Published in final edited form as:

Trends Biochem Sci. 2017 February ; 42(2): 90–97. doi:10.1016/j.tibs.2016.11.004.

Speed limits for non-vesicular intracellular sterol transport

Jeremy S. Dittman and Anant K. Menon

Department of Biochemistry, Weill Cornell Medical College, 1300 York Avenue, New York, NY 10021, USA

Abstract

Sterol transport between the endoplasmic reticulum (ER) and plasma membrane (PM) occurs by non-vesicular mechanisms requiring sterol transport proteins (STPs). Here we examine the idea that transport is enhanced at membrane contact sites where the ER is closely apposed to the PM. We conclude that sterol desorption from the membrane, rather than STP-mediated diffusion, is rate limiting in the cellular context, so there is no apparent kinetic benefit to having STP-mediated sterol transfer occur at contact sites. Contact sites may instead compartmentalize lipid synthesis or transport machinery, providing opportunities for regulation.

Keywords

Sterol transport protein; diffusion; membrane contact site; endoplasmic reticulum; Osh4; StARD4

Cholesterol has been called ‘the central lipid of mammalian cells’ [1], in part because of its profound ability to regulate membrane structure [2, 3]. Cells acquire cholesterol by synthesis and/or import, and the distribution of cholesterol in cellular membranes is strictly controlled [4, 5]. While many aspects of cholesterol homeostasis are well understood, much less is known about intracellular cholesterol transport mechanisms that are needed to move cholesterol from its point of entry or synthesis to other cellular compartments, and to guarantee that variations in cholesterol distribution within the cell are rapidly sensed and corrected [6]. Here we discuss the particular problem of sterol transport between the endoplasmic reticulum (ER) and plasma membrane (PM) in the context of new ideas about the role of subcellular architecture in this process.

Extraction from a membrane or diffusion through the cytoplasm: what sets the time scale of non-vesicular sterol transport?

Sterols are synthesized in the ER and transported by non-vesicular mechanisms to other cellular locations [7–10], especially the PM where they represent almost 40% of all PM lipids. In all cells, the ER is closely associated with the PM. In yeast this is an exceptionally

Correspondence: jed2019@med.cornell.edu (J.S. Dittman). akm2003@med.cornell.edu (A.K. Menon).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

prominent feature of cell architecture as almost half the PM retains a cortical ER membrane ~30 nm away (Fig. 1)[11, 12]. It is widely assumed that these regions of close proximity enhance the rate of sterol transport between the ER and PM [13]. However, is this really the case? Here we consider a few basic principles to calibrate discussion of this point, using the budding yeast *Saccharomyces cerevisiae* as our example.

We begin with some numbers. A yeast cell has a radius of 2.5 μm , and each cell has 10^8 ergosterol molecules (ergosterol is the yeast counterpart of mammalian cholesterol) [14]. For simplicity, we assume that the ER and PM are the only membranes relevant to our discussion. The rate at which ergosterol moves between the ER and PM is known from pulse-chase experiments. To interpret these experiments, it is useful to think of the yeast cell as a closed system at equilibrium: the system is perturbed by generating a small bolus of [^3H]ergosterol in the ER, and then relaxes back to equilibrium during the chase period as the [^3H]ergosterol is distributed within the cell. Measurements indicate that the relaxation process has a time constant $\tau \sim 6$ min [15]. Assuming that ergosterol is transported between the ER and PM with similar forward and reverse rate constants, both equal to k , then $k = 1/2\tau$ or $\sim 0.001 \text{ s}^{-1}$. Although a living yeast cell is not a closed system (ongoing ergosterol synthesis in the ER sets up a gradient in ergosterol activity that is dissipated by non-vesicular transport), we can use this estimate of the rate constant as a guide. With 10^8 ergosterol molecules per cell, $k = 0.001 \text{ s}^{-1}$ implies a net rate of ergosterol transport between the ER and PM of 10^5 molecules s^{-1} under steady-state conditions.

Sterols can exchange slowly between populations of vesicles in the test tube on the time scale of a few hours [16, 17], but sterol exchange between the ER and PM is undetectable over a period of 90 minutes in yeast cells that have been treated with energy poisons [10]. Thus the spontaneous rate of sterol exchange - slow *in vitro* and undetectable *in vivo* - is simply not sufficient to account for the measured rate of ergosterol transport between the ER and PM. Therefore, non-vesicular transport of sterol between the ER and PM is presumed to require carriers, sterol transport proteins (STPs) that exchange ergosterol between the cytoplasmic face of the ER and that of the PM. In the simplest case, an STP picks-up an ergosterol molecule from the ER (or PM), diffuses through the cytoplasm and drops-off its cargo at the PM (or ER) (Fig. 2). In the following sections, we look at two extreme scenarios to clarify the contributions of diffusion and extraction to the intracellular transport of ergosterol. We first consider the situation where the pick-up and drop-off events occur so rapidly that transport times are set entirely by diffusion of the STP in the cytoplasm.

Diffusion-limited ergosterol transport

Consider a yeast cell devoid of organelles, containing only cytoplasm (Fig. 3A). If an ergosterol-loaded STP were to be dropped anywhere within this cell and allowed to diffuse freely, the average time it would take for it to arrive at (and be captured by) the PM is $t_c = R^2/15D$ [18], where R is the radius of the cell and D is the diffusion coefficient of the STP. (If the STP were to be placed at the cell center, then the mean time for its capture by the PM would be $t_c = R^2/6D$.) Using $D = 10 \mu\text{m}^2 \text{ s}^{-1}$ for green fluorescent protein in the cytoplasm [19], and $R = 2.5 \mu\text{m}$, the mean time to capture $t_c = 40$ msec. Extrapolating, we conclude that each STP would be able to deliver 25 ergosterol molecules to the PM per second, and

that 4,000 perfectly efficient STPs acting in parallel would be sufficient to account for the measured rate of sterol transport (10^5 s^{-1}).

Next, we model the close association between the ER and PM and ask how this geometry would impact the average capture time. Imagine that the cortical ER is a spherical surface, concentric with the PM and separated from it by a gap $\epsilon = 0.03 \mu\text{m}$ (Fig. 3B). Assume further that whereas the PM can capture STPs, the ER acts as a reflector. If an STP were to be dropped into the annular space between the ER and PM, it would be captured by the PM in an average time $t_c \sim \epsilon^2/3D = 30 \mu\text{sec}$ [18]. If both the ER and PM were able to accept ergosterol from an STP within the annulus (Fig. 3B), then the mean time to capture would be 4x faster than if the PM alone was accepting ergosterol. However, the mean time to capture of an STP at the PM would still be 30 μsec . With this very fast capture time, it would require just a few (<10) STPs within ER-PM contact sites, each delivering $>30,000$ ergosterol molecules per second, to account for the measured transport rate! Note that t_c responds nonlinearly to ϵ : doubling the gap between the ER and PM at contact sites would result in a 4-fold increase in the number of STPs needed to maintain the transport rate.

These simple calculations make it clear that if non-vesicular sterol transport is rate-limited by diffusion, then a few thousand STPs operating widely through the cytoplasm or fewer than 10 STPs operating within the confines of an ER-PM contact site would be needed to achieve the measured transport rate of 10^5 s^{-1} . Stated differently, a fixed number of STPs operating within the confines of ER-PM contact sites would significantly increase the transport rate compared with the same number of STPs operating in the bulk cytoplasm, as long as transport is diffusion-limited.

When ergosterol transport is rate-limited by acquisition, rather than diffusion

We now turn to the converse situation where sterol pick-up is rate-limiting. How difficult is it to extract a sterol from a membrane (Fig. 2)? This question can be answered by measuring the rate of sterol transfer between populations of synthetic phospholipid vesicles at different temperatures and analyzing the data according to the Arrhenius equation in which the rate constant for intermembrane sterol transfer $r = A \cdot \exp(-E_a/k_B T)$. In this equation, k_B is the Boltzmann constant, T is the absolute temperature, E_a is the energy barrier that must be overcome for a sterol to exit the membrane and enter the aqueous phase (see Box 1), and A is a prefactor representing the reaction rate if there was no energy barrier (Fig. 4).

Text Box 1

Characteristics of Sterol Exchange

When sterol exchange between vesicle populations is quantitatively measured in vitro, the kinetics are well described by a first order process that is independent of acceptor vesicle concentration [16]. What is the mechanism of exchange and which step in the exchange process is rate-limiting (hence first order)? If exchange relied on direct collisions between donor and acceptor membranes, the transfer rate would have scaled with the concentration of acceptor vesicles. Thus, transfer must occur by desorption of

sterol into the aqueous medium (step 1)(sterols have some solubility in water, ~100 nM), followed by its capture by another vesicle (step 2). If step 2 were rate-limiting, then transfer would again depend on acceptor vesicle concentration. Thus, sterol desorption (step 1) is rate-limiting, and the overall kinetics of sterol exchange are set by the energy barrier of desorption.

E_a turns out to be in the range ~16–17.4 kcal/mole [16, 17], which, in units of $k_B T$ at room temperature, corresponds to 27–30 $k_B T$. This is quite a high barrier (compare it with the standard free energy of ATP hydrolysis which is 12 kcal/mol or 20 $k_B T$), and can be explained by realizing that many interactions have to be disrupted in order for a sterol to be torn out of the membrane. Because the extent and nature of these interactions depends on the characteristics of the membrane from which the sterol is to be desorbed, the exact value of E_a will vary. For example, $E_a \sim 27\text{--}30 k_B T$ was measured for cholesterol desorption from an egg phosphatidylcholine bilayer (a loosely packed phospholipid bilayer with a mix of saturated and unsaturated fatty acyl chains) and would be expected to be lower for a membrane with a higher complement of unsaturated acyl chains or a membrane with significant lipid packing defects. In the following calculations we use $E_a = 27 k_B T$, the lower end of the measured range of E_a values corresponding to the fastest extraction rate possible.

The pre-factor A is complicated [20, 21] (See Box 2). Accounting for the pre-factor in the usual Arrhenius analysis is not always straightforward, but it has a significant effect on the actual desorption rate. Assuming a generously large prefactor of $A \sim 10^{10} \text{ s}^{-1}$ [20], and using $E_a = 27 k_B T$, the desorption rate constant calculated from the Arrhenius equation is a mere $\sim 0.02 \text{ s}^{-1}$. Lowering E_a by ~32% ($\sim 9 k_B T$) will cause a 5000-fold increase in the desorption rate to $100 \text{ molecules s}^{-1}$, making it comparable to the rate of 25 s^{-1} for diffusion-controlled transport of sterols in the bulk cytoplasm model discussed above (Fig. 2A)). This rate enhancement could be accomplished by STPs that lower E_a by disrupting sterol-membrane interactions and providing a hydrophobic binding pocket to receive the extracted sterol. In principle, STPs could enhance the rate of sterol transport by accelerating the prefactor rather than lowering the energy barrier. Kinetic measurements at different temperatures would be required to distinguish between these possibilities, and no such data currently exist. For the purposes of discussion, we will only consider changes in the energy barrier, but the arguments are entirely general and the simple models described in Figure 5 include this possibility. Thus, if an STP can lower the energy barrier by $>10 k_B T$, while maintaining a large prefactor, then diffusion rather than desorption will be rate-limiting. Under such conditions, there would be an obvious rate benefit in having STPs that operate within a contact site.

Text Box 2

Prefactor A

The prefactor A represents several aspects of the sterol extraction ‘reaction’ including the energy landscape of the STP-membrane interaction, the rate of diffusion-limited collisions between the STP and sterols in the membrane, the viscous drag of the solvents (both cytoplasmic and membrane), and according to some theories, the entropy of the

transition state. This complex amalgam of factors contributing to the reaction rate renders it nearly impossible to produce a simple analytical expression for the prefactor, but theoretical considerations and experimental measurements limit its maximum to something on the order of $10^9 - 10^{10}$ per second for an aqueous-phase reaction.

What is known about the E_a -lowering ability of an STP? We can deduce this from measurements of the rates of STP-catalyzed sterol exchange between populations of vesicles *in vitro*. These rates fall in the range $0.1-0.3 \text{ s}^{-1}$ per STP, for mammalian StARD4 [22] and yeast Osh4 [23]. If we use $E_a = 27 \text{ k}_B\text{T}$ and $A \sim 10^{10} \text{ s}^{-1}$ (as above, assuming that the value of A is not affected by the STP), then E_{STP} , the reduction in E_a achieved by the STP, is $2-3 \text{ k}_B\text{T}$ for Osh4 and StARD4.

In the previous section, we estimated the number of STPs that would be needed to support the measured rate of sterol transport under diffusion-limited conditions. What happens to the STP requirement when transport is desorption-limited? This depends on A , E_a , and E_{STP} . The relationship between the number of STPs (N), A , and E_{STP} is graphed in Fig. 5A. For example, with $A \sim 10^{10} \text{ s}^{-1}$, $E_a = 27 \text{ k}_B\text{T}$ and $E_{\text{STP}} = 3 \text{ k}_B\text{T}$, we infer that $N=300,000$ (red dot in Fig. 5A). As there is some uncertainty in the value of E_{STP} (approx $\pm 2 \text{ k}_B\text{T}$), N could be anywhere from $35,000 - 2 \text{ million}$ STPs per cell (error bar, Fig. 5A). This is not an unreasonable spread of values as the lower end of this range corresponds to the number of molecules/cell of the yeast sterol binding/transport protein Osh4 [24, 25] (dotted line in Fig. 5A), while the higher end is well within the range for levels of some of the more abundant yeast cytoplasmic proteins. For example, phosphoglycerate kinase and glyceraldehyde phosphate dehydrogenase isozymes are found at $200,000-1.5 \times 10^6$ molecules/cell [25].

Beyond yeast

The calculations that we have presented for a yeast cell suggest that for reasonable values of A and E_{STP} , for example 10^{10} s^{-1} and $3 \text{ k}_B\text{T}$, respectively, the rate-limiting step in STP-mediated sterol transport is desorption rather than diffusion. Only in the case of extraordinarily effective STPs, capable of lowering E_a by $>10 \text{ k}_B\text{T}$ (while maintaining a large prefactor), would transport become diffusion-limited. But what about large mammalian cells where STPs have to travel further in order to deliver sterols? In this case does STP-mediated transport become diffusion-limited, and consequently is there now a benefit to having transport occur over a short distance at a membrane contact site? We can answer this by defining a point of transition between desorption-limited and diffusion-limited transport. For example, we can reasonably postulate that transport is diffusion-limited when the desorption rate constant, $A \cdot \exp(-(E_a - E_{\text{STP}})/k_B\text{T})$, is more than 10 times the reciprocal of the mean time to capture, $10/t_c (=10 \cdot 15D/R^2)$. This scenario is graphed in Fig. 5B for a yeast cell ($R = 2.5 \text{ }\mu\text{m}$) and a large mammalian cell ($R = 20 \text{ }\mu\text{m}$) over a realistic range of the parameters A and E_{STP} . The graph shows that STP-mediated sterol transport in the yeast cell falls almost entirely in the desorption-limited regime, whereas diffusion-limited transport may occur in mammalian cells, depending on precise values of A and E_{STP} .

Concluding remarks

The analysis presented here describes the interplay between two fundamental aspects of non-vesicular intracellular sterol transport: diffusion, and a rate-limiting energy barrier. Which is more relevant in an actual cell for interpreting the known rate of sterol transport? In the absence of evidence for an STP with the ability to catalyze a large drop in the energy barrier of sterol desorption ($> 10 k_B T$), desorption likely sets the speed limit for sterol transport making contact sites irrelevant for transport enhancement. This is also likely to be the case for mammalian cells despite their size, although the corresponding energy barrier drop necessary may be closer to $> 6 k_B T$. If sterol transport is desorption-limited, how do contact sites influence transport as has been suggested? We speculate that the true advantage of contact site-restricted sterol transport lies in compartmentalization. Restricting transport to subdomains of the ER and PM may allow local concentration gradients to be maintained, prevent deleterious redistribution of particular lipids, allow specific exchange of lipids via counterflow mechanisms (for example, exchange of sterol and phosphatidylinositol-4-phosphate), and increase the efficiency of lipid transport for particular classes of lipids that are consumed in signaling processes [23, 26–30]. Given that several STPs readily bind to more than one type of lipid, compartmentalization is a straightforward means of limiting STP promiscuity. These conclusions apply not only to ER-PM contact sites but also to contacts between the ER and other organelles such as mitochondria, lysosomes, and endosomes. Finally, contact sites may allow for independent regulation of distinct classes of lipids rather than simply altering the global rates of transport for all lipids.

Acknowledgments

We thank Yves Sere and Lee Cohen-Gould (Weill Cornell Medical College) and William Rice (Simons Electron Microscopy Center at New York Structural Biology Center) for the model shown in Figure 1. A.K.M. wishes to acknowledge the usual insights provided by S. Canis and B. Dylan. This work was supported by research grants from the Qatar National Research Fund (NPRP 7-082-1-014 (AKM)) and the National Institutes of Health (GM095674 (JSD)).

References

1. Maxfield FR, van Meer G. Cholesterol, the central lipid of mammalian cells. *Curr Opin Cell Biol.* 2010; 22:422–429. [PubMed: 20627678]
2. Holthuis JC, Menon AK. Lipid landscapes and pipelines in membrane homeostasis. *Nature.* 2014; 510:48–57. [PubMed: 24899304]
3. Mesmin B, Maxfield FR. Intracellular sterol dynamics. *Biochim Biophys Acta.* 2009; 1791:636–645. [PubMed: 19286471]
4. Brown MS, Goldstein JL. Cholesterol feedback: from Schoenheimer's bottle to Scap's MELADL. *J Lipid Res.* 2009; 50(Suppl):S15–27. [PubMed: 18974038]
5. Brown MS, Goldstein JL. A century of cholesterol and coronaries: from plaques to genes to statins. *Cell.* 2015; 161:161–172. [PubMed: 25815993]
6. Wüstner D, Solanko K. How cholesterol interacts with proteins and lipids during its intracellular transport. *Biochim Biophys Acta.* 2015; 1848:12–28.
7. Urbani L, Simoni RD. Cholesterol and vesicular stomatitis virus G protein take separate routes from the endoplasmic reticulum to the plasma membrane. *J Biol Chem.* 1990; 265:1919–1923. [PubMed: 2153669]

8. Heino S, et al. Dissecting the role of the golgi complex and lipid rafts in biosynthetic transport of cholesterol to the cell surface. *Proc Natl Acad Sci U S A*. 2000; 97:8375–8380. [PubMed: 10890900]
9. Li Y, Prinz WA. ATP-binding cassette (ABC) transporters mediate nonvesicular, raft-modulated sterol movement from the plasma membrane to the endoplasmic reticulum. *J Biol Chem*. 2004; 279:45226–45234. [PubMed: 15316012]
10. Baumann NA, et al. Transport of newly synthesized sterol to the sterol-enriched plasma membrane occurs via nonvesicular equilibration. *Biochemistry*. 2005; 44:5816–5826. [PubMed: 15823040]
11. Pichler H, et al. A subfraction of the yeast endoplasmic reticulum associates with the plasma membrane and has a high capacity to synthesize lipids. *Eur J Biochem*. 2001; 268:2351–2361. [PubMed: 11298754]
12. West M, et al. A 3D analysis of yeast ER structure reveals how ER domains are organized by membrane curvature. *J Cell Biol*. 2011; 193:333–346. [PubMed: 21502358]
13. Lahiri S, et al. Membrane contact sites, gateways for lipid homeostasis. *Curr Opin Cell Biol*. 2015; 33:82–87. [PubMed: 25569848]
14. Sullivan DP, et al. Sterol trafficking between the endoplasmic reticulum and plasma membrane in yeast. *Biochem Soc Trans*. 2006; 34:356–358. [PubMed: 16709160]
15. Georgiev AG, et al. Osh proteins regulate membrane sterol organization but are not required for sterol movement between the ER and PM. *Traffic*. 2011; 12:1341–1355. [PubMed: 21689253]
16. McLean LR, Phillips MC. Mechanism of cholesterol and phosphatidylcholine exchange or transfer between unilamellar vesicles. *Biochemistry*. 1981; 20:2893–2900. [PubMed: 7195733]
17. Phillips MC, et al. Mechanisms and consequences of cellular cholesterol exchange and transfer. *Biochimica et Biophysica Acta*. 1987; 906:223–276. [PubMed: 3297153]
18. Berg, HC. *Random Walks in Biology*. Princeton University Press; 1983.
19. Di Rienzo C, et al. Probing short-range protein Brownian motion in the cytoplasm of living cells. *Nat Commun*. 2014; 5:5891. [PubMed: 25532887]
20. Evans E. Probing the relation between force--lifetime--and chemistry in single molecular bonds. *Annu Rev Biophys Biomol Struct*. 2001; 30:105–128. [PubMed: 11340054]
21. Daune, M. *Molecular Biophysics: Structures in Motion*. Oxford University Press; 1999.
22. Iaea DB, et al. STARD4 Membrane Interactions and Sterol Binding. *Biochemistry*. 2015; 54:4623–4636. [PubMed: 26168008]
23. von Filseck JM, et al. A phosphatidylinositol-4-phosphate powered exchange mechanism to create a lipid gradient between membranes. *Nat Commun*. 2015; 6:6671. [PubMed: 25849868]
24. Ghaemmaghami S, et al. Global analysis of protein expression in yeast. *Nature*. 2003; 425:737–741. [PubMed: 14562106]
25. Kulak NA, et al. Minimal, encapsulated proteomic-sample processing applied to copy-number estimation in eukaryotic cells. *Nat Methods*. 2014; 11:319–324. [PubMed: 24487582]
26. Stefan CJ, et al. Osh proteins regulate phosphoinositide metabolism at ER-plasma membrane contact sites. *Cell*. 2011; 144:389–401. [PubMed: 21295699]
27. Tavassoli S, et al. Plasma membrane--endoplasmic reticulum contact sites regulate phosphatidylcholine synthesis. *EMBO Rep*. 2013; 14:434–440. [PubMed: 23519169]
28. Gatta AT, et al. A new family of StART domain proteins at membrane contact sites has a role in ER-PM sterol transport. *Elife*. 2015;4.
29. Saheki Y, et al. Control of plasma membrane lipid homeostasis by the extended synaptotagmins. *Nature Cell Biology*. 2016; 18:504–515. [PubMed: 27065097]
30. de Saint-Jean M, et al. Osh4p exchanges sterols for phosphatidylinositol 4-phosphate between lipid bilayers. *J Cell Biol*. 2011; 195:965–978. [PubMed: 22162133]
31. Raychaudhuri S, et al. Nonvesicular sterol movement from plasma membrane to ER requires oxysterol-binding protein-related proteins and phosphoinositides. *J Cell Biol*. 2006; 173:107–119. [PubMed: 16585271]

Outstanding Questions Box

- What are the actual values of the key parameters (diffusion coefficient, kinetic prefactor, desorption energy barrier for extraction from realistic membranes, and STP-catalyzed energy barrier drop) involved in STP-mediated sterol transport?
- If sterol transport is desorption-limited, how do contact sites influence transport as has been suggested?

Trends Box

- Sterols are transported within cells mainly by non-vesicular mechanisms requiring sterol transport proteins (STPs), and this is believed to be accelerated by contact sites between the endoplasmic reticulum (ER) and plasma membrane (PM).
- STPs must extract sterols from membranes and transfer them to a distinct membrane by diffusion through the cytoplasm.
- Recent transport estimates of STP-catalyzed sterol transport provide an opportunity to compare rate constants for extraction versus diffusion and thereby reveal the rate-determining step in cellular sterol transport.

Figure 1. Juxtaposition of the plasma membrane (PM) and endoplasmic reticulum (ER) in a yeast cell

Images obtained from focused ion beam – scanning electron microscopy (FIB-SEM) were used to generate this model showing a cut-away view of a yeast cell revealing select structural elements: PM (*purple*), vacuole (*vac, red*), nucleus (*nuc, yellow*) and ER (*green*). The image highlights the extent to which the ER is in close (~ 30 nm) apposition to the PM. The image was generated by Yves Y. Sere, Lee Cohen-Gould, William Rice and one of the authors (A.K.M.).

Figure 2. Sterol transport protein (STP)-mediated sterol transport

An STP (*pink*) picks up a sterol molecule (*green*) from the ER, diffuses through the cytoplasm and deposits the sterol at the PM. All steps depicted are reversible. In the models considered in this article, all regions of the ER and PM are equally likely to desorb or adsorb a sterol molecule.

Figure 3. Models of STP-mediated sterol transport

A. An STP (*pink*) with sterol (*green*) bound is dropped into a yeast cell that contains cytoplasm but is devoid of organelles. The mean time for the STP to arrive at the PM via random walk is $t_c = 40$ msec. **B.** Membrane contact site model. The ER is depicted as a spherical shell, concentric with the PM and spaced 30 nm away. An STP dropped into the annular shell between the ER and PM would arrive at the PM with a mean time $t_c = 30$ μ sec.

Figure 4. Energy barrier for sterol extraction

The activation energy (E_a) of spontaneous desorption of a sterol molecule (*green*) is shown in *black*. In the presence of an STP (*pink*), a new reaction pathway emerges with a smaller associated activation energy ($E_a - E_{STP}$) as shown in *blue*. Note that small changes in the activation energy of desorption correspond to exponentially larger changes in the rate of desorption. In this scenario, the rate-determining step of the process is set by the activation energy barrier.

Figure 5. Relationship between various parameters that define nonvesicular intracellular sterol transport

A. The overall sterol transport rate equals $N \cdot A \cdot \exp(-(27 k_B T - E_{STP})/k_B T)$. This expression was used to generate a set of parameters N , A , and E_{STP} for which the rate is equal to 10^5 s^{-1} . The prefactor A ranged over 2 orders of magnitude, with a generous maximum of 10^{10} s^{-1} while N increased from 10 to 10 million. A series of E_{STP} values was used to generate the individual isochrones, starting with $3 k_B T$ corresponding to the value calculated in the text for an STP such as StARD4 or Osh4. The red dot corresponds to such an STP: with $E_{STP} = 3 k_B T$, $A = 10^{10} \text{ s}^{-1}$, $N \sim 300,000$. Since the experimental error for E_a was $\sim 2 k_B T$ [10], the true correspondence of the StARD4 data to the isochrones was assigned an uncertainty of $\pm 2 k_B T$ (error bars). The number of molecules of Osh4 in a yeast cell, 32,000, is shown as the red dashed line (Osh4 has been shown to be dispensable for ER-PM sterol transport [15, 31] but it is convenient to mark it on this plot to illustrate the quantitative relationship between copy number and transport rate). The combination of parameters that reflects the reality within a cell likely falls within the shaded zone. **B.** The transition from diffusion-limited transport to desorption-limited transport depends on cell size as well as the parameters A and E_{STP} . We define the transition from desorption-limited to diffusion-limited transport when the desorption rate constant is more than 10 times the reciprocal of the mean time to capture: $A \cdot \exp(-(E_a - E_{STP})/k_B T) < 150 \cdot D/R^2$. This transition is shown for a yeast cell ($R = 2.5 \mu\text{m}$) and a large mammalian cell ($R = 20 \mu\text{m}$) over a realistic range of the parameters A and E_{STP} . Note that this transition does not

depend on the number of STPs assuming that each STP functions independently of the others.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript