A systematic review of Sec24 cargo interactome

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Abstract
Endoplasmic reticulum (ER)-to-Golgi trafficking is an essential and highly conserved cellular process. The coat protein complex-II (COPII) arm of the trafficking machinery incorporates a wide array of cargo proteins into vesicles through direct or indirect interactions with Sec24, the principal subunit of the COPII coat. Approximately one-third of all mammalian proteins rely on the COPII-mediated secretory pathway for membrane insertion or secretion. There are four mammalian Sec24 paralogs and three yeast Sec24 paralogs with emerging evidence of paralog-specific cargo interaction motifs. Furthermore, individual paralogs also differ in their affinity for a subset of sorting motifs present on cargo proteins. As with many aspects of protein trafficking, we lack a systematic and thorough understanding of the interaction of Sec24 with cargoes. This systematic review focuses on the current knowledge of cargo binding to both yeast and mammalian Sec24 paralogs and their ER export motifs. The analyses show that Sec24 paralog specificity of cargo (and cargo receptors) range from exclusive paralog dependence or preference to partial redundancy. We also discuss how the Sec24 secretion system is hijacked by viral (eg, VSV-G, Hepatitis B envelope protein) and bacterial (eg, the enteropathogenic Escherichia coli type III secretion system effector NleA/EspI) pathogens.

KEYWORDS
cargo, COPII, ER export motif, ER Golgi transport, Sec24, Sec24 paralog

1 | INTRODUCTION

Gene duplication is one of the key drivers of functional diversification during evolution. Closely related gene pairs often exhibit functional redundancy but over time also evolve to execute distinct functions. One such well-studied example of gene family duplication and functional diversity is seen in the coat protein complex-II (COPII) members. COPII subunits form vesicles that package and transport proteins from the endoplasmic reticulum (ER) to the Golgi, the first transport step in the classical secretory pathway. COPII cargoes include a diverse range of proteins comprising of secretory (eg, cytokines), extracellular matrix (ECM), organellar and signalling proteins as well as large complexes such as lipoproteins (~500 nm). Accurate conveyance of newly synthesised proteins is a prerequisite for all aspects of cellular functions, including organellar maintenance and response to the extracellular environment.

Structurally, the core COPII complex is an icosidodecahedral coat consisting of an inner, Sar1-Sec23-Sec24 lattice and outer layer of Sec13-Sec31 heterotetramers. The GTPase Sar1, activated by Sec12, is recruited at the ER membrane, which is followed by binding of Sec23-Sec24 heterodimer. Sec23-Sec24 acts as cargo selection platform and forms the pre-budding complex. Sec13-Sec31 joins the complex after cargo capture. Sec23 stimulates Sar1-GTP hydrolysis leading to vesicle fission. The fact that there is a highly diverse repertoire of cargo needed to maintain both steady-state and tissue-specific demand, as well as cater to changing cellular requirements,
Sec24 (Figure S2). The other records include Sec24 paralogs in yeast, Drosophila, Escherichia coli, Plasmodium, and plants. Considering the vast literature on ER-Golgi export studies, to comprehensively identify all known Sec24 cargo proteins and their Sec24-binding motifs, we conducted a systematic search using a PRI-SMA approach followed by a qualitative assessment of the studies. We have extended our analysis to obtain a thorough overview of cargo recognition specificity by synthesising the existing data of ER export motifs in a paralog-specific manner. In all, this study represents a comprehensive analysis to capture both redundancy and diversity displayed by Sec24 paralogs in context of cargo recognition.

2 | RESULTS

2.1 | Search results and overview of literature distribution

Sec24 plays the vital role of cargo recruiter of COPII complex and it either binds directly to the cargo proteins or indirectly via adaptor proteins called cargo receptors (Figure 1). To identify the known Sec24 paralog-specific cargo interactome, a literature search was performed on PubMed, Scopus and Web of Science to extract papers that study the interaction between Sec24 and its cargoes. Briefly, the title, abstract and keywords were searched with a curated combination of entry terms, described in Methods, to obtain research articles on Sec24-binding proteins and motifs. Papers focused on COPII coat organisation and structure or on ER exit regulation were excluded because they were not of interest to this review. Highly specific results comprised Sec24 papers that contained keywords of ‘interaction’ or ‘binding’ of ‘cargo’ or ‘motifs’ and of ‘cargo sorting’ or ‘ER export’. The title and abstract of the 136 records obtained from the database searches and the 33 records obtained from complementary manual searches were screened according to the exclusion criteria detailed in Methods, to obtain a total of 76 articles included for full-text review (Figure S1).

Species-wise categorisation of the 76 articles revealed that 45 records studied mammalian Sec24 while 31 records studied non-mammalian Sec24, of which 20 studied Saccharomyces cerevisiae yeast Sec24 (Figure S2). The other records include Sec24 paralogs in Trypanosoma brucei,16 Drosophila,17,18 Plasmodium,19 plants20-25 and zebrafish26 (Figure S2 and Table S1).

Classification of the yeast Sec24-binding proteins based on cellular function revealed that the field is heavily skewed towards ER-Golgi transport proteins such as SNAREs and cargo receptors followed by proteins involved in ion exchange (Figure 2A and listed in Table 1).
Comparatively, as shown by protein location analysis, a minority of the yeast studies were done on specific Sec24 cargoes that are secreted or localised to the plasma membrane (PM), cytosol or vacuole (Figure 2B) such as PM ATPases Pma1p and Yor1p.

For the mammalian homologue, most studies focused on cargoes, mainly PM proteins while fewer focused on secreted, endosomal or cytosolic proteins (Figure 3A). Approximately one-fifth of the mammalian studies of Sec24-binding proteins were done on non-cargo ER-Golgi transport proteins (Figure 3B). The most represented functional groups of the identified mammalian Sec24 cargoes were ion exchange channels and compound transporters (Figure 3B). The remaining PM or ECM cargo proteins identified in this systematic review included cell-cell adhesion and ECM proteins (E-cadherin, claudin-1, collagen VII and procollagen), G protein-coupled receptors (GPCRs; α2B- and β2-adrenergic receptors, CCR5 chemokine receptor, GPR15 and autotaxin), amyloid proteins and an immune system protein (glycoprotein CD59). Interestingly, several proteins broadly involved in lipid synthesis and regulation were identified, including apolipoprotein B-100 involved in lipoprotein formation, PCSK9 involved in plasma cholesterol homeostasis and Sac1 (phosphatidylinositol-3-phosphatase) involved in lipid level regulation.

2.2 | Methodological characteristics of the Sec24 studies

To better interpret the collective findings, the 76 studies were further subjected to methodological assessment. Most of the yeast studies...
| Sec24 paralog | Sec24-binding protein* | Binding motif | Paper references |
|--------------|-----------------------|---------------|-----------------|
| Sec24p       | Atg9 (autophagy machinery component) | – | [27] |
|              | BMV 1a (Brome mosaic virus) via Erv14 (cargo receptor) | – | [28] |
|              | Shr3p (chaperone to package Gap1p) | – | [29] |
|              | Erv41p-Erv46p complex | Dihydrophobic motifs (IL in Erv41p and FY in Erv46p) both required together for export of complex | [30] |
|              | Axl2 (TM secretory protein cargo) via Erv14p (cargo receptor) | IFRTL in Erv14 | [31] |
|              | Erv14 | IFRTL | [32] |
|              | Yor1p (ATPase) | Dihydrophobic (DxE) | [32,33] |
|              | Prm8p | Dihydrophobic: FF in C terminus | [34] |
|              | Gap1p, Bos1p | – | [35] |
|              | Sec22p (SNARE) | – | [35] |
|              | – | – | [36] |
|              | Sys1p | Diacidic: DxE | [34] |
|              | – | Diacidic: DxE at C terminus | [38] |
|              | – | Diacidic: DxE | [37] |
|              | Bet1p (SNARE) | LxxLE | [37] |
|              | – | – | [34] |
|              | – | – | [35] |
| Sec24p and Iss1p | Sed5p (SNARE) | Bipartite signal: YNNSNSPF (primary) and LxxME (secondary) | [37] |
|              | – | YxxoNPF (in open conformation) | [39] |
|              | – | – | [40] |
| Sec24p and Lst1p and Iss1p | – | – | [41] |
| Lst1p        | Atg40 (ER-phagy receptor) | – | [12] |
|              | Gas1p (GPI-anchored PM protein) | – | [41] |
|              | Pma1p (ATPase) via Ydl121c (cargo receptor) | – | [42] |
|              | Emp24 and Env25 | Dihydrophobic (FF on Emp24, YF on Env25) | [43] |
|              | Pma1p (ATPase) | – | [44] |
| Sec24p and Lst1p | – | – | [35] |
|              | – | – | [45] |
|              | Sit4p (regulator of COPII coat depolymerisation) | – | [46] |
|              | Û-factor precursor | – | [35,45] |
|              | – | – | [36] |
| Sec24p and Iss1p | – | – | [35] |
| Sec24p and Lst1p and Iss1p | CPY (carboxypeptidase) | – | [41] |
|              | ALP (alkaline phosphatase), invertase | – | [41] |

*aFor cargoes that interact with Sec24 by binding a cargo receptor which itself binds Sec24, the cargo receptor is introduced in italics.*
relied on in vitro COPII vesicle formation assay and pulse-chase techniques to test paralog specificity. This was complemented with X-ray crystallography and affinity titrations by fluorescence polarisation to measure the effects of alanine substitutions (Table S1). One study of plant Sec24 (Vicia faba) used in vivo FRET to confirm Sec24 binding.21

Based on the methodological assessment (Table S2), the mammalian Sec24 studies were categorised based on the cell types used (Figure 4A,B), the binding of proteins to Sec24 (Figure 4C,D), their paralog specificity (Figure 4E,F) and binding motifs (Figure 4G,H). In all, 22 epithelial cell types were used, mostly human cell lines and few non-primate cells (Figure 4A). The diversity of cell types benefits the field of Sec24 interactome studies, as proteins traversing the secretory pathway may vary with cell type. For instance, a study by Chen et al implicated that only Sec24A and B are involved in PCSK9 secretion in 293T cells whereas a later study by Deng et al reported that Sec24A, B and C mediate PCSK9 secretion in Huh7 cells.47,48 Although 50% of the mammalian studies used one cell line,
often HEK293 or HeLa transfected with the protein of interest (POI), 36% of the studies used two cell lines (Figure 4B), complementing the use of transfected cells with the use of another cell line with endogenous POI expression.

To identify Sec24-binding to the POI, most studies (60%) used a single technique, while a quarter of the studies did not experimentally verify physical Sec24-POI binding (Figure 4C). In such studies, Sec24-POI binding was either deduced from previous literature, or by confirming Sec24-driven ER export as cargoes are known to be sorted for ER export by direct or indirect interactions with Sec24. The most frequently used techniques to verify Sec24-POI binding were immunoprecipitation (IP) and pulldown assays, coupled with immunoblotting of the POI or liquid chromatography-mass spectrometry (LC-MS) or MS/MS for identification of the binding protein (Figure 4D and listed in Tables S2 and S3). Few studies used two techniques such as pulldown combined with fluorescence resonance energy transfer (FRET) or with X-ray crystallography, or a combination of molecular homology modelling with FRET or with IP. One study validated binding using pulldown, yeast two-hybrid (Y2H) assays and protein-protein interaction ELISA while another confirmed binding through four assays, consisting of pulldown followed by LC-MS/MS or by Western blot to confirm the LC-MS/MS findings, complemented by IP performed in two different cell types.

To delineate paralog specificity, most studies (50%) tested all four Sec24 paralogs (Figure 4E). For such studies, the most common method to verify the Sec24 specificity was RNA interference (RNAi) silencing of the paralogs (Figure 4F) followed by a downstream assay to test the function or location of the POI. Studies that tested only two of the four paralogs either did not provide an explanation for their choice, or in the case of one study, justified the use of Sec24A and Sec24C as representatives of Sec24A/B and Sec24C/D, respectively, because of their sequence identities.

To identify Sec24-binding motifs, approximately a third of the studies relied on motifs that were previously identified in literature or identified new binding motifs from empirical testing from untargeted mutations or from targeted mutations after identification of conserved sequences (Figure 4G). Upon identification of a motif, a variety of assays were used to confirm the motif’s role in export, most commonly functional assays of cargo packaging into COPII or localization assays (Figure 4H). Further, few studies relied on X-ray crystallographic and biochemical assays to dissect mechanistic details of cargo discrimination by specific paralogs.

2.3 Sec24-paralog specificities of cargo proteins

From compiling of all known Sec24-binding cargoes obtained from this review, we first aimed to identify groups with differing Sec24 paralog specificities. Sec24p binds specifically to many cargoes such as the autophagy machinery component Atg9, Erv14, Sys1p and Shr3p chaperone (Table 1). Specific cargoes for Lst1p include PM proteins Pma1p and Gas1p, as well as ER-Golgi resident proteins Emp24 and Erv25. Carboxypeptidase, alkaline phosphatase and inverterase were shown to be clients of all three yeast paralogs.

For mammals, as shown in Table 2, cargoes specific for Sec24 paralogs have been reported, for example, E-cadherin reported to be a Sec24A client, Vangl2 a Sec24B client, and various compound transporters and ion channels specific for Sec24 A, C or D. The neurotransmitters, noradrenaline and glycine and γ-aminobutyric acid (GABA) transporters (DAT, NET, GLYT1 and GAT1 respectively) were identified to exclusively bind to Sec24D while serotonin transporter (SERT) is an exclusive client of Sec24C (Table 2).

