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*Insights*

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# Studying the Cytoskeleton: Case of Intermediate Filaments



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# STUDYING THE CYTOSKELETON: CASE OF INTERMEDIATE FILAMENTS

*The cytoskeleton is a dynamic intracellular structure in charge of major cellular functions. Its architectural organization is the main determinant of its intracellular function. A methodology based on experimental data and mathematical modelling is detailed to study the cytoskeleton. As an illustration, some examples are given in the case of intermediate filaments.*

## What? The Cytoskeleton

The cytoskeleton is an intracellular structure consisting of three interconnected networks: the actin, intermediate filament and microtubule networks. Each cytoskeletal network is composed of structural proteins assembled in filaments that crosslink to form networks. Each network has specific architectures and cellular functions. These are now detailed.

A microtubule is a cylindrical tube of about 25 nanometers in diameter. The subunits of microtubules are heterodimers of αß-tubulin. Heterodimers assemble head to tail to form protofilaments that associate laterally to form a cylinder; a microtubule comprises typically about 13 protofilaments. Assembly of tubulin is energy-dependent. The assembly dynamics of microtubules is regulated by microtubule-associated proteins (MAPs). Microtubules exhibit dynamic instability characterized by frequent switches between growing and shrinking phases (Mitchison and Kirschner, 1984). The architecture of the microtubule network is also controlled by MAPs and motor proteins. Most microtubules are initiated/nucleated from the microtubule-organizing centers (MTOCs), usually located near the nucleus in interphasic cells, and radiate to the cell periphery. Microtubules are polar polymers, as are actin filaments. Their minus ends (slow-growing ends) are embedded at the MTOC, whereas their plus ends (fast-growing ends) are at the cell periphery. Microtubules are used as tracks to transport organelles and proteins stored in vesicles within cells. Motor proteins such as dyneins and kinesins 'walk' along microtubules while they are attached to a cargo (vesicles or organelles). Microtubule-dependent transport is bidirectional; kinesin walks from the minus end to the plus end providing transport from the interior of the cell to its periphery, whereas dynein walks from the plus end to the minus end resulting in transport from the periphery to the center of cells. Microtubules are the highway transporting information in the cells. Owing to their hollow cylindrical shape, microtubules are stiffer polymers than actin and intermediate filaments and are highly resistant to compressive forces. Microtubules also form the mitotic spindle, a dynamic structure that segregates chromosomes to daughter cells during cell division (Helmke et al., 2013).

Actin filaments are helical polymers of five nanometers in diameter formed from actin globular monomers (G-actin). Similarly to microtubules, the assembly of actin is energy-dependent. The architecture and functions of the actin network are regulated by a large number of actin binding proteins. The binding proteins involved shape the actin network; they define its architecture. The architecture of the actin network can range from branched networks, linear structures, bundles and contractile structures to crosslinked networks (Rohn and Baum, 2010). The actin network is in charge of major cellular functions such as cell motility, endo- and exocytosis, dynamics (change and maintenance) of cell shape and muscle contraction, and is the propelling system of cells (Blanchoin et al., 2014). Misregulations or mutations of actin are associated with neurodegeneration, cancer or myopathies. The actin cytoskeleton is also associated with infectious diseases; pathogens exploit the host cell actin network to invade cells (Rottner et al., 2004).

