Sec24D-positive ER exit sites sort raft-preferring proteins for rapid ER export.

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The specific determinants of subcellular trafficking are unknown for many membrane proteins. Although motifs responsible for cargo integration into envelope/adapter-mediated sorting schemes have been identified, these are insufficient to explain the kinetics of protein movement between organelles. Another potential factor in the organization and traffic of membrane proteins is the membrane nanodomains known as lipid rafts. These domains are small, dynamic assemblies of lipids and proteins that preferentially interact with each other. They are often associated with the plasma membrane but are probably also present in various endo-membranes. To assess the role of membrane nanodomains in the early secretory pathway, we use a robust tool for synchronized protein traffic known as RUSH (Retention Using Selective Hooks), in which tagged proteins can be retained by a resident “hook” in specific organelles and then rapidly released. We applied RUSH to a library of transmembrane domains (TMDs) to investigate the role of raft affinity in ER exit kinetics and the machinery involved. We found that raft-preferring TMD probes exit the ER faster than those without raft affinity and that they have different preferences for ER exit sites characterized by specific isoforms of sec24, the cargo adaptors that form the inner envelope of ER export vesicles. Namely, probes with raft affinity tend to localize to sec24D-positive exit sites, whereas probes without raft affinity tend to localize to sec24A-positive sites. Surprisingly, probes with identical COPII recognition motifs are sorted differently into the sec24D or sec24A exit sites based on the lipid raft affinity of their transmembrane domains. Consistent with this, sec24D, but not sec24A, ER exit sites accumulate a fluorescent cholesterol analog. These observations suggest that despite relatively low cholesterol concentrations in the ER, cholesterol-rich domains can sort proteins for rapid export from the ER.