Sugar transporters from bacteria, parasites and mammals: structure–activity relationships

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Sugar transport across the plasma membrane is one of the most important transport processes. The cloning and expression of cDNAs from a superfamily of related sugar transporters that all adopt a 12-membrane-spanning-domain structure has opened new avenues of investigation, including pre-steady-state kinetic analysis. Structure–function analyses of mammalian and bacterial sugar transporters, and comparisons of these transporters with those of parasitic trypanosomatids, indicate that different environmental pressures have tailored the evolution of the various members of the sugar-transporter superfamily. Subtle distinctions in the function of these proteins can be related to particular amino acid residue substitutions.

MOST HETEROTROPHIC CELLS use sugars such as D-glucose, D-fructose and D-galactose as principal sources of carbon and energy, and must use specialized transporter proteins to transport these molecules across the plasma membrane. Mammalian glucose transport has received much attention because of the need to understand diseases associated with defective glucose homeostasis, such as diabetes mellitus. Five human sugar transporters have been cloned (GLUT1–GLUT5; see Table I)[1,2]. These isoforms transport sugars passively (i.e. transport is driven by the concentration gradient of the sugar) and are characterized by moderate-affinity D-glucose binding (K_m in the mM range) and a rapid rate of transport ($k_{cat} > 1\ ms^{-1}$).

Bacteria use the proton gradient generated across the cell membrane by respiration to accumulate sugars actively through proton symporters[3]. For example, the GalP, AraE and XylE proteins are responsible for the active accumulation of D-galactose/D-glucose, L-arabinose and D-xylose, respectively, in *Escherichia coli*. These transporters all bind sugars with relatively high affinity (K_m in the μM range) but transport sugars relatively slowly ($k_{cat} < 10\ s^{-1}$; see Table I) in the absence of a proton motive force (pmf).

Collectively, these transporters form one of 18 groups of a superfamily termed the major facilitator (MF) superfamily[4]; 133 members of the sugar-permease branch have been sequenced to date (see PROSITE PS00216). The identification of several sequence-related sugar transporters that differ in their modes of operation (e.g. passive versus secondary-active transport) but that have overlapping specificities for sugars and inhibitors [such as cytochalasin B (CB)] has allowed investigation of the molecular basis for their function, in the absence of a crystal structure, by comparative site-directed mutagenesis.

A group of parasitic protozoa (the kinetoplastida) that cause a variety of tropical diseases, including sleeping sickness, Leishmaniasis and Chagas disease, also have sugar transporters that belong to this superfamily[5,6]. These transporters are more distantly related to mammalian or bacterial transporters than the latter two groups are to one another[7] (Fig. 1); however, comparisons with other family members might have implications for the development of new anti-parasite chemotherapy. For example, *Trypanosoma brucei* causes sleeping sickness, which is prevalent in Africa. Tsetse flies transmit these parasites between mammalian hosts, where they live free in the bloodstream. The tsetse-fly midgut and mammalian plasma present two very different environments, in terms of sugar composition, and distinct parasite transporters have evolved to deal with these environments. In the mammalian bloodstream, the parasite has an absolute requirement for D-glucose, which it consumes at a rate exceeding even that of mammalian active muscle and takes up via the transporter THT1 (Table I)[8]. This transporter therefore represents a perfect target for chemotherapy – particularly because it is present at the parasite surface and, as such, does not require drugs to traverse any cell membranes. However, drugs must be specific for THT1; hence, the more we understand about transporter structure, the greater the likelihood that such a compound will be identified.

The two-dimensional structure of major-facilitator transporters – lessons from GLUT1

The availability of copious amounts of purified GLUT1 from human erythrocytes has allowed extensive biophysical manipulation of GLUT1, and we have obtained detailed structural information on this transporter[9]. The transporter contains 12 membrane-spanning helices, which are separated by hydrophilic loops (see Fig. 2); the N- and C-termini are intracellular[9,10]. The similarity in the primary sequences of the MF-family members suggests a conserved architecture.

Recently crystallization of membrane proteins has provided three-dimensional structural data for a range of proteins, including ion-channels[11] and light-harvesting complexes[12]. So far, however, no structural information on any transporter of a carbon substrate is available. Consequently, crystallization of members of the MF family is a major target of current research.

