Functioning and Evolutionary Significance of Nutrient Transceptors

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The discovery of nutrient transceptors, transporter-like proteins with a receptor function, suggests that receptors for chemical signals may have been derived in evolution from nutrient transporters. Several examples are now available for nutrient transporters with an additional nutrient signaling function, nutrient receptors with a transporter-like sequence and structure but without transport capacity, and G protein–coupled receptors (GPCRs) that have nutrients as ligands. Recent results have revealed that transceptor signaling requires a specific ligand-induced conformational change, which indicates that transceptors function in a similar way as regular receptors. Advanced bioinformatic analysis for detection of homology in distantly related proteins identifies the nontransporting glucose transceptor Rgt2 as the closest homologue of the glucose-sensing GPCR Gprl in yeast. This supports an intermediate position for nutrient transceptors in evolution, between nutrient transporters and classical receptors for chemical signals.

Introduction

Nutrient transporters and receptors for chemical signals are two major types of plasma membrane proteins, which have shown until recently very little overlap. The discovery of transporter-related receptors, now called transceptors, has raised interesting questions with respect to their functioning and their significance for the evolutionary origin of receptors.

Different Types of Transceptors

Transporting Transceptors

A nutrient sensor protein with residual transport activity has been discovered in Escherichia coli. One copy of a duplicated gene encodes a glucose-6-phosphate (Glc6P) transporter, UhpT, whereas the other copy encodes a sensor for external Glc6P, UhpC (Schwöppe et al. 2003). UhpC displays low residual Glc6P transport activity and triggers induction of UhpT expression.

In yeast, several transceptors with normal transport activity were discovered as playing a role in the rapid adaptation of fermenting yeast cells to the presence of essential nutrients like nitrogen and phosphate in the medium (Hirimburegama et al. 1992; Holsbeeks et al. 2004). These transceptors were previously considered to be regular nutrient transporters induced during starvation for their nutrient. Readaptation of such a missing essential nutrient stimulates fermentation and growth, which is triggered by transceptor-mediated stimulation of the protein kinase A (PKA) pathway (Holsbeeks et al. 2004), Gap1, the “general amino acid permease” of yeast, transports all common amino acids and also many o-amino acids and nonmetabolizable amino acid analogs. Nearly all these substrates trigger rapid activation of the PKA pathway (Donaton et al. 2003). Activation by ammonium in nitrogen-starved cells is mediated by Mep2 (and Mep1), a channel-type transporter (Van Nuland et al. 2006). In phosphate-starved fermenting cells, the Pho84 phosphate carrier is strongly induced and readaptation of phosphate triggers rapid Pho84-mediated activation of the PKA pathway (Giots et al. 2003). In these cases, nutrients exert a hormone-like effect similar to the hormone-like stimulation of cAMP synthesis by glucose in glucose-deprived yeast cells which is triggered by the glucose-sensing G protein–coupled receptor (GPCR) Gprl (Kraakman et al. 1999). However, rapid activation of the PKA pathway by the transceptors is not mediated by cAMP as second messenger (Hirimburegama et al. 1992; Durme et al. 1994; Giots et al. 2003).

Evidence for transporters functioning as transceptors has also been obtained in other systems. The yeast Mep2 ammonium transporter and homologues in other fungi, for example, Ustilago maydis and Candida albicans, have been implicated in control of filamentation (Pan et al. 2000; Smith et al. 2003; Biswas and Morschhäuser 2005). Also in animal and plant cells, evidence has been found for the existence of transceptors (Holsbeeks et al. 2004; Hundal and Taylor 2009; Taylor 2009). In Drosophila, two amino acid transporters were found to affect growth independent of their amino acid transport function (Goberdhan et al. 2005). The mammalian EAAT1 glutamate transporter stimulates extracellular signal-regulated kinases signaling in astrocyte cultures in response to its substrate as well as to transported, unmetabolizable analogs (Abe and Saito 2001). A transceptor function has also been suggested for the SNAP2 amino acid transporter in mammalian cells (Hyde et al. 2007). In Arabidopsis, a nitrate transporter was suggested to have a direct nitrate-sensing role in a nitrate signaling pathway affecting root architecture (Walch-Liu and Forde 2008).

Nontransporting Transceptors

The discovery of proteins with strong sequence similarity to nutrient transporters but without detectable transport activity suggested that these might function as nutrient sensors. In yeast, three such proteins have been discovered, which are all three involved in nutrient regulation of the expression of their homologous regular transporters. Snf3 and Rgt2 are high-affinity and low-affinity glucose transceptors, respectively (Özcan et al. 1998), and Ssy1...
is an amino acid transceptor (Didion et al. 1998; Iraqui et al. 1999; Klasson et al. 1999). They all lack transport activity and trigger the induction of regular glucose and amino acid transporters, respectively (Forsberg and Ljungdahl 2001). Clear homologues of these proteins have been identified in several other fungi (Madi et al. 1997; Betina et al. 2001; Brega et al. 2004; Stasyl et al. 2004; Brown et al. 2006; Stasyl et al. 2008) but not in higher organisms. On the other hand, the mammalian SGLT3 glucose carrier–like protein is apparently also unable to transport glucose and has been suggested to function as a glucose sensor instead (Diez-Sampedro et al. 2003).

The Functioning of Transceptors

A major issue concerning the functioning of transceptors is the relationship between transport and signaling. For the nontransporting transceptors, a major question is whether their nutrient-sensing mechanism bears any relationship to the transport mechanism of their evolutionary ancestors. Do these sensors use the same substrate-binding site as the homologous transporters, does the substrate move through the same passageway and do the conformational changes associated with this movement play a role in the signaling mechanism? Alternatively, the sensor may have kept only the over-all structure of the transporter and developed a new substrate-binding site and signaling mechanism independent of the previous transport mechanism.

Initially, a major issue for the nontransporting transceptors was whether the nutrient truly bound directly to the transceptor and not to another protein. The main argument for direct binding was the strong sequence similarity with regular transporters carrying the same nutrient. Subsequently, mutations were isolated in Ssy1, which made the sensor hyper- or hyporesponsive to amino acids, strongly suggesting direct binding of the amino acid to the protein (Gaber et al. 2003; Poulsen et al. 2008). However, whether the nutrients bind to the sensor in a similar way, for example, in an equivalent binding site, as in the homologous regular transporters still remain unclear.

In case of the _E. coli_ UhpT transporter and UhpC transceptor for Glic6P, the similarity between the duplicated genes suggests that the sensor functions in a very similar way as the transporter and that therefore (part of) the conformational changes occurring during transport are in some way used for signaling. The residual transport activity of UhpC allowed to explore the relationship between transport and signaling experimentally (Schwöppe et al. 2003). Specific site-directed mutagenesis of UhpC abolished residual transport without affecting signaling. This indicates that complete transport of the substrate is not required for signaling but does not exclude that part of the initial conformational changes occurring during transport are responsible for triggering signaling. Specific mutations were also found that had no effect on transport but abolished signaling. Because the transceptor has to interact with a downstream signal transducer, it seems plausible that certain amino acid residues are important for this interaction and not important for transport. The authors also found several mutations that caused constitutive signaling and did not affect transport and concluded that transport could still function normally when UhpC is locked in the signaling conformation (Schwöppe et al. 2003). This would appear to contradict that part of the conformational changes occurring during transport are responsible for signaling to a downstream effector. However, it cannot be excluded that the mutations caused a signaling domain on the surface of the protein to adopt a conformation that is normally triggered by conformational changes occurring in the transport cycle of the substrate.

Transporters exist in an outward-facing conformation with their substrate-binding site oriented toward the extracellular environment and an inward-facing conformation with the substrate-binding site oriented toward the inside of the cell (fig. 1). (This is not the case for the channel-type Mep2 ammonium carrier.) When a substrate binds to the outward-facing binding site, the transporter undergoes a series of conformational changes creating a passageway for the substrate to move through the carrier until the inward-facing conformation is obtained and the substrate can be released. Recent crystallographic studies have provided evidence that the transporter temporarily acquires a so-called occluded conformation, in which the substrate is shielded from both the extracellular and intracellular environment (Yamashita et al. 2005; Holyoake and Sansom 2007; Faham et al. 2008; Shi et al. 2008).

The group of Morten Kielland-Brandt proposed a model for the sensing mechanism of Ssy1, the nontransporting yeast amino acid transceptor, based on the assumption that the unloaded Ssy1 protein also switches between an outward- and inward-facing conformation like regular transporters (Wu et al. 2006). Because Ssy1 has lost transport capacity, it cannot switch to the inward-facing conformation in the loaded state. They suggested that the outward-facing conformation was the signaling conformation, whereas the inward-facing conformation was not signaling. As a result, binding of the nutrient would stabilize the outward-facing conformation and stimulate signaling. They conceived an experimental test of this model based on the assumption that stabilization of the nonsignaling inward-facing conformation, by artificially increasing the intracellular amino acid concentration, should reduce the availability of the signaling outward-facing conformation and thus reduce the affinity of Ssy1 for signaling by external amino acids. Enhancement of the intracellular level of leucine indeed resulted in a drop in the affinity of signaling with extracellular amino acids, which was taken as support for the proposed model. However, the model has other implications that are more difficult to rationalize. If it were correct, it would be impossible to find competitive inhibitors of signaling because they would also stabilize the outward-facing conformation and thus trigger signaling. Actually, any molecule that binds to the outward-facing conformation, including noncompetitive inhibitors, would stabilize it and trigger signaling. This could easily compromise the specificity of Ssy1 as an amino acid sensor.

Transporting transceptors like Gap1 offer more possibilities to investigate transceptor functioning because they still display transport. We have screened for competitive inhibitors of the transport function of Gap1 because such compounds should bind to the amino acid–binding site
of Gap1 similarly to regular substrates (Van Zeebroeck et al. 2009). If the outward-facing conformation would be the signaling conformation, all competitive inhibitors of Gap1 should stabilize it and thus trigger signaling.

We actually found both competitive and noncompetitive inhibitors of transport with and without signaling capacity. Moreover, some of the signaling agonists were not transported by Gap1. The discovery of competitive inhibitors without signaling capacity and of signaling agonists that are not transported indicates that the ligand molecule has to induce a specific conformational change in the transceptor, which may be part of the transport cycle but clearly does not need the complete transport cycle (fig. 1). Hence, Gap1 appears to function like a regular receptor for its signaling mechanism. We also identified by substituted cysteine accessibility method (SCAM) analysis two residues exposed with their side chain into the amino acid–binding site of Gap1. Blockage of the binding site in the two cysteine substitution alleles with a sulfhydryl-reactive compound inactivated both transport and signaling, indicating that the transceptor uses the same amino acid–binding site for transport and signaling (Van Zeebroeck et al. 2009). Also for the phosphate transceptor Pho84, we have identified a nonsignaling competitive inhibitor as well as nontransported signaling agonists. SCAM analysis of Pho84 also indicates that the same binding site is used for transport and signaling. Hence, the phosphate transceptor Pho84 seems to work in a very similar way as the amino acid transceptor Gap1 (Popova Y, Thayumanavan P, Lonati E, Agrochão AM, Thevelein JM, in preparation).

It appears plausible that the nontransporting transceptors Snf3, Rgt2, and Ssy1 use a similar mechanism as the transporting transceptor Gap1 (fig. 1). Screening for competitive inhibitors of their signaling function would reveal whether such compounds can be identified and thus whether also in this case binding to the transceptor is not enough to trigger signaling. Constitutively activating alleles have been obtained for Snf3, Rgt2, and Ssy1 (Ozcan et al. 1996; Gaber et al. 2003). They may lock the transceptor in the special signaling conformation. For these nontransporting transceptors, it is clear that the complete transport cycle is not required for signaling. However, it needs to be clarified whether there remains any relationship between the action mechanism of these transceptors and the transport mechanism of their homologous transporters. For instance, whether the outward-facing substrate-binding site in the transporters has been conserved in the homologous transceptors.

The transport function of Gap1 has helped to gain insight into the mechanism of its receptor function. Maybe the signaling function of transceptors may also help to understand how transport functions. It has been notoriously difficult to elucidate the precise conformational changes happening during the transport of substrates through transporters. The signaling function in transceptors provides a new readout for the conformational changes happening during the transport. The intensity of Gap1 signaling for instance clearly differs with different amino acid substrates, possibly indicating differences in conformational changes of Gap1 induced by these amino acids. This may be due to differences in their contact points with the amino acid residues lining the transport passageway through Gap1. Certain conformations may be more potent than others for induction of signaling and/or for conveying the signal to downstream components.

**Fig. 1.—**Conformational changes in transporters, transporting and nontransporting transceptors. Like transporters, transceptors are assumed to switch between an outward- and an inward-facing conformation. Binding of a nutrient substrate to the transceptor induces a conformational change that triggers activation of a signaling pathway. The signaling conformation is possibly one of the initial structural intermediates that accompany substrate transport in transporters.
As opposed to regular transporters, the Mep ammonium carriers are channel-type proteins that are not considered to undergo major conformational changes during transport (Khademi et al. 2004). However, it cannot be excluded that also channel-type transporters can undergo local conformational changes that could trigger a signal transduction pathway. Recent work on the *S. cerevisiae* and *C. albicans* Mep2 transceptors has identified mutant alleles in which transport and signaling for filament formation were uncoupled (Rutherford et al. 2008; Dabas et al. 2009). Mutational analysis of *S. cerevisiae* Mep2 suggests that binding of ammonium to specific residues in Mep2 during its translocation over the plasma membrane results in a conformational change that triggers signaling. For *C. albicans* Mep2, mutant alleles were identified that could still induce filamentous growth but had reduced transport capacity, indicating that signaling does not depend on (complete) transport of the ammonium substrate.

**Transceptor Downregulation**

Transceptors that are expressed in the plasma membrane during starvation for a specific nutrient are rapidly downregulated when the nutrient is added again to the cells. Addition of amino acids to Gap1-expressing cells triggers its ubiquitination, endocytic internalization, and degradation in the vacuole (Roberg et al. 1997; Helljwell et al. 2001; Soetens et al. 2001). A similar process appears to be responsible for phosphate-induced internalization and degradation of Pho84 (Petersson et al. 1999; Lau et al. 2000; Lundh et al. 2009). The nutrient-induced downregulation process of transceptors is reminiscent of ligand-induced internalization and breakdown of classical receptors (Sorkin and Von Zastrow 2002). Moreover, in the case of Gap1, it seems important for the cell that the protein does not remain in the plasma membrane in the presence of external amino acids because newly synthesized Gap1 within Golgi-derived vesicles is directly routed to the vacuole when amino acids are present in the medium (Rubio-Texeira and Kaiser 2006).

The sophisticated controls operating on Gap1 trafficking have been studied in great detail, but until recently, they were only interpreted in terms of Gap1 functioning as an amino acid transporter (Risinger et al. 2006). The discovery of the transceptor function of Gap1 suggests that just like regular receptors, Gap1 is downregulated to avoid overstimulation of the PKA pathway. The latter is well known to cause loss of viability under conditions of poor growth and starvation (Thevelein and de Winde 1999). Cells starved for multiple essential nutrients, like nitrogen and phosphate, and replenished only with amino acids would undergo Gap1-mediated stimulation of the PKA pathway while being unable to initiate growth because of the absence of the other essential nutrients. Hence, the presence of Gap1 would be toxic under such conditions. Moreover, the different nutrient transceptors, which are induced under different starvation conditions, may use the same downstream signaling pathway. This would be akin to distinct receptors expressed in different mammalian cell types that make use of the same downstream signaling pathway. Hence in this case, it would be essential to remove the transceptor from the plasma membrane once its nutrient substrate appears in the medium. Otherwise, it would continue stimulating the signaling pathway and prevent proper functioning of the other nutrient transceptors when the cells are deprived of their substrate.

**Physiological Relevance**

Rapid transceptor signaling clearly serves to stimulate the initiation of growth and fermentation (Donaton et al. 2003; Giots et al. 2003; Van Nuland et al. 2006). This appears contradictory with the observation that also unmetabolizable nitrogen compounds, like D-amino acids, can stimulate rapid Gap1 signaling. However, in nature, microorganisms are unlikely to encounter pure unmetabolizable nitrogen compounds. When a microorganism experiences a new nutrient condition, in all likelihood, it will encounter a mixture of nutrients. For a microorganism, rapid response to changes in the nutrient condition is essential for survival given the tremendous competition for food in the microbial world. Hence, it makes sense that nitrogen-starved cells react immediately to the presence of any nitrogen source with stimulation of the growth machinery. Whatever the type of nitrogen compound detected, the probability that there is also metabolizable nitrogen present is very high. The same is true for other essential nutrients. Hence, yeast cells use Gap1 and other transceptors to rapidly stimulate fermentation and growth irrespective of the nutritive value of the compound detected. When it turns out that no metabolizable nitrogen sources are present, the PKA pathway and the growth machinery will simply be downregulated again because their continued activation requires active metabolism (Thevelein and de Winde 1999).

**Evolution of Receptors from Transporters**

It seems plausible that in evolution nutrients were used by cells prior to signaling molecules and thus that transporters existed before receptors. Transporters have evolved the capacity to recognize extracellular molecules and to respond with a conformational change (allowing the passage of the molecule into the cell). Receptors in part do the same: they detect an extracellular molecule and change their configuration in response. In evolution, receptors may have arisen from nutrient transporters that gained a receptor function and then gradually lost their transport capacity (fig. 2). This idea is now receiving support with the discovery of intermediate forms between pure transporters and pure receptors: proteins like Gap1 and Pho84 which combine a transport and receptor function, proteins like UhpC with a receptor function and residual transport activity, and transporter-related proteins like Snf3 and Ssy1 with a receptor function and no transport at all. These different types of transporter-related receptors strongly suggest that transporters gained at some point in evolution a receptor function to signal the presence of nutrients in the environment. Some of these transceptors then (gradually) lost their transport function to become pure sensors and afterward may have
evolved into receptors for diverse chemical signaling molecules (fig. 2). A similar model has previously been proposed for the evolutionary origin of neurotransmitter receptors, based on the observation that many established neurotransmitter molecules are minimally modified nutrient molecules taken up in many systems by ion-coupled transport systems (Boyd 1979).

For the nontransporting transceptors Rgt2/Snf3 and Ssy1, it has been shown that they interact with downstream signaling components via their long C-terminal and N-terminal tails, respectively (Özcan et al. 1998; Liu et al. 2008). Although evidence has been obtained implicating the C-terminal tail of Gap1 in signaling to PKA (Donaton et al. 2003), no specific signaling domains or directly interacting downstream components have yet been identified for the transporting transceptors Gap1 and Pho84. Because both transceptors signal to the PKA pathway, they may use a similar or even the same downstream signaling mechanism. The latter would explain why the proteins are rapidly removed from the plasma membrane once they sense their substrate ligand in the medium, so as not to interfere with the sensing by the other transceptors. Identification of the downstream effector components of Gap1 and Pho84 may shed further light on their proposed function as evolutionary precursors of classical receptors (Donaton et al. 1998). Possibly, this unusual insert played a role in the cytosolic extrusion of transmembrane domains 6–10 in the glucose-/sucrose-sensing transceptor that may have served as evolutionary ancestor for Gpr1.

In general, GPCRs display little sequence conservation with other members of this protein family. Gpr1 itself was classified in a distantly related subfamily (Graul and Sadee 2001). Hence, it appeared rather futile to search with regular sequence alignment programmes for conserved sequence similarity between Gpr1 and glucose transporters to gain further evidence for the proposed evolutionary scheme. However, recent much more sensitive bioinformatics programmes allow to identify distant homologous relationships between proteins based on comparisons of sequence profiles of protein families rather than individual sequences and also taking predicted structural information into account. Structures diverge much more slowly in evolution than sequences, the number of protein structures is much more limited, and proteins may remain structurally very similar long after their sequence similarity has disappeared (Kinch and Grishin 2002).

The online bioinformatics programme HHpred (http://toolkit.tuebingen.mpg.de/hhpred) uses pairwise comparison of profile Hidden Markov Models (HMMs) to infer distant homologous relationships between proteins (Söding 2005; Söding et al. 2005). Profile HMMs are similar to sequence profiles but next to amino acid frequencies, they also take into account the likelihood of amino acid insertions and deletions at a specific position along the alignment. Moreover, it is also possible to include the

**Fig. 2.** Evolution of receptors from nutrient transporters via intermediate transceptor stages. Some nutrient transporters have apparently gained in evolution an additional function in signaling to the cellular machinery the presence of the nutrient. The signaling domain could be located in an extended terminus or elsewhere in the protein. These transporters became transceptors. Subsequently, loss of the transport function created nontransporting transceptors (nutrient sensors). They may have evolved into nutrient receptors by deficient insertion of part of the transmembrane domains (creating for instance an unusually large third intracellular loop). The nutrient receptors subsequently diversified into the vast array of currently known chemical signal receptors.
(predicted) secondary protein structure as an extra parameter in the search and to make local or global alignments. This makes HHpred a suitable tool to identify possible evolutionary links between distantly related proteins.

We have applied the HHpred programme to search for proteins related to the low-affinity glucose-sensing GPCR Gpr1 in the \textit{S. cerevisiae} genome. Remarkably, using the local alignment programme, the low-affinity glucose-sensing nontransporting transceptor Rgt2 was the most closely related protein, whereas the mating pheromone GPCR Ste3 and the high-affinity glucose-sensing nontransporting transceptor Snf3 were second and fourth, respectively. The \( P \) values ranged from \( 4.7 \times 10^{-08} \) for Rgt2 to \( 3.6 \times 10^{-06} \) for Snf3, and the calculated probability of homology is 97.3\% for Rgt2 and 92.8\% for Snf3. This indicates a very high probability of a common evolutionary origin. Most of the other closely related proteins were nutrient transporters, including Gap1. These results substantiate the hypothesis that nutrient-sensing GPCRs have evolved from nontransporting nutrient transceptors and thus provide further support for the proposed evolutionary scheme from nutrient transporters to receptors for chemical signals (fig. 2).

**Perspectives**

The discovery of nutrient transceptors has created a new layer of cellular regulation, in between the classical regulation of metabolism with its many allosteric interactions and the regulation of metabolism by signaling cascades emanating from extracellular chemical and physical signals. Future research will reveal how extensive nutrient-induced signaling impacts on cell metabolism and on other processes like cellular growth, which is and must be intimately connected to nutrient availability. Three-dimensional structure determination and further application of programmes for remote homology detection between nutrient transporters, nutrient transceptors, and classical (G protein-coupled) receptors may provide further support for a common evolutionary origin of these major groups of integral membrane proteins. Particularly, appealing is the possibility that nutrient transceptors may provide novel insight into the conformational changes occurring during nutrient transport because of the new readout provided by the signaling function. Careful comparison of transport rate, signaling capacity, and the structure of the transported nutrient in transporting transceptors, with possibly also the discovery of transported substrates without agonist function, may provide new information on the passageway, that is, the precise physical path, followed by different types of nutrients through transporters.

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