**Commentary. Re-visiting the intracellular pathway of transferrin on board of a mathematical simulation.**

**Franco Nieto and Luis S. Mayorga**

* **Short Description of the Modeling Strategy**

The simulation combines an Agent-Based Model (ABM, implemented in Repast [1]) with Ordinary Differential Equations (ODE) managed by COPASI [2]. More details can be found in references [3–5]. The parameter sets provided in the tables below were used for the three processes that govern trafficking: vesicle/tubule formation, fusion among organelles, and maturation. We did not perform a systematic sensitivity analysis for these parameters; instead, the values were adjusted to maintain stable areas for all membrane domains over an extended period (for instance, 180,000 ticks corresponding to 3 hours of cellular time [4]). In addition, we verified that the total area of the endosomal compartment—as well as the proportions of endosomes (EE + SE + RE) and late endosomes (LE)—approximately match those reported in [6]. For this, we considered that we have modeled a section of 15x15x1 m (with the 1 μm thickness chosen arbitrarily). This section is enclosed by 60 m2  of plasma membrane area, which correspond to a 1/37th of the area of a completed Baby Hamster Kidney cell as reported in [6].

**Table 1. Cell area, volume, and transferrin receptors comparing experimental data** [6,7] **with a simulation**

|  |  |  |  |
| --- | --- | --- | --- |
| **parameters** | **complete cell** | **0.027 fraction** | **model\*** |
| **plasma membrane area (m2)** | 2200 | 60 | 60 |
| **endosomes area (m2)** | 430 | 12 | 14 |
| **lysosomes area (m2)** | 370 | 10 | 11 |
| **volume internalizedm3/min)** | 0.57 | 0.02 | 0.03 |
| **plasma membrane internalized m2/min)** | 35 | 1 | 1.5 |
| **receptors at the PM (number)** | 50,000 | 1350 | 2052 |

\*Values are from a representative 30 min simulation

**Table 2. Probability of forming a vesicle/tubule**

|  |  |
| --- | --- |
| **compartments** | **probability** |
| **EE** | 0.8 |
| **SE** | 1 |
| **RE** | 1 |
| **LE** | 0.1 |
| **TGN** | 0.8 |
| **tGolgi** | 1 |
| **mGolgi** | 1 |
| **cGolgi** | 1 |
| **ERGIC** | 1 |

**Table 3. Fusion probabilities among compartments and recycling probabilities** (meaning fusion with the PM or the ER)

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **compartments** | **EE** | **SE** | **RE** | **LE** | **TGN** | **tGolgi** | **mGolgi** | **cGolgi** | **ERGIC** | **PM** | **ER** |
| **EE** | 1 | 0.8 |  |  |  |  |  |  |  | 0.2 |  |
| **SE** | 0.8 | 1 | 0.5 | 0.01 |  |  |  |  |  | 0.3 |  |
| **RE** |  | 0.5 | 1 |  | 0.1 |  |  |  |  | 0.5 |  |
| **LE** |  | 0.01 |  | 1 |  |  |  |  |  |  |  |
| **TGN** |  |  | 0.1 |  | 1 | 0.5 |  |  |  | 0.5 |  |
| **tGolgi** |  |  |  |  | 0.5 | 1 | 0.5 |  |  |  |  |
| **mGolgi** |  |  |  |  |  | 0.5 | 1 | 0.5 |  |  |  |
| **cGolgi** |  |  |  |  |  |  | 0.5 | 1 | 0.5 |  |  |
| **ERGIC** |  |  |  |  |  |  |  | 0.5 | 1 |  | 0.06 |

**Table 4. Maturation probabilities of membrane domains listed in the rows to membrane domains in columns**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **compartments** | **EE** | **SE** | **RE** | **LE** | **TGN** | **tGolgi** | **mGolgi** | **cGolgi** | **ERGIC** |
| **EE** |  | 0.85/0.1\* |  | 0.15/0.8 |  |  |  |  |  |
| **SE** |  |  | 1/0.08 |  |  |  |  |  |  |
| **RE** |  |  |  |  |  |  |  |  |  |
| **LE** |  |  |  |  |  |  |  |  |  |
| **TGN** |  |  |  |  |  |  |  |  |  |
| **tGolgi** |  |  |  |  | 1/0.8 |  |  |  |  |
| **mGolgi** |  |  |  |  |  | 1/0.8 |  |  |  |
| **cGolgi** |  |  |  |  |  |  | 1/0.8 |  |  |
| **ERGIC** |  |  |  |  |  |  |  | 1/0.8 |  |

\*The numbers indicate “maturation probability” / “proportion of the area that matures”

* **Vesicle and Tubule Budding and Cargo Sorting**

Vesicles and tubules may bud from an organelle. These structures carry a single membrane domain that facilitates the sorting of both membranes and cargoes. Whenever a fission event occurs, membrane-associated cargoes (e.g., R, R-Tf, and R-Tf.Fe) are partitioned among the resulting organelles according to their affinity for specific membrane domains. In cases where no specific affinity is defined, the cargoes are distributed proportionally to the area of the resulting organelles. Soluble cargoes (Fe, Tf, Tf.Fe) are distributed according to the volume of the resulting organelles.

**Table 5. Properties of molecules or complexes for sorting during fission**

|  |  |
| --- | --- |
| **Molecule/complex** | **affinity** |
| **R-Tf.Fe** | SE, RE |
| **R-Tf** | SE, RE |
| **R** | SE, RE |
| **Labelled R** | SE, RE |
| **Tf.Fe** | soluble |
| **Tf** | soluble |
| **Fe** | soluble |

* **Organelle Movement in 2D Space**

In two-dimensional space, organelles move randomly unless they are near a microtubule (MT). When an organelle is close to an MT, it attaches with a given probability and then moves either toward the plasma membrane or toward the microtubule organizing center. Note that budding tubules or vesicles and the remaining organelle may exhibit distinct movement behaviors.

**Table 6. Probability of movement on microtubules.** Positive values indicate movement toward the plasma membrane, whereas negative values indicate movement toward the microtubule organizing center.

|  |  |  |
| --- | --- | --- |
| **compartments** | **tubule/vesicle** | **organelle** |
| **EE** | 1 | 0 |
| **SE** | 1 | 1 |
| **RE** | 1 | -0.1 |
| **LE** | -0.5 | -1 |
| **TGN** | 1 | 1 |
| **tGolgi** | -1 | -1 |
| **mGolgi** | -1 | -1 |
| **cGolgi** | -1 | -1 |
| **ERGIC** | -1 | -1 |

1. North MJ, Collier NT, Ozik J, Tatara ER, Macal CM, Bragen M, Sydelko P. Complex adaptive systems modeling with Repast Simphony. Complex Adaptive Systems Modeling 2013;1:1–26. doi.org/10.1186/2194-3206-1-3

2. Hoops S, Sahle S, Gauges R, Lee C, Pahle J, Simus N, Singhal M, Xu L, Mendes P, Kummer U. COPASI--a COmplex PAthway SImulator. Bioinformatics 2006;22:3067–3074.

3. Quiros DN, Nieto F, Mayorga LS. From cartoons to quantitative models in Golgi transport. Biology of the Cell 2021;113:146–164. doi.org/10.1111/boc.202000107

4. Nieto F, Garrido F, Dinamarca S, Cebrian I, Mayorga LS. Kinetics of antigen cross-presentation assessed experimentally and by a model of the complete endomembrane system. Cellular Immunology 2022; 382:104636. doi.org/10.1016/j.cellimm.2022.104636

5. Mayorga LS, Cebrian I, Verma M, Hoops S, Bassaganya-Riera J. Reconstruction of endosomal organization and function by a combination of ODE and agent-based modeling strategies. Biology Direct 2018;13:25. doi.org/10.1186/s13062-018-0227-4

6. Griffiths G, Back R, Marsh M. A quantitative analysis of the endocytic pathway in baby hamster kidney cells. The Journal of Cell Biology 1989;109:2703–2720. doi.org/ 10.1083/jcb.109.6.2703

7. Ciechanover A, Schwartz AL, Dautry-Varsat A, Lodish HF. Kinetics of internalization and recycling of transferrin and the transferrin receptor in a human hepatoma cell line. Effect of lysosomotropic agents. Journal of Biological Chemistry 1983;258:9681–9689. PMID: 6309781.