The kinetics of sugar binding: a comparison of GLUT1 and GalP

Two dominant schools of thought on the kinetic mechanism of sugar transport by GLUT1 exist. In both, the transporter has inward- (endofacial) and outward-facing (exofacial) sugar-binding sites. One model proposes that these sites are exposed simultaneously and that sugars are translocated between these sites[13,14]. The

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Table I. Comparison of the kinetic properties of a range of sugar transporters

Transporter	K_m	Turnover number[a] (min^{-1})	K_i CB	K_m (D-Gal)	K_m (D-Fruct)	Other substrates	Is transport asymmetric?	Proton coupled	Function
GLUT1	~20 mM[b]	10 000–27 000	0.1–0.2 μM	~17 mM	>5 M	Mannose	Yes	No	Constitutive glucose transporter
GLUT2	~42 mM[b]	High	>2 μM	>50 mM	66 mM	Mannose	No	No	Trans-epithelial movement
GLUT3	~10 mM[b]	51 000	~0.4 μM	8.5 mM	–	Mannose	?	No	Neuronal transporter
GLUT4	~2 mM[b]	~25 000	0.1–0.2 μM	>50 mM	–	Mannose	No	No	Insulin-responsive transporter
GLUT5	–	–	Not sensitive	–	6–14 mM	–	?	No	Fructose transporter
THT1	~0.6 mM[c]	–	~0.4 mM	>250 mM	~0.9 mM	Mannose	Yes	No	Constitutive bloodstream form
THT2	~0.05 mM[c]	–	~0.4 mM	~33 mM	~0.05 mM	Mannose	?	Maybe	Constitutive procyclic form
TcrHT1	~0.05 mM[c]	–	~0.4 mM	~61 mM	~0.06 mM	Mannose	?	No	Constitutive – all stages
TvHT1	~0.6 mM[c]	–	~0.4 mM	–	–	Mannose	?	No	Constitutive bloodstream (and possibly all) forms
GalP	0.034 mM[d] 0.236 mM[c]	–	1.1 μM	0.103 mM	–	Various	Yes, if coupled	Yes	

[a]The turnover number is the number of sugar molecules transported per minute. [b]3-*O*-methyl-D-glucose. [c]2-Deoxy-D-glucose. [d]D-Glucose. Abbreviations used: CB, cytochalasin B; D-Gal, D-Galactose; D-Fruct, D-fructose.

other, more generally accepted, model proposes that the transporter exists in one of two conformations, which expose either the endofacial or the exofacial binding site[9]; sugar translocation occurs as the transporter oscillates between these conformations (Fig. 3). In this model, the binding of sugars at the exofacial and endofacial sites is rapid, compared to the reorientation of the binding sites. Reorientation of the unloaded transporter is believed to be slower than that of the sugar-loaded transporter and is the rate-limiting step in the translocation cycle.

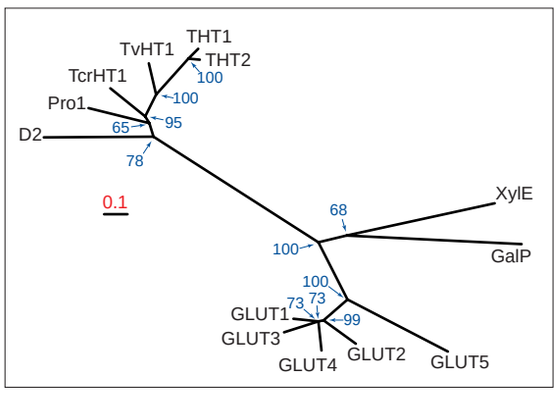

Figure 1

Evolutionary relationships of bacterial, mammalian and kinetoplastid transporters. We used PROTDIST with the maximum-likelihood estimate to generate a matrix; we used FITCH with an additive tree model to estimate phylogenies, and TREEVIEW to generate the image. The numbers shown are the bootstrap percentages calculated for each node. D2, *Leishmania donovani* transporter; Pro1, *Leishmania enrietti* transporter; TcrHT1, *Trypanosoma cruzi* transporter; THT1 and THT2, *Trypanosoma brucei* bloodstream form and procyclic forms, respectively; TvHT1, *Trypanosoma vivax* transporter.

Kinetic studies of GLUT1 are reviewed elsewhere[9,13,15]; hence, we provide only a brief synopsis here, focusing on transient-kinetics studies of ligand binding and sugar translocation because these provide a more rigorous mechanistic analysis than steady-state measurements.

Stopped-flow fluorescence spectroscopy can measure changes in tryptophan fluorescence as transporters switch between the exofacial (T_o) and endofacial (T_i) conformations (Fig. 3). Application of this technology to GLUT1 (Ref. 16) and GalP (Ref. 17) supports the alternating-conformation model. The binding of D-glucose to GLUT1 accelerates reorientation, and the rate constants for this process have been determined (Table II)[16,18]. In addition, measurement of the temperature dependency of transporter function allowed estimation of the distribution of GLUT1 between the T_o and T_i conformers (Table II)[15]. Similar approaches[17] allowed the rate constants for re-orientation, and the dissociation constant for the binding of sugar to the endofacial site of GalP, to be determined (Table II).

Comparison of the kinetic mechanisms of GLUT1 and GalP. Transient-kinetics studies have provided a detailed mechanism for the sugar-translocation cycle (Fig. 3). For GalP, the additional mechanistic steps pertaining to the binding of protons and changes in membrane potential have yet to be studied, and will not be considered here. Nonetheless, important insight into transporter function can be obtained from consideration of GalP function in the absence of a pmf. A comparison of the kinetic parameters for sugar binding and translocation by the GLUT1 and GalP transporters is presented in Table II. Kinetic analyses have shown that there is an asymmetrical distribution of conformational forms[15]. In the absence of glucose, most GLUT1 adopts the endofacial conformation whereas most GalP transporters adopt the exofacial form. Under non-energizing conditions, the rate constants for reorientation of the unloaded and sugar-loaded transporters are much lower for GalP than for GLUT1. The binding of sugars to GLUT1 catalyses reorientation by providing an increase of about an order of magnitude in the reorientation rate constants. By contrast, binding of sugar to GalP slightly hinders reorientation, in the absence of an accompanying membrane pmf, and the rate of reorientation of the D-glucose–GalP complex is over two orders of magnitude lower than that of the D-glucose–GLUT1 complex. The presence of a pmf increases the sugar-binding affinity of GalP by two orders of magnitude (K_m = 10 μM for D-glucose). Dissociation of the proton from GalP reduces affinity for the sugar (K_d = 1.4–1.5 mM for D-glucose); hence, D-glucose binds with high affinity at the exofacial site, translocates rapidly and then

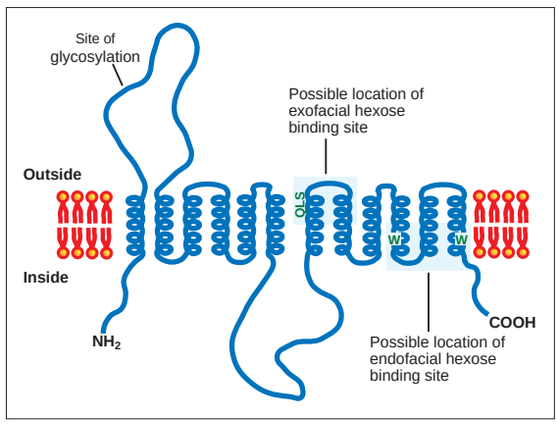

Figure 2

Structural model of major-facilitator transporters, showing the 12 membrane-spanning helices (I–XII; numbered from N- to C-terminus). The regions thought to make up the exofacial and endofacial substrate-binding sites, together with important amino acid residues discussed in the text – notably the tryptophan residues involved in CB binding – and the glutamine residues thought to be important for substrate selection, are shown.

dissociates from the endofacial site after the loss of the proton.

By contrast, GLUT transporters bind sugars with moderate affinity, which allows rapid sugar translocation[9,19]. This behaviour can be explained thermodynamically[20]. Binding of sugar decreases the free energy of the system – the tighter the sugar is bound the greater the decrease in free energy. Reorientation of the transporter–sugar complex requires a transition state in which neither binding site is exposed. The difference between the free energy of the initial transporter–sugar complex and that of the complex in the transition state defines the activation energy for reorientation, which in turn governs the rate constant for reorientation – the more tightly a sugar is bound, the greater the activation energy and the slower the reorientation.

Similar models, in which a transporter oscillates between inward- and outward-facing conformations, exist for a several secondary-active-transport systems[21]. The most extensively studied is the Na^+-dependent D-glucose cotransporter (SGLT1), for which electrophysiological measurements reveal further kinetic properties[22,23]. These studies indicate that the binding of the cation (Na^+, Li^+ or H^+) to the transporter induces a conformational change in the sugar-binding pocket and thereby increases affinity for the sugar. Once the sugar is bound, it is transported at a rate that is also determined by the cation[23]. These examples provide a clear illustration of how a common kinetic mechanism has been modified through evolution.

The binding of sugars to GLUT1, GalP and the bloodstream form trypanosome transporter, THT1

Several groups have investigated the sugar specificity of the GLUT1 and GalP proteins in detail, using pre-steady-state kinetics[9,15,24]. Such approaches have allowed an analysis of the substrate specificity of GalP and, more significantly, a means of examining the role of the individual hydroxyl groups of D-glucose in substrate recognition[17]. Sugar recognition and substrate specificity arise during the initial interaction of sugar with GalP rather than during translocation. No individual hydroxyl group is essential for transport, although the formation of a hydrogen bond between the ring oxygen and the protein during translocation is necessary. The C-3 hydroxyl group is of paramount importance in providing high-affinity binding to GalP (the K_d for 3-fluoro-deoxy-glucose binding is 1/124 of that for D-glucose binding[17]). Mutagenesis studies have identified particular amino acid side chains that are important in the binding and translocation phases of the transport process[17].

GLUT1 and GalP proteins have similar specificities and a similar relative importance of individual sugar hydroxyl groups. The fact that the transporters can bind the 1-deoxy-D-glucose analogue indicates that the ring structure is accepted by the sugar-binding site. Some important differences also exist, including the ability of GLUT1, but not GalP, to transport 5-thio-D-glucose; this suggests that a hydrogen bond forms between the sugar-ring oxygen and GalP – but not between the sugar-ring oxygen and GLUT1 – perhaps during translocation.

The glucose molecule has a specific orientation during transport by GLUT1 (Ref. 25): it enters the exofacial site C-1 first, and the C-4 and C-6 regions enter last; the molecule leaves the endofacial binding site in the same orientation. Other GLUT isoforms also bind glucose with similar polarity[26]. The distinct nature of the exofacial and endofacial binding sites has been established for both GLUT1 and GLUT4[9,27].

Sugar analogues in which there are changes in the groups attached to each carbon atom have also been used to determine which hydroxyl groups are involved in the liaison between the trypanosome transporters and their substrates. In the case of the *T. brucei* bloodstream-form transporter, THT1, the hydroxyls at the C-6 and C-2 positions do not appear to be important for binding[28]. Addition of relatively bulky groups at the C-2 position yielded compounds that are still recognized by the transporter. This led to speculation that addition of toxic moieties at the C-2 position might be a method for producing cytotoxic molecules that would be carried into the parasites through the glucose transporter[5]. However, although such compounds inhibit glucose transport, which implies that they interact with the exofacial binding site, compounds bearing groups significantly larger than the hydroxyl group are not transported (M. P. Barrett, unpublished). The size of the molecule that can be transported by the MF-superfamily members seems to be restricted, presumably by the dimensions of the translocation pathway.

Subtle alterations in amino acid residues radically alter transporter properties

Studies of the structure–activity relationships of the MF transporters have been aimed mainly at GLUT1 and GalP. Here, we briefly review some of the crucial mutagenesis studies of substrate and antibiotic binding. Figure 4 shows a partial sequence alignment of members of the transporter superfamily. Helices X and XI form part of the endofacial site and are involved in CB binding; helix VII forms part of the exofacial binding site and is involved in substrate selection. Other regions of the transporter also play a crucial role(s) in transport catalysis[27,29–32]. Some aspects of the functional behaviour of other members of the transporter superfamily can be predicted on the basis of sequence information within these regions.

Cytochalasin-B binding: the role of tryptophan residues in helices X and XI. The antibiotic fungal metabolite CB is a potent inhibitor of GLUT1 (Ref. 33) and GalP (Ref. 34), but its affinity for the trypanosome transporters is two orders of magnitude lower. CB competitively inhibits sugar efflux, rather than influx; this suggests that it competes for the endofacial binding site. The binding of CB quenches the fluorescence of GLUT1 and GalP[16,18,35,36]. Stopped-flow spectroscopy revealed that the affinity of the endofacial site for CB was less than the overall affinity measured by steady-state approaches. This is consistent with the proposal that there is an equilibrium between the T_o

and T_i conformers. An additional conformational form could exist (the T_i-CB to $T_{i/o}$-CB transition in Fig. 3), in which the transporter attempts to re-orientate but manages to do so only partially because of the occlusion of the (relatively) large antibiotic by the transporter. This occluded state gives rise to high-affinity binding.

UV light induces CB to bind covalently to GLUT1. A region between helices X and XII is involved, and D-glucose inhibits this binding. By contrast, sugar derivatives that bind to the exofacial binding site label in the region of helices VII to VIII; this demonstrates the functional separation of the binding sites[9,27]. Because CB binding quenches the tryptophan fluorescence of GLUT1, Oka and co-workers[37,38] and Mueckler and colleagues[39] studied the role of the two tryptophan residues in this region – Trp388 and Trp412 (which is preceded by an asparagine that is also implicated in binding; see Fig. 4) – replacing either or both tryptophan residues with a leucine residue. A Trp388→Leu mutant exhibited decreased affinity for CB, whereas the Trp412→Leu mutant exhibited decreased glucose transport but no alteration in CB affinity[38,39]. Mutation of Trp388 or Trp412 (to a leucine residue) decreased photolabelling by CB, but only when both tryptophan residues were replaced was photolabelling abolished[37]; this double mutant could still catalyse CB-sensitive sugar transport. These data argue that both tryptophan residues are close to the endofacial site, and imply that Trp388 is involved in antibiotic binding and Trp412 is involved in transport.

Mutation of either of the corresponding tryptophan residues of GalP (Trp371 and Trp395) does not prevent photolabelling by CB. In fact, the Trp371→Phe and Trp395→Phe mutations only cause modest changes (compared to the wild type) in the K_d values for CB. However, transient-kinetics studies reveal that the affinity of the endofacial site actually decrease by factors of 10 and 43, respectively[40]. The Trp371→Phe mutation reduced the affinity of the endofacial sugar-binding site for D-galactose by a factor of 15, but the Trp395→Phe mutation had no effect upon sugar binding. Such data suggest that both the Trp residues form part of the CB-binding site and that Trp371, but not Trp395, contributes to the sugar-binding site.

Of the mammalian GLUTs, GLUT5 alone is insensitive to CB (Table I)[41]. This correlates with the lack of the Asn-Trp motif in helix XI (the asparagine residue is absent) and with the lack of the tryptophan residue in helix X (Fig. 4). This illustrates that we can predict functional attributes of the protein from its primary sequence.

We can explain, in part, the lack of high-affinity binding of CB to the trypanosome transporters[42] by the replacement of the critical tryptophan residues by phenylalanine residues, along with the loss of the asparagine residue in helix XI (Fig. 4). Such structural distinctions between the endofacial sites of mammalian and trypanosome transporters allow specific binding of CB. Other inhibitors might bind specifically to the trypanosome proteins. A rational approach based on the structure of the transporters might become possible in the future – when we have obtained three-dimensional structures of the proteins and molecular models have been made widely available. However, as we move into a new era of drug design by combinatorial chemistry, discovery of molecules that kill trypanosomes by binding specifically to the glucose transporter might precede determination of these three-dimensional structures.

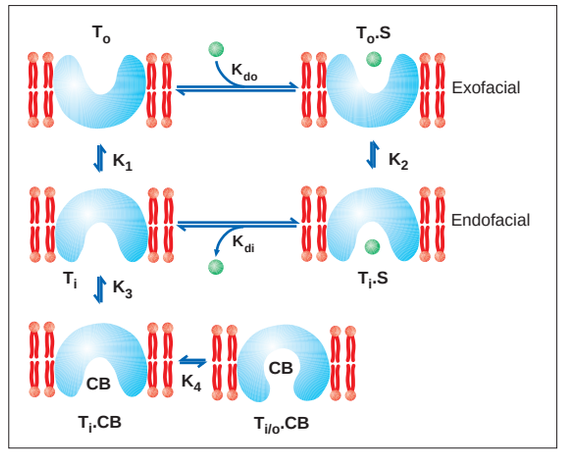

Figure 3

The sugar-translocation cycle. The sugar transporter (shown in blue) undergoes reorientation between exofacial [outward-facing (T_o)] and endofacial [inward-facing (T_i)] conformations in the presence and absence of sugar (shown in green). The antibiotic cytochalasin B (CB) binds to the endofacial binding site and induces a further conformational change, in which the transporter undergoes a partial reorientation ($T_i/_o$.CB). The rate constants defining each step are indicated (see Table II).

Glutamine residues in Helix VII – part of the exofacial binding site? Mutation of glutamine residues in GLUT1 has offered further insight into the mechanism of transporter action. A highly conserved QLSQQLS sequence (residues 279–285 in GLUT1) is present in GLUT1, GLUT3 and GLUT4, the glucose-specific mammalian transporters (Fig. 4). The Gln282→Leu substitution results in a loss of photolabelling by the exofacial-site-specific ligand ATB-BMPA and has little other effect on steady-state transport parameters, which provides evidence that helix VII constitutes part of the exofacial substrate-binding site[43].

GLUT1, GLUT3 and GLUT4 are relatively high-affinity glucose transporters, whereas GLUT2 has a much lower affinity, and GLUT5 does not transport D-glucose at all. The latter two species are, however, capable of transporting D-fructose[19]. Analysis of the regions of

Table II. A comparison of the kinetic parameters for the GLUT1 and GalP translocation cycles

Complex (constant)	GLUT1	GalP
T_i–CB (K_d)	1.63 μM	0.41 μM
T_i–T_o (k_1)	148 s^{-1}	≥ 10.2 s^{-1}
T_o–T_i (k_{-1})	600 s^{-1}	2.8 s^{-1}
T_i–T_o (K_1)	0.25	≥ 3.63
% T_o	20.0	≥ 78.4
T_o–Glu ($K_{d(o)}$)	7.1 mM	1.5 mM
T_i–Glu ($K_{d(i)}$)	13.4 mM	1.4 mM
Asymmetry $K_{d(o)}/K_{d(i)}$	0.53	1.07
T_i–Glu–T_o–Glu (k)	1273 s^{-1}	3.9 s^{-1}
T_o–Glu–T_i–Glu (k)	2887 s^{-1}	1.3 s^{-1}
T_i–Glu–T_o–Glu (K_{eq})	0.44	1.28
% T_o	30.5	56.0

Shown are estimated kinetic parameters for GLUT1 and GalP. Note that the transporters differ in their affinities for substrate, asymmetry and the relative distribution of conformers. The data for GalP were collected under non-energizing conditions [in the absence of a proton motive force (pmf)]. Clearly, the presence of a pmf will alter many of these rate constants. However, to date, the influence of pmf on pre-steady-state kinetics has not been addressed. A pmf increases the affinity for substrate; this is discussed in the text. Mammalian transporters are characterized by moderate affinity and rapid transport rates, whereas bacterial transporters typically exhibit higher affinity but slower translocation cycles.

Figure 4

Sequence alignment of functionally important domains in major-facilitator transporters. Helices VI–XI of five mammalian GLUTs, six trypanosomatid transporters and two bacterial transporters are shown. Regions discussed in the text are shown in red. The PileUp program was used to construct this alignment. D2, *Leishmania donovani* transporter; Pro1, *Leishmania enrietti* transporter; TcrHT1, *Trypanosoma cruzi* transporter; THT1 and THT2, *Trypanosoma brucei* bloodstream form and procyclic forms, respectively; TvHT1, *Trypanosoma vivax* transporter.

GLUT2 responsible for conferring the ability to transport D-fructose, using GLUT2/GLUT3 chimeras, demonstrated that information within helix VII of GLUT2 is crucial for D-fructose transport[44].

GLUT1, GLUT3 and GLUT4 all contain a QLS motif in helix VII (QLSQQLS in GLUT1, residues 279–281; Fig. 4); while GLUT2 and GLUT5 do not. Addition of the QLS motif from GLUT3 to GLUT2 revealed that the motif probably interacts with the C-1 position of D-glucose and acts as a molecular filter capable of discriminating between sugars at the exofacial site[45]. Gln279 might therefore define the interaction between the C-1 hydroxyl of D-glucose and a key component of the exofacial binding site. Given that D-fructose is a C-1 isomer of D-glucose, recognition by transporters that can carry both sugars presumably also involves other regions of the transporter. No D-fructose carrier contains this QLS motif (Fig. 4). The *T. brucei* transporters lack the QLS C-1 filter, and can also recognize and transport D-fructose[46]. In fact, THT1, transports D-fructose analogues with far higher affinity than its mammalian glucose–fructose-co-carrying counterpart GLUT2 ($K_m \approx 4$ mM – compared to $K_m \approx 66$ mM, for GLUT2). Fructose analogues that bind to and block the transporter could be a further means of generating anti-trypanosomal drugs.

Concluding remarks

The sugar-permease branch of the MF family encompass a series of transporters that have different substrate specificities and affinities, antibiotic-binding profiles and other functional attributes. This has allowed us to build up a picture of how these molecules recognize and transport substrate – despite the lack of X-ray-structural data. This picture might help us to design new molecules that can act against the plasma-membrane sugar permeases of parasitic trypanosomatids and thereby help us to develop new and urgently required drugs.

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Structural organization of MAP-kinase signaling modules by scaffold proteins in yeast and mammals

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MAP-kinase signaling pathways are activated by multiple extracellular stimuli. The specificity of activation and function of MAP-kinase signaling modules is determined, in part, by scaffold proteins that create multi-enzyme complexes. In *Saccharomyces cerevisiae*, two MAP-kinase-scaffold proteins have been identified. Recent studies of mammalian cells have also led to the identification of putative scaffold proteins. These scaffold proteins appear to facilitate MAP-kinase activation, in response to specific physiological stimuli, and to insulate the bound MAP-kinase module against activation by irrelevant stimuli. Scaffold proteins are therefore critical components of MAP-kinase modules and ensure signaling specificity.

MITOGEN-ACTIVATED PROTEIN (MAP) kinases phosphorylate Ser-Pro and Thr-Pro motifs in substrate proteins and are regulated by numerous extracellular stimuli, including growth factors, mitogens, cytokines and environmental stress[1]. MAP-kinase signaling pathways have been implicated in the regulation of the physiological responses of many organisms (e.g. plants, fungi, slime moulds, insects and mammals)[1–3]. In the budding yeast *Saccharomyces cerevisiae*, MAP-kinase signaling pathways control diverse cellular processes, such as sporulation, cell-wall integrity, invasive growth, pseudohyphal growth, osmoregulation and mating[2]. In *Drosophila melanogaster*, MAP-kinase signaling pathways are required for embryonic development and the immune response[3], whereas in mammalian cells these signaling pathways have been implicated in cell growth, oncogenic transformation, cell differentiation and apoptosis[1,3].

MAP-kinases are activated by dual phosphorylation on threonine and tyrosine residues within the T-loop adjacent to the catalytic cleft by a signaling module that includes a MAP-kinase kinase (MKK) and a MAP-kinase-kinase kinase (MKKK)[1]. Distinct signaling modules activate different MAP-kinase groups. However, these groups can be activated by overlapping sets of extracellular stimuli, and some components of these modules can participate in more than one signaling pathway. Therefore, mechanisms that allow cells to achieve signaling specificity and to respond correctly to changes in the extracellular environment must exist. Indeed, in *S. cerevisiae*, genetic analysis has demonstrated that the different MAP-kinase modules are functionally distinct, irrespective of whether they use the same protein kinases[4].

One important mechanism that regulates signal transduction pathways is the formation of complexes, either between different components of particular signaling pathways or between signaling molecules and anchor or scaffold proteins[5]. Anchor proteins localize their binding partners to specific subcellular compartments or to specific substrates, whereas scaffold proteins can bind several signaling molecules to create multi-enzyme complexes. These proteins include components of the I-κB-kinase complex, the receptor for activated C kinase (RACK) group of proteins, and the A-kinase-anchoring protein (AKAP) family[5]. Scaffold proteins have also been identified for two *S. cerevisiae* MAP-kinase pathways: the pheromone mating-response pathway; and the osmoregulatory pathway.

The scaffold protein Ste5p coordinates the yeast MAP-kinase module that regulates mating

The Ste5p scaffold binds components of the pheromone mating-response MAP-kinase module (Fig. 1). Distinct regions of Ste5p interact with the MKKK Ste11p, the MKK Ste7p, and the MAP-kinases Fus3p and Kss1p (Ref. 6). These protein kinases co-sediment with Ste5p

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