Interestingly, cargoes specific for more than one paralog were also identified (Table 2). Some cargoes were found to specifically bind either to Sec24A/B or Sec24C/D, which may be explained by the higher sequence identities within each paralog pair. Exceptions to these A/B or C/D pair-specific interactions can be seen for the Sec24A- and D-specific potassium channel Kv1.3 and the Sec24B-specific and D-specific GPCR ER exit regulator CNIH4. However, it should be noted that both these studies did not test all four paralogs, hence Sec24 paralog specific binding cannot be concluded. Intriguingly, the finding on Sec24B-specificity and D-specificity of CNIH4 by Sauvageau et al contrasted with the SILAC-based proteomics study by Adolf et al which identified CNIH4 to be preferentially sorted into Sec24A vesicles.

Non-cargo proteins involved in the biogenesis of COPII vesicles were also found to possess Sec24-paralog specificities, as highlighted in Table 3. The binding motifs, location, function and full methodological assessment for non-cargo proteins have been summarised in Table S3. The vesicle trafficking protein Sec22b was consistently found to bind exclusively to Sec24A and B.14,62,81 The Q-SNARE proteins syntaxin-5, GS27 and Bet1 are sorted into COPII vesicles through the formation of a complex where syntaxin-5 directly interacts with Sec24C or D (Table 3). The cargo receptor ERGIC53 was identified as a Sec24A-dependent client in one study and a B-dependent client in another (Table 3), whereas ERGIC1 was identified to be a Sec24C/D-dependent client.15 From all the systematically collected studies in this review, Sac1 and procollagen was experimentally shown to equally interact with all four Sec24 paralogs. Efficient ER export of Sac1 is mediated by the adaptor protein TMEM39A/SUSR2.

2.4 Sec24-binding motifs

Because of the complexity of Sec24 paralog cargo specificities, better understanding of the binding at the molecular level is needed, which could be achieved through identification of binding motifs on the cargoes. Sec24 binds to cargo through different ER export recognition sites. Many motifs have been identified, ranging from specific short amino acid sequence motifs to conformational features such as a correct folding or a certain signature chemical property on a tertiary structure. To elucidate the Sec24 paralog specificity of the
### TABLE 2  Sec24 paralog-specific cargo proteins and the associated binding motif from the 45 mammalian Sec24 studies

| Sec24 paralog | Sec24-binding protein | Binding motif | Paper references | Sec24 paralog tested<sup>b</sup> |
|---------------|-----------------------|---------------|------------------|---------------------------------|
| Sec24A        | PC-I (procollagen)<sup>c</sup> | Diacidic (DxE) | [53]             | All 4                           |
|               | E-cadherin            | Diacidic (DxE) | [53]             | All 4                           |
|               | Kv1.3 (potassium voltage-gated channel) | Triacidic (ExExE) | [55]             | All 4                           |
|               | PS1 (presenilin-1)<sup>d</sup> | -             | [54]             | All 4                           |
|               |                      | Diacidic (DxE) | [63]             | All 4                           |
| Sec24A and B  | PCSK9                 | -             | [47]             | All 4                           |
| Sec24A, B and C |                        | -             | [48]             | All 4                           |
| Sec24B        | Vangl2                | Looptail D255 and S464 | [10]           | All 4                           |
| Sec24C        | ATB0+ (amino acid transporter) | RlnK          | [64]             | All 4                           |
|               | Claudin-1              | YV            | [61]             | All 4                           |
|               | ATX (autotaxin) via p23 | -             | [49]             | All 4                           |
|               | AE1 (anion exchanger)  | Hydrophobic motif (ΦXΦXΦ) (V/L/F)X(I/L)X(M/L) | [65] | All 4                           |
|               | SERT (serotonin transporter) | RlnK          | [51]             | All 4                           |
|               | Cholesterol and triglycerides via CTAGES (Mia2) | Proline-rich domain | [66] | C – none                         |
|               | Collagen VII export via TANGO-1 (Mia3) | Proline-rich domain | [67] | C – none                         |
|               | APP (amyloid precursor protein) | C-terminal region | [68] | C – none                         |
|               | FAM134B/RTN3           | -             | [12]             | All 4                           |
| Sec24D        | ENaC (epithelial sodium channel) assisted by ERp29 | -             | [69]             | D – [58]                        |
|               | ENaC (epithelial sodium channel) assisted by Hsp70 | -             | [70]             | D – [59]                        |
|               | DAT (dopamine transporter)  | KW and PYKR   | [72]             | D – representing               |
|               | NET (noradrenaline transporter) | -             | [52]             | All 4                           |
|               | GLYT1 (glycine transporter) | RLX8D, that is, R575 L576 (X8) D585 | [73] | D – [37]                        |
|               | GAT1 (GABA transporter) | RL            | [57]             | D – [61]                        |
|               | VMI and AYI            | VMI and AYI   | [74]             | D – representing               |
|               | GPR15 (GPCR)           | Dibasic (RR)  | [75]             | D – [92]                        |
| Sec24A and D  | Kv1.3 (potassium voltage-gated channel) | Diacidic signal (EE) | [58] | A,D – none                       |
| Sec24C and D  | α2B-AR (α2B-adrenergic receptor) | Triacidic (RRR) | [76] | All 4                           |
|               | CD59 via p24-p23 complex | -             | [50]             | All 4                           |
|               | Fibronectin 1          | -             | [90]             | All 4                           |
|               | Nicastriń<sup>d</sup> | -             | [63]             | All 4                           |
| Sec24B and D  | β2-AR (β2-adrenergic receptor) and CCR5 (chemokine receptor) via CNIH4 | -             | [77]             | B,D – none                       |
| All 4         | Sac1 (phosphatidylinositol-3-phosphatase) | -             | [87]             | All 4                           |
|               | PC-1 (procollagen)<sup>c</sup> | -             | [90]             | All 4                           |
| Unspecified   | ApoB100 (apolipoprotein B100) via CideB | -             | [91]             | ?                                |
|               | CRTR (chloride ion channel) | Diacidic (DxD) | [59,92] | ?                                |

<sup>a</sup>For cargoes that interact with Sec24 by binding a cargo receptor which itself binds Sec24, the cargo receptor is introduced in italics.

<sup>b</sup>For studies that did not test all four Sec24 paralogs, the paralogs used are indicated (A-D) and the justification for the choice of specific paralogs used is shown as citations of the relevant previous studies, or indicated as ‘none’ if there was no justification, or as ‘representing’ if the paralog was empirically chosen to represent all 4.

<sup>c</sup>Same cargo.

<sup>d</sup>Subunits of γ-secretase.
various ER export recognition sites, sequence motifs on cargoes and non-cargo proteins binding to Sec24 have been listed alongside the proteins in Tables 1 to 3.

In yeast, the most commonly found motifs include the diacidic (DxE) and dihydrophobic motifs. The PM ion-exchange protein Erv14 utilises the unique IFRTL motif to bind to Sec24p. In plants, both diacidic and triacidic motifs are the most studied ones (Table S1).

In mammals, 14 sequence motifs and 2 conformational motifs have been identified so far (Table 4). Diacidic motifs (D/E-x-D/E), which were found to be involved in the ER export of a diverse range of mammalian proteins including transmembrane proteins, potassium channels and the cystic fibrosis transmembrane conductance regulator (CFTR). Many dihydrophobic motifs such as FF, FY, LL or VV have also been identified, found on cargoes such as ion exchangers and non-cargo ERGIC53. Dihydrophobic motifs show redundancy in Sec24 paralog selectivity except LL motif mediated transport, which is dependent on Sec24A. In case of SERT, a single substitution of lysine to tyrosine in the Sec24-binding motif switched its binding preference from Sec24C to D.51 Further, cargo receptor-specific motifs were also identified, such as the IxM motif on syntaxin-5 and the YxxCE motif on Bet1 (Table 3). IxM motif binds specifically to a groove on Sec24C and D, demonstrating functional overlap between Sec24C and D.

TABLE 3  Sec24 paralog-specific non-cargo proteins and their associated binding motif from the 45 included studies

| Sec24 paralog | Sec24-binding protein | Binding motif | Paper references | Sec24 paralog tested |
|---------------|-----------------------|---------------|-----------------|---------------------|
| Sec24A        | HBV (Hep B virus) envelope S domain | Dibasic (RR) | [78]            | All 4               |
|               | VSV-G                 | Diacidic (DxE) | [53]            | All 4               |
|               | Sec22                 | –             | [62]            | A,C                 |
|               | ERGIC-53              | Hydrophobic (FF) | [79]          | All 4               |
| Sec24B        | ERGIC-53              | Dihydrophobic (FF/YY). Less strong binding to the functionally substituted (I/L)(I/L) and VV | [80] | B,C |
| Sec24A and B  | NleA                  | I1Q           | [60]            | All 4               |
|               | Bet1                  | YxxCE         | [14]            | All 4               |
|               | VSV-G                 | Diacidic (DxE) | [14]          | All 4               |
|               | Sec22                 | Folded epitope | [81]          | All 4               |
| Sec24C        | Syntaxin5 (complexed with GS27, Bet1) | IxM + open conformation of syntaxin5 | [62] | A,C |
|               | NleA                  | –             | [82]            | C                   |
| Sec24D        | Syntaxin5 (complexed with GS27, Bet1) | IxM         | [15]            | All 4               |
|               | Rab1                  | –             | [83]            | C,D                 |
| Sec24C and D  | NleA                  | IxI           | [60]            | All 4               |
|               | Syntaxin5 and membrin | IxM          | [14]            | All 4               |
| Unspecified   | Yip1A                 | –             | [84]            | ?                   |

TABLE 4  Occurrence of various Sec24 paralog-specific binding motifs of host proteins in the 45 mammalian Sec24 studies

| Sec24-binding motif | Sec24 paralog |
|---------------------|----------------|
|                     | A  | B  | C  | D  |
| Diacidic (D/E-x-D/E) |  6 |  1 |  2 |  1 |
| Triacidic (ExExE)   |  1 |  1 |
| Di-hydrophobic (F/Y-F/Y) |  1 |  1 |
| Tri-hydrophobic (ΦxΦxΦ) |  2 |  2 |
| Dibasic (RR)        |  1 |  1 |
| Tribasic (RRR)      |  2 |  1 |
| YxxCE               |  1 |  1 |
| Looptail D...S      |  1 |  1 |
| Proline-rich        |  1 |  1 |
| R/I/L               |  3 |  2 |
| YV                  |  1 |  1 |
| KW + PYRKR          |  1 |  1 |
| VMI                 |  1 |  1 |
| No motif: folded epitope |  1 |  1 |
| No motif: C-terminus |  1 |  2 |

Other unique motifs have been reported for specific cargoes, such as YV on claudin-1, the looptail comprising of D and S residues in Vangl2 and two synergistic motifs KW and PYRKR found on dopamine transporter (Table 2). It is yet to be studied whether these motifs
are also found on other cargoes or cargo receptors and whether the specificity of recognition is based on a particular physico-chemical characteristic rather than the specific amino acid sequences. Thus far, conformational motifs have been reported for Sec22 and syntaxin-5 (Table 3). Additionally, the ER export of cholesterol and collagen depend on the proline-rich domain of the related cargo receptors Mia2 and Mia3 (Table 2). Thus, as with the diversity of Sec24-paralog specificities of different cargoes, we further highlight the varying Sec24-paralog specificities of different motifs revealing both specificity and partial redundancy of Sec24 paralogs that is both cargo-dependent and motif-dependent (Table 1).

Further, from the studies included in this systematic review, binding motifs on cargoes that bind cargo receptors instead of Sec24 were also identified: the ‘FF’ motif on autotaxin binds p23,49 the ‘KEEL’ motif on ERp29 binds the KDEL receptor69 and the ‘LS’ motif on SAC1 binds the phosphoregulatory protein 14-3-3.87 These findings highlight an increased complexity in the Sec24-cargo interactions that involve various mediators such as cargo receptors that themselves bind to a wide repertoire of cargoes through specific motifs.

2.5 | Sec24 is a target of viral and bacterial virulence factors

Among the non-cargo proteins, along with cargo receptors and ER-Golgi transport proteins, we have included non-mammalian proteins such as vesicular stomatitis virus glycoprotein (VSV-G),14,53 Hepatitis B envelope protein (HBV-S)78 and NleA (aka EspI)60,82 which is injected into mammalian cells via a type III secretion system injectisome, expressed on the bacterial cell wall of enteropathogenic and enterohemorrhagic E. coli (EPEC and EHEC)88 (Table 3). Notably, these were shown to bind to mammalian Sec24 by mimicking the sorting motifs present in host cargo. Seminal work on VSV-G paved the way for studies on selective ER export. DxE motif on the cytoplasmic tail of VSV-G was one of the first motifs to be discovered and it was demonstrated to confer specificity for human Sec24A/B.89 HPV protein mimics a dibasic (RR) ER-export motif thus enabling direct interaction with Sec24A, which promotes HBV envelope trafficking and egress.79 Further, NleA/Espl interacts with Sec24 paralogs via dihydrophobic motifs, thus possibly interfering with secretion of cargoes. Further, mutations in Sec24 binding motifs in NleA greatly diminished the ability of the natural mouse pathogen Citrobacter rodentium to colonise and infect mice, thus underpinning the importance of NleA mediated inhibition of COPII in EPEC and EHEC pathogenesis.95 However, the nature of cargoes affected by NleA and the downstream effects of NleA-Sec24 interaction remains elusive.

3 | CONCLUSIONS AND FUTURE PERSPECTIVES

In this critical review, we have carried out a comprehensive, systematic analysis of Sec24 clients and their sequence motifs. Studies on yeast Sec24 pioneered the research on COPII coat structure and organisation as well as binding to SNAREs, inspiring later studies on mammalian homologues. Currently, interaction studies of Sec24 and ER-Golgi transport proteins are more abundant than Sec24-cargo. This distribution of Sec24-binding protein studies was reflected in our search results, whereby despite the search strategy being tailored to include cargo proteins, 33% of the included studies were on non-cargo proteins. Because our aim was to only include studies on Sec24 paralog-associated cargo export, the current search would have overlooked studies on cargoes that were found to bind to other COPII components but were not yet disproven to bind Sec24 too, making them interesting putative Sec24-binding cargoes that would require experimental verification. Considering that approximately one-third of all mammalian proteins rely on the COPII-mediated secretory pathway for membrane insertion or secretion,95 and the largest fraction of the tissue-enriched transcriptome codes for secretory proteins,94 a lot still remains to be discovered.

Delineating Sec24 paralog specific clients is experimentally challenging because of the low copy number of cargo molecules being transported at steady state levels, with <1 copy per vesicle required to maintain the most abundant transmembrane proteins.15 Only one study which opted for proteomics of the human Sec24 paralog-specific interactome observed that the identified proteins were not cargo proteins but cargo receptors that reside in the ER-Golgi compartments and proteins involved in tethering and fusion of vesicles such as SNAREs and GOT1B, thus highlighting the technical difficulty to assay Sec24 and cargo interactions. Further, Sec24B is prone to proteolytic degradation, so Sec24B paralog specific COPII proteome could not be elucidated.15 The methodological assessment of the included studies revealed that a high diversity of epithelial cell types from various tissues and mammalian species were used, although, importantly, not polarised cells except for MDCK cells, which were used in five papers.69-71,73,74 As polarity determines protein destination, it might also affect expression and binding specificities of the cargoes. Cellular factors could also impact the binding of two proteins, making methods that use cell lysates to test binding, such as co-IP and pulldowns, more relevant than Y2H assays to show that interactions occur in cellulo. Furthermore, protein overexpression studies in transfected cells may not guarantee that the same binding occurs with endogenous expression in physiological conditions. Because RNAi silencing methods do not lead to complete silencing, accurate assessment of paralog specific transport becomes technically challenging. Another limit is that when Sec24-dependent transport is halted, some proteins may rely on bulk flow to be transported albeit at a slower pace, as was shown for SERT. These findings underline a need for more sophisticated proteomics techniques such as cross-linking, proximity labelling and high-throughput data studies to elucidate the COPII cargo interactome.

Among the ER export motifs, diacidic and dihydrophobic motifs are the best characterised ones in both yeast and mammals. However, presence of existing ER export motifs should not be solely used to predict export of proteins. For instance, a study identified 100 cargoes that contained putative diacidic motifs and proposed that the functional ER export diacidic motifs seem to appear in a region with ordered structure and higher hydrophobicity.95 The importance of the dihydrophobic nature, rather than the specific residues, of the ER exit signal ‘FF’ in ERGIC-53 was found by replacing the di-phenylalanine...
FF' motif by di-tyrosine 'YY', di-valine 'VV', di-isoleucine 'II' or di-leucine 'LL' which mediated transport as efficiently if not more than the 'FF' motif.\(^\text{50,95}\) Indeed, the possession of a motif does not guarantee ER export or Sec24 binding, as was seen in a study where a Sec24C/D-binding 'KxM' motif nested in the 'DxE' diacidic motif of VSV-G did not bind Sec24C/D.\(^\text{34}\) Moreover, even with four different diacidic motifs identified in the potassium channel KAT1, only one was important for transport of KAT1 to the PM.\(^\text{23}\) Although this review focused on short sequence-based motifs that bind Sec24, other ER export recognition sites that rely more on conformational features exist, such as a correct folding or a certain signature chemical property on a tertiary structure. Indeed, Sec22 binds Sec24 with a folded epitope,\(^\text{81}\) syntaxin-5 binds Sec24 only in its open conformation,\(^\text{62}\) and Erv14 was suggested to recognize its specific cargos by their common TM domain length.\(^\text{86}\) Even the well-described Sec24-binding 'RI' motif in SERT was found to have a crucial role in the correct folding of the transporter, as the defective ER export resulting from the motif's mutation was because of impaired protein folding.\(^\text{97}\)

Nonetheless, characterisation of Sec24-cargo interactome is an ongoing, complex field of research and given the diversity of proteins trafficking via ER-Golgi secretory pathway, many more specific interactions and motifs are yet to be characterised. However, several factors need to be accounted for, which includes (a) diversity of cargo, (b) flux of ER export, (c) steady state concentration of cargoes, (d) uncharacterized binding motifs and (e) sensitivity of current analytical methods given the transient nature of interaction. Interestingly ER export motifs are often mirrored by pathogens to capitalise upon the secretory pathway and hence their understanding is crucial to decipher the dynamic interaction between host and pathogens. For instance, Hepatitis B virus also selectively intercepts the COPII pathway for intracellular transport via a direct interaction between di-arginine motif of HBV and Sec24A.\(^\text{78}\) Gastrointestinal pathogens such as EPEC and EHEC use NleA to compromise host trafficking by directly binding to Sec24,\(^\text{60}\) the functional consequences of which remain largely unknown. Nonetheless, deleting nleA from the EPEC-like mouse pathogen C. rodentium causes significant attenuation,\(^\text{60}\) illustrating the key role of hijacking the COPII pathway for infection. Therefore, NleA is an invaluable tool to study the physiological roles of Sec24 paralog and associated cargo. Manipulation of COPII export by selectively blocking a subset of cargo, for example, by cell-permeable peptides designed to block Sec24 paralog specific function, may be useful in treatment of ER stress-related diseases and cancer. A complete blueprint of Sec24 paralog specific cargo clientele and dissecting the binding motifs that may confer paralog specificity will enhance our understanding of the fundamental process of selective ER export.

4 | MATERIAL AND METHODS

4.1 | Search strategy

To conduct the systematic review, the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) search strategy was adopted according to its guidelines. The PRISMA diagram (Figure S1) depicts the different phases of the systematic review. An initial PubMed search using the term 'Sec24' yielded 364 articles. To increase the stringency of search, a finetuned combination of selected search terms were designed and optimised to extract the papers with the most potential for relevance to this review's research questions about Sec24-binding cargoes and motifs in the context of ER export and the secretory pathway: (sec24*) and (motif or cargo*) and (interact* or bind* or 'cargo-binding' or signal* or 'exit signal*' or 'export signal*') and (secretory pathway/cargo/protein or cargo sort*/adaptor/transport or traffic* or selective* export/sort* or export). This search query was then applied to Scopus and Web of Science. The literature search in PubMed was performed on April 12, 2020, and in Scopus and Web of Science on June 5, 2020.

To find all other relevant papers that would not have been found in the search strategy (for instance if the paper happened to not mention Sec24 in the title or abstract), literature cited in the papers collected from the above search strategy and reviews on COPII cargo export were manually searched, and potentially relevant studies were added to the study collection. The manual search was stopped when a loop was reached whereby no new studies were found. Of the total of 171 papers obtained from the three database searches and the manual search, 95 papers were excluded based on the exclusion criteria detailed in Figure S1, with the aim to only include studies on Sec24-dependent cargo export and Sec24-binding. The remaining 76 included articles underwent full-text review. A limitation in this review's search strategy is that, despite optimising the query terms combination, articles that studied relevant topics but did not have the searched terms in at least one of the titles, abstract or search engine keywords, would have not been identified.

4.2 | Data synthesis and analysis

To assess the findings of the included studies, the following information was extracted from each study: (a) the Sec24 paralog-specific binding protein(s) identified, (b) the binding sequence motif if applicable and (c) the binding protein's location and cellular function.

The methodological characteristics of the techniques used in the 76 articles were critically assessed by extracting the following information from each article: (a) the cell lines used, (b) the Sec24 paralogs tested, (c) the techniques used to test Sec24-cargo protein binding, and (d) the techniques used to identify the binding motif. Bar graphs were plotted using GraphPad Prism version 8.3.0 (GraphPad Software Inc., USA) and pie charts were plotted using Microsoft Excel in Office 365.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.
AUTHOR CONTRIBUTIONS
Sharanya Chatterjee: Conceptualised the manuscript. Ana Jeemin Choi: Performed the systematic search and data collection under supervision by SC. Gad Frankel: Conceptualised the manuscript, critically reviewed the manuscript. All authors participated in data analysis and manuscript writing. All authors read and approved the final manuscript.

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DATA AVAILABILITY STATEMENT
All data analysed in the current study are included in the manuscript and its supplementary information files.

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