

The Coherence of the Vesicle Theory of Protein Secretion

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Key words: Vesicle, Transport, Mathematical model, Secretion, Exocytosis

The well established vesicle theory of protein secretion [Palade, 1975, Hirschberg et al., 1998, Alberts et al., 2002] has been called into question by a recent article [Rothman, 2007]. Rothman [2007, p.150 top left] claims that “the vesicle theory ... has a fatal quantitative failing. The transport mechanisms it proposes are unable to balance forces.” We disagree with the author and demonstrate below the coherence of the questioned vesicle theory of protein secretion.

The vesicle theory explains constitutive protein secretion as it operates in all eukaryotic cells as a sequence of packaging of luminal and trans-membrane proteins into and discharge from transport vesicles. Specifically, newly synthesized proteins that have entered the endoplasmic reticulum (ER) and are destined for secretion are first packaged into small COPII-coated transport vesicles. These transport vesicles bud from specialized regions of the ER, called ER exit sites. After tethering and SNARE-mediated fusion with the target membrane of the *cis* Golgi network the synthesized proteins traverse the Golgi apparatus, a stack of several cisternae, where proteins and lipids are being modified. Transport vesicles destined for the plasma membrane depart from the *trans* Golgi network. The membrane proteins and the lipids in these vesicles provide new components for the cell’s plasma membrane, while the soluble proteins inside the vesicles are constitutively secreted into the extracellular space. Specialized secretory cells also possess a regulated secretory pathway in which proteins are accumulated within secretory vesicles close to the plasma membrane and massively discharged in response to a stimulus.

How can all these interacting, random microscopic events take place at the right frequencies such that an ordered macroscopic behavior emerges? This is the intriguing question raised by Rothman [2007].

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The author argues that ordered macroscopic behavior cannot be explained by the vesicle theory and claims that the vesicle theory suffers from the following three problems.

- 1. The continuous flux of synthesized proteins in the ER needs to be balanced by protein-loaded vesicles detaching from the ER. How should individual vesicles adjust their individual contribution to the total efflux from the ER in the size required to maintain the balance between synthesis flux and total efflux?
- 2. How could a balance of the total vesicle-bound influxes and effluxes of intermediate compartments of the secretory pathway be established? This balance appears as an improbable coincidence [Rothman, 2007, Fig.1].
- 3. When multiple protein species are transported simultaneously by the same transport carriers [e.g. the secretory granule considered in Rothman, 2007, Fig.2] but synthesized with different fluxes, how could synthesis match secretion for every species individually?

The author claims that a solution of these problems would require additional “intelligence” about the accumulating, macroscopic consequence of the random microscopic processes and that such coordination cannot emerge within the vesicle theory. He therefore abandons the vesicle theory of protein secretion.

In the following we show that the author’s claims are based on a misunderstanding of the role of the protein concentration in intermediate compartments: “If the difference between the two [influx and efflux] is zero, then of course so is the concentration of the protein in the cisterns, and being zero it could not possibly provide information about the rate of synthesis or anything else.” [Rothman, 2007, p.154 bottom left]. The vesicle theory regards proteins as being contained or stored in their carrying vesicle during transport [Lauenburger and Linderman, 1993, Heinrich and Rapoport, 2005]. The number of proteins contained in a vesicle is the system’s response to the fluctuating microscopic events and a key quantity to be determined below.

For the problems 1., 2. and 3. we demonstrate in the corresponding sections 1, 2 and 3 that random, elementary local processes are sufficient to balance total fluxes. Therefore we consider the vesicle theory as consistent.

1 Continuous synthesis balanced by discontinuous transport

Consider a continuous flux $j_{ps}(t)$ of proteins of a single species due to synthesis at the ER, measured in particles per time unit.¹ $j_{ps}(t)$ shall already be compensated for endocytosis and degradation or other sources and sinks of the

¹ We perform the calculations in terms of particle numbers as opposed to concentrations since the variable volumes of vesicles render the latter more complex, albeit as consistent.

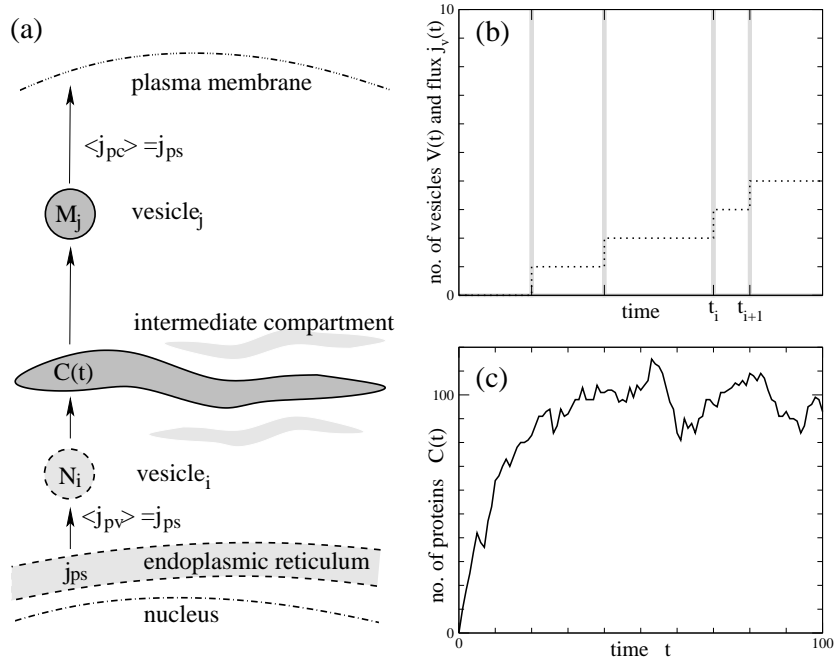


Fig. 1. (a) Schematic view of the exocytic pathway of a eukaryotic cell. We consider proteins being produced with flux j_{ps} in the endoplasmic reticulum. Then vesicle i shall carry N_i proteins to an intermediate compartment that contains a number $C(t)$ of proteins and can be related to a cisterna of the Golgi stack. Vesicle j carries M_j proteins from the intermediate compartment to the plasma membrane and secretes the proteins. (b) The cumulative number $V(t)$ of vesicles i that have departed from the endoplasmic reticulum is sketched as a dotted curve. The flux $j_v(t)$ of vesicles i is defined as the temporal derivative of $V(t)$ which equals zero between the steps of $V(t)$ and amounts to a set of δ -distributions (represented by vertical gray lines) at the steps of $V(t)$. (c) Numerical simulation of the number of proteins $C(t)$ contained in the intermediate compartment according to Eq. 2. During each time step of the simulation we let one vesicle i arrive and one vesicle j depart from the compartment. The random numbers N_i of arriving proteins are drawn from the Poisson distribution $p(N_i, \lambda) = e^{-\lambda} \lambda^{N_i} / N_i!$ with $\langle N_i \rangle_i = \lambda = 10$. The random fractions $\alpha_j \in (0, 1]$ of the total protein number in the compartment before budding are here drawn with uniform probability density from the interval $(0, 0.2]$ and with vanishing probability density from $(0.2, 1]$ such that $\langle \alpha_j \rangle_j = 0.1$. Starting from an empty compartment, $C(t)$ approaches an average $\langle C(t) \rangle_t = 100 = \langle N_i \rangle_i / \langle \alpha_j \rangle_j$ as predicted by Eq. 5.

considered protein. Transport vesicles (chronologically ordered and numbered by $i \in \mathcal{N}$) bud from the ER at discrete time points $t_i \in (t_0, \infty)$ and carry the proteins (Fig. 1(a)). Each budding process shall be completed after a short time period ε . Then the cumulative number $V(t)$ of vesicles which budded within the interval $(t_0 + \varepsilon, t)$ is a discontinuous function with steps of height 1 at a set of $t = t_i$, see the dotted curve in Fig. 1(b). The instantaneous flux of vesicles is given by the temporal derivative of $V(t)$ as $j_v(t) = dV(t)/dt = \sum_{i=1}^{\infty} \delta(t - t_i)$ with $\delta(t - t_i) = 0$ for $t \neq t_i$ being the δ -distribution that yields $\int_{t_i-\varepsilon}^{t_i+\varepsilon} \delta(t - t_i) dt = 1$, see Fig. 1(b). Hence $V(t_n + \varepsilon) = \int_{t_0+\varepsilon}^{t_n+\varepsilon} j_v(t) dt = n$ as defined.

The vesicle-bound flux of proteins $j_{pv}(t)$ (summed over the whole surface of the ER) inherits its discontinuous distribution from the carrier process $j_v(t)$ with the additional information of the number N_i of proteins carried by vesicle i , hence $j_{pv}(t) = \sum_{i=1}^{\infty} N_i \delta(t - t_i)$. We assume the forming vesicle i obtains its protein load with continuous flux $j_{ps}(t)$ during the time interval (t_{i-1}, t_i) between the budding of the previous vesicle and its own budding from the ER. Then $N_i = \int_{t_{i-1}}^{t_i} j_{ps}(t) dt$. Here we restrict ourselves to the special case of constant $j_{ps}(t) = j_{ps}$ which suffices to demonstrate the emergent macroscopic behaviour.² Then $N_i = \int_{t_{i-1}}^{t_i} j_{ps} dt = j_{ps}(t_i - t_{i-1})$ can be inserted into $j_{pv}(t)$ and the *time average* (denoted by $\langle \cdot \rangle_t$) of the vesicle-bound protein flux becomes

$$\begin{aligned} \langle j_{pv}(t) \rangle_t &= \frac{\int_{t_0+\varepsilon}^{t_n+\varepsilon} j_{pv}(t') dt'}{(t_n + \varepsilon) - (t_0 + \varepsilon)} = \frac{\sum_{i=1}^n N_i}{t_n - t_0} \\ &= \frac{\sum_{i=1}^n j_{ps}(t_i - t_{i-1})}{t_n - t_0} = \frac{j_{ps} \sum_{i=1}^n t_i - t_{i-1}}{t_n - t_0} \\ &= j_{ps} \end{aligned} \tag{1}$$

and hence is equal to the continuous synthesis flux. The time average is performed over any arbitrarily chosen time interval (t_0, t_n) bounded by the budding events of the vesicles with numbers 0 and n . Hence the vesicular transport flux is balanced by the continuous synthesis flux within finite physiological time spans.

If multiple carriers are being filled simultaneously by fractions of the synthesis flux then these fractions add up again in the sum of the time averaged vesicle flux.³ Moreover, the balance of fluxes always remains satisfied whatever mechanism is employed to regulate the time points t_i of budding events, for example in response to the protein number $N(t) = j_{ps}(t - t_{i-1})$ within the presently filled carrier.

² The following arguments carry over to temporally varying fluxes.

³ Note, the order of both linear operations, summation over flux fractions and summation over vesicles, is interchangeable.

2 Balanced discontinuous influxes and effluxes of intermediate compartments

Consider an intracellular compartment as an intermediate store in the secretory pathway with a discontinuous influx of vesicles (again numbered by i) that fuse with the compartment at times t_i and discharge their protein content N_i . A different set of vesicles (numbered by j) bud from the compartment at times t_j and carry proteins of number M_j . This yields the efflux from the compartment j_{pc} . In the time interval $(t_0 + \varepsilon, t_m + \varepsilon)$, m vesicles have budded and $n(m)$ vesicles have fused with the compartment. As a result, the number of proteins $C(t)$ contained in the intermediate compartment fluctuates as

$$C(t_m + \varepsilon) = C(t_0 + \varepsilon) + \sum_{i=1}^{n(m)} N_i - \sum_{j=1}^m M_j \quad . \quad (2)$$

To assure stability of the steady state balance below, it is necessary that the time-averaged flux of departing proteins is an increasing function of $C(t)$. Several microscopic mechanisms may yield this dependence, including receptor-mediated vesicle formation where the probability of protein-receptor interactions increases with $C(t)$ given the bounded volume of the intermediate compartment. Here we assume the volume of the intermediate compartment fluctuates between a lower and an upper limit and consider a stochastic mechanism of vesicle budding at random time points t_j . Moreover, we regard the lumen of the intermediate compartment as a well mixed solution of proteins such that the local density of proteins is (at the time of budding) proportional to the total number $C(t_j - \varepsilon)$ of proteins.⁴ Then the protein load $M_j = \alpha_j C(t_j - \varepsilon)$ shall be a random fraction $\alpha_j \in (0, 1]$ of the total protein number in the compartment before budding. This is yet another consequence of the law of mass action. For a numerical simulation of Eq. 2 see Fig. 1(c).

For the macroscopic variable $C(t)$, the described microscopic process yields the equation $C(t_m + \varepsilon) = C(t_0 + \varepsilon) + \sum_{i=1}^{n(m)} N_i - \sum_{j=1}^m \alpha_j C(t_j - \varepsilon)$. This equation couples the load of the next budding vesicle to the history of fused and budded vesicles. Performing the time average over the budding events at t_m yields

$$\langle C(t) \rangle_t = \langle C(t_m + \varepsilon) \rangle_m$$

⁴ We excluded the pathological case in which the volume of the intermediate compartment is allowed to grow indefinitely which otherwise could allow an excess accumulation of synthesized proteins despite a low local density and low protein secretion flux.

$$\begin{aligned}
&= C(t_0 + \varepsilon) + \left\langle \sum_{i=1}^{n(m)} N_i \right\rangle_m - \left\langle \sum_{j=1}^m \alpha_j C(t_j - \varepsilon) \right\rangle_m \\
&= C(t_0 + \varepsilon) + \left\langle \sum_{i=1}^{n(m)} N_i \right\rangle_m - m \langle \alpha_j \rangle_j \langle C(t_j) \rangle_j
\end{aligned} \tag{3}$$

since here α_j and $C(t_j - \varepsilon)$ are not correlated. Rearranging the terms yields

$$\frac{\langle C(t) \rangle_t - C(t_0 + \varepsilon)}{m \langle t_j - t_{j-1} \rangle_j} = \frac{\langle \sum_{i=1}^{n(m)} N_i \rangle_m}{m \langle t_j - t_{j-1} \rangle_j} - \frac{\langle \alpha_j \rangle_j \langle C(t) \rangle_t}{\langle t_j - t_{j-1} \rangle_j} . \tag{4}$$

Hence $C(t)$ fluctuates around the steady state (set l.h.s. of Eq. 4 to zero and rewrite the r.h.s., then insert N_i from Sec. 1)

$$\langle C(t) \rangle_t = \left\langle \frac{1}{m} \sum_{i=1}^{n(m)} N_i \right\rangle_m / \langle \alpha_j \rangle_j = \frac{\langle t_j - t_{j-1} \rangle_j}{\langle \alpha_j \rangle_j} \times j_{ps} , \tag{5}$$

which is a fixed quantity and proportional to the synthesis flux. Eq. 5 predicts that longer average waiting times between budding events, larger synthesis flux and smaller average *fractions* of departing proteins will result in larger amounts of proteins being stored in the intermediate compartment. Note, the average absolute number of departing proteins per vesicle is independent of the average fraction $\langle \alpha_j \rangle_j$. As a result, the average influx of proteins $\langle \frac{1}{m} \sum_{i=1}^{n(m)} N_i \rangle_m / \langle t_j - t_{j-1} \rangle_j = j_{ps}$ equals the average efflux

$$\langle j_{pc} \rangle_t = \langle \alpha_j \rangle_j \langle C(t) \rangle_t / \langle t_j - t_{j-1} \rangle_j = j_{ps} , \tag{6}$$

which follows from Eq. 4 when initial transients are neglected and $C(t_0 + \varepsilon)$ is set to $\langle C(t) \rangle_t$. This balance is a stable state since a below(above)-average choice of α_j yields a larger(smaller) $C(t_j + \varepsilon) = C(t_{j+1} - \varepsilon)$ and hence an above(below)-average loss at the next budding event.

Altogether, the number of proteins contained in the well-mixed compartment constitutes the missing link between the influx and efflux as opposed to the statement ‘‘If the difference between the two [influx and efflux] is zero, then of course so is the concentration of the protein in the cisterns, and being zero it could not possibly provide information about the rate of synthesis or anything else.’’ in [Rothman, 2007, p.154 bottom left].

3 Parallel protein fluxes are balanced individually

The calculations in Sec. 1 and 2 can easily be repeated for multiple protein species. As noted in Sec. 1 the balance of average fluxes is independent of the mechanism selecting the budding events. In Sec. 2, the budding events could, for instance, be regulated by one specific protein species and yet all other species would be carried along with individually balanced average fluxes. The balance is guaranteed as long as protein partitioning into the vesicle is an increasing function of the protein's total number inside the compartment. As above, a bounded compartment volume suffices together with the law of mass action. Different proteins' total numbers inside the compartment will, on average, have the same ratio as their synthesis fluxes (Eq. 5). Specifically, the content of a large secretory granule will be made up of different secretory proteins according to the relative sizes of their synthesis fluxes. Averaged over time, simultaneous exocytosis of multiple proteins by means of secretory granules therefore occurs with fluxes equal to the individual proteins' synthesis fluxes.

4 Summary

We have addressed in detail three putative problems of the vesicle theory of protein secretion as identified in [Rothman, 2007] and have shown that they can all be solved within the framework of the vesicle theory. Specifically,

- 1. The continuous flux of synthesized proteins in the ER is balanced by the *temporally averaged* vesicle-bound protein efflux from the ER.
- 2. The *temporally averaged* vesicle-bound influxes and effluxes of intermediate compartments of the secretory pathway balance as a result of a dynamically stable steady state of the protein number in the intermediate compartment of bounded volume.
- 3. Fluxes of multiple, co-transported proteins are balanced simultaneously as the emergent protein numbers in the intermediate compartment assume values specific for each protein and proportional to the individual synthesis fluxes.

We didn't specify the dynamics of membrane turnover through the secretory, recycling and endocytic pathways. Intermediate compartments recycle part of the incoming membrane by retrograde traffic of protein-poor vesicles. We suggest that budding of such retrograde vesicles reduces the volume of the intermediate compartment but (almost) not its protein content. The volume of the intermediate compartment may thereby fluctuate between lower and upper bounds as assumed in Sec. 2. Another open problem that awaits further studies concerns experiments that seem to contradict the vesicle theory [see

Rothman, 2007, and references therein]. Therein the secretion flux depends on the extracellular protein concentration and this evidence could point to a feedback loop controlling protein synthesis by extracellular demand or to regulated fluxes of endocytosis and protein degradation.

In summary, continuous and discontinuous transport processes affect the number of proteins contained in the transport carriers or intermediate compartments and therewith establish a stable steady state in which protein secretion fluxes balance protein synthesis fluxes. Thus we find emergent deterministic, macroscopic behaviour in a system with random discontinuous, microscopic processes. This finding is by no means a rare exception but typical for living and non-living systems. For instance, molecules in metabolic pathways often follow concentration-dependent kinetics although the enzyme-catalyzed reactions that constitute a pathway microscopically operate in a stochastic and discontinuous fashion. The well-known diffusion equation for the spatio-temporally varying concentration of particles, as another example, captures the emergent macroscopic behavior of the individual particles performing microscopic random walks.

5 Acknowledgements

We thank F. Peruani, M. Kücken and E. Flach for fruitful discussions and acknowledge support by the systems biology network HepatoSys of the German Ministry for Education and Research through grant 0313082J. Andreas Deutsch is a member of the DFG Research Center for Regenerative Therapies Dresden - Cluster of Excellence - and acknowledges support by the Center.

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