
  Edited by: RP Vries, IB Gelber, MR Andersen (2016)
  www.caister.com/aspergillus2
- **The Bacteriocins: Current Knowledge and Future Prospects**
  Edited by: RL Dorit, SM Roy, MA Riley (2016)
  www.caister.com/bacteriocins
- **Omics in Plant Disease Resistance**
  Edited by: V Bhadauria (2016)
  www.caister.com/opdr
- **Acidophiles: Life in Extremely Acidic Environments**
  Edited by: R Quatrini, DB Johnson (2016)
  www.caister.com/acidophiles
- **Climate Change and Microbial Ecology: Current Research and Future Trends**
  Edited by: J Marxsen (2016)
  www.caister.com/climate
- **Biofilms in Bioremediation: Current Research and Emerging Technologies**
  Edited by: G Lear (2016)
  www.caister.com/biorem
- **Microalgae: Current Research and Applications**
  Edited by: MN Tsaloglou (2016)
  www.caister.com/microalgae
- **Gas Plasma Sterilization in Microbiology: Theory, Applications, Pitfalls and New Perspectives**
  Edited by: H Shintani, A Sakudo (2016)
  www.caister.com/gasplasma
- **Virus Evolution: Current Research and Future Directions**
  Edited by: SC Weaver, M Denison, M Roossinck, et al. (2016)
  www.caister.com/virusevol
- **Arboviruses: Molecular Biology, Evolution and Control**
  Edited by: N Vasilakis, DJ Gubler (2016)
  www.caister.com/arbo
- ***Shigella*: Molecular and Cellular Biology**
  Edited by: WD Picking, WL Picking (2016)
  www.caister.com/shigella
- **Aquatic Biofilms: Ecology, Water Quality and Wastewater Treatment**
  Edited by: AM Romaní, H Guasch, MD Balaguer (2016)
  www.caister.com/aquaticbiofilms
- **Alphaviruses: Current Biology**
  Edited by: S Mahalingam, L Herrero, B Herring (2016)
  www.caister.com/alpha
- **Thermophilic Microorganisms**
  Edited by: F Li (2015)
  www.caister.com/thermophile

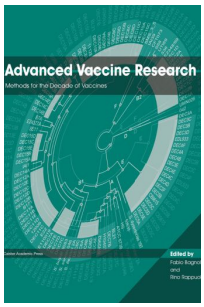


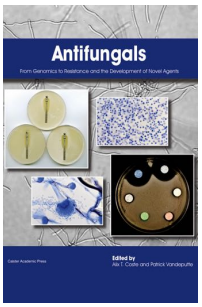












- **Flow Cytometry in Microbiology: Technology and Applications**
  Edited by: MG Wilkinson (2015)
  www.caister.com/flow
- **Probiotics and Prebiotics: Current Research and Future Trends**
  Edited by: K Venema, AP Carmo (2015)
  www.caister.com/probiotics
- **Epigenetics: Current Research and Emerging Trends**
  Edited by: BP Chadwick (2015)
  www.caister.com/epigenetics2015
- ***Corynebacterium glutamicum*: From Systems Biology to Biotechnological Applications**
  Edited by: A Burkovski (2015)
  www.caister.com/cory2
- **Advanced Vaccine Research Methods for the Decade of Vaccines**
  Edited by: F Bagnoli, R Rappuoli (2015)
  www.caister.com/vaccines
- **Antifungals: From Genomics to Resistance and the Development of Novel Agents**
  Edited by: AT Coste, P Vandeputte (2015)
  www.caister.com/antifungals
- **Bacteria-Plant Interactions: Advanced Research and Future Trends**
  Edited by: J Murillo, BA Vinatzer, RW Jackson, et al. (2015)
  www.caister.com/bacteria-plant
- ***Aeromonas***
  Edited by: J Graf (2015)
  www.caister.com/aeromonas
- **Antibiotics: Current Innovations and Future Trends**
  Edited by: S Sánchez, AL Demain (2015)
  www.caister.com/antibiotics
- ***Leishmania*: Current Biology and Control**
  Edited by: S Adak, R Datta (2015)
  www.caister.com/leish2
- ***Acanthamoeba*: Biology and Pathogenesis (2nd edition)**
  Author: NA Khan (2015)
  www.caister.com/acanthamoeba2
- **Microarrays: Current Technology, Innovations and Applications**
  Edited by: Z He (2014)
  www.caister.com/microarrays2
- **Metagenomics of the Microbial Nitrogen Cycle: Theory, Methods and Applications**
  Edited by: D Marco (2014)
  www.caister.com/n2

Order from caister.com/order

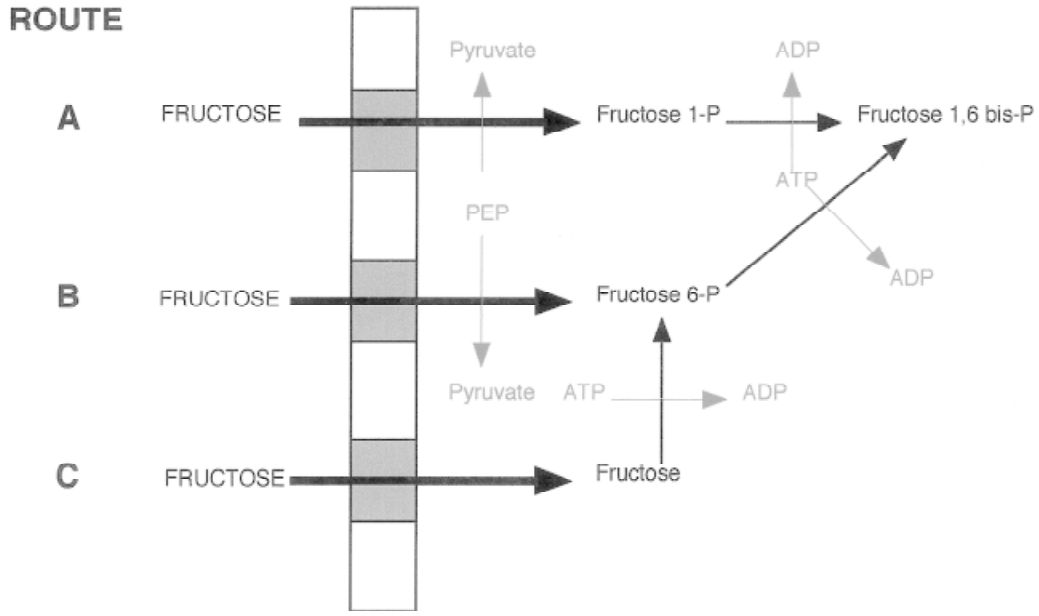


Figure 1. Routes for the uptake and utilization of fructose. Routes A and B necessitate the action of the PTS; in Route C, fructose enters the cells by faclitated diffusion via an isoform of the principal Enzyme II for glucose transport, but without involvement of the PTS.

all other routes form the 6-phosphofructose (Fraenkel, 1968; Ferenci and Kornberg, 1974; Jones-Mortimer and Kornberg, 1974, 1976).

Secondly, the gene *fruA* that specifies the membrane-spanning protein differs markedly both in its size and in the arrangement of its functional domains from other membrane-spanning proteins of the PTS (Prior and Kornberg, 1988). It has been pointed out by Saier *et al*, (1985) that the sugar-specific proteins of the PTS consist of either an Enzyme II of approximately 65 kDa, or of an Enzyme II / III pair with a combined molecular mass of 65 kDa. The calculated molecular mass of Enzyme II^{fru}, comprising 563 aminoacids, is 57.5 kDa, a value similar to the 54.0 kDa calculated for Enzyme II^{gut} (Yamada and Saier, 1987); this latter enzyme has been shown to function in conjunction with a 13.3 kDa Enzyme III^{gut}. The analogous Enzyme III^{fru} function is supplied by the DTP protein, of molecular mass 39.6 kDa and comprising 376 aminoacids (Reizer *et al.,* 1994), which combines both Enzyme III and HPr-like activities within this one large protein (Geerse *et al.,* 1989). Moreover, in most enzymes II, phosphate from phosphorylated HPr is transferred initially to a histidine residue and thence to a cysteine linked to the carboxy-terminal end of the membrane-spanning protein: these are generally described as the A and B regions respectively of Enzyme II (reviewed by Postma *et al.,* 1996). In FruA, however, the 100 residue B region is duplicated (Charbitt *et al.,* 1996) and transposed to form the amino-terminal end of this membrane-spanning protein.

A third unusual feature is that the small histidine-containing carrier protein HPr, which effects the transfer of phosphate from phosphorylated Enzyme I of the PTS to a variety of membrane-spanning or membrane-associated proteins, is not needed for the phosphorylation of fructose via Route A: mutants devoid of HPr (designated *ptsH*) grow on fructose with doubling times only slightly different from those of wild-type *E.coli* though they do not grow on other PT-sugars (Saier *et al.,* 1970). Prior growth on fructose allows *ptsH* - mutants also to utilize other PT-sugars for a while but this ability is diluted out during subsequent growth, which then ceases. Clearly, the cellular component that functions in lieu of HPr is induced by fructose but can act pleiotropically only if present in adequate cellular concentration: this cellular component is DTP. As expected from its dual role as the immediate acceptor of phosphate from phosphorylated Enzyme I and as the agent for its transfer to FruA, the C-terminal region of DTP exhibits 35 % sequence identity with the HPr of both Gram-positive and Gram-negative bacteria.

The fourth unusual feature of Route A is that the *fruA* gene that specifies the membrane-spanning protein (Prior and Kornberg, 1988) and the gene *fruB* that specifies the diphosphoryl transfer protein DTP closely associated with it (Geerse *et al.,* 1986; Reizer *et al*. 1994a) are located in one operon but are not contiguous (Rudd, 1998): they are separated by the gene *fruK* that acts on the product of the other two and that specifies a soluble 1-phosphofructokinase (Ferenci and Kornberg, 1974; Orchard and Kornberg, 1990). This operon, located at a region spanning min. 48.7 on the chromosome, is under negative regulation from a repressor protein FruR (Geerse *et al.* 1986, Kornberg and Elvin, 1987) which appears to have a multitude of functions: it also serves as a general regulator of gluconeogenesis (Chin *et al.,* 1987; Ramseier *et al.,* 1993, Vartak *et al.,* 1991). Moreover, and unlike many other regulatory genes of transport proteins which are often found in close proximity to the structural genes they regulate, the *fruR* gene is located diametrically opposite the *fru* operon, at min. 1.9 on the *E.coli* linkage map (Leclerc *et al.,* 1990; Jahreis *et al.,* 1991; Rudd, 1998).

The 1-phosphofructose, formed inside the cell from PEP and external fructose through the combined action of the FruA and DPT proteins, is next converted to fructose 1,6-*bis*phosphate by ATP and a 1-phosphofructokinase. This enzyme, specified by *fruK*, exhibits little sequence similarity to the major 6-phosphofructokinase of *E. coli*, specified by *pfkA,* and the 6-phosphofructokinases present in anumber of pro- and eukaryotic organisms, but does show some (27%) identity of sequence with the minor 6-phosphofuctokinase of *E.coli* specified by *pfkB.* FruK comprises 312 aminoacids and has a molecular mass of 33.8 kDa; the paper reporting its sequence (Orchard and Kornberg, 1990) also recorded parts of the sequences of the adjacent *fruB* and *fruA* genes, which confirmed the location of *fruK* between these two.

Route B

A number of permeases of the PTS recognize and translocate fructose but do so with affinities much lower than those of the proteins of Route A. These permeases fall into two groups: those that are formed constitutively and that therefore normally play some role in the utilization of fructose for growth, and those that take up fructose to any significant extent only if the normally inducible transport protein is overproduced through mutation of some regulatory component. A prime example of the first class is the mannose-transport system, specified by the genes *manXYZ* which, in *E. coli*, readily effects the uptake and phosphorylation also of fructose, glucose, and 2-deoxyglucose but which requires fructose to be present at >2mM if that ketohexose is to serve as sole carbon source for growth at doubling times less than 4 h. (Jones - Mortimer and Kornberg, 1974). Mutants devoid of ManXYZ activity grow at rates virtually identical with wild-type organisms whereas *fruA* - mutants, in which fructose utilization has to proceed via the mannose permease (all other possible routes having been blocked), grow much more slowly and at rates acutely dependent on the concentration of fructose in the external environment (Ferenci and Kornberg, 1974).

PT - permeases of the second group, which are capable of transporting and phosphorylating fructose but require to be present in extraordinarily large activities, are those for mannitol (Grisafi *et al.,* 1989; Lolkema *et al.,* 1990) and for glucitol (Jones - Mortimer and Kornberg, 1976; Yamada and Saier, 1987). In one mutant of this latter class, the mutation conferring both constitutivity of glucitol uptake and the ability to grow on fructose in the absence of the *fru* operon and of *manXYZ* was found to be associated with a G215T mutation in the regulatory gene *srlR* (H. L. Kornberg, A. A. Sproul and B. Thibodeau, unpublished experiments).

In an analogous manner, mutants devoid of the membrane-spanning proteins PtsG and ManXYZ which normally effect the PTS-dependent uptake of mannose and of glucose, do not grow on either sugar, but can mutate to take up mannose with low efficiency (K_m > 5mM); such mutants were found to be de-repressed for the *fru-* operon (Kornberg and Lambourne, 1992). These mutants now effect the uptake and phosphorylation of mannose via the over-produced FruA protein but form the 6-phospho ester of mannose, which is subsequently converted to 6-phosphofructose and, via ATP and PfkA, to fructose 1,6-*bis* phosphate

Route C

Strains of *E.coli* and of *S. typhimurium* devoid of the cytosolic proteins of the PTS or of functional components of both Routes A and B have been shown to give rise to further mutants that can grow - albeit rather poorly - on fructose (Saier *et al.,* 1971; Aulkemeyer *et al.,* 1991); such mutants also have elevated levels of manno(fructo)kinase (Mak^+), an enzyme first purified and characterized by Sebastian and Asensio (1972). This implies that fructose is taken up by a membrane - spanning protein capable of effecting the transport of free fructose, that the hexose appears inside the cells initially in an unphosphorylated form, and that it is converted only thereafter to the 6-phospho-ester by ATP and Mak.

Studies on fructose-positive mutants of *E. coli* in which the utilization of fructose by Routes A and B was abolished by deletions in the *fru* and *gut* operons as well as by insertions of a chloramphenicol-resistance transposon into *manXYZ* revealed that the uptake of free fructose was effected by an isoform of PtsG, designated PtsG-F, in which the G at position 34 in the published nucleotide sequence (Blattner *et al.,* 1997) has been replaced by T; mutants that do not contain this V12F isoform take up fructose to only a negligible extent (Kornberg *et al*. 2000). It is of interest that the predominant type of mutant of *E.coli* selected from continuous cultures subjected simultaneously to glucose and oxygen limitation (Manché *et al*. 1999; Notley-McRobb and Ferenci, 2000) exhibits the identical V12F change in PtsG; this mutation appears to result in rates of glucose transport greater than those observed with wild-type strains.

Several enzymes that can catalyse the ATP-dependent phosphorylation of fructose to 6-phosphofructose in *E.coli* have been described. One is a fructokinase designated ScrK which has been characterized by Aulkemeyer *et al.,* (1991) and which is plasmid-encoded; it enables sucrose-negative strains of *E.coli* to grow upon that disaccharide. In this process, sucrose is taken up via the PTS and appears inside the cells as 6-phosphosucrose, which is subsequently hydrolysed by an invertase-like hydrolase to 6-phosphoglucose and free fructose; this latter product is then acted upon by ATP and ScrK. Cloning and sequencing of the *scrK* gene carried by the sucrose plasmid pUR 400 revealed an ORF of 924 bp, equivalent to a peptide of 307 amino acids with a calculated M_r of 33.4 kDa (Aulkemeyer *et al.,* 1991). The synthesis of ScrK is repressed by fructose, which renders unlikely that it plays any role in the growth of *E.coli* on that hexose.

A chromosomally - encoded operon for sucrose utilization (*csc*) was discovered by Bockmann *et al.* (1992) in an isolate of *E.coli* able to grow upon sucrose. They observed that the transfer of the *csc* - genes to *E. coli* K-12 led both to acquisition of sucrose positivity and to the loss of D - serine deaminase activity, confirming an earlier report by Alaeddinoglu and Charles (1979) and indicating the location of *csc* close to *dsdA* at min. 51 on the *E. coli* linkage map (Berlyn, 1998). Bockmann *et al*. (1992) established that the *csc* - regulon is inducible and is controlled by a sucrose-specific repressor; the uptake of sucrose via this route is not dependent upon the PTS but is mediated by proton - symport. Hydrolysis of the sucrose

taken up by an invertase releases free glucose and free fructose, both of which are then phosphorylated to their 6 - phospho esters. The D - fructokinase activity specified by the *cscK* - gene in this operon is co-ordinately induced by sucrose but not by fructose, which indicates that it also is unlikely to play any role in the uptake and utilization of fructose by *E. coli* K12.

The third fructokinase, Mak, acts equally well on mannose and, to a lesser degree, on glucose, 2 - deoxyglucose and glucosamine (Sebastian and Asensio, 1972). It is normally present in only trace amounts in *E. coli* and these organisms were consequently designated "Mak-o" by Aulkemeyer *et al.* (1991), the Mak-rich mutants to which they give rise being termed "Mak$^+$". The conclusion that this enzyme is indeed the effector of growth on fructose by mutants of *E. coli* K12 in which Routes A and B have been rendered inoperative rests on the observations (H. L. Kornberg, A. A. Sproul, L. M. T. Lambourne and J. Jean-Jacques D., to be published) that

(i) strains of *E.coli*, in which Routes A and B have been deleted and which carry the PtsG-F isoform grow upon fructose, albeit poorly (with K_m for growth approx. 8 mM and V_{max} for generation time approx. 70 min.; Kornberg *et al.,* 2000), but only if they are also Mak$^+$;
(ii) such strains lose the ability to grow on fructose, and also lose approx. 90 % of their Mak activity, when DNA closely linked to (but not identical with) *araJ* from wild-type *E.coli* is introduced either by Hfr-mediated conjugation or by P1-transduction. Conversely, Mak-o strains acquire the ability to grow on fructose after phage-mediated transduction of this region from Mak$^+$ donors;
(iii) growth of Mak$^+$ strains on fructose is abolished by introduction of the *pfkA* gene, which shows that it is indeed the 6-phospho ester that is formed by the kinase;
(iv) Mak activity is specified by an ORF, designated *yajF* on the physical map of *E.coli* adjacent to *araJ* and mapping at min. 8.8 (Rudd, 1998). Introduction of this region, cloned from a Mak$^+$ donor into a multi-copy plasmid, into Mak-o mutants enables the resultant organisms to grow upon fructose and raises their Mak activity by several hundred-fold.

The difference between Mak-o and Mak$^+$ appears to reside in a change of GCC to GAC in codon 24 of the 1032 bp. region specified by the *mak* - gene, which would result in a change from alanine (in *mak-o*) to aspartate (in *mak*$^+$) in the deduced 344 aminoacid polypeptide. This difference is manifested not only in the Mak activity but also in the heat stabilty of these two forms: at 61 ^{o}C, the cloned *mak-o* activity decays with a half-life of 6 min. whereas the cloned *mak*$^+$ loses half of its activity only after 32 min. This indicates that the mutation from Mak-o to Mak$^+$ involves a major change in the conformation of the enzyme: the nature of this change remains to be elucidated.

Envoi

There is no reason to assume that the three routes discussed in this review represent the limits to which *E. coli* are constrained in their ability to take up and utilize fructose. The elucidation of the complete nucleotide sequence of the *E. coli* genome (Blattner *et al.,* 1998) has served also to reveal the existence of numerous ORFs which are not known to play any metabolic role but which show a high degree of sequence similarity to genes that do. The identification of such "paralogues" owes much to the work of Jonathan Reizer and his co-workers (for example, see Reizer and Reizer, 1996). In the present context, it is particularly relevant to draw attention to the recognition of analogues of FruA, specified by *frvA* and *frvB* at min. 88.1 (Plunkett *et al.,* 1993; Reizer *et al.,* 1994b), and *frwC, frwB* and *frwD* at min. 89.3 (Blattner *et al.,* 1993; Reizer *et al.,* 1995). Whether these ORFs actually specify, or can be made to specify, metabolic pathways alternative to the Routes A, B and C here discussed remains to be seen. This will require study of strains of *E. coli* in which *fruA* is deleted but in which the PTS is not impaired and which are also able to utilize any 1-phosphofructose that may be formed by permeases other than the FruA protein. In undertaking such studies, we shall be ever conscious of, and grateful for, the impetus and inspiration provided by Jonathan's pioneer work.

References

Alaeddinoglu, N. G., and Charles, H. P. (1979). Transfer of a gene for sucrose utilization into *Escherichia coli* K12, and consequent failure of expression of genes for D-serine utilization. J. Gen. Microbiol. 110: 47-59.

Aulkemeyer, P., Ebner, R., Heilenmann, G., Jahreis, K., Schmid, K., Wrieden, S., and Lengeler, J. W. 1991. Molecular analysis of two fructokinases involved in sucrose metabolism of enteric bacteria. Molecular Microbiol. 5: 2913-2922.

Berlyn, M. 1998. Linkage map of *Escherichia coli* K-12, edition 10: the traditional map. Microbiol. Mol. Biol. Revs. 62: 814-984.

Blattner, F. R., Burland, V., Plunkett III, G., Sofia, H. J., and Daniels, D. L. 1993. Analysis of the *Escherichia coli* genome. 4. DNA sequence of the region from 89.2 to 92.8 minutes. Nucleic Acids Res. 21: 5408-5417.

Blattner, F. R., Plunkett III, G., Bloch, C. A., Perna, N. T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J. D., Rode, C. K., Mayhew, G. F., Gregor, J., Davis, N. W., Kirkpatrick, H. A., Goeden, M. A., Rose, D. J., Mau, B., and Shao, Y. 1997. The Complete Genome Sequence of *Escherichia coli* K-12. Science 277: 1453-1474.

Bockmann, J., Heuel, H., and Lengeler, J. W. 1992. Characterization of a chromosomally encoded, non-PTS metabolic pathway for sucrose utilization in *Escherichia coli* EC3132. Mol. Gen. Genet. 235: 22-32.

Charbitt, A., Reizer, J., and Saier, M. H., jr. 1996. Function of the duplicated IIB domain and oligomeric structure of the fructose permease of *Escherichia coli*. J. Biol. Chem. 271: 9997-10003.

Chin, A. M., Feucht, B. U., and Saier, M. H., jr. 1987. Evidence for regulation of gluconeogenesis by the fructose phosphotransferase system in *Salmonella typhimurium*. J. Bacteriol. 169: 897-899.

Ferenci, T., and Kornberg, H.L. 1974. The role of phosphotransferase-mediated syntheses of fructose 1-phosphate and fructose 6-phosphate in the growth of *Escherichia coli* on fructose. Proc. Roy. Soc. Lond. B 187: 105-119

Fraenkel,D.G. 1968. The phosphoenolpyruvate-initiated pathway of fructose metabolism in *Escherichia coli*. J.Biol.Chem. 243: 6458-6463.

Geerse, R.H., Ruig, C.R.,Schuitema, A.R.J., and Postma, P.W. 1986. Relationship between pseudo-HPr and the PEP:fructose phosphotransferase system in *Salmonella typhimurium* and *Escherichia coli*. Mol. Gen. Genet. 203: 435-444.

Geerse, R.H., Izzo, F., and Postma, P. W. 1989. The PEP:fructose phosphotransferase system in *Salmonella typhimurium*. FPr combines enzyme III^{Fru} and pseudo-HPr activities. Mol. Gen. Genet. 216: 517-525.

Grisafi, P. L., Scholle, A., Sugiyama, J., Briggs, C., Jacobson, G. R., and Lengeler, J. W. 1989. Deletion mutants of the *Escherichia coli* K-12 mannitol permease: dissection of transport-phosphorylation, phospho-exchange and mannitol- binding activities. J. Bacteriol. 171: 2719-2727.

Jahreis, K., Postma, P.W., and Lengeler, J.W. 1991. Nucleotide sequence of the *ilvH-fruR* region of *Escherichia coli* K12 and *Salmonella typhimurium* LT2. Mol. Gen. Genet. 226: 332-336

Jones-Mortimer, M.C., and Kornberg, H.L. 1974. Genetical analysis of fructose utilization by *Escherichia coli*. Proc. Roy. Soc. Lond. B 187: 121-131

Jones-Mortimer, M.C., and Kornberg, H.L. 1976. Uptake of fructose by the

sorbitol phosphotransferase of *E. coli* K12. J. gen. Microbiol. 96: 383-391.

Kornberg, H. L., and Elvin, C. M. 1987. Location and function of *fruC*, a gene involved in the regulation of fructose utilization by *Escherichia coli*. J. gen. Microbiol. 133: 341-346.

Kornberg, H. L., and Lambourne, L. T. M. 1992. Role of the phosphoenolpyruvate-dependent fructose phosphotransferase system in the utilization of mannose by *Escherichia coli*. Proc. Roy. Soc. Lond. B 250: 51-55.

Kornberg, H. L., Lambourne, L. T. M., and Sproul, A. A. 2000. Facilitated diffusion of fructose via the phosphoenolpyruvate / glucose phosphotransferase system of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 97: 1808-1812.

Lolkema,J. S., Dijkstra, D. S., Ten Hoeve-Duurkens, R. H., and Robillard, G. T. 1990. The membrane-bound domain of the phosphotransferase enzyme II ^{Mtl} of *Escherichia coli* constitutes a mannitol translocating unit. Biochemistry. 29: 10659-10663.

Manché, K., Notley-McRobb, L., and Ferenci, T. 1999. Mutational adaptation of *Escherichia coli* to glucose limitation involves distinct evolutionary pathways in aerobic and oxygen-limited environments. Genetics 153: 5-12.

Notley-McRobb, L., and Ferenci, T. 2000. Substrate specificity and signal transduction pathways in the glucose-specific Enzyme II (E II^{Glc}) component of the *Escherichia coli* phosphotransferase system. J. Bacteriol. 182: 4437-4442.

Orchard, L.M.D., and Kornberg, H. L. 1990. Sequence similarities between the gene specifying 1-phosphofructokinase (*fruK*), genes specifying other kinases in *Escherichia coli* K12, and l*acC* of *Staphylococcus aureas*. Proc. Roy. Soc Lond. B 242: 87-90.

Plunkett, G., Burland, V. D., Daniels, D. L., and Blattner, R. R. 1993. Analysis of the *Escherichia coli* genome. III. DNA sequence of the region from 87.2 to 89.2 minutes. Nucleic Acids Res. 21: 3391-3398.

Postma, P.W., Lengeler, J.W., and Jacobson, G.R. 1996. Phosphoenolpyruvate: carbohydrate phosphotransferase systems. In *Escherichia coli* and *Salmonella*, 2nd Ed. ASM Press: Washington, DC.

Prior, T.I., and Kornberg, H.L. 1988. Nucleotide sequence of *fruA*, the gene specifying enzyme II^{fru} of the phosphoenolpyruvate-dependent sugar phosphotransferase system in *Escherichia coli* K12. J. Gen. Microbiol.134: 2757-2768.

Ramseier, T.M., Negre, D., Cortay, J.-C., Scarabel, M., Cozzone, A.J., and Saier, M. H., jr. 1993. In vitro binding of the pleiotropic transcriptional regulatory protein, FruR, to the *fru, pps, ace, pts*, and *icd* operons of *Escherichia coli* and *Salmonella typhimurium*. J. Mol. Biol. 234: 28-44.

Reizer, J., and Reizer, A. 1996. A voyage along the bases: novel phosphotransferase genes revealed by *in silico* analyses of the *Escherichia coli* genome. Res. in Microbiol. 147: 458-471.

Reizer, J., Reizer, A., and Saier, M.H., jr. 1995. Novel phosphotransferase genes revealed by bacterial genome analysis-a gene cluster encoding a unique Enzyme I and the proteins of a fructose-like permease system. Microbiology, 141: 961-971.

Reizer, J., Reizer, A, Kornberg, H.L., and Saier, M.H., jr. 1994a. Sequence of the *fruB* gene of *Escherichia coli* encoding the diphosphoryl transfer protein (DTP) of the phosphoenolpyruvate:sugar phosphotransferase system. FEMS Micobiol. Lett. 118: 159-162.

Reizer, J., Michottey, V., Reizer, A., and Saier, M. H., jr. 1994b. Novel phosphotransferase genes revealed by bacterial genome analysis: unique, putative fructose-and glucoside-specific systems. Protein Sci. 3: 440-450.

Rudd, K. 1998. Linkage map of *Escherichia coli* K-12, Edition 10: The physical map. Microbiol. Mol. Biol. Revs. 62: 985-1019.

Saier, M. H., jr., Simoni, R. D., and Roseman, S. 1970. The physiological behavior of enzyme I and heat-stable protein mutants of a bacterial phosphotransferase system. J. Biol. Chem. 245: 5870-5873.

Saier, M. H., jr., Scott Young III, W., and Roseman, S. 1971. Utilization and transport of hexoses by mutant strains of *Salmonella typhimurium* lacking enzyme I of the phosphoenolpyruvate-dependent phosphotransferase system. J. Biol. Chem. 246: 5838-5840.

Saier, M. H., jr.,Grenier, F. C., Lee, C. A., and Waygood, E. B. 1985. Evidence for the evolutionary relatedness of the proteins of the bacterial phosphoenolpyruvate: sugar phosphotransferase system. J. Cellular Biochem. 27: 43-56.

Sebastian J., and Asensio, C. 1972. Purification and properties of the mannokinase from *Escherichia coli*. Arch. Biochem. Biophys. 151: 227-233.

Vartak, N. B., Reizer, J., Reizer, A., Gripp, J. T., Groisman, E. A., Wu, L.-F., Tomich, J. M., and Saier, M. H., jr. 1991. Sequence and evolution of the FruR protein of Salmonella typhimurium: a pleiotropic transcriptional regulatory protein possessing both activator and repressor functions which is homologous to the periplasmic ribose-binding protein. Res. Microbiol. 142: 951-963.

Yamada, M., and Saier, M. H., jr. 1987. Glucitol-specific enzymes of the phosphotransferase system in *Escherichia coli*. Nucleotide sequence of the *gut* operon. J. Biol. Chem. 262: 5455-5463.