Toward a computer-aided methodology for topology-based simulation of the Golgi apparatus

Mathieu Poudret[1,4], Jean-Paul Comet[2], Pascale Le Gall[1,3]
François Képès[1], Agnès Arnould[4], Philippe Meseure[4],
Jean-Marc Verbavatz[5], Alain Rambourg[5]

[1] Epigenomics Project, Genopole® and University of Evry, F-91000 Evry
[2] I3S, UMR 6070 CNRS, Univ. of Nice-Sophia-Antipolis, F-06903 Sophia-Antipolis
[3] MAS, Ecole Centrale Paris, Grande Voie des Vignes - F-92295 Châtenay-Malabry
[4] XLIM/SIC, UMR 6172 CNRS, University of Poitiers, F-86962 Futuroscope
[5] IBITEC-S CEA and CNRS URA2096, F-91191 Gif-sur-Yvette
and LRA17V, University of Paris-Sud 11, F-91405 Orsay

Abstract

The Golgi apparatus is the place in the cell where proteins remain for a maturation phase before their excretion. Several hypotheses on its dynamics have been formulated under the form of schematic models. The GolgiTop working group initiated by the Epigenomics Project (Genopole, Evry) aims at studying the 3-dimensional structure and dynamics of the Golgi apparatus by building 3D structures with the help of computer graphics tools and by animating them using transformation rules which depend on topological, geometric and/or biochemical data. Finally, we introduce a first proposition for a computer-aided methodology for helping biologists to understand and choose hypotheses about the topology and dynamics of the Golgi apparatus.

1 Introduction

The Golgi apparatus is the place in the cell where proteins remain for a maturation phase before their excretion. The generic shape of the 2-dimensional projection of the Golgi apparatus is widely known and rather easily recognizable since it is often located near the cell nucleus and composed of a stack of 5 or 6 flattened cisternae surrounded with numerous vesicles. Moreover, biochemical studies have established that the main role of the Golgi apparatus is the maturation and transportation of proteins. In particular, the Golgi apparatus is the central point for ensuring the excretion of proteins towards the cell environment. In fact, this few and basic biological knowledge about the Golgi apparatus is shared by the whole scientist community.

It remains some open questions about the Golgi apparatus. In particular, answering both following interlinked questions would be essential for a better understanding of the functioning of the Golgi apparatus: first, what are the plausible 3-dimensional structure of the Golgi apparatus both from a topological and geometric point of view? Secondly, what are the dynamic topological transformations of the Golgi apparatus which ensure the protein excretion?

These questions are widely studied and several hypotheses have been formulated under the form of schematic models. These last ones give some intuitions on plausible transformation rules likely to explain the functioning of the Golgi apparatus. In fact, since no experiments allow one to fully access neither the 3D Golgi structure nor its dynamics, some information is missing or misleading to discriminate among the ongoing hypotheses proposed by biologists which one is the most convincing with respect to the biological observations. Numerous experiments, in particular concerning locations or flow of enzymes or various proteins, are achieved to complete the biological knowledge about the Golgi apparatus. Results issued from these experiments can be interpreted according to each hypothesis. As a consequence, the controversy is continuously sustained since supporters of a given hypothesis emphasize the biological results which better fit with their favorite hypothesis and neglect the other ones. At the moment, it is then not possible to discriminate between these hypotheses. Finally, the large amount of works on the Golgi apparatus indicates that the 3D structure and the dynamics of the Golgi apparatus involve really complex and subtil mechanisms.

The GolgiTop working group initiated by the Epigenomics Project (Genopole, Evry) aims at studying both the 3D structure and the dynamics of the Golgi apparatus by building 3D structures with the help of computer graphics tools and by animating them using transformation rules which depend on geometric and/or biochemical data. For each considered hypothesis, we build a 3D structure and animate it with rules which capture the essence of the underlying Golgi apparatus hypothesis. An analysis of 2-dimensional sections helps us in calibrating them according to available biological observations. The initial postulate of the GolgiTop working group is that using computational tools to model and simulate such systems is essential. This entails recognition of the main characteristics of the phenomenon, choice of the appropriate level of abstraction, and comparison of different models. In the case of the Golgi apparatus modelling, compartmentalization is an important issue and a spatial representation of the compartments is needed to describe both static and dynamic characteristics [10].

A variety of approaches have been used to model cellular systems. In particular, rule-based modelling has already been advocated for biochemical reactions since biochemical reactions can be easily translated into transformation rules. In the case of rule-based modelling, formal methods like model checking [1] or symbolic execution [7] have been fruitfully applied to verify that the model satisfies a known property of the biological system. However, many rule-based models ignore compartmentalization and treat the system, unrealistically, as a homogeneous environment. Recent rule-based modelling takes into account different compartments (see Brane calculi [3], Bioambients [13] and BioCham [2]). In these models, the compartmentalization only captures static topology or simple topological modifications (resulting, for example, from endocytosis or exocytosis) but not geometric aspects (such as

the position and shape of the objects).

Topology-based geometric modelling [6] is particularly adapted to represent compartmentalization and is widely advocated for computer graphics. It deals with the representation of the structure of objects (their decomposition into topological units: vertices, edges, faces and volumes) and of the neighbourhood relations that exist between topological units. It treats topological structure and geometry separately; this means that the topological properties of objects can be studied without knowledge of their geometry. In a previous work [9], we formally expressed basic topological operations in terms of generic rules that can be applied to a large family of topological objects and we illustrated this topology-based approach using as examples, resp. a simple interaction between two cells.

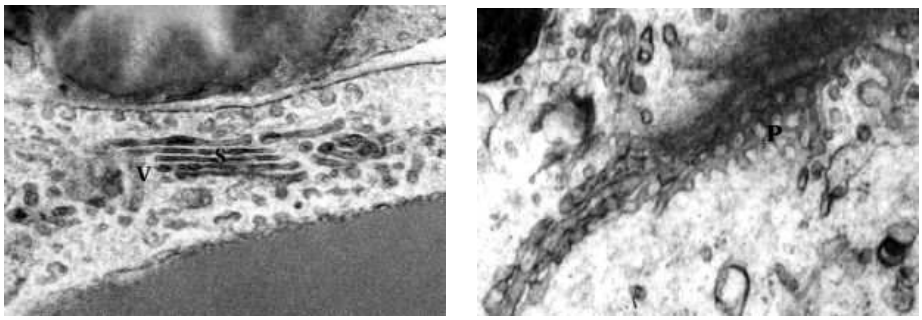
The paper is a short version of [8]: in this paper, we intentionally discard all technical elements related to topology-based geometric modelling (see [8] or [6]). On the contrary, we focus on our methodological approach devoted to help biologists to analyse their hypothesis with computer-aided model animation. The main difficulty is to find an appropriate trade-off between simplicity and fullness. Indeed, models should be sufficiently simple to allow manipulation and reasoning on them but also sufficiently complete to incorporate pertinent elements which are involved in the phenomenon under modelling.

Section 2 introduces some basic features of the Golgi apparatus. Section 3 presents three ongoing hypotheses on the dynamics of the Golgi apparatus. Section 4 describes our rule-based topological approach for the simulation of the models. Finally, in Section 5, we briefly present our computer-aided methodology for helping biologists to understand and choose hypotheses about the topology and dynamics of the Golgi apparatus.

2 The Golgi Apparatus : general description

Discovered by Camillo Golgi in 1898 in the cytoplasm of nerve gaglion cells, the Golgi apparatus (or dictyosome in plants) is an organelle that formed an extensive perinuclear network. Thanks to the use of electron microscope, [4] confirmed in 1954 that in the juxtanuclear area of mammalian cells, the Golgi apparatus usually appears as a system of stacks of closely apposed lamellae also known as saccules or cisternae. It is now widely known that the Golgi apparatus is present in most cells as an organelle made up of stacked flattened saccules and vesicles. As an illustration, let us observe the electron micrographs 1(a) and 1(b) (this last one can also be found in [12]). The Golgi apparatus appears on 1(a) as a stack of 5 disconnected cisternae (the saccules) bounded with a phospholipidic membrane (see the image part **S**). This saccule stack is usually surrounded by small vesicles that bud out from the saccules (see the image part **V** on Figure 1(a)). Notice that on some pictures like the one given by Figure 1(b), the saccules appear perforated : the image part **P**

depicts such regularly perforated saccules.