Lastly, intermediate filament proteins are encoded by about 70 genes (Eriksson et al., 2009); the expression of intermediate filament protein types depends on the cell type. For instance, epithelial cells mainly express keratin, while mesenchymal cells express vimentin. All intermediate filament proteins are fibrous molecules sharing the same tripartite structure: a rod domain flanked by head and tail domains. Modes of intermediate filament assembly differ but all filaments are non-polar cables of about 10 nanometers in diameter. Intermediate filament networks are resistant to breakage when they are mechanically stressed; they can be stretched more than three times their resting length before breaking and are predominantly elastic (Sivaramakrishran et al., 2008). The networks exhibit a property of strain hardening; the more they stretch, the stiffer they get (Janmey et al., 1998). These mechanical properties are related to the cellular functions of the intermediate filament networks; these functions are structural and mechanical. Intermediate filaments are in charge of maintaining cell integrity, mechanical resistance of cells and physical resilience of tissues. More functions, such as in cell signalling, have now been identified in relation to diseases for which defaults in intermediate filament networks are associated. More and more human diseases, such as skin fragility diseases, some myopathy and cardiomyopathy and neurodegenerative diseases, have been associated with mutations of intermediate filament proteins (Eriksson et al., 2009). Intermediate filaments are regulated by post-translational modifications such as phosphorylation (Snider and Omary, 2014). In interphasic cells, intermediate filament proteins are found mainly assembled in filaments and networks, the so-called insoluble pool. The pool of soluble intermediate filament proteins composed of tetramers is very small in comparison to the insoluble pool (Soellner et al., 1985). The disassembly/assembly dynamics *in vivo* is regulated by the activities of kinases/phosphatases mediating the phosphorylation/dephosphorylation. Those kinases/phosphatases are regulated by mitosis or stress-related signals. Intermediate filaments are stress absorbers.

The organization of a cytoskeletal network is the main determinant of its function in cells. The cytological signature of some human diseases is the misorganization of cytoskeletal networks. Hence, understanding mechanisms determining the cytoskeleton organizations in cells is essential and could lead to new therapies.

## *How? Combining Experimental Data and Mathematical Modelling Approaches*

The organization of cytoskeletal networks *in vivo* is a very complex problem, it can be observed with different types of microscopy (Cooper, 2000); observations can be quantified, for instance using image analysis, thereby generating quantitative experimental data (see, e.g., Portet et al., 1999; Danuser, 2011; Moch et al., 2013). Multiple processes contribute to the cytoskeletal organization; all are not well understood and the interplay between some of these processes can result in emergent behaviours. To gain a better understanding of the factors contributing to cytoskeletal organization, combining experimental data and mathematical modelling is a powerful tool of investigation. An outline of this methodology is given in Figure 1 and is now detailed.

First of all, the question to be investigated is defined. Modellers need properly to define the question at hand, i.e. choose what type of features the map that they provide will represent. Once the question has been well posed, the quantities under investigation that will constitute the variables of the models are identified. The model variables must be related to the experimentally observed or observable quantities.

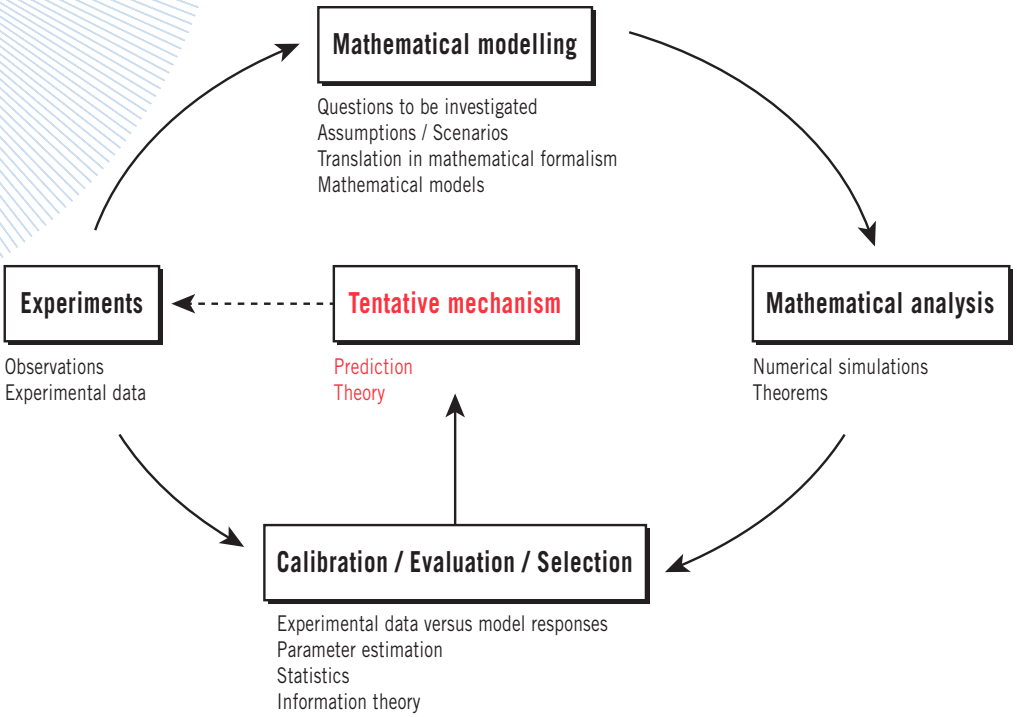


Figure 1: Approach to study of the cytoskeleton.

Then, the model assumptions have to be decided on; the important processes governing the observed problem are identified. The questions of what mechanisms or processes to neglect and what mechanisms or processes to consider must be answered. For instance, do quantities studied react to or are submitted to a transport? All assumptions have to be physically or biologically plausible.

Given model assumptions, the basic principles that govern or link the quantities investigated are then identified. The questions of interest here are the following: What types of interactions, relationships or reactions link the quantities studied? What physical laws govern the quantities considered? These principles are then expressed mathematically, i.e. they are translated into mathematical models using an appropriate mathematical formalism. A mathematical model is a mathematical object consisting of an equation, a system of equations or a graph. The formalism used depends on the intrinsic properties of the observed problem and the question being investigated. The formalism can be static or dynamic, deterministic or stochastic, homogeneous or heterogeneous in space, continuous or discrete in space or time, etc. Once the formalism is decided upon, the translation of each individual process assumed to represent the observed problem takes place. For instance, a saturating process can be described by several mathematical functions; the more adequate form of the function to represent the specificity of the observed problem must be chosen. When no information is available, an educated guess is made or several possible functions are considered to represent the same process, leading to different models that then have to be compared. Furthermore, different hypotheses for a same assumption can be accounted for. For instance, is transport passive or active? Combining the different hypotheses, a collection of models is then created to represent the different possible hypotheses, scenarios or synopses leading to the observed problem. The end product of the modelling phase is thus a mathematical model or a collection of mathematical models.

After the mathematical modelling phase, mathematical and computational analysis take place. Depending on the formalism used for the mathematical models, theories exist to tackle the behaviour of the models. For instance, a dynamic system formalism such as differential or

difference equations allows the use of powerful theories and tools to investigate the long-term (asymptotic) behaviour of models. Computational analogues of mathematical models are designed to run numerical simulations approximating the solutions of the models. Then, qualitative and quantitative model responses are confronted with experimental data and observations. Model responses are compared to experimental data to calibrate, evaluate and select the best models if a collection of models is considered (Johnson and Omland, 2004). Each model is defined with a set of parameters, the values of which are unknown and have to be estimated from comparison with experimental data. For each model, parameter values that give the best fit of experimental data are determined. For instance, the estimation of parameters can be carried out using the least squares method, which minimizes the distance (the sum of the squared residuals) between the numerical model responses and the experimental data. Minimization might require methods of global optimization such as genetic algorithms or particle swarming. The use of the least squares method allows for the estimation of the likelihood for each model, which is useful for comparing models when needed. When a collection of models is considered, once the parameter values are estimated and the best fit for each model is obtained, selection and evaluation are carried out to identify the 'best of the best' models. If models are nested, statistical approaches such as the likelihood ratio test or F-test can be used to compare models pairwise. Two models are nested if one of them is a special case of the other one: the reduced model is nested in the complete model. Parsimonious models (models with fewer parameters) with similar predictive powers are preferred. Nested or non-nested models can be compared by information theoretic approaches such as the Akaike Information Criterion (AIC) (Burnham and Anderson, 2002). This criterion provides an indicator of the performance of each model by accounting for the goodness of the fit as well as the number of estimated parameters and the size of the experimental data set considered. From the AIC, an Akaike weight can be derived for each model, providing the probability that the model is the best given the experimental data and the collection of models considered (Johnson and Omland, 2004). This approach allows not only for a global ranking of the models in the collection but also an evaluation of all the models with respect to selecting the best one, i.e. the model having the smallest AIC value.

The best of the best models represents a tentative description of the mechanism, a chain of events that could lead to the observed problem. The last step is to conduct actual biological experiments to confirm or invalidate the tentative mechanism proposed by the approach.

As mentioned previously, a single mathematical model can be considered. However, the use of a collection of models allows for the weighting of the contribution of each process to the observed problem and the characterization of the main contributors, the required or mandatory and the non-significant processes. When only one model is considered, sensitivity analysis, i.e. the study of the effect of parameter values on model responses, gives some insights into the contribution of each process; however, the predictive power of the approach is reduced.

## *Why?*

Using the type of approach outlined previously, the *in vitro* assembly of intermediate filaments was investigated with specific focus on the mechanisms of filament elongation (Kirmse et al., 2007). Experimental data gave the mean length of vimentin filaments observed by scanning force and electron microscopies at different time points of assembly. Different hypotheses, based on previously published biological evidence (see, e.g., Herrmann et al., 1999), were made about the possible mechanisms of filament elongation: addition of tetramer to the filament end, addition of unit-length-filament, composed of eight laterally-aggregated tetramers, to the filament tip or fusion (longitudinal annealing) of two filaments. Considering all

possible combinations of these three on- or off- events, eight scenarios of filament elongation were derived. After studying numerical solutions giving the mean length of filaments for the eight scenarios and comparing them to the experimentally-measured mean lengths, major conclusions were made. It was found that tetramer addition to the filament tip is a non-significant event in the elongation of intermediate filaments but that the process of filament fusion must be included in order to explain the experimentally-observed mean length of vimentin filaments. The occurrence of filament fusion was confirmed experimentally a few years later (Çolakoglu and Brown, 2009; Winheim et al., 2011). More investigation using the same approach but on the distributions of filament lengths demonstrated the importance of the flexible nature of intermediate filaments on their assembly process (Portet et al., 2009; Portet, 2013).

In a recent work studying the *in vivo* assembly of keratin networks, this approach was used again. This investigation concerned the effects of the interplay between the turnover and transport of keratin material on the organization of its networks in epithelial cells (Portet et al., 2015). Experimental data published in Moch et al., 2013, represented the spatial distributions of the assembled keratin proteins along the diameter of a cell at 24 and 48 hours. The keratin material was categorized in two pools: the soluble pool and the insoluble pool, the latter being the observable quantity in this case. It was postulated that the rate of change of both pools at any location of the cell results from assembly-disassembly processes and transport of assembled and soluble proteins in the cell. For the soluble pool, it was assumed that the only mode of transport of soluble proteins within the cell was through diffusion. On the other hand, three modes of transport were hypothesized for the motion of the insoluble pool. First, only a wiggling motion of filaments due to thermal agitation was considered. Then, in addition to the wiggling motion, an inward drift due to microtubule-dependent transport was assumed. The inward drift was considered with both almost constant speed and variable speed. For the turnover, i.e. the assembly of soluble pool units resulting in the growth of the insoluble pool (filaments) and the disassembly of the insoluble pool into units of soluble pool, two types were assumed: a linear exchange between pools and enzymatic activities of the Michaelis Menten type for both the assembly and disassembly processes. Finally, based on experimental data (Moch et al., 2013), preferential regions for assembly and disassembly were identified, and the rates of assembly and disassembly used in the both turnover terms were taken to be constant over the cell or space dependent. Two types of assembly rates and three types of disassembly rates were assumed. Taking all these hypotheses into account, a total of 36 scenarios was therefore considered. After estimating parameters in these scenarios, the best profiles for each scenario were identified. Using an information-theory approach, the best scenario was identified and a tentative mechanism for explaining keratin organization in epithelial cells was proposed. This tentative mechanism predicts the diffusion of soluble keratin proteins, an inward motion for the assembled keratin, enzyme-activated assembly and disassembly processes with a spatialization of the disassembly. Thus, this tentative mechanism confirms in part a previous biological model hypothesized in Leube et al., 2011, and Windoffer et al., 2011. Experimental protocols must now be designed to validate or invalidate the major conclusion of the tentative mechanism, namely that the disassembly process is localized in the perinuclear region.

## Conclusion

Mathematical modelling is an inexpensive and non-invasive tool that allows for the testing of a large number of different scenarios to determine which one best reproduces the observed problem. Because the tentative mechanisms suggested by the best scenