The bacterial phosphotransferase system (PTS) consists of two energy-coupling soluble proteins (enzyme I and HPr) and a large number of inner membrane transporters (enzymes II) that mediate concomitant phosphorylation and translocation of sugars and hexitols. The transporters consist of three functional units (IIA, IIB, IIC), which occur either as protein subunits or domains of a multidomain polypeptide. The membrane-spanning IIC domain contains the substrate binding site; IIA and IIB are phosphorylation domains that transfer phosphate from HPr to the transported sugar. The transporter complexes of the PTS are good examples for variation of design by modular assembly of domains and subunits. The domain order is IIC-IIIB in the membrane subunit of the *Escherichia coli* glucose transporter (IICBGlc) and IIB-IIIC in *Salmonella typhimurium* sucrose transporter (IIBCScr). The phosphorylation domain of IICBGlc was translocated from the carboxyl-terminal to the amino-terminal end of the IIC domain, and the activity of the circularly permuted form was optimized by variation of the length and the composition of the interdomain linker. HBapCGlc, with an alanine-proline-rich interdomain linker has 70% of the control specific activity after purification and reconstitution into proteoliposomes. These results indicate that the amino-terminal end of IICBGlc must be on the cytoplasmic side of the inner membrane, that membrane insertion of the IIC domain is insensitive to the modification of its amino-terminal end, and that a domain swap as it could occur by a single DNA translocation event can rapidly lead to a functional protein. However, IIB could not be substituted for by glucokinase. Fusion proteins between the IIC domain and glucokinase do not transport and phosphorylate glucose in an ATP-dependent mechanism, although the IIC moiety displays transport activity upon complementation with soluble subclonal IIB, and the glucokinase moiety retains ATP-dependent non-vectorial kinase activity. This indicates that IIC and IIB are two cooperative units and not only sequentially acting upon a common substrate, and that translocation of glucose must be conformationally coupled to the phosphorylation/dephosphorylation cycle of IIB.

**The Glucose Transporter of *Escherichia coli* with Circularly Permutated Domains Is Active in Vivo and in Vitro**

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The bacterial phosphotransferase system (PTS) is involved in transport and phosphorylation of carbohydrates and in regulation of the metabolism in response to the availability of these carbohydrates as nutrients (1–3). The PTS is almost ubiquitous in bacteria but with one exception, has not been found in eukaryotic cells. It consists of two cytoplasmic phosphoryl carrier proteins termed enzyme I and HPr, which sequentially transfer phosphoryl groups from FEP to the different substrate-specific transporters. The transporters comprise three independently folding units of function, IIA and IIB are hydrophilic and contain one phosphorylation site each, and IIC spans the membrane and contains the sugar binding site. The three units occur either as domains in a single polypeptide chain or as subunits of a complex. The number of carbohydrate-specific transporters varies from species to species. There are 38 PTS-related genes in *Escherichia coli* and 26 PTS genes in *Bacillus subtilis* (EMBL/SwissProt data base), most of which code for PTS transporters and their subunits. Only two open reading frames with homologies to known PTS transporters were found in *Hemophilus influenzae* (4) and only one in *Mycoplasma genitalium* (5). The transporters differ in amino acid sequence (from insignificant to more than 90% amino acid similarity), substrate selectivity, and in the structure of the phosphorylation site (histidine and cysteine phosphorylation).

Lengeler et al. (6) grouped the transporters into five main families (glucose-sucrose, mannitol-fructose, lactose-cellobiose, mannose, glucitol) according to amino acid sequence similarity. The EMBL/SwissProt data base lists 24 homologous proteins from 12 different Gram-positive and -negative bacteria in the glucose-sucrose family. Nine have the domain order CB, and 15 have the order CB (Fig. 1). CB and BC can be considered as circularly permuted variants. The hydrophilic B domains contain the strongly conserved sequence N(I/L)X4DTRLRXLXR, with the active-site cysteine, which is phosphorylated by the cognate IIA domains (7). The CB forms, in addition, contain the highly conserved sequence KTPTGRED (residues 368–388 of IICBGlc), This motif is 35 residues upstream of the active-site cysteine, and it serves as a hinge of constrained mobility between the IIC and the IIB domain (8). It is the only region at which a functional hybrid protein between the homologous transporters for GII and GIIAuc could be constructed (9). The

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1 The abbreviations used are: PTS, phosphoenolpyruvate-dependent carbohydrate-phosphotransferase system (EC 2.7.1.69); IICBGlc, transmembrane subunit of the glucose transporter; IICBGlc, transmembrane domain of IICBGlc; IIBCGlc, cytoplasmic domain of IICBGlc; HBapCGlc, IICBGlc, and IHapCglc, circularly permuted forms of IICBGlc with a short (x) and a long Ala-Pro-rich (ap) linker; Glk, glucokinase (EC 2.7.1.2); IIC-Glk, GlkxIIC, and GlkapIIC, fusion proteins between IICBGlc and glucokinase with short(x) and long (ap) linkers; IICGlc, hydrophilic subunit of the glucose transporter (EC 2.7.1.69); HPr, histidine-containing phospho carrier protein of the PTS; EL, enzyme I of the PTS; PEP, phosphoenolpyruvate; NTA, nitrilotriacetic acid.

2 http://protein.toulouse.inra.fr/cgi-bin/ReqProtomII.pl?acc_seq2=P05053.
BC variants have a conserved sequence GNXXVXXX(F/Y) or GXGXXVXXX(F/Y) 42 residues downstream of the active-site cysteine. It is unique to the BC subgroup and, as judged from the spacing, could be part of a linker between the B and C domains.

The different transporters of the PTS are a good example for variation of design by modular assembly of domains and subunits (10). It has been speculated that the different types of PTS transporters are circularly permuted forms of a consensus structure envisaged to consist of two transmembrane portions and of two large loops, forming cytoplasmic subdomains (11).

Saier, Reizer, and co-workers produced a number of sophisticated analyses that suggest that the multidomain proteins of the PTS arose during evolution by repeated recombinational gene rearrangements (12) and document the combinatorial nature and modular design of the PTS transporters (13–19). Here we show that the C and B domains of the glucose transporter of E. coli can be circularly permuted with only minor effects on the activity and without detectable effects on membrane insertion.

The wild-type form of the E. coli glucose transporter consists of two subunits IIA\textsuperscript{Glc} and IIC\textsuperscript{Glc}. IIA\textsuperscript{Glc} is a monomeric soluble 18-kDa protein that transfers the phosphate group from HPp to the IIC\textsuperscript{Glc} subunit and at the same time plays an important role in allosteric regulation of adenylate cyclase, glycerol kinase, and carbohydrate transporters for lactose, maltose, and other non-PTS sugars (2). IIC\textsuperscript{Glc} is a homodimeric membrane protein. The 52-kDa subunits consist of two domains. The C domain contains six plus two membrane-spanning (residues 17–210 and 280–325), and two extended hydrophilic segments (residues 211–279 and 326–386) on the cytoplasmic face (20). The B domain on the cytoplasmic face (residues 386–477) has a split α/β sandwich fold composed of an antiparallel sheet and three α-helices superimposed onto one side of the sheet (21, 22). The C domain contains the sugar binding site (9), whereas the B domain contains the phosphorylation site (Cys-421 (7)). This B domain was translocated from its natural location at the carboxyl-terminal end to the amino-terminal end of the C domain. The two domains were coupled by peptide linkers of 4 and 22 residues. The recombinant IIBC\textsuperscript{Glc} subunit with the shorter linker displayed 20%, and the protein with longer Ala-Pro-rich linker (11) displayed 70% of wild-type activity.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Growth Conditions—** E. coli K-12 ZSC112ΔHIC (ptsG manZ glk ptsH ptsI crv) (23) and E. coli K-12 ZSC112LG (ptsG:cat manZ glk) (24) were used as hosts for in vivo complementation assays on McConkey plates and for protein expression. The transformed cells were grown on a rotary shaker in LB broth at 37 °C. When the culture had reached A\textsubscript{600} = 1.0, protein expression was induced with 0.1 mM isopropyl-1-thio-β-D-galactopyranoside, and growth continued overnight.

**Plasmid Construction—** Plasmids pJFBxC and pJFBapC (Fig. 2) encode under the control of the Ptac promoter the circularly permuted IIBC\textsuperscript{Glc} variants with a hexahistidine tag at the carboxyl termini. They were constructed from plasmids pJBH and pJCH (24) encoding the IIB gene rearrangements (12) and document the combinatorial nature and modular design of the PTS transporters (13–19). Here we show that the C and B domains of the glucose transporter of E. coli can be circularly permuted with only minor effects on the activity and without detectable effects on membrane insertion.

**Fig. 2.** Plasmid map of pJFBxC, linker sequences, and polyacrylamide gel of purified proteins. A, plasmids pJFBxC and pJFBapC encode IIBx\textsuperscript{Glc} or IIBapC\textsuperscript{Glc} (filled) under control of the Ptac promoter. The nucleotide and amino acid sequences of the two linkers (x and ap) between the B and C domains are indicated. BglII, NsiI, AsuII, and NdeI sequences are underlined. B, lane 1, wild-type IIBC\textsuperscript{Glc}; lane 2, IIBx\textsuperscript{Glc}; lane 3, IIBapC\textsuperscript{Glc}; lane 4, IIC-Glk; lane 5, Glk\textsubscript{ap} after Ni\textsuperscript{2+} chelate affinity chromatography. Glk\textsubscript{HIC} could not be purified.
**Fig. 3. Phosphotransferase activities of IICB (circles), IIbapC (squares) IIbC (triangles).** A, in vivo transport activity of E. coli ZSC112LAG expressing plasmid-encoded proteins induced with isopropyl-1-thio-b-D-galactopyranoside (20 μM, dotted lines; 50 μM, broken lines; 80 μM, solid lines). B, in vitro import of Glc into proteoliposomes reconstituted from purified proteins and E. coli lipids and loaded with enzyme I, HPr, IIA^C^, and PEP. C, nonvectorial phosphorylation of Glc. Enzyme I, HPr, IIA^C^, and PEP were added to the outside of the proteoliposomes. Aliquots of 75 μl containing 5 to 6 μg of purified protein were assayed per time point. Where shown, means and S.D. are of three independent experiments.

**Fig. 4. Dependence of nonvectorial phosphotransferase activity of IIbCGlc and IIbxC^Glc^ on IIA^C^ concentration.** The activities are given in nmol of Glc 6-phosphate/30 min per 1 ng of IICB^Glc^ (circles) and per 15 ng of IIbxC^Glc^ (triangles), respectively. The calculated K_{cat} values are 2.3 ± 0.5 μM for IICB^Glc^ and 0.56 ± 0.8 μM for IIbxC^Glc^, respectively. Shown are means and S.D. of three independent experiments. The concentrations of IICB^Glc^ and IIbxC^Glc^ were 2.5 and 29 nm, respectively.

**RESULTS**

**Gene Reconstruction and Protein Purification**—The genes for the two circularly permuted IICb^Glc^ variants of the glucose transporter (IICb^Glc^) were reconstructed from the ptsG gene fragments encoding the two domains and two heteroduplexes encoding linker sequences of different length and amino acid composition (Fig. 2A). Both IICb^Glc^ and IIbapC^Glc^ could be solubilized quantitatively with 2% N-decyl-β-D-maltopyranoside and loaded with enzyme I, HPr, and glucose uptake was assayed as described (34).

**Other Techniques**—Protein samples were not boiled in sample buffer before electrophoresis on standard 15% polyacrylamide gels (32). Gels were stained with Coomassie Blue. Protein concentrations were determined by a modified Lowry assay (35) with bovine serum albumin as the standard.

**Protein Purification**—Membranes were prepared, and the proteins were purified as described (24) with the following modifications. Membranes from 500-ml cell cultures were suspended in 10 ml of buffer A (10 mM Tris-HCl, pH 9.3, 10 mM β-mercaptoethanol) and solubilized by the addition of N-decyl-β-D-maltopyranoside to 1% final concentration. The suspension was mixed with 2 ml of Ni^{2+}-nitritotriacetic acid agarose (Qiagen) equilibrated with buffer B, pH 8 (50 mM NaPi, 50 mM NaCl, 10 mM β-mercaptoethanol, 0.2% N-decyl-β-D-maltopyranoside), and incubated for 30 min at room temperature. The suspension was packed into a column at 4 °C, and excess solution was drained off. The column was washed at 4 °C with, consecutively, 10 ml of buffers B, pH 8 and pH 6, and the bound protein was eluted with buffer C (100 mM imidazole, 50 mM NaPi, pH 7.5, 500 mM NaCl, 10 mM β-mercaptoethanol, 0.2% N-decyl-β-D-maltopyranoside), and incubated for 30 min at 4 °C. The active fractions were concentrated to 3 ml and loaded onto a nickel-resin column (30) and pTSGH11 (8).

**Sugars Phosphorylation Activity**—Sugar phosphorylation activity was assayed by the ion exchange method (31, 32). A cell-free cytoplasmic extract from E. coli ZSC112LAG(pTSHC19) overexpressing enzymes I, HPr, and IIA^C^ or the proteins purified from this extract were used to complement phosphotransferase activity (39). Either Glc or α-methyl-D-glucopyranoside was added at 50 μM.
Glucose Transporter of E. coli

Expression and subcellular distribution of glucokinase and glucokinase-IIGlc fusion proteins

Cells from 800-ml overnight cultures were fractionated, and glucokinase activity was determined. 100% activity corresponds to 1.29 × 10^9 nmol of Glc 6-phosphate formed in 30 min at 37 °C. The total activity of the fusion proteins in percent of the activity of wild-type glucokinase is given in the last column. After centrifugation, the supernatant was withdrawn by aspiration. The top 90% of the supernatant and the 10% of the bottom layer over the membrane sediment were collected separately.

| Membranes          | Glucokinase (0.4) | GlkxIC fusion (2) | GlkapIC fusion (50) | IIIC-Glk fusion (42) |
|--------------------|-------------------|-------------------|---------------------|---------------------|
| Distribution (%)   | 94 (top 90%)      | 89 (top 90%)      | 13 (top 90%)        | 6 (top 90%)         |
| Glucokinase activity (%) | 2               | 2                 | 33                   | 35                   |
| Cell debris        | 100               | 2                 | 0.1                 | 0.9                 |

**TABLE II**

IIC-dependent phosphotransferase activity of glucokinase-IIGlc fusion proteins

The activities in the membrane and soluble fractions were determined as Glc 6-phosphate formed upon complementation with the subclonal IIBGlc domain. The specific activity of [1^4C]Glc was 1000 cpm/nmol. Protein was determined according to Ref. 35.

| Phosphotransferase activity | In membrane fraction | In soluble fraction (supernatant) |
|-----------------------------|----------------------|----------------------------------|
|                            | −IIBC | +IIBC | −IIBC | +IIBC |
| Glucokinase (munol Glc 6-phosphate/min) | ND | 3.1 | 5.7 |
| GlkxIC fusion | 10.4 | 11.1 | ND | ND |
| GlkapIC fusion | 10.5 | 27.4 | 1.5 | 1.8 |
| IIIC-Glk fusion | 19.7 | 245 | 1.8 | 16.8 |
| IIIC-Glk (C421S) | 41.5 | 456 | 3.5 | 14.7 |

* ND, not detected.

TABLE I

Distribution of glucokinase activity (%)

| Membranes          | Glucokinase (0.4) | GlkxIC fusion (2) | GlkapIC fusion (50) | IIIC-Glk fusion (42) |
|--------------------|-------------------|-------------------|---------------------|---------------------|
| Supernatant (top 90%) | 94 (top 90%) | 89 (top 90%) | 13 (top 90%) | 6 (top 90%) |
| Glucokinase activity (%) | 2               | 2                 | 33                   | 35                   |
| Cell debris | 100               | 2                 | 0.1                 | 0.9                 |

**ylen glycolyl ether for 3 weeks at 4 °C until they started to lose activity. Freezing at −20 °C resulted in precipitation and partial inactivation. About 55% of the phosphotransferase activity originally present in the membranes was recovered after metal affinity chromatography. Part of the activity was lost due to incomplete binding to the column. Between 1.5 and 2 mg of pure IIBC^Glc^ could be recovered from 1 liter of an overnight cell culture. IIBC^Glc^ (M_r 53,229) with the short linker had an increased electrophoretic mobility (Fig. 2B) relative to wild-type IICB^Glc^ (M_r 51,864), suggesting that it binds more SDS and/or has a different shape in the partially unfolded form. Attempts to completely unfold the proteins by boiling in sample buffer resulted in protein aggregation. Proteolytic degradation can be excluded as the cause of the increased mobility since Edman degradation demonstrated an intact amino terminus (results not shown), and the intactness of the carboxyl terminus could be confirmed by successful binding to the N_i^2^-NTA column. IIBapC^Glc^ (M_r 54,768) with the 22 residues linker rich in alanine and proline has an only marginally decreased electrophoretic mobility.

Phosphotransferase Activity of the Mutants with Circularly Permutated Domains—E. coli ZSC112ΔG expressing IIBapC^Glc^ and wild-type IICB^Glc^ under the control of the leaky Ptac promoter fermented glucose on McConkey plates already without induction (results not shown). In contrast, cells expressing IIBC^Glc^ fermented glucose only after induction of gene expression with 10 μM isopropyl-1-thio-b-D-galactopyranoside, indicating that IIBC^Glc^ with the short linker is less active than its counterpart with the longer linker and wild-type IICB^Glc^.

The difference was confirmed by in vivo transport assays. E. coli expressing IIBC^Glc^ displayed a reduced transport activity at all levels of induction (Fig. 3A).

IICB^Glc^ catalyzes vectorial transport coupled to phosphorylation of the substrate (vectorial phosphorylation) as well as substrate phosphorylation without transport (nonvectorial phosphorylation). Active transport was measured with IICB^Glc^ containing proteoliposomes that were loaded with PEP, enzyme I, HPr, and IIA^Glc^ and to which glucose was added from the outside. The initial rates of glucose transport into these proteoliposomes were similar for wild-type IICB^Glc^, IIBxGlc^, and IIBapC^Glc^, but the level to which glucose was accumulated was only 55% for IIBxGlc^ and 75% for IIBapC^Glc^ (Fig. 3B). Nonvectorial phosphorylation was measured with IICB^Glc^-containing proteoliposomes to which glucose, PEP, and the soluble components were all added from the outside. Purified IIBxGlc^ was displayed between 20 and 30% wild-type nonvectorial PTS activity in the presence of saturating concentrations of enzyme I, HPr, IIA^Glc^, and glucose. IIBapC^Glc^ had between 50 and 70% wild-type activity (Fig. 3C).

It is not clear whether docking of IIA^Glc^ to the IIB domain, phosphate transfer between IIA and IIB and between IIB and Glc, or translocation of Glc is affected by the circular permutated IIIC-Glk fusion. Comparing the K_m for IIA^Glc^ of the two forms allows estimation of differences of affinity for IIA^Glc^, IIA^Glc^ was titrated in the presence of excess Ei and HPr, and a rate-limiting concentration of IICB^Glc^ and IIBxGlc^, respectively. The K_m for IIA^Glc^ determined as IICB^Glc^ concentration necessary for half maximal phosphotransferase activity was 2.3 ± 0.5 μM for wild-type IICB^Glc^ and 0.36 ± 0.8 μM for IIBxGlc^ (Fig. 4). Assuming that the overall rate of phosphate transfer from IIA to Glc is faster than the dissociation rate of the IIA-IIB complex, the reduced K_m can be accounted for by the observed reduction of V_max (Fig. 3C). Therefore it appears that the affinity of IIA for IIBxGlc^ is comparable with the affinity to wild-type IICB^Glc^.

Substitution of the IIB Domain by Glucokinase—The comparable activities of IICB^Glc^ and IIBapC^Glc^ could suggest that the main function of IIB is to remove by phosphorylation the tightly bound glucose from the cytoplasmic face of the IIC domain and that covalent binding of IIB to IIC would serve to maintain a high concentration of the phosphoryl donor at the binding site. If this were so, glucokinase (Glk) might be capable of complementing IIB activity. To test this hypothesis, fusions of the 35-kDa E. coli glucokinase (26) to the amino and carboxyl terminus of the IIC domain were constructed, and the fusion proteins were expressed in E. coli ZSC112ΔHIC lacking glucokinase, IICB^Glc^, and IIA^Glc^ as well as the general PTS enzymes I and HPr. The transformants formed yellow colonies on McConkey glucose indicator plates containing different isopropyl-1-thio-b-D-galactopyranoside concentrations. To test for function of the IIC domain, the fusion proteins were expressed in ZSC112ΔG(pACYCB). This strain lacks IICB^Glc^ and glucokinase but expresses the chromosomally encoded enzyme I, HPr, IIA^Glc^ and the plasmid-encoded subclonal IIB^Glc^ domain. Cells coexpressing IIC-Glk and subclonal IIB^Glc^ formed red colonies on McConkey glucose plates, whereas cells expressing GlkxIIC or GlkapIIC and IIC^Glc^ were red-centered, indicating that the IIC domain of the fusion protein was functional.

Since the fermentation phenotype on McConkey plates was
variable and did not allow for quantitative analysis of function, lysates from cells expressing Glk, IIC-Glk, GlkxIIC, and GlkapIIC were fractionated, and the membrane and cytoplasmic fractions were assayed for glucokinase and IIC-dependent phosphotransferase activity (Tables I and II). The IIC-dependent phosphotransferase activity was assayed with membranes in the presence of increasing concentrations of purified subclonal IIB Glc (3 mg/µl). Activities are given in pmol of Glc 6-phosphate (Glc-6P) formed in 30 min/µg of total membrane protein. IIC-Glk (square), GlkapIIC (inverted triangle), GlkxIIC (triangle), IICB Glc (C421S) (circle).

**DISCUSSION**

The PTS transporters of the glucose family consist of two domains, the transmembrane IIC domain and the cytoplasmic IIB domain. The domain order can be either CB as in the glucose transporter or BC as in the sucrose transporter IIBC Scr of Salmonella typhimurium (36). Here we show that the two domains of the IICB Glc subunit of the glucose transporter can be circularly permuted, thus mimicking domain shuffling as it might have occurred during the evolution of the PTS transporters from common ancestors. The circularly permuted form (IIBC Glc) complemented glucose transport and phosphorylation in vivo and could be purified and functionally characterized in vitro. The permuted form without additional structural adjustments had already between 10 and 20% wild-type activity. Increasing the length of the interdomain linker from 4 to 20 residues (Fig. 1) increased the activity to 70% of the wild-type reference. At the same time, the phosphorylation domain IIB could not be functionally substituted for by glucokinase. This indicates that IIC and IIB are two cooperative units and not only two proteins that sequentially act upon a common substrate and that translocation of glucose must be conformationally coupled to the phosphorylation/dephosphorylation cycle of IIB. However, despite the high specificity of the interaction between IIC and IIB, binding is not so tight that the covalently bound but inactive IIB cannot be displaced. For example, the soluble subclonal IIB Glc unit (10 kDa) can successfully displace an inactive IIB domain (in the C421S mutant of IICB Glc, Table II) or the even larger glucokinase moiety (35 kDa) in a fusion protein between the IIC domain and glucokinase. Coupling between the two domains is likely to be mediated by parts of the antiparallel β-sheet, which carries the active site Cys-421 and the essential Arg-424 and Arg-426 (8). The helices that form a dome-like structure on the face of the antiparallel β-sheet opposite to the active site (21) mediate the covalent links to the neighboring domains in multidomain proteins. In IICB Glc, helix 1 connects the β-sheet to the carboxyl terminus of the IIC domain, whereas helix 3 is free. In the homologous transporter for GlcNAc (IICB GlcNAc) (34), helix 3 connects the β-sheet with the IIA domain. This same helix 3 is able to link the sheet to the IIC domain in the circularly permuted form of the IICBGlc subunit.

The successful fusion of a cytoplasmic domain to the amino terminus of the IICB Glc domain confirms the prediction from PhoA and LacZ fusion experiments that the amino terminus of IICB Glc is located on the cytoplasmic face of the membrane (20).
However, insertion of the IIC fusion protein with modified amino-terminal ends is less efficient than the insertion of fusion proteins with the native amino terminus. Insertion is also less efficient with increasing size of the attached domain, but this inhibitory effect partially can be compensated by increasing the length of the interdomain linker.

The interdomain linker was chosen from among the well characterized alanine-proline-rich sequences that form flexible hinges in multidomain proteins (37–39). They occur naturally in the E2 chain of the pyruvate dehydrogenase, in the proteolytic processing site of the endo-β-N-acetylglucosaminidase preprotein, and in the IiABMan subunit of the E. coli mannose transporter (summarized in Ref. 40). This type of linker has been successfully used to construct a multidomain protein from the four subunits of the glucose PTS (23). The length of the linker appears important for membrane insertion of the IIC domain.

When transformants expressing soluble glucokinase or IIC-glucokinase fusion proteins were plated on McConkey plates containing maltose (a non-PTS sugar), only cells expressing IIC-Glk formed strongly fermenting colonies, whereas cells expressing soluble glucokinase and GlkapIIC remained yellow (results not shown). Meyer et al. (26) report that high concentrations of soluble glucokinase interfered with the expression of the maltose system. It is likely that expression of soluble glucokinase from a multicopy plasmid resulted in a nonfermenting phenotype, whereas the approximately 100-fold reduced glucokinase activity of IIC-Glk was just right for the phosphorylation of intracellular glucose but did not yet repress the maltose fermentation under the chosen conditions.

The results of this study indicate that a domain swap as it could occur by a single DNA translocation event can immediately lead to a functional protein and that only minor structural adjustments, such as duplicating the fusion joint, might be necessary to compensate for a partial loss of activity due to a structural mismatch between the reoriented domains.

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