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Review

The KDEL receptor: New functions for an old protein

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ABSTRACT

The KDEL receptor is a seven-transmembrane-domain protein that was first described about 20 years ago. Its well-known function is to retrotransport chaperones from the Golgi complex to the endoplasmic reticulum. Recent studies, however, have suggested that the KDEL receptor has additional functions. Indeed, we have demonstrated that chaperone-bound KDEL receptor triggers the activation of Src family kinases on the Golgi complex. This activity is essential in the regulation of Golgi-to-plasma membrane transport. However, the identification of different KDEL receptor interactors that are inconsistent with these established functions opens the possibility of further receptor activities.

1. Introduction and historic perspective

The secretory pathway includes a number of anatomically separate compartments that constantly exchange their membranes and proteins in an organised sequence of events [1,2]. Newly synthesised proteins enter the endoplasmic reticulum (ER) and move on to the Golgi complex, where they are modified prior to being distributed to their final destinations [3].

In the 1980s, the concept that newly synthesised proteins fold spontaneously began to change in favour of a chaperone-driven mechanism. This followed the identification and cloning of endoplasmic reticulum (ER)-resident proteins that are involved in the folding of nascent transmembrane and secretory proteins. These findings, however, led to more questions, such as how and where these ER-resident proteins are sorted out from newly synthesised secretory proteins.

Pelham and co-workers [4] later identified a C-terminal tetrapeptide motif (the KDEL motif in mammals, HDEL in yeast) that is shared by several ER chaperones, including glucose-regulated protein-78 (GRP78), glucose-regulated protein-94 (GRP94) and protein disulphide isomerase (PDI). They hypothesised that the KDEL/HDEL motif acts as an ER retention/retrieval signal to keep the chaperones in the ER.

The proof of concept was provided with the addition of the last six amino-acid residues of GRP78 to the C-terminus of an exogenous protein (lysozyme) engineered to be luminal and to cross the secretory pathway; this prevented its secretion, with its consequent accumulation in the ER [4]. Analyses of the carbohydrate modifications of an artificial HDEL-tagged yeast secretory protein (invertase) suggested that ER-resident chaperone proteins can leave the ER, undergo Golgi-specific modifications, and then return to the ER [5–7]. This thus suggested that the HDEL motif is part of a retrieval, rather than a retention, mechanism. Indeed, overexpression of an HDEL-tagged exogenous protein leads to increased secretion of the endogenous yeast chaperone GRP78, indicating that the HDEL retention system can be saturated by HDEL ligands and that chaperone retrieval into the ER is mediated by a specific receptor [7].

To identify the HDEL receptor and the components involved in the ER-retention system, several ER-retention defective (*erd*) yeast mutants were obtained by UV-induced random mutagenesis [6]. These mutants failed to retain HDEL-tagged invertase and endogenous GRP78 in the ER. Functional complementation of these mutants led to the identification of two genes, *ERD1* and *ERD2* [8,9].

Abbreviations: ER, endoplasmic reticulum; ERGIC, intermediate ER-Golgi compartment; *erd*, ER-retention defective mutant; SED, suppressors of *erd2*-deletion; GRP78, glucose-regulated protein-78; GRP94, glucose-regulated protein-94; PDI, protein disulphide isomerase; SFKs, Src family kinases; PKA, cAMP-dependent protein kinase A; BFA, brefeldin A; JNKs, c-Jun amino-terminal kinases; MAPKs, mitogen-activated protein kinases; COP, coat protein complex; ARF1, ADP-ribosylation factor 1; ARFGAP1, ADP-ribosylation factor GTPase-activating protein 1; ARFGEF, ADP-ribosylation factor guanine nucleotide exchange factor; GPI, glycosylphosphatidylinositol; GPCR, G-protein-coupled receptor

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The *ERD1* gene encodes an integral membrane protein, the deletion of which indeed leads to secretion of HDEL-tagged proteins, although unexpectedly, these yeasts show defects in the N-glycosylation of their secreted proteins [8]. The *ERD2* gene also encodes an integral membrane protein that is localised in the ER and the Golgi complex, the deletion of which leads to secretion of HDEL-tagged proteins. The overexpression of the *ERD2* gene in *erd2*-deleted mutant cells restored their ability to retain GRP78 and artificial HDEL-containing fusion proteins in the ER. Furthermore, in a yeast strain with deletion of its endogenous *ERD2* and carrying one copy of the *ERD2* gene on a centromere-containing expression vector, an HDEL-tagged invertase fusion protein was not retained in the ER, indicating that the efficiency of this retrieval system depends on the level of the HDEL receptor, Erd2p [9].

In addition to these alterations to the HDEL retention system, *erd2*-deletion mutant yeast also accumulate intracellular membranes, concomitant with the inhibition of secretory protein transport through the Golgi complex. This suggests that there are transport defects in the ER and the Golgi complex. It has been hypothesised that both defects (in the retention of HDEL-containing ER proteins, and in secretion) are related, since *ERD2* might be necessary not only for retrieval of ER proteins, but also for recycling of other components that are necessary for anterograde trafficking [9]. Notably, *erd2*-deficient yeast gradually become quiescent and cease to grow.

To determine the role of Erd2p in the Golgi complex and why it is essential for yeast growth, multicopy suppressor screening of the growth phenotype induced in *erd2*-deletion mutant cells has been carried out [10]. This led to the identification of six genes, called suppressors of *erd2*-deletion (*SED*) 1–6. *SED1* is a stress-response gene that encodes a cell-wall protein that appears to be involved in the maintenance of mitochondrial genome stability [11]. *SED2* is also known as *SEC12*, and it is a guanine nucleotide exchange factor (GEF) that is involved in the exit of cargo from the ER, as an activator of the small GTPase Sar1, the master regulator of COPII coat complex formation [12]. *SED3* is also known as *DPM1*, and it is a dolichol-phosphate mannose synthase, an enzyme that is involved in protein glycosylation and formation of glycosylphosphatidylinositol (GPI) anchors [13]. *SED4* is a transmembrane protein that has been suggested to work in concert with *SED2* to regulate the activation/ inactivation of Sar1 [14,15]. *SED5* is a t-SNARE syntaxin protein that is involved in ER-to-Golgi transport [16,17]. Finally, *SED6* is also known as *ERG6*, and it is a methyltransferase that is involved in ergosterol synthesis [18].

How these genes compensate for the lack of the *ERD2* gene, however, is not obvious, although it has been proposed that they can counteract the membrane transport imbalance that results from the loss of Erd2p [10]. Although the secretory and growth phenotypes that have been observed for *erd2*-deleted yeast cells remain to be explained, we have recently reported on and defined the mechanisms behind a similar secretion defect in eukaryotic cells expressing a dominant-negative mutant of human *ERD2* [19].

2. Cloning and intracellular distribution of the human KDEL receptor

The recognition of the *ERD2* gene as the HDEL receptor in the yeast *Saccharomyces cerevisiae* and *Kluyveromyces lactis* [9,20] was rapidly followed by the cloning of mammalian orthologues. By 1990, Lewis and Pelham had identified the first human HDEL receptor homologue, referred to as *hERD2* or the KDEL receptor, since the KDEL motif is the most common ER-retrieval sequence in mammals [21]. The *hERD2* gene encodes a protein of 214 amino acids that has about 50% identity with *S. cerevisiae* and *K. lactis* Erd2p. Interestingly, the identity between these two yeast *ERD2* genes is only around 60%, suggesting that strong evolutionary pres-

Table 1
Protein identities and similarities across the different KDEL/HDEL receptor isoforms. The analysis was performed using the EMBOSS algorithm (matrix, Blosum62; Open gap penalty, 10.0; Gap extension penalty, 0.5) at www.ebi.ac.uk.

Identity / Similarity

	HDEL receptor	KDEL receptor 1	KDEL receptor 2	KDEL receptor 3a	KDEL receptor 3b
HDEL receptor (Ac. No. CAA84860)		50,2% / 70,3%	49,5% / 68,0%	47,5% / 69,7%	43,9% / 65,2%
KDEL receptor 1 (Ac. No. NP_006792)	50,2% / 70,3%		83,5% / 93,9%	72,0% / 86,0%	65,5% / 79,5%
KDEL receptor 2 (Ac. No. NP_006845)	49,5% / 68,0%	83,5% / 93,9%		75,7% / 87,9%	69,1% / 81,4%
KDEL receptor 3a (Ac. No.NP_006846)	47,5% / 69,7%	72,0% / 86,0%	75,7% / 87,9%		91,4% / 92,3%
KDEL receptor 3b (Ac. No. NP_057839)	43,9% / 65,2%	65,5% / 79,5%	69,1% / 81,4%	91,4% / 92,3%	

sure was applied to this gene to preserve its function from yeast to human.

Two independent laboratories eventually identified a second human KDEL receptor that is functionally identical to first human KDEL receptor, with about 83% identity (Table 1) [22,23]. This new gene was named KDEL receptor 2 and ELP-1.

The KDEL receptor localises preferentially to the Golgi complex, the ER (similar to yeast) and the intermediate ER-Golgi compartment (ERGIC). The expression of an artificial ligand, such as KDEL-tagged lysozyme, results in redistribution of the KDEL receptor into the ER, a phenomenon that is reminiscent of the ligand-induced internalisation of the G-protein-coupled receptors (GPCRs).

Electron microscopy studies have confirmed and better defined the presence of the KDEL receptor in the ER, the ERGIC and the Golgi complex [24]. Quantitative examination has revealed that the KDEL receptor is mainly localised in the ERGIC and the *cis*-side of the Golgi complex, which contains fivefold more KDEL receptor labelling than the medial and *trans* parts of the Golgi complex [25]. Instead, the endosomal compartment is almost devoid of KDEL receptor staining [24]. Of note, the distribution of the KDEL receptor generally overlaps with that of the chaperones [24].

Under stress conditions, such as with virus infection, temperature shifts or increased cargo loading of the secretory pathway, the distribution of the KDEL receptor is modified, so that high levels are then found even on the TGN [24]. This can be explained considering that in situations with heavy traffic loads, the sorting of the KDEL receptor at the Golgi complex is not efficient, and as a result its localisation on the TGN is increased. It would be interesting to investigate the consequences of this redistribution on the functioning of the KDEL receptor. Thus far, the role of cell stress with KDEL receptor expression is still controversial, since it has been reported that cell stress causes receptor overexpression in yeast and plants [26,27], whereas it might remain unchanged in mammals [28].

A third KDEL receptor was identified in the late 1990s, during the genome and ORFeome sequencing project [29]. Although lacking formal experimental evidence, it appears that KDEL receptor 3 exists in two spliced isoforms (a and b, or alternatively, 1 and 2). These encode two proteins of ~220 amino acids that differ one from the other in their last 19 C-terminal amino acids, due to a fourth intron in the longer isoform (b or 2) [30]. However, thus far, only KDEL receptor 3a has been partially characterised, and it shows a Golgi localisation, as seen for the other KDEL receptors [31].

Along the years, the KDEL receptor class of proteins has been associated with different names; in Table 2, we have summarised the different definitions that have been attributed to the human and yeast KDEL receptors. Here, we propose to use the gene names given in the first column of Table 2 to indicate these genes (e.g. KDELR1), and the protein names given on the third column to indicate these proteins (e.g. KDEL receptor 1).

Sequence analyses of the human KDEL receptor proteins show a significant degree of conservation, since their identities range from ~65.0% to ~83.5% (Table 1), while their homologies increase from ~80% to ~94% (Table 1), suggesting that they might have redundant functions, although with some degree of specificity possible.

During evolution from yeast to human, the gene coding for the KDEL receptor has undergone triplication, and there are indications

Table 2
KDEL receptor nomenclature in human and *Saccharomyces cerevisiae*.

Source	Gene name	Synonymous gene name	Common protein name	Alternative protein name
S.cerevisiae	ERD2	YBL040C	HDEL receptor	ER lumen protein retaining receptor, Erd2p
Homo Sapiens	KDELR1	ERD2.1, ERD2, HDEL, PM23	KDEL receptor 1	ER lumen protein retaining receptor 1, Putative MAPK-activating protein P23, KDEL endoplasmic reticulum protein retention receptor 1
	KDELR2	ERD2.2, ELP-1, FLJ45532	KDEL receptor 2	ER lumen protein retaining receptor 2, ERD2-like protein 1, KDEL endoplasmic reticulum protein retention receptor 2, ELP-1
	KDELR3	ERD2.3, ERD2L3	KDEL receptor 3a	ER lumen protein retaining receptor 3 isoform 1, KDEL endoplasmic reticulum protein retention receptor 3, KDEL receptor 3 isoform a
	KDELR3	ERD2.3, ERD2L3	KDEL receptor 3b	ER lumen protein retaining receptor 3 isoform 2, KDEL endoplasmic reticulum protein retention receptor 3, KDEL receptor 3 isoform b

that each of the three genes can be expressed as different splice variants (http://harvester.fzk.de/harvester/ and [31]), emphasising the importance of this protein for cell function. As expected for proteins committed to basic cell functions (retrieval of ER chaperones), each of the KDEL receptor isoforms are expressed in all human tissues, although to different extents; this generally follows a common pattern, with *KDELR1* more transcribed than *KDELR2*, which is in turn more transcribed than *KDELR3* [31,32] (http://www.genecards.org).

The specificities of the three KDEL receptors have been addressed more recently by Ruddock and co-workers [31], who applied bimolecular-fluorescence-complementation-based screening. They demonstrated that each of the KDEL receptor isoforms binds preferentially to a specific set of KDEL-like sequences [31]. This study also showed that KDEL receptor 1 and KDEL receptor 3 are less specialised than KDEL receptor 2 in their abilities to bind and retrieve into the ER any KDEL-motif variants. Within this general classification, and taking into consideration the two most frequent C-terminal motifs in the human ER chaperones (KDEL and HDEL), KDEL receptor 1 preferentially binds chaperones bearing the KDEL over the HDEL motif, whereas KDEL receptor 3 strongly favours the HDEL motif over the KDEL motif. In contrast, the specialised KDEL receptor 2 preferentially recognises variants of the HDEL motif over any other. The functional significance of these different binding specificities, however, still remains to be defined. We can at present hypothesise that each KDEL receptor can recognise a sub-population of KDEL ligands, the expression and presence of which outside the ER can change in response to cellular stimuli or physiopathological conditions; as a result, each individual KDEL receptor isoform might generate a specific outcome.

3. Structural and functional relationships of the KDEL receptor

As an initial attempt to reveal the structural organisation of the KDEL receptor, hydropathy analyses of the yeast and human KDEL receptors were carried out. These have provided evidence of an integral membrane protein with seven-transmembrane domains [9]. This prediction has since been confirmed experimentally in different studies, which have also located the N-terminal region of the KDEL receptor to the lumen of the organelles and the C-terminus to the cytosol [33,34].

An alternative topology has been reported for the KDEL receptor: six membrane-spanning domains with both the N-terminus and the C-terminus located in the cytoplasm; this was seen by tagging the KDEL receptor with c-myc and the N-glycosylation sites along the protein [35]. This six-transmembrane-domain model has been little considered, however, as a number of subsequent studies have generated consensus for the seven-transmembrane-domain model by analysing their findings with a view to this latter topology. The presence of this seven-transmembrane-domain architecture (which is reminiscent of the GPCR family; see below) promoted in vitro ligand-binding studies, which have been aimed at demonstrating that the KDEL receptor can bind KDEL peptides. In these studies, microsomal membranes bearing the KDEL receptor were shown to have specific binding activities for KDEL-like-containing peptides. The affinities were higher for the KDEL and HDEL motifs, and lower for other KDEL variants, such as DDEL and HDEV. In addition, from these studies, it has emerged that the affinity of the KDEL receptor for KDEL ligands does not depend on a specific lipid environment [36], while it does dependent on pH. Indeed, under acid conditions, KDEL receptor–KDEL ligand binding is very efficient; however, at neutral or basic pH, this binding becomes significantly weaker [37]. This would suggest that the association of the KDEL-containing proteins with the KDEL receptor in the Golgi complex and their release into the ER can be explained by the pH difference between these two organelles, as the *cis*-Golgi pH is lower than that in the ER [38,39].

The relationships between the KDEL receptor structure and function have been investigated by mutational analysis. In the yeast *K. lactis*, it has been reported that the KDEL receptor N51 residue is involved in the specificity of ligand recognition. Indeed, an N51D mutation impaired recognition of the HDEL motif, but not of the DDEL motif, indicating that a single amino-acid residue can influence the binding specificity of the KDEL receptor [40].

The human KDEL receptor has been extensively studied according to more than 80 different mutations [34]. In general, mutations in the cytoplasmic loops of the KDEL receptor affect transport between the ER and the Golgi complex, while they have minor effects on ligand binding. In contrast, mutations in the luminal regions or in the peri-luminal moiety of the transmembrane domains usually alter KDEL receptor binding, but not the KDEL receptor distribution, unless the altered distribution is a consequence of the binding defects. It is worth noting that mutation of D193 in the KDEL receptor, which is localised in the seventh transmembrane region, does not impair KDEL binding activity in vitro, but fails to redistribute the KDEL receptor into the ER and prevents the KDEL receptor from retrieving KDEL-containing proteins. This suggests that D193 is a part of an essential inter-molecular or intra-molecular interaction(s) that is required for retrograde transport of the KDEL receptor. More recently, our group has demonstrated that the KDEL receptor D193N-mutant also acts as a functional dominant negative towards endogenous KDEL receptors [19].

Using a sulphydryl-specific labelling approach, the ligand-binding pocket of the KDEL receptor has been defined, with the identification of four essential amino-acid residues: R5, D50, Y162 and N165 [33]. These amino acids are well conserved among all of the KDEL receptors, they are part of the first, second and sixth transmembrane regions of the KDEL receptor, and they are involved in the binding of the KDEL ligands. Interestingly, the D50C mutation in the human KDEL receptor impairs the binding of KDEL-, HDEL- and RDEL-containing peptides. Conversely, the binding affinity for a DDEL peptide was not affected, indicating that residue D50 is involved in mediating the ligand specificity of the KDEL receptor. This presumably occurs through an interaction with a positively charged amino acid at position −4 of the ligands. In our opinion, several structural and functional features that have been observed for the KDEL receptor are particularly reminiscent of the GPCR superfamily. The GPCRs are a large family of proteins that have a characteristic seven-transmembrane-domain, helical organisation that is involved in the transduction of extracellular signals into the cell [41].

The GPCR ligand-binding sites are generally formed of charged amino-acids embedded inside a hydrophobic seven-helix bundle [41,42]. Agonist binding induces conformational changes to GPCRs, which in some cases involve dimerisation [43], and these lead to the activation of heterotrimeric G-proteins on the cytoplasmic side of the plasma membrane, and in turn, to the production of second messengers [41]. This signalling is quenched upon receptor phosphorylation by second-messenger-dependent protein kinases (PKA and PKC) and GPCR kinases [44,45]. Receptor phosphorylation promotes uncoupling of the G proteins and recruitment of adaptor proteins (arrestins), which initiates receptor internalisation into the endosomal compartment [44]. These internalised GPCRs release their ligands and are recycled back to the plasma membrane [44].

As indicated above, and as with the GPCRs, the KDEL receptor has a seven-transmembrane-domain organisation with a ligand-binding site formed by charged amino acids embedded in a hydrophobic core [33]. Ligand binding induces a conformational

change/dimerisation of KDEL receptor [46], which probably then interacts with heterotrimeric G proteins (our unpublished observations, and [47]). Furthermore, the KDEL receptor can be phosphorylated by PKA, which promotes recruitment of the adaptor proteins (coat protein complex (COP) I) that are important for receptor translocation [48]. Finally, the KDEL receptor ligands detach upon their arrival in the destination compartment (ER), and the KDEL receptor is recycled back to the original membranes (in the Golgi) [9,49].

These similarities also extend to the functional aspects of the KDEL receptor, as the properties of the KDEL receptor indicated above make it a signalling protein [19,50] (see below for details).

4. Molecular machinery involved in KDEL-receptor-dependent retrieval of ER chaperones

Overexpression of the KDEL receptors has revealed that their intracellular distributions are dynamic and relate to their levels of expression. At low levels, the KDEL receptors preferentially localise on the Golgi complex, whereas at high levels they have reticular ER-like patterns. This suggests that high expression levels of the KDEL receptor can induce ligand-independent auto-activation, which in turn enhances retrograde transport from the Golgi complex to the ER [23]. This massive receptor auto-activation can promote the cytosolic redistribution of ADP-ribosylation factor 1 (ARF1, the master regulator of membrane trafficking) and COPI (a set of seven proteins under the control of ARF1 that is necessary for formation of retrograde transport carriers), and the collapse of the Golgi complex into the ER. This phenotype induced by the overexpression of the KDEL receptor is similar to that generated by treatment of cells with the fungal toxin brefeldin A (BFA) [51]. BFA inhibits the ARF guanine nucleotide exchange factors (ARFGEFs), which reduces the activation of ARF, prevents recruitment of COPI to the Golgi complex, and promotes redistribution of the Golgi membranes into the ER.

An extensive study of the KDEL receptor has led to the identification of several interactors through which the KDEL receptor can affect the early secretory pathway. Indeed, the KDEL receptor can physically interact with ARF1, ARF GTPase-activating protein 1 (ARF-GAP1), β-COP (a subunit of COPI), the p24 family of Golgi/ER transmembrane proteins (components of the ARF1 complex) and several SNARE proteins, including mSec22b, mUse1 and mSec20/BNIP1 [46,52–54].

To shed more light on the behaviour of different KDEL receptor interactors, the spatio-temporal relationships between these interactors and the KDEL receptor have been investigated following the binding of KDEL ligands. This binding induces KDEL receptor dimerisation/oligomerisation [46], strongly increases its interactions with ARFGAP1, ARF1 and COPI, and decreases its interactions with the p24 family proteins [52].

Of note, since the formation of KDEL receptor interaction complexes depends on the activation status of the KDEL receptor [46,55], this might explain the BFA-like phenotype that can be seen in cells overexpressing the KDEL receptor. Here, KDEL receptor auto-activation recruits large amounts of ARFGAP1, which leads to the complete inactivation of ARF1. As indicated, ARF1 inactivation is at the basis of the Golgi redistribution phenotype promoted by BFA [51].

To summarise, the available data demonstrate that during membrane transport, chaperones enter the secretory pathway in cargo-containing carriers and arrive at the Golgi complex. Here, they bind to the KDEL receptor, for the recruitment of a number of interactors, which leads to the formation of retrograde transport carriers, and finally to the retrieval of KDEL-containing chaperones and the KDEL receptor to the ER.

5. Exploitation of the KDEL receptor for cell intoxication

As an endogenous system for retrograde transport of proteins, the KDEL receptor can be hijacked by toxins that need to be transported retrogradely along the secretory pathway to the ER. Some of the bacterial toxins enter the cell through the endocytic pathway, and cross the secretory system to reach the ER, where they use the ER translocon sec61p complex to enter the cytosol and bind to their targets [56,57]. Among these, some protein toxins, like the A-fragment of cholera toxin, the heat labile toxin LT1 produced by *Escherichia coli*, and *Pseudomonas* exotoxin A, have evolved a KDEL or a KDEL-like motif at their C-terminus to exploit the KDEL receptor as a shuttle for more efficient transport from the Golgi complex to the ER. Although important, this transport mechanism is not essential for some toxins; indeed, mutation of the KDEL sequence in the A-fragment of cholera toxin does not completely inhibit its toxicity. This suggests that retrograde transport of the cholera toxin A-fragment can also use a pathway that is independent of the KDEL receptor [58].

A particular system of retrograde transport is seen in *Pseudomonas* exotoxin A. This toxin ends with an RDELK sequence, and as such, it cannot bind to the KDEL receptor. However, prior to entry into host cells, the lysine of the RDELK sequence is removed by proteases, and thus the toxin acquires the ability to bind to the KDEL receptor in the Golgi complex, for its retrograde transport to the ER [59]. Furthermore, intoxication by *Pseudomonas* exotoxin A is strictly dependent on the KDEL receptor retrieval system, since mutations in the RDELK signal, microinjection of antibodies against the KDEL receptor, or expression of lysozyme-KDEL (which saturates the KDEL receptor retrieval activity) protect against *Pseudomonas* exotoxin A [60]. It would therefore be worth investigating the possibility of pharmacologically antagonising the KDEL receptor as a tool to reduced intoxication by these toxins.

6. Regulation of KDEL receptor cycling

The retrograde transport of the KDEL receptor from the Golgi complex to the ER can be modulated by the tyrosine kinase Src and by serine/threonine protein kinase A (PKA). Src is the founding member of the Src family kinases (SFKs), which are involved in the regulation of several cellular functions, including cell proliferation, survival, differentiation, migration, cytoskeletal rearrangements, secretion and other biological activities. SFKs have also been shown to be downstream effectors of GPCRs and tyrosine kinase receptors [61,62]. Interestingly, Src participates in different aspects of KDEL receptor functioning, including modulation of its retrograde transport [63]. Specifically, the overexpression of a constitutively active Src redistributes the KDEL receptor out of the Golgi complex, and the trafficking rate of the *Pseudomonas* exotoxin from the Golgi complex to the ER is reduced. It has thus been argued that decreased availability of the KDEL receptor in the Golgi complex slows the rate of retrograde transport of the *Pseudomonas* exotoxin [63].

PKA is a central player in the cAMP–PKA signalling pathway, and it is involved in the regulation of many trafficking steps, including Golgi-to-ER retrograde transport, intra-Golgi transport and Golgi-to-plasma membrane transport [64,65]. Of note, retrograde transport of the KDEL receptor depends on its phosphorylation by PKA (Fig. 1), in a model again reminiscent of the regulation/internalisation of GPCRs. Phosphorylation of the KDEL receptor by PKA on S209 exposes a hidden dilysine motive that is necessary for the interaction of the KDEL receptor with the COPI/ARFGAP1 complex and subsequent retrograde transport. Replacement of this S209 with the non-phosphorylatable alanine markedly affects retrograde transport of the KDEL receptor [48]. Many questions, how-

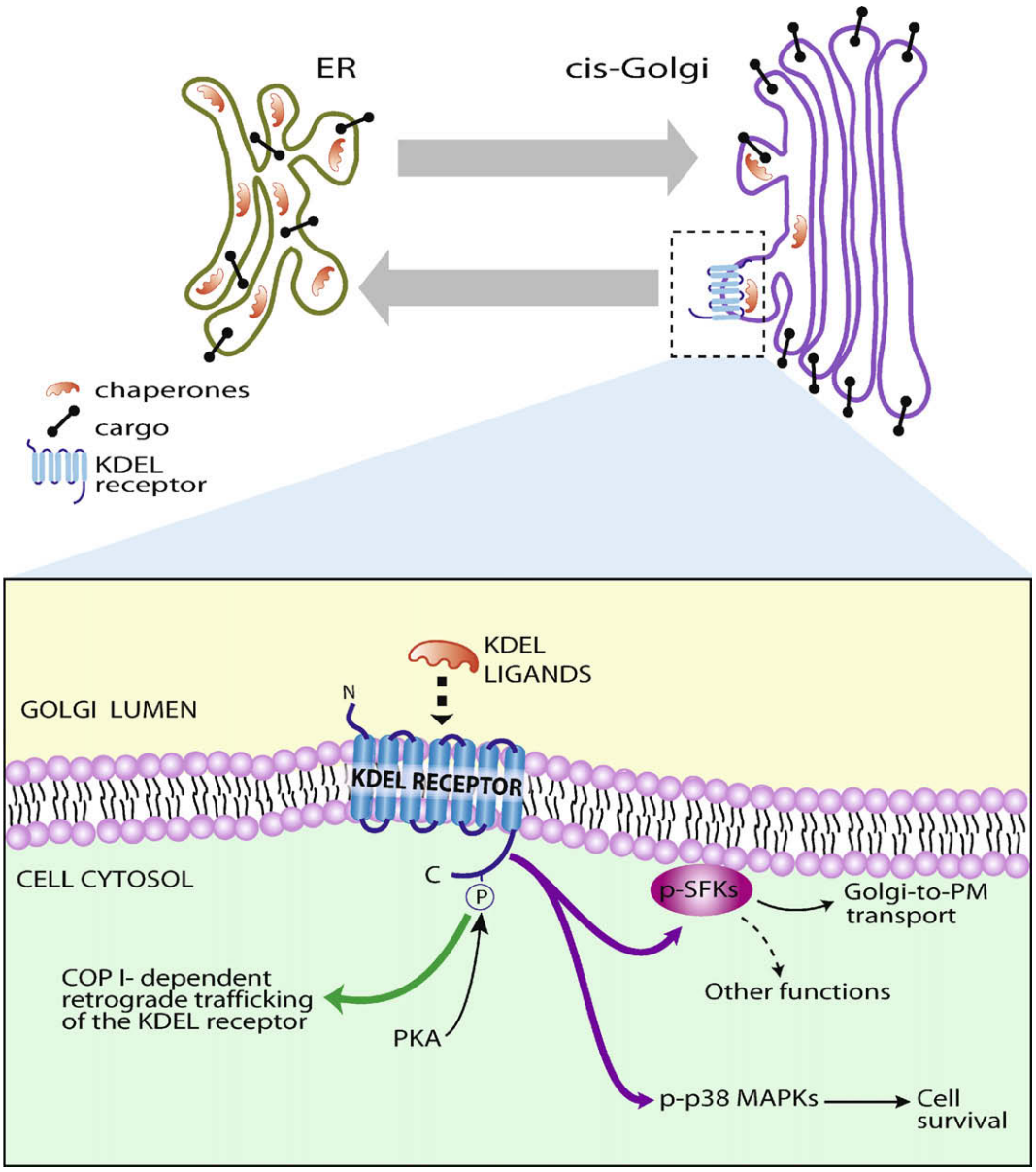

Fig. 1. Model of the KDEL receptor signalling functions and their regulation. Chaperones (the KDEL ligands) containing KDEL sequences (red, umbrella-shaped) can leave the ER during normal anterograde transport or cell stress. On arrival at the *cis*-Golgi, they bind to the KDEL receptor, triggering phosphorylation of SFKs (p-SFKs) and p38 MAPKs (p-p38 MAPKs). SFK activation is required for Golgi-to-plasma-membrane transport, and it appears relevant for the regulation of other cellular functions, while activation of MAPK signalling can promote cell survival. Cross-talk of the KDEL receptor with PKA arises via PKA-dependent phosphorylation of the C-terminal of the KDEL receptor, which also appears to be relevant for the modulation of COPI-dependent retrograde transport of the KDEL receptor.

ever, still remain unanswered. For instance, how and when PKA is activated, how KDEL receptor phosphorylation is coordinated with the needs of retrograde transport, and what are the players involved in this signalling pathway.

7. The KDEL receptor participates in the ER stress response

Since its discovery in yeast, it has become apparent that the KDEL receptor has additional functions besides its chaperone-retrieval activity. This also arose as the *erd2*-deleted mutant yeast phenotypes (Golgi-transport impairment, glycosylation defects, lethality) cannot be explained on the basis of chaperone leakage alone.

The functions that are now attributed to the KDEL receptor include a role in the regulation of ER quality control [66], which is a mechanism to ensure that only correctly folded proteins are exported from ER. An increase in misfolded proteins in the ER results in the ER stress response, which activates several signalling pathways, leading to overexpression of ER chaperones, inhibition of protein synthesis, and increased ER-associated degradation of misfolded proteins. As a result, activation of the ER stress response might be either cytoprotective or proapoptotic for a cell, although the molecular mechanisms that discriminate between these two fates are poorly understood.

Aoe and co-workers (2003) reported that the KDEL receptor is associated with stress-dependent activation of p38 mitogen-activated protein kinases (MAPKs; Fig. 1) and c-Jun amino-terminal kinases (JNKs) [67]. Indeed, expression of the R169N-mutated KDEL receptor suppresses ER-stress-response-dependent activation of p38 and JNK1, but not of JNK2, indicating that besides its retrieval activity, the KDEL receptor can modulate MAPK signalling. These cells cannot recognise and retrieve chaperones to the ER [34] be-

cause this mutant KDEL receptor is also more sensitive to ER stress [67].

Since MAPK signalling has been implicated in a variety of processes, such as cell development, differentiation, survival and death [68], it is conceivable that KDEL-receptor-dependent modulation of the MAPK signalling pathway is important in both physiological and pathological processes.

8. KDEL receptor function in the animal model

Although the role of the KDEL receptor in yeast and mammalian cells has been widely investigated, its function in a wider context such as in whole organisms remains poorly explored. To address the function of the KDEL receptor in vivo, Aoe and co-workers (2004) generated a transgenic mouse that stably expressed the D193N-mutant of the KDEL receptor, which acts as a dominant negative versus the wild-type KDEL receptor [19,34,69]. This D193N-mutant KDEL receptor is characterised by impaired trafficking from the Golgi complex to the ER, and it also impairs intra-Golgi and post-Golgi transport [19,34]. Furthermore, in line with its involvement in ER quality control, this KDEL receptor also accumulates ubiquitinated protein aggregates, and perturbs ER-to-Golgi transport [69].

Transgenic mice expressing this D193N mutant KDEL receptor have problems in breathing, show reduced movement, and generally die at about 14 months. These symptoms arise from a dilated cardiomyopathy that is characterised by increased ventricular chamber size, reduced contractility of the heart, and enlarged cardiomyocytes. The cardiomyocytes show different signs that are typical of chronic heart failure, including proliferation of the sarcoplasmic reticulum, formation of abundant misfolded protein aggregates, reduced density of functional L-type Ca^{2+} channels, and overexpression of atrial natriuretic factor. Further to this, the presence of interstitial fibrosis and the overproduction of the proapoptotic transcription factor CHOP indicate that mutation of the KDEL receptor can render the heart more sensitive to ER stress, thus promoting apoptotic cell death and the development of a dilated cardiomyopathy [69].

9. The KDEL receptor triggers activation of Src family kinases

A recent study from our laboratory has provided conclusive evidence of the signal transduction abilities of the KDEL receptor. We have demonstrated that upon arrival of incoming traffic at the Golgi complex, KDEL receptor engagement triggers activation of SFKs on the Golgi complex (Fig. 1) [1,19,70]. The central role of the KDEL receptor in this process was revealed by using multiple approaches, including the use of the D193N dominant-negative mutant of the KDEL receptor, microinjection of an antibody against the cytosolic tail of the KDEL receptor, and knock-down of the KDEL receptor using siRNAs. These approaches all markedly reduced the activation of SFKs on the Golgi complex that accompanied the arrival of incoming traffic from the ER. We also provided evidence that activation of the KDEL receptor is sufficient to stimulate SFKs on the Golgi complex, for example, by ligands arriving at the Golgi complex anterogradely from the ER or retrogradely from the endocytic compartment, or with the simple overexpression of the KDEL receptor (a condition previously shown to self-activate the KDEL receptor).

In our model, the signal generated by the incoming traffic at the Golgi complex is provided by the chaperones that exit the ER during the secretory process. When these reach the *cis*-Golgi compartment, they bind to the KDEL receptor, which thus triggers activation of SFKs, the tyrosine kinase activities of which are essential for the progression of cargo molecules through and from the Golgi complex to the plasma membrane (Fig. 1) [1,19].

In summary, we believe that a system is in place in the cell that can sense the amount of incoming traffic at the Golgi complex. Although we cannot at this stage formally exclude that other cellular compartments are targeted by this signalling, the transmission of this information from the incoming cargo prepares the Golgi complex itself to handle the arriving cargo and to ultimately send it on towards the correct final destinations. Thus far, the molecular players involved in the signal transduction from the KDEL receptor to the SFKs remain unknown, as do the SFK effectors that are involved in the regulation of Golgi transport. However, considering the topological organisation of the KDEL receptor and our unpublished data that heterotrimeric G-proteins can interact with the KDEL receptor, we believe that the KDEL receptor transduces signals through a mechanism similar to that of the GPCRs.

10. Puzzling KDEL receptor interactors

Most of the known KDEL receptor interactors reported above are consistent with the established cycling of the KDEL receptor between the ER and the Golgi complex for the recovery of KDEL-containing chaperones that have 'escaped' from the ER. However, systematic analyses in yeast and human have revealed unexpected genetic and physical KDEL receptor partners, which would suggest additional functional roles for the KDEL receptor. Here, we report the identification of some of these unexpected KDEL receptor partners.

The first genetic screen aimed at identifying the suppressor of *ERD2* deletion (see above) identified three proteins involved in the ER-Golgi transport pathway (*SED2, SED4, SED5*), along with a stress-response gene (*SED1*), a gene involved in the synthesis of ergosterol (*SED6*), and a gene involved in the formation of GPI anchors (*SED3*) [10]. Fields and co-workers [71] later revealed seven additional KDEL receptor physical partners: three cell-membrane permeases (TAT1, TPO3 and CAN1, specific for tryptophan, polyamine and arginine, respectively); a cell membrane glucanosyltransferase (GAS3); a subunit of the signal peptide recognition system (SPC1); a protein involved in the formation of GPI anchors (GPI8); and a protein involved in ergosterol synthesis (ERG25). Notably, the latter two interactors are consistent with previously reported genetic screening results [10].

With the aim of analysing the functioning of the early secretory pathway in yeast, another study reported that two subunits of the co-chaperone complex involved in the folding of microtubules (GIM3 and GIM4) have epistatic relationships with the KDEL receptor [72]. In addition, the KDEL receptor has a synthetic lethal relationship with Ire1p, an ER protein crucial for the unfolded protein response [73].

Regarding the mammalian KDEL receptor, a mass-spectrometry-based study that was aimed at providing a better understanding of the functions of potentially disease-associated human genes found the KDEL receptor as a partner of four new proteins [74]: Serinc3, a protein involved in the metabolism of serine at the plasma membrane; BZW1, a leucine-zipper protein potentially involved in transcription regulation; NEK6, a serine/threonine kinase involved in mitosis progression that regulates the formation of the mitotic spindle; and WBP5, the function of which remains to be clarified.

In conclusion, there are at least 14 KDEL receptor partners for which the potential functions do not appear to be compatible with the known primary role of the KDEL receptor, thus suggesting that it has novel roles in the functioning of the cell. We can, for instance, hypothesise involvement of the KDEL receptor in lipid metabolism, through its relationships with proteins that participate in the formation of GPI anchors and in cholesterol synthesis. Fur-

thermore, its relationships with different amino-acid transporters and with NEK kinase would suggest an involvement in cell growth.

A word of caution is needed, however, as many of these interactors arise from genetic studies, and might thus represent very indirect relationships. Physical interaction data has been obtained through large-scale, two-hybrid screening, which again is prone to generating false-positive results. Furthermore, the interactors of the human KDEL receptor identified by mass spectrometry analysis and co-immunoprecipitation of overexpressed baits could still represent false-positives, despite all of the possible controls that have been included; in addition, this approach identifies direct and indirect KDEL receptor partners as parts of large complexes.

Despite these potential pitfalls, we believe that a strong case can be made for additional functions of the KDEL receptor, although these require further investigation.

11. Concluding remarks

The KDEL receptor represents an important class of proteins that is necessary for the recovery of ER-resident chaperones from the late secretory compartments back to the ER. This role can be envisaged as part of a wider function in the coordination of ER quality control and the unfolded protein response. In addition, the KDEL receptor synchronises the whole secretory system, since the binding of chaperones also triggers a signalling cascade that is involved in the regulation of secretion at the Golgi complex. At present however, some of the identified KDEL receptor interactors remain difficult to position within this scenario, thus suggesting additional KDEL receptor function(s).

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