(a) Saccules stack **S** and vesicles **V** (b) Saccules perforation **P**

Figure 1: The Golgi apparatus

Using histochemical techniques at the light microscope scale, [11] has observed in 1969 an irregular presence of carbohydrates within the Golgi apparatus. Indeed, a carbohydrate gradient appears from the face close to the nucleus, (which contains few such carbohydrate stains) to the other face (which contains a lot of carbohydrate stains). Thus, the Golgi apparatus is a polarised object: the *cis* face is directed to the endoplasmic reticulum while the opposite *trans* face is often directed to the plasma membrane. Subsequent radioautographic and biochemical studies revealed that the Golgi apparatus is involved in the elaboration of complex carbohydrates, by progressively adding carbohydrate elements from its *cis* face to its *trans* face. This may be detailed according to the two following points:

- The main function of the Golgi apparatus is to sort proteins synthesized by the cell and then to transport them from the endoplasmic reticulum to adapted locations as the plasma membrane or lysosomes.

- During the transportation inside the Golgi apparatus, proteins are subjected to a maturation phase by the means of loss of peptidic sequences and addition of sugars (glycosylation) or sulfate (sulfatation).

3 Three hypotheses on the dynamics of Golgi apparatus

Because of observation limitations, the complete structure of the Golgi apparatus is not precisely known. Indeed, with optical microscopy techniques, biologists observe the dynamics at the cost of a small resolution that does not allow them to observe the structure. By contrast, electron microscopy provides high resolution pictures but the observation is done on thin and inert sections of the Golgi apparatus. Last but not least, those thin sections lead to many interpretation mistakes when a 3-dimensional reconstruction is performed (for instance, both spheres and tubes section can appear as discs on a picture).

In particular, the path that proteins follow from the endoplasmic reticulum to the plasmic membrane or lysosomes is not well known. Consequently, three main hypotheses exist [5].

3.1 Vesicular excretion

The first hypothesis views the Golgi apparatus as a static organelle composed of a stack of disconnected flattened saccules surrounded with numerous vesicles. Vesicles are supposed to play a major role in the excretion of proteins. In this vesicular secretion hypothesis (see Figure 2), an aggregate of endoplasmic reticulum (**ER**) fragments generates disconnected saccules (**S**). Proteins migrate through the stack by means of vesicles (**V**) that jump from one saccule to another. They are finally evacuated by the means of secretory granules (**G**) that bud out from the *trans* face. We know that enzymes in charge of the activation and the maturation of proteins are located near the *cis* face of the Golgi apparatus. In this first hypothesis, those enzymes may stay in the first saccules that are motionless by definition.

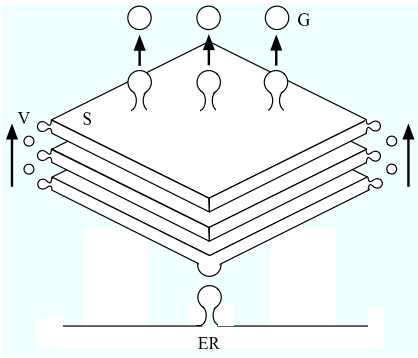


Figure 2: Vesicular excretion

3.2 Saccule maturation

The second hypothesis appears quite similar to the first one since they both suppose that vesicles play a major role in the excretion of proteins. In the saccule maturation hypothesis (see Figure 3), saccules are still disconnected but follow an anterograde movement from the *cis* face to the *trans* face which supports the transport of proteins. Here, vesicles move along a retrograde flow in order to return enzymes that are useful at the beginning (near the *cis* region), of the protein pathway to ensure protein maturation.

Vesicles issued from the endoplasmic reticulum fuse together to form the *cis* saccules. Saccules are then shifted forward to the *trans* face when new saccules are created at the *cis* face.

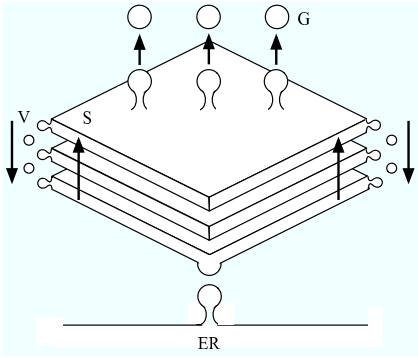


Figure 3: Saccule maturation

3.3 Continuous membrane flow

The third hypothesis promotes a continuous 3D structure for the Golgi apparatus. The saccules are no more isolated but connected together to form a unique continuous structure. Such a view can be represented by the Figure 4. This continuous hypothesis does not rely on any vesicle transportation. On the contrary, it considers a continuous membranes flow (see Figure 4) emerging from the endoplasmic reticulum. Indeed, observed endoplasmic reticulum fragments and vesicles are interpreted in this hypothesis as small sections of a tubular network that connects the saccules (**T**). In this case, proteins may follow the membrane flow and diffuse from one saccule to another along the tubes while enzymes may diffuse following a retrograde movement. Moreover, in this last hypothesis, the saccules perforation may explain the creation of the secretory granules by the rupture of the junctions resulting from the perforation.

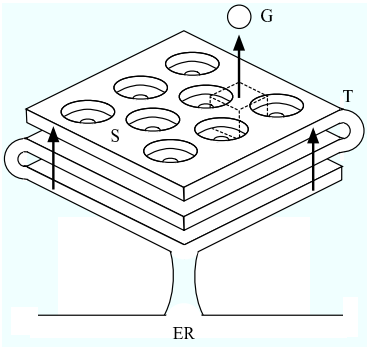


Figure 4: Continuous membrane flow

4 Topology-based simulation technics

4.1 Topology-based geometric modelling

It seems clear that among the numerous features involved in the Golgi apparatus (from the precise shape of the object to the different molecule flows), the role played by the topology is decisive. Thus, a relevant abstraction of the previous Golgi Apparatus hypotheses must handle this component. In [9, 8], we have already proposed a topology-based abstraction dedicated to the animation of simple biological processes.

In order to take the biological compartments in our model into account, we rely on the topology-based geometric modelling (topological modelling for short). This field of the computer graphics deals with the representation of the object structure (their decomposition into topological units: vertices, edges, faces and volumes) and of the neighbourhood relations that exist between topological units. Among numerous topological models, we choose the n-dimensional generalised map [6] (n-G-maps for short). It defines the topology of an n-dimensional space subdivision and allows the representation of a large class of objects[1]. This topological model has the advantage of providing a homogeneous mathematical definition for all dimensions. In this paper, we do not give details about the G-maps model for describing topological structures.

4.2 Topology-based abstraction of Golgi Apparatus hypotheses

In Section 3, we introduced three hypotheses that may explain the behavior of the Golgi apparatus. Two of these hypotheses implicate vesicles in the transport of proteins while the third hypothesis involves a continuous membrane flow in a tubular network. In this section, we use our topology-based approach to model on one hand both the vesicular excretion and the saccule maturation hypotheses and on the other hand the continuous membrane flow hypothesis. Indeed, the vesicular excretion and saccule maturation hypotheses are strictly identical from the topological point of view while the continuous membrane flow introduces significant topological differences (connected and perforated saccules).

Figure 5 illustrates the 3-G-map topological representation of the vesicular excretion and saccule maturation hypotheses. We call it the plate stack model. Figure 6 illustrates the 3-G-map topological representation of the continuous membrane flow. We call it the tower model.

The topology-based geometric modelling allows one to easily abstract geometry and to focus on pure topology which is, as we said, the most relevant distinction between studied Golgi apparatus hypotheses. When it is necessary, geometric shapes can be associated with the topological units. On Figure 5 and 6, the geometry is basic (here, the object are said to be polyhedric) but the topological differences between hypotheses are captured.

[1] Quasi-manifolds, orientable or not.

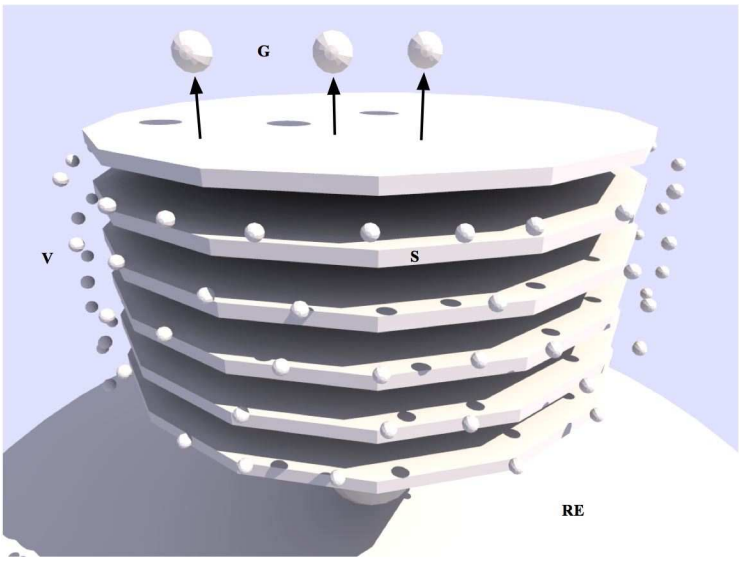


Figure 5: Plate stack model

The first distinction is the connection between the saccules (**S**). The proteins are transported through vesicles (**V**) in the plate stack while they diffuse into tubes (**T**) that connect the saccules in the tower model. As we see, we choose to abstract both saccules and vesicles with volumes with which we associate concentrations that abstract proteins (concentration gradients are modelled by subdividing volumes and associating different concentrations with each subdivision). Moreover, from a topological point of view, a tube between two saccules is represented with a volume stuck between the connected saccules. Because topological models allow one to handle border of volumes, we abstract the transport of proteins by associating permeability on faces that connect saccules to tubes. The second topological distinction concerns the creation of the secretory granules. In the plate stack, the secretory granules (**G**) bud out from the *trans* face (see arrows of Figure 5) while in the tower model, they are constituted of saccule pieces that result from the rupture (framed on Figure 6) of the bee nest structure that abstracts the perforation (according to the biologists, the perforation appears progressively from the *cis* face to the *trans* face). Finally, small parts of the endoplasmic reticulum aggregate into saccules in the first model, while the endoplasmic reticulum is connected to the *cis* face in the second one.

4.3 Topological transformation rules

In order to edit topological objects, computer scientists have defined many topological operations on the n-G-maps. In [9], we have formally expressed the basic operations as graph transformation rules. In order to model biological cellular processes we may want to attribute different kinds of information to the topological units. For instance, we may want to attach types, biochem-

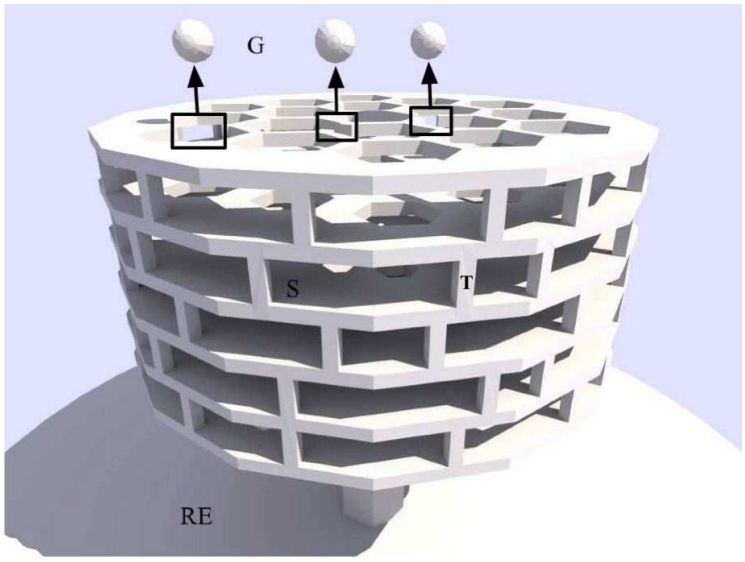
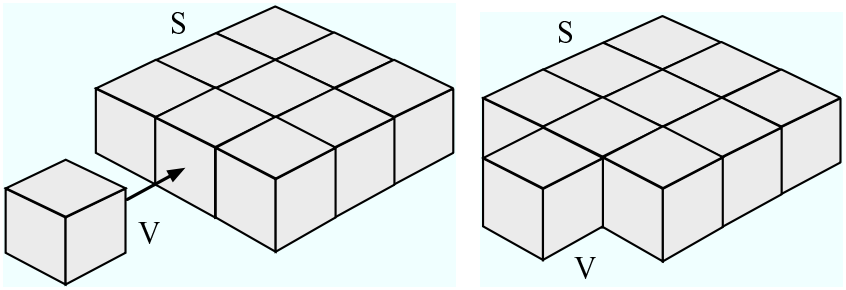


Figure 6: Tower model

ical data, geometric data (when the biological observation allows it), etc. to the volumes that abstract the biological compartments. Thus, we may want to write transformation rules whose application depends on values of these different data and that modify them.

In order to animate the plate stack model and the tower model, we have to write the rules that capture their dynamics. We succinctly give two examples of such rules. They are detailed in [8] and are illustrated in Figure 7 and Figure 8.

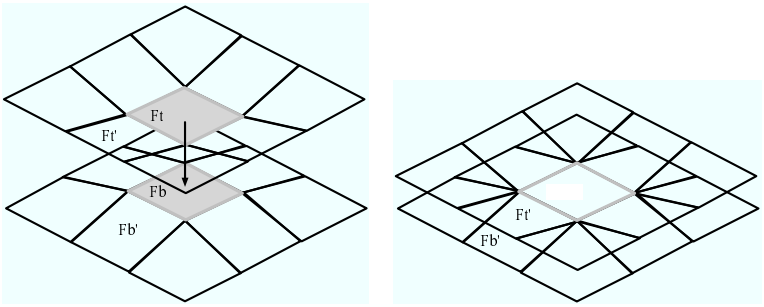


(a) Matched pattern (b) Result

Figure 7: Gluing a vesicle with a saccule

The first rule is dedicated to the plate stack model. It models the gluing of a vesicle with a saccule which initiates their fusion. Figure 7(a) introduces a simplified representation of the matched pattern, it contains a vesicle (**V**) close to a saccule (**S**). The transformation rule glues them and updates the position of the glued vesicle (see Figure 7(b)).

The second rule is dedicated to the tower model. It provides a means to



(a) Matched pattern (b) Result

Figure 8: Perforating a saccule

perforate the saccules (which is one of the behaviors at the root of the continuous membrane flow hypothesis). The matched pattern (see Figure 8(a)) contains two close faces (**Ft**) and (**Fb**) that belong to the same saccule (one is on the top, the other on the bottom). The rule executes the perforation removing faces (**Ft**) and (**Fb**) and linking their neighbours (see Figure 8(b)).

These two examples are well-representative of the transformation rules we need in order to animate the plate stack and tower models. For instance, in the plate stack model, most of the topological operations consist in sticking (it is the case of the example rule) or unsticking topological objects. Notice that the stick operation can also be used to aggregate the pieces of endoplasmic reticulum that constitute a new saccule while the unstick operation is used to abstract the budding out of vesicles and secretory granules.

We should notice that geometry plays a decisive role in animation processes. The geometric data that influence the biological function we are abstracting are handled in the condition associated with the rules. For instance, when a rule is applied it can take the proximity of objects into account. Other phenomena, e.g. collision detection between vesicles or secretory granules, should be ignored. In fact, many of them only influence the visual rendering, and we do not consider this issue as significant in our context.

5 Toward a topological discrimination of Golgi Apparatus hypotheses

5.1 Iterative construction of the topological models of the Golgi apparatus

Both models, the plate stack one and the tower model, have been elaborated by following a loop of topological model refinements. Biologists have deeply analysed intermediate models by proposing many topological updates. This is particularly true for the continuous membrane flow for which the tower model presented in Figure 6 gives a first insight of its precise topological

structure. The study of vertical sections of tower model has played an important role in the biologists' validation process (see [8] for an illustration of such a vertical section). Our tower model, which has been initiated by the biologist observation of perforations and by the interpretation of vesicles as small sections of tubes, is fully compatible with the electron micrographs that are usually used to promote the vesicular excretion and saccule maturation hypotheses. Thus, the tower model is a first original contribution of our topological abstraction process since it has been shown to be consistent with the biological observations.

5.2 Discrimination methodology

The definition of adequate transformation rules is mandatory for animating the topological models but is not sufficient to simulate such complex systems. The rules only define the syntactic part of the simulations, in other words, they define what kind of transformations the simulator performs. Our ongoing work consists in exploring what kind of strategies have to be taken into account when applying transformation rules in order to play simulations. In order to help the biologists to better understand a given biological complex system, we furthermore aim at introducing a computer-aided methodology for analysing topology and dynamics of different hypotheses associated to the biological system. Our goal is not to build an accurate model, but instead, we would rather discriminate between the different models and choose the one which best approximates the observed phenomena. However, to properly define our discrimination methodology, we need at first to define the parameters of the models.

Table 1 gives an example of parameters that have been discussed with biologists about discrimination of Golgi apparatus hypotheses. The first column displays the name of the parameters. An approximation of their value when they exist (according to the biological state of the art) appears in the second column. A value OUT means that the parameter is computed within the simulation. The third and fourth columns tell whether a given parameter makes sense in respectively, the plate stack model and the tower model. The given set of parameters is not exhaustive but contains the parameters considered by the biologists as the most relevant for the comparison of the two models.

The first six parameters are input parameters (their values are given by the biologists, according to the observations) and are used to initialize the topological models. They are associated with the topological units at the beginning of the simulation and can be refined as we discuss in the next section. The vesicle and tube diameter are also input parameters but fit to only one topological model (respectively the plate stack model and the tower model). Let us remember that depending on the hypothesis we are considering (vesicular excretion, saccule maturation or continuous membrane flow hypothesis), the same pieces of an electron micrograph can be considered as vesicle or tube section. Thus, vesicle and tube diameter must be the same. In

Table 1: Simulation parameters

Parameters	Value	Plate stack model	Tower model
Membrane tickness	$7\eta m$	Yes	Yes
Number of saccules	6	Yes	Yes
Saccule thickness	$30\eta m$	Yes	Yes
Saccule length	$50 \times 30\eta m$	Yes	Yes
Secretory granule diameter	$120\eta m$	Yes	Yes
Number of proteins in a granule	600	Yes	Yes
Vesicle diameter	$60\eta m$	Yes	No
Tube diameter	$60\eta m$	No	Yes
Membrane quantity	OUT	Yes	Yes
ATP consumption	OUT	Yes	Yes

other words, updating one of them implies to update the other one. Finally, the last two parameters: membrane quantity and ATP (it is the energetic unit of the cell) consumption have been chosen among others to discriminate the topological models. Biologists think that the quantity of membrane within the Golgi apparatus (vesicles and secretory granules take part of it) must be constant in time. Thus, if reaching a wanted quantity of transported proteins within the Golgi implies to break this property in one model, this could allow the discrimination of the two models. In the same manner, the fact that the quantity of consumed ATP reaches a critical level could be discriminating too.

The proposed methodology, which is based on successive simulations of the topological models that implement the hypotheses, is illustrated on Figure 9. The figure only takes into account two topological models but can easily be extended. The different kinds of parameters described in the previous sections are introduced. The input parameters (IN_1 and IN_2) are used to initialize the simulations of, respectively, topological models M_1 and M_2 that implement the selected hypotheses. Note that, as discussed earlier, some parameters can be specific to only one model, but are correlated to parameters of the other model. This consistency between parameters of M_1 and M_2 is necessary for the models discriminating process. OUT_1 and OUT_2 parameters result from the simulations of, respectively, M_1 and M_2 (for instance, the flow of excreted proteins are output parameters for both models). The results of the simulations are compared with biological experimental observations (OBS on the figure). Our methodology then consists in a refinement process, that is modifying the set of input parameters according to the observations. Note that the models must not be refined independently: the updates still guarantee the consistency between IN_1 and IN_2. Thereafter, we reiterate the simulation, comparison and refinement processes. This loop aims at making

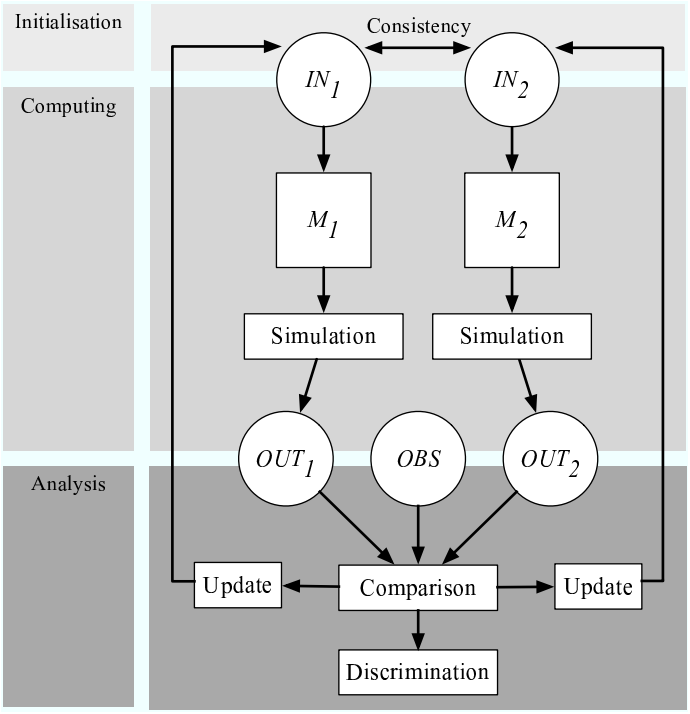


Figure 9: Models discrimination loop

both models converge toward the experimental observations. We then select the model that better approximates the observation or eliminate the one that do not converge toward the desired output values. More precisely, a model is given up when one can no longer update parameters in a satisfactory manner. If such a model is not consistent with available biological data, it is refuted and only the other model is considered for further analysis. Our methodology is intentionally simplistic. Our goal is to consider models as simple and abstract as possible to be analysed by biologist experts. When necessary, according to observations or in order to discriminate models, detailed elements are gradually incorporated in the models in order to make models more complex, up until the moment biologist experts can discard one of the models. Thus, there is a trade-off between abstraction of the models and the need of information to discriminate models. The development of finer models becomes useless even if these finer models would be more plausible with respect to the real biological processes under consideration.

Conclusion

In this paper, the Golgi apparatus is widely recognized as a complex biological system where topology plays a key (but poorly-understood) role. In the GolgiTop working group, we develop a topology-based method of modelling cellular processes. This method puts in place transformation rules

that allow simultaneous simulation of topological, geometric and biochemical mechanisms. This facilitates a better understanding of the dynamics of these cellular processes that strongly depend on compartmentalization. We first study two very different topological representations based on the three principal hypotheses about the topology of the Golgi: in the plate stack model corresponding to both vesicular excretion and saccule maturation hypotheses, saccules are disconnected and proteins move from one saccule to another *via* vesicles, while in the tower model corresponding to the so-called continuous membrane flow hypothese, saccules are connected with tubes that allow proteins to cross the Golgi. Finally, these topological models can be animated using transformation rules that are determined by the geometric and biochemical data and that determine both these data and the topology itself.

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