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REVIEW ARTICLE

How are signals transduced across the cytoplasmic membrane? Transport proteins as transmitter of information

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Abstract In order to adapt to ever changing environmental conditions, bacteria sense environmental stimuli, and convert them into signals that are transduced intracellularly. Several mechanisms have evolved by which receptors transmit signals across the cytoplasmic membrane. Stimulus perception may trigger receptor dimerization and/or conformational changes. Another mechanism involves the proteolytic procession of a receptor whereby a diffusible cytoplasmic protein is generated. Finally, there is increasing evidence that transport proteins play an important role in transducing signals across the membrane. Transport proteins either directly translocate signaling molecules into the cytoplasm, or transmit information via conformational changes to their interacting partners such as membrane-integrated or soluble components of signal transduction cascades. Employing transport proteins as sensors and regulators of signal transduction represents a sophisticated way of interconnecting metabolic flux and transcriptional regulation in cells.

Keywords Trigger enzymes · ToxR · Histidine kinase · Secondary transporter · ABC transporter

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Introduction

Bacteria are able to cope with and to regulate their metabolism in response to a wide range of environmental growth conditions. As prerequisite bacteria have to sense environmental stimuli and transduce this information across the cytoplasmic membrane into the cytoplasm. In most cases, these processes lead to alterations in gene expression triggered by modulation of transcriptional regulator proteins. Bacterial signal transduction is usually mediated by two components: a membrane-integrated sensor protein that perceives a stimulus and converts it into an internal signal, and a response regulator that receives this signal (Mascher et al. 2006; Szurmant et al. 2007). This response regulator interacts with regulatory DNA sequences to activate or repress gene expression. It is well conceivable that sensory proteins contain extracytoplasmic or periplasmic domains which directly sense external stimuli, but it is less obvious how the corresponding information is transmitted to the cytoplasmic components. Here we will describe general mechanisms how integral membrane sensors are able to transduce information across the membrane to the subsequent signaling components. We will especially focus on the recently discovered function of transport proteins as transmitter of information.

Signal transduction across the membrane

Upon stimulus perception sensory proteins undergo structural changes to be detected by their cytoplasmic partners. These changes can either be of reversible nature, allowing the sensor to cycle between an inactive and an active state, or of irreversible nature (Fig. 1). Reversible modulations might be alterations in the conformation or the oligomeric

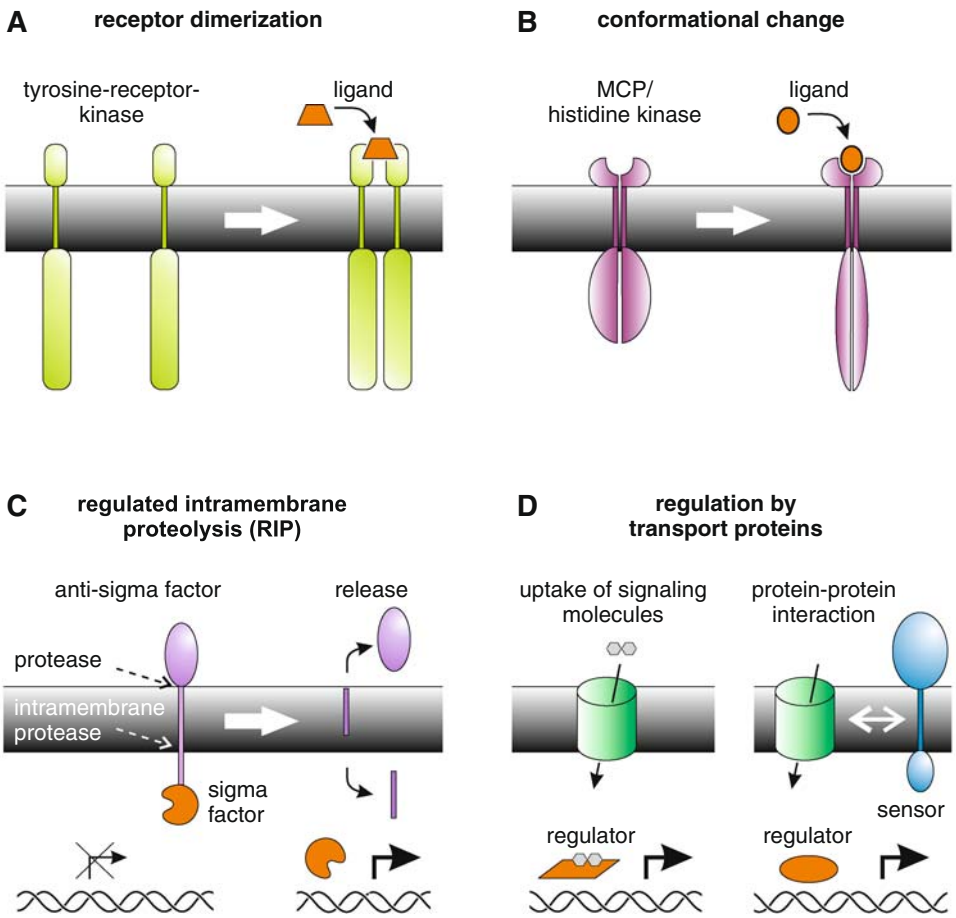

Fig. 1 Signal transduction across the cytoplasmic membrane. After perception of an external stimulus, signals are transmitted across the cytoplasmic membrane in different ways. **a** Eukaryotic tyrosine receptor kinases dimerize upon stimulus perception, and this dimerization leads to autophosphorylation. **b** Histidine kinases or the methylaccepting proteins (MCP) transduce information across the membrane by conformational changes. **c** An external stimulus activates regulated intramembrane proteolysis, and a soluble domain is released. **d** Transport proteins act as transmitter of information in two ways: they either take up signaling molecules necessary for induction of gene expression in the cytoplasm or they co-sense signaling molecules in an interplay with membrane-integrated sensors

state. Receptor dimerization is common in eukaryotic cells while this mechanism of regulation is rarely found in prokaryotes (Khorchid and Ikura 2006). Tyrosine receptor kinases that play an important role in eukaryotic signal transduction are inactive in their monomeric form and dimerize upon binding of ligands with the formation of either homo- or heterodimers (Fig. 1a) (Cunningham et al. 1991; Hurwitz et al. 1991). The transcriptional regulator TraT of *Agrobacterium tumefaciens* uses a similar mechanism as it is inactive as a monomer and active as a dimer (Qin et al. 2000). In this case dimerization is induced by binding of an acyl-homoserine lactone as signaling molecule. Subsequently, the previously membrane-associated protein is released into the cytoplasm. Certainly, transitions of the oligomeric state require conformational changes upon ligand binding of the respective receptor proteins, too. Membrane-integrated receptors such as the histidine kinases of two-component systems undergo conformational changes upon perceiving a stimulus, but remain in a dimeric state regardless of the activation state of the protein (Fig. 1b) (Khorchid and Ikura 2006; Heermann et al. 1998). Similarly, the methylaccepting chemotactic receptors, such as Tar, undergo conformational changes upon chemotactic stimuli which then trigger the formation of a ternary complex between receptor, the histidine kinase CheA and the adapter protein CheW (Fig. 1b) (Manson et al. 1985). It is still discussed how information is transduced across the membrane by these proteins but helix sliding, tilting, rotation or altered helix dynamic are conceivable possibilities (Bass and Falke 1999; Hulko et al. 2006; Kwon et al. 2003). Both, receptor dimerization of eukaryotic tyrosine kinases and conformational changes of bacterial histidine kinases lead to autophosphorylation of the respective sensors. Phosphorylation is an elegant way to transmit the signal to a soluble cytoplasmic protein via phosphotransfer.

Another way to transmit signals across the cytoplasmic membrane is an irreversible process called regulated intramembrane proteolysis (RIP) which involves the proteolytic degradation of membrane-integrated proteins (Ehrmann and Clausen 2004). RIP, which occurs in response to a specific regulatory signal, results in the liberation of a functional domain, and allows its distribution in the cytoplasm (Fig. 1c). RIP proteases are found in bacteria, archaea and eukarya. Their substrates are transmembrane proteins, often with only one transmembrane domain, that are in an inactive state prior to cleavage. In eukarya RIP proteases play a role in various signaling

events as their substrates include human amyloid precursor protein, or Notch (Käsbauer et al. 2007; Steiner et al. 2008). A well examined bacterial example of a RIP-regulated protein is the anti-sigma factor RseA in *Escherichia coli* that keeps the alternative sigma factor RpoE in an inactive state under normal growth conditions (Akiyama et al. 2004). Upon extracytoplasmic stress measured by the amount of abnormal envelope proteins, the periplasmic protease DegS cleaves within the periplasmic domain of RseA. Release of the periplasmic domain leads to conformational changes of the anti-sigma factor in a way that a second cleavage site within the transmembrane domain becomes accessible to the intramembrane protease RseP. Upon cleavage by RseP, SigmaE is released and thus freely diffusible. It should be noted that RseA is not a sensor by itself but relies on the protease DegS as sensor for extracytoplasmic stress (Hasselblatt et al. 2007). A similar system is found in *Bacillus subtilis* where the anti-sigma factor RsiW is degraded in a RIP-like manner following alkaline stress (Heinrich and Wiegert 2006). In *Vibrio cholerae* the protease YaeL, a homolog to RseP, is involved in the regulation of the ToxR-dependent signal transduction cascade (Matson and DiRita 2005). YaeL in combination with a second protease rapidly degrades TcpP, a membrane protein with one transmembrane domain, unless it is stabilized by interaction with the membrane-integrated TcpH. The TcpP/TcpH pair interacts with the ToxR/ToxS pair supporting ToxR-dependent transcriptional activation by a mechanism that is not completely understood to date.

Recently, transport proteins were recognized as transmitter of information across the membrane. These proteins are perfect information carriers as they sense signaling molecules in the external medium, transport them into the cytoplasm and provide dynamic information about the metabolic flux. Therefore, it is advantageous for the cells to employ these proteins in signal transduction (Fig. 1d). In this review we will focus on the role of transport proteins as trigger for transcriptional regulation. For stress sensing transport proteins, like MscL, ProP, BetP or OpuA, we refer the reader to other reviews (Heermann and Jung 2004; Morbach and Kramer 2005; Poolman et al. 2004; Wood 2006).

The influence of transport proteins on signal transduction

The most and obvious function of transport proteins is the translocation of molecules that cannot cross the cytoplasmic membrane by diffusion. Although transport can be accomplished in both directions—substrates are taken up from the extracellular medium or metabolic products are excreted—in the context of signal transduction proteins that facilitate passage of substrate from the outside to the inside are of particular interest. These proteins have two features predestining them for signal transduction. First, they transport molecules that bind as inducer or repressor to cytoplasmic transcriptional regulators in the cytoplasm. Secondly, these proteins are able to sense the extracellular concentration of the transported molecules. A good example for the transport of inducer molecules is the *lac* operon in *E. coli* (Lewis 2005). The operon encodes three proteins, the lactose permease (*lacY*), the β-galactoside-transacetylase (*lacA*) and the β-galactosidase (*lacZ*). LacI, a repressor encoded by a gene upstream of the operon, binds to the *lac* operator thereby inhibiting transcription of the *lac* operon. In the absence of glucose and concurrent presence of lactose, transcription of *lacZYA* is activated by allolactose. Lactose is taken up by the lactose permease LacY (Kaback 2005). In the cytoplasm lactose is not only cleaved into two monosaccharides, but is also converted into allolactose by β-galactosidase. Subsequently, allolactose binds to LacI, which abolishes its DNA-binding properties, and the *lac* operon becomes fully induced (Fig. 1d).

While transport proteins for inducers, like lactose or arabinose, are solely performing their intrinsic function as transporters and are therefore only indirectly involved in gene regulation (Megerle et al. 2008), others have acquired additional regulatory functions. These proteins have gained the capability to directly interact with soluble or membrane-integrated components of signal transduction. An excellent example for the combination between transport and regulation provides the phosphotransferase system (PTS) (Görke and Stülke 2008; Postma et al. 1993). The *E. coli* system is composed of a membrane-integrated part (EIICB, PstG) that functions as glucose-specific translocator, and the cytoplasmic components, EIIA, HPr and EI. During transport, glucose is phosphorylated to glucose-6-phosphate (Glc6P). The phosphoryl group is donated by phosphoenolpyruvate (PEP) and transferred via EI, HPr and EIIA to glucose. The PTS measures the PEP to pyruvate ratio, and thereby the flux through glycolysis by monitoring the phosphorylation state of EIIA. In the presence of glucose EIIA exists predominantly in the dephosphorylated form. In this state EIIA directly inhibits other sugar permeases, e.g. for maltose and lactose, a process commonly known as inducer exclusion. At the same time metabolic pathways for other sugars are turned off, a phenomenon known as carbon catabolite repression. This glucose-dependent repression results from the need of the cAMP receptor protein (CRP) to bind cAMP in order to activate expression of genes whose products are involved in exploitation of alternative carbon sources. The signal molecule cAMP is produced by adenylate cyclase which again is activated by phosphorylated EIIA.

In addition to this, in the model organisms *E. coli* and *B. subtilis* several operons for the catabolism of PTS sugars are under the control of transcriptional regulators that contain so called PTS-regulatory domains (PRDs). These domains become phosphorylated in a PTS-dependent way, and thereby perceive information on the availability of glucose and other PTS sugars. This type of regulation, called induction prevention, allows the hierarchical use of PTS sugars because PRD-containing transcriptional factors are inhibited in the presence of a preferred carbon source. PRD-mediated regulation has been studied most intensively for the *B. subtilis* LicT anti-terminator, which controls expression of the *bglPH* operon for the use of aryl-*β*-glucosides (Görke and Stülke 2008).

Regulatory influence of transport proteins on soluble transcriptional regulators

The PTS participates in an additional way in the regulation of gene expression. Mlc is a global regulator and repressor of several genes involved in sugar degradation and in particular of PTS genes (Plumbridge 1999). It belongs to the so-called ROK family, which contains two identified classes of proteins, transcriptional activators and glucose/fructose kinases (Titgemeyer et al. 1994). Binding sites for Mlc were identified in all of the regulated operons. However, all efforts to identify an inducer that would inactivate Mlc-binding to its operator failed. As transport of glucose via PstG resulted in derepression of Mlc-controlled genes, it was concluded that PTS-dependent glucose transport was the beginning of a signal transduction cascade leading to the inactivation of Mlc (Plumbridge 1999). Eventually, it was shown that Mlc becomes sequestered to the membrane by binding to PstG under conditions of active transport thereby depleting the cytoplasm from the active repressor that is no longer able to bind to its operator sequences (Fig. 2a) (Lee et al. 2000). Both components of PstG, EIIB and EIIC, are needed for sequestration but binding is controlled by phosphorylation of the EIIB subunit. Thus, Mlc measures the activation state of the PTS by monitoring the phosphorylation state of PtsG and thereby also in an indirect way the flux through glycolysis. It might be interesting to note that Mlc also exists in *Thermus thermophilus* although the bacterium like other thermophiles lacks a PTS (Chevance et al. 2006). In this bacterium, Mlc acts as transcriptional regulator for a glucose/mannose ABC transporter. Interestingly, Mlc is controlled by direct glucose binding which reduces its binding to the corresponding operator region.

Another example of PTS-dependent regulation in *E. coli* is the BglG–BglF system (Lopian et al. 2003). BglF, an enzyme EII of the PTS, is a sugar sensor that catalyzes

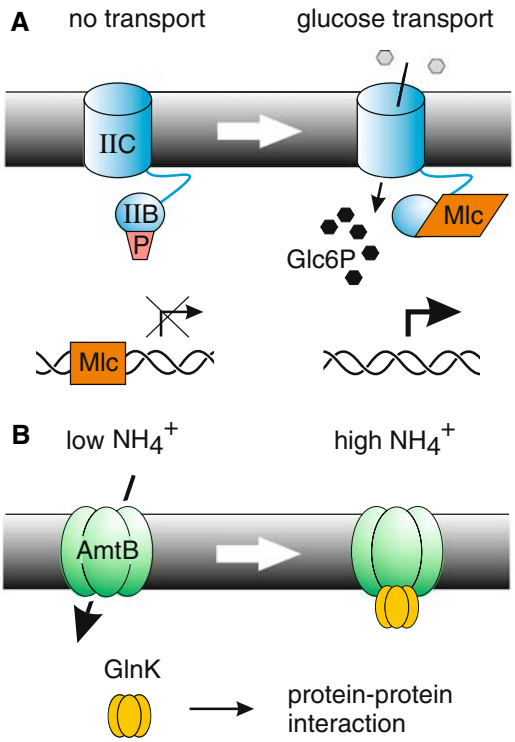

Fig. 2 Regulatory influence of transport proteins on soluble transcriptional regulators. A common principle of interaction between a transporter and soluble transcriptional regulators is sequestration of transcriptional regulators to the membrane which leads to their inactivation. **a** In *E. coli* the translocating subunits of the PTS, PtsG (EIICB), regulates the transcriptional repressor Mlc. In the absence of glucose, PtsG is phosphorylated, Mlc is unable to bind to PtsG, and acts as transcriptional repressor in the cytoplasm. In the presence of external glucose, PtsG acts as glucose permease and is concurrently dephosphorylated. The dephosphorylated PtsG binds Mlc and subsequently gene expression can take place. **b** The trimeric high-affinity ammonium transporter AmtB efficiently scavenges ammonium at low ammonium concentrations. Under this condition the soluble regulator GlnK is uridylylated, and cannot bind to AmtB. At high ammonium concentrations AmtB is inactive as a transporter, and binds non-uridylylated GlnK

transport and phosphorylation of *β*-glucosides. In addition to that, it controls the activity of the transcriptional anti-terminator BglG by reversible phosphorylation depending on *β*-glucoside availability (Amster-Choder and Wright 1990). It was demonstrated that in the absence of *β*-glucosides the membrane-integrated BglF binds BglG, which sequesters the anti-terminator to the membrane. This interaction requires phosphorylation of either BglF or BglG (Lopian et al. 2003). BglG is released to the cytoplasm when BglF detects and binds *β*-glucosides because this results in dephosphorylation of BglF. This mechanism is similar to the one described for the sequestration of Mlc by PstG (Lee et al. 2000), but in contrast to PstG and Mlc, that assemble upon glucose sensing, the BglF–BglG complex preassembles in the absence of a stimulus and BglG is released upon stimulus perception (Lopian et al. 2003).

In *Listeria monocytogenes* the PTS influences expression of virulence genes by interaction with the major transcriptional regulator PrfA (Mertins et al. 2007). PrfA is the central regulator for virulence genes in *L. monocytogenes* (Bohne et al. 1996) and belongs to the CRP/FNR family of transcriptional activators (Lampidis et al. 1994). The synthesis of PrfA is autoregulated by a PrfA-dependent promoter. In addition to that, two other promoters assure constitutive expression at low levels (Freitag et al. 1993) and also other listerial factors are known to modulate PrfA activity (Böckmann et al. 1996). Interestingly, when PrfA was overproduced or constitutively active, growth of *L. monocytogenes* was strongly inhibited in defined medium with glucose as carbon source (Marr et al. 2006). This growth defect was not observed with sugars that were not transported by the PTS which led to the conclusion that PrfA interferes with the PTS-dependent sugar uptake and that limitation of the carbon source accounts for the growth defect. At the same time, it was observed that PrfA activity was repressed by an unknown mechanism when *L. monocytogenes* was cultivated in the presence of PTS sugars, and it was found that this effect was due to inhibition of PrfA by components of the PTS (Gilbreth et al. 2004). To date it has not yet been elucidated which component of the PTS interacts with PrfA but it could be excluded that the cellular level of HPr in the phosphorylated form plays a role (Mertins et al. 2007). Likewise, a direct role of the central regulatory carbon catabolite repression protein CcpA (catabolite control protein) had been ruled out (Behari and Youngman 1998). Therefore, it was suggested that PrfA itself is affected by the phosphorylation state of other components that are phosphorylated by HPr $\sim$ P.

Another example for the interconnection between transport and transcriptional regulation is represented by the MalK–MalT system in *E. coli*. MalK, the energy-transducing subunit of the maltodextrin ABC transporter, downregulates the activity of MalT, a transcriptional activator that controls expression of the maltose regulon (Böhm and Boos 2004). Inhibition of MalT ensures that the maltose regulon is not induced by endogenous maltotriose in the absence of external maltodextrin (Bukau et al. 1986). There are some hypotheses how MalK exerts its inhibitory effect on MalT. A first indication for the interconnectivity between the two proteins was the observation that MalT specifically binds to MalK in vitro (Panagiotidis et al. 1998). Since the soluble form of MalK, which is normally associated with the membrane-integrated translocating subunit MalF of the ABC transporter, was able to affect MalT just as well as the membrane-associated form, it was suggested that membrane sequestration does not play a role in this case (Joly et al. 2004). Some MalT mutants exhibited a higher affinity for maltotriose, while they were less sensitive to inhibition by MalK revealing that the interaction between MalK and MalT prevents MalT from binding maltotriose. Thus, MalK antagonizes binding of the inducer to MalT. During transport, MalK energizes maltose transport via ATP hydrolysis. Thereby MalK generates a conformational signal which is transduced to MalT (Davidson et al. 2008). Under this condition MalT is released, binds the inducer maltotriose, and activates its target genes (Böhm and Boos 2004).

The last example of this group concerns the complex regulation of nitrogen metabolism. Nitrogen is essential for bacterial growth, and therefore, nitrogen metabolism is highly regulated. The Amt proteins are high-affinity ammonium transporters that probably function to scavenge ammonium ions and to recapture ammonia lost from cells by diffusion across the cell membrane (Coutts et al. 2002; Javelle et al. 2007). Amt proteins are a unique and ubiquitous group of integral membrane proteins found in all domains of life. Remarkably, most genes encoding the ammonium transporter AmtB are found in an operon together with a second gene which encodes the small signal transduction protein GlnK. GlnK belongs to the P_{II} family whose members are sensors for the cellular nitrogen level in prokaryotes and in plants. In *E. coli* two P_{II} proteins exist, GlnK and GlnB, that share homology on the primary sequence level which is also reflected in their tertiary and quaternary structure (Xu et al. 1998). They form trimers and are covalently uridylylated in response to nitrogen deprivation while this process is reversed under nitrogen surplus. P_{II} proteins regulate the activities of other proteins by protein–protein interactions as exemplified by the role of GlnB in modulating the activity of the histidine kinase/response regulator system NtrB/NtrC (Pioszak et al. 2000). The genetic linkage of *amtB* and *glnK* in an operon is reflected by a physical interaction between the corresponding proteins as AmtB is able to sequester GlnK to the cytoplasmic membrane (Fig. 2b) (Coutts et al. 2002). The affinity between these proteins was found to be higher for the non-uridylylated GlnK which exists predominantly under nitrogen-sufficient conditions when AmtB does not work as an ammonium scavenger. Therefore, the interaction between GlnK and AmtB is promoted under conditions of excess ammonium. At the same time the transport activity of AmtB decreases when GlnK is bound, indicating that GlnK acts as a negative regulator of the transporter (Fig. 2b). This regulatory scheme fulfills two functions. First, the activity of the transporter is modulated by GlnK so that it is only fully active under nitrogen-limiting conditions. Secondly, by sequestering GlnK to AmtB, the cytoplasmic GlnK pool is depleted, and GlnK is no longer able to regulate its targets when excess ammonium is available. In *B. subtilis* this regulation is even more complex in that the transcriptional activator TnrA, which activates expression of genes for ammonium uptake and

assimilation under nitrogen-limiting conditions, is sequestered to the membrane by binding to GlnK that is associated with the membrane-integrated AmtB (Heinrich et al. 2006).

Regulatory influence of transport proteins on membrane-integrated sensors

Transport proteins have been shown to interact with two types of membrane-integrated sensor proteins, the histidine kinases of two-component systems (Stock et al. 1990), and a member of the ToxR-like transcriptional activators (Miller et al. 1987). All types of transporters, secondary and primary ones, and even single subunits of primary ABC transporters are found to be involved in regulation. Some regulatory events necessitate transport whereas in other cases sensing and transport activities are uncoupled to the point of complete loss of transport activities for the benefit of sensing.

Sensors of the ToxR-like protein family combine sensory and DNA-binding function in one single polypeptide (Miller et al. 1987). These proteins are composed of an N-terminal DNA-binding domain and a C-terminal periplasmic sensory domain which are connected by one transmembrane domain. Furthermore, these proteins mediate signal transduction without chemical modification. The activation mechanism of these one-component transcriptional activators has not been elucidated yet and in particular it remains elusive how a membrane-integrated protein binds to the DNA. Recently it has been shown that CadC of *E. coli* co-senses external stimuli in an interplay with a secondary transporter (Tetsch et al. 2008) (Fig. 3a). CadC, the transcriptional activator of the *cad* operon, is inhibited by the lysine-specific permease LysP in the absence of lysine (Neely et al. 1994). The Cad system is involved in the acid tolerance response of *E. coli* and is activated under conditions of low pH (5.8) and the concurrent availability of lysine in the extracellular medium. Upon stimulus perception CadC binds to the promoter of the *cadBA* operon inducing the transcription of *cadA* and *cadB* which encode the lysine decarboxylase CadA and the lysine/cadaverine antiporter CadB, respectively. CadA decarboxylates lysine to cadaverine and CadB is an antiporter that takes up lysine and excretes cadaverine. Both, consumption of a cytoplasmic proton during decarboxylation, and excretion of a polyamine that is more basic than the substrate lysine, result in an increase of the intra- and extracellular pH. LysP, a lysine-specific permease with 12 transmembrane helices (Ellis et al. 1995), represses *cadBA* expression in the absence of lysine (Neely et al. 1994). It was suggested that this repression was either due to substrate competition between the transcriptional activator and the lysine permease that exhibits high affinity for lysine (Rosen 1971) or to direct interaction between CadC and LysP. Earlier, the periplasmic domain of CadC was proposed to be a lysine sensor (Dell et al. 1994), but recent biochemical studies clearly indicated that CadC binds cadaverine but not lysine. These and further results led to the suggestion that CadC senses the external lysine concentration solely by an interplay with LysP (Tetsch et al. 2008). Cross-linking studies demonstrated a direct interaction between the two membrane-integrated proteins CadC and LysP. Moreover, this interaction was found to be exerted via the transmembrane domain of CadC. A cluster of aromatic amino acids located within the transmembrane helix of CadC was found to be involved in this regulatory process, an effect that could be ascribed predominantly to one single amino acid residue, phenylalanine 165. Aromatic residues have already been shown to mediate the self-assembly of soluble proteins through π–π interactions of the planar aromatic rings and also seem to promote the dimerization of transmembrane proteins (Sal-Man et al. 2007). The current working model suggests the following (Fig. 3a): in the absence of lysine LysP interacts with CadC and prevents activation probably by locking CadC in an inactive conformation. In the presence of lysine, LysP releases CadC which thereupon undergoes pH-dependent conformational changes and induces *cadBA* expression (Tetsch et al. 2008). It is an open question whether binding of lysine to LysP is sufficient for the release of CadC or whether only a transport-active LysP releases CadC.

A good example for a secondary transporter that has lost most of its transport function in favor of sensing is UhpC, the sensor for external Glc6P in *E. coli* (Schwöppe et al. 2003). UhpC is a membrane-integrated protein required for recognizing the extracellular concentration of Glc6P. Upon sensing of Glc6P, UhpC interacts with the membrane-integrated histidine kinase UhpB and stimulates phosphorylation of the UhpB/UhpA system that in turn induces expression of *uhpT* encoding the transport protein UhpT (Wright and Kadner 2001). UhpC and UhpT are homologous to each other and belong to the Major Facilitator Superfamily (Lalonde et al. 1999). Although no transport activity was determined for UhpC when the gene was chromosomally encoded, experiments with overproduced protein revealed that UhpC mediates a low but detectable Glc6P/P_i antiporter activity. Interestingly, UhpT contains an intrahelical salt bridge that is indispensable for transport but missing in UhpC. Insertion of this salt bridge in UhpC increased the specific transport activity, but resulted in a complete loss of the sensory function and hence *uhpT* induction. Removal of the bridge in UhpT, however, did not transform the protein into a Glc6P sensor. Mutation of arginine residues known to be important for transport activity in UhpT had hardly any effect on transport activity

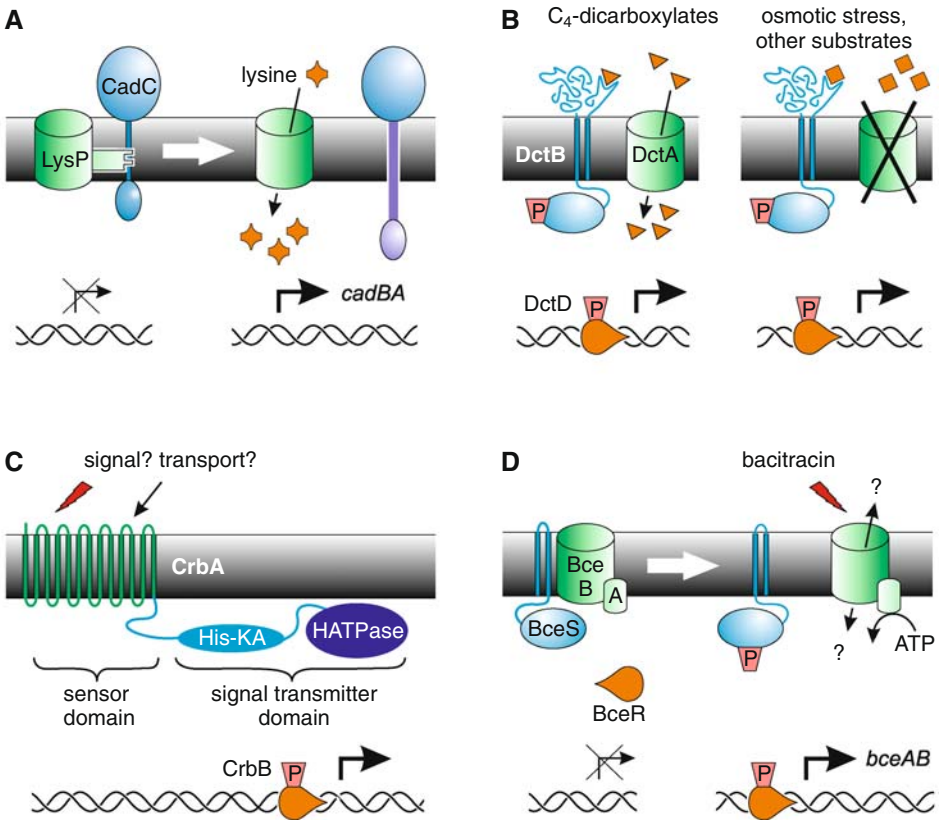

Fig. 3 Regulatory influence of transport proteins on membrane-integrated sensors. Secondary and primary transporters influence membrane-integrated sensors by direct interaction. **a** The lysine permease LysP inhibits the ToxR-like transcriptional regulator CadC in the absence of lysine via an interaction with the transmembrane domain of CadC. This interaction prevents CadC from activating *cadBA* expression. In the presence of lysine, LysP transports lysine and the interaction between LysP and CadC is released. CadC becomes susceptible to activation by low pH, the second stimulus needed for activation, and induces *cadBA* expression. **b** The C_4-dicarboxylate uptake system DctA influences the two-component system DctB/DctD. In the presence of C_4-dicarboxylates DctB phosphorylates the response regulator DctD which induces gene expression. DctB functions as a sensor for dicarboxylates, but it is also regulated by DctA. When DctA is absent, DctB has a broader substrate spectrum, and it is also activated by other stresses such as osmotic stress. Thus, DctA increases the specificity of DctB/DctD. **c** The CrbA/CbrB two-component system of various *Pseudomonas* species participates in the adjustment of the intracellular carbon/nitrogen ratio of the cells, but the direct stimuli sensed are not yet identified. CbrA is composed of a sensor domain with high homology to Na^+/solute symporters (SSS) which is covalently linked to the transmitter domain. It is still unclear whether the symporter domain mediates active transport or whether it is transformed into a pure sensing domain. **d** The bacitracin ABC transporter BceAB inhibits the histidine kinase BceS in the absence of bacitracin. In the presence of bacitracin the inhibitory effect is released, and BceS phosphorylates the response regulator BceR which in its phosphorylated state induces expression of the *bceAB* operon. The exact inhibitory mechanism is not elucidated yet, but transport of bacitracin is crucial for the activation of BceS. The direction of bacitracin transport by BceAB remains elusive so far

of UhpC but in contrast had a drastic effect on UhpC-mediated transcriptional activation of *uhpT* expression which in several cases became constitutive. These results indicated that sensing and transport of the substrate are uncoupled in UhpC and that binding of Glc6P induces a conformational change which leads to activation of UhpC without the need to translocate the substrate. Similarly, in the eukaryote *Saccharomyces cerivisiae* transcriptional induction of amino acid transporter genes is dependent on the amino acid sensor Ssy1 that itself belongs to a family of secondary amino acid transport proteins, but has completely lost the ability to transport (Didion et al. 1998; Wu et al. 2006). Ssy1 is located in the plasma membrane, and generates a signal by sensing leucine in the medium with high sensitivity.

The next example describes the interaction between the active secondary dicarboxylate transporter DctA and the two-component system DctB/DctD in *Rhizobium leguminosarum* (Fig. 3b) (Reid and Poole 1998). Rhizobia use C_4-dicarboxylates such as L-malate, fumarate and succinate as carbon source during nitrogen fixation in legume nodules, and these substrates are transported by the secondary uptake system DctA (Engelke et al. 1987; Engelke et al. 1989). Strains with a dysfunctional DctA fail to transport dicarboxylates and are unable to fix nitrogen. In the presence of dicarboxylates the membrane-integrated histidine

kinase DctB is phosphorylated, and transfers the phosphoryl group to the response regulator DctD which in turn induces transcription of *dctA*. The fact that strains mutated in *dctA* exhibited constitutive *dctA* expression indicated that the transporter interferes with the signal transduction process of the histidine kinase/response regulator pair by acting as a sensor for dicarboxylates thereby controlling its own synthesis (Yarosh et al. 1989). Consequently, it had been proposed that in the absence of substrate, DctA might interact with DctB within the cytoplasmic membrane thereby preventing DctB activation. Binding of C_4-dicarboxylates to DctA would lead to a release of DctB and enable the subsequent activation of the DctB/DctD pair (Fig. 3b). This model does not exclude the possibility that DctB rather detects the C_4-dicarboxylate dependent conformational state of DctA instead of acting as a sensor for dicarboxylates. In this case, DctA would completely take over the sensory function from the histidine kinase. However, expression of *dctA* was still inducible in a Δ*dctA* strain. This induction was dependent on the presence of DctB and DctD indicating that DctB itself must be able to sense dicarboxylates and does not solely detect the solute-binding state of DctA (Reid and Poole 1998). Further studies revealed that DctA and DctB can indeed recognize different substrates (Yurgel and Kahn 2004). Whereas DctA prefers substrates which adopt a stretched conformation with the carboxyl groups in *cis* position, DctB recognizes predominantly substrates in folded conformation with the carboxyl groups in *trans* position. In addition to C_4-dicarboxylate-dependent activation, *dctA* expression is also activated under nitrogen-limiting conditions regardless of the presence of DctA (Reid and Poole 1998). This regulatory process is under the additional control of the regulator NtrC. Interestingly, in the absence of DctA the *dctA* promoter is additionally induced by osmotic stress and calcium limitation in a DctB/DctD dependent manner. It can be summarized that in the absence of DctA the *dctA* promoter becomes highly sensitive to induction, especially to cross-induction by stimuli other than dicarboxylate substrates. Furthermore, the specificity for dicarboxylates changes and DctB is activated by a much broader spectrum of substrates. Thus, DctB must be able to sense its substrates autonomously, which is consistent with the existence of a large periplasmic loop. There are two possibilities for DctA to exert its inhibitory effect on DctB. By interacting with DctB in the membrane and changing its conformation, DctA could either influence the specificity for ligands or the signaling ability of the histidine kinase by modulating the kinase and/or phosphatase activity. Inspite of intensive research it remains unsolved thus far if active transport of dicarboxylates by DctA is required to relieve the repression on DctB and if this repression is due to a direct protein–protein interaction between DctA and DctB.

Recently a similar system has been described in *E. coli* (Kleefeld et al. 2008). In this organism the fumarate/succinate antiporter DcuB is required for fumarate respiration under anoxic conditions. Expression of *dcuB* is regulated by the DcuS/DcuR two-component system. Deletion of *dcuB* resulted in a fumarate-independent induction of gene expression indicating that the DcuS/DcuR pair in turn is regulated by the antiporter. Amino acid replacements in DcuB revealed that DcuB is indeed a bifunctional protein with a regulatory function independent from transport. Amino acids important for transport are clustered in the N-terminal half, whereas amino acids important for regulation are predominantly located in the C-terminal half of the protein. Since the histidine kinase DcuS contains a periplasmic binding site for C_4-dicarboxylates, which was characterized by structural and mutational studies (Kneuper et al. 2005), it was concluded that the DcuS/DcuR pair contains a second signal input site. While DcuS itself senses the C_4-dicarboxylate concentration in the periplasm, the DcuB transporter rather senses C_4-dicarboxylate transport and metabolism, and both inputs are integrated at the transcriptional level (Kleefeld et al. 2008).

The histidine kinase CbrA of *Pseudomonas* species represents an extraordinary example for the interconnectivity between transport and signal transduction. CbrA contains an Na^+/solute symporter (SSS) domain as input domain (Jung 2002), which is covalently linked to the transmitter domain (Fig. 3c) (Nishijyo et al. 2001). The CrbA/CbrB two-component system participates in the adjustment of the intracellular carbon/nitrogen ratio of the cells, but the direct stimuli sensed are not yet identified. Moreover, it is still unclear whether the SSS domain senses during simultaneous transport or is solely responsible for sensing.

In contrast to secondary transporters, bacterial ABC transporters are primary transporters using the hydrolysis of ATP as energy source (Davidson et al. 2008). They are usually composed of two membrane-spanning subunits acting as translocases and two ATP-hydrolyzing subunits. In addition, substrate-binding proteins may be coupled to the translocase or are freely diffusible in the periplasmic space. In *B. subtilis* sensing of the cell wall antibiotic bacitracin depends on the bacitracin ABC transporter BceAB that acts as a bacitracin detoxification pump, and regulator of the two-component system BceS/BceR (Rietkötter et al. 2008) (Fig. 3d). To date it is still unclear in which direction bacitracin is transported. Originally, BceAB was described as an efflux pump because of the lack of a substrate-binding protein (Bernard et al. 2007), but its architecture and the existence of a large extracytoplasmic loop important for stimulus perception allude to active bacitracin uptake (Poolman et al. 2004; van der Heide and Poolman 2002). Import of the bacitracin as a

detoxification mechanism might make sense when the antibiotic solely acts on the outside of cells as discussed (Rietkötter et al. 2008) but this remains to be elucidated. The two-component system BceS/BceR, activated by BceAB upon stimulus perception, itself induces expression of *bceAB* in the presence of bacitracin. Response to cell envelope stress as induced by antibiotics like bacitracin is mediated by four different two-component systems, of which BceS/BceR is the most sensitive sensor system. In the interplay between transporter and two-component system, ATP-binding and -hydrolysis by BceAB and thus active transport are crucial for stimulus perception. The coupling of transport and stimulus perception is reasonable because the detoxification by the most efficient bacitracin resistance determinant simultaneously removes the stimulus thereby switching off the system once the bacitracin concentration decreases under the sensory threshold. At the same time instant resistance is ensured because the ABC transporter is regulated by the most sensitive regulatory system BceS/BceR and thus activated at very low concentrations of bacitracin. A connection of ABC transporters and two-component systems seems to be quite frequent in the Firmicutes like *Bacillus* because to date about 70 examples of two-component systems encoded by genes adjacent to those encoding ABC transporters are described (Mascher et al. 2006).

Single components of ABC transporters also may play a role in signal transduction as it was shown for several substrate-binding proteins. The maltose binding protein MalE of *E. coli* binds maltose and acts in concert with the membrane-integrated transporter MalF and MalG and the cytoplasmic ATP hydrolase MalK to transport maltose across the membrane (Manson et al. 1985). In addition to that, MalE possesses a regulatory function in chemotaxis by interplaying with the chemoreceptor Tar (Hazelbauer 1975). Tar is able to directly sense the attractant L-aspartate as well as repellents such as the heavy metals cobalt and nickel. Additionally, maltose is sensed by direct interaction with the maltose-binding protein MalE. Another substrate-binding protein involved in the regulation of gene expression is PtsS, the substrate-binding protein of the high-affinity phosphate transporter PstSCAB in *E. coli* (Makino et al. 1989). Under conditions of low external phosphate, expression of genes belonging to the PHO regulon such as the genes for the PstSCAB transporter are induced by a two-component system consisting of the histidine kinase PhoB and the corresponding response regulator PhoR. Under excess of extracellular P_i, PstSCAB functions as a repressor of the PHO regulon via an interaction with the PhoB–PhoR system in a manner that is independent of its transport activity. The nature of this regulatory process still remains elusive, and there are indications that either PstS interacts with the extracellular domain of the histidine kinase PhoB or a component of the Pst transporter might interact directly with the cytoplasmic response regulator PhoR (Cox et al. 1989; Steed and Wanner 1993).

Trigger transporters

The examples discussed vary in different aspects, but they all have in common that transport proteins are involved in regulation. These transporters have—in addition to their traditional function—acquired an accessory capacity, which is the ability to regulate the function of another protein. As this regulation is directly connected to the regulation of gene expression, the transporters can be compared to trigger enzymes (Commichau and Stülke 2008). Trigger enzymes have been defined as enzymes that have acquired the ability to regulate gene expression by various mechanisms in addition to their catalyzing function. Against this background it does not seem too ambitious to call the transporters involved in gene regulation trigger transporters. Trigger transporters interact both with soluble cytoplasmic transcriptional factors, such as Mlc or GlnK and with membrane-integrated histidine kinases like UhpB, DctB, BceS or ToxR-like sensors, like CadC. On the other hand, transporters involved can be the PTS, secondary transporters like LysP, DctA and UhpC or primary ABC transporters, like BceAB. Even single subunits of ABC transporters can be involved in regulation. For some regulatory processes transport is indispensable as for the PTS-dependent regulation of PrfA and Mlc. Other transporters as UhpC or the yeast protein Ssy1 have partially or completely lost their ability to transport in favor of the ability to sense. In some cases components of the signal transduction systems maintain their sensory function and utilize the transport protein only to modulate their specificity as has been demonstrated for DctB, whereas others like CadC completely depend on the transport protein for sensing their ligand. Direct interaction between the partners of the regulatory processes has been postulated for all examples, whereby either formation of the complex or release of the components results in gene expression. The recently discovered interaction between signal transduction and transporters seems to be of great importance, and more widely distributed than thought to date. With the upmost probability the examples of such interactions will increase the more they are searched for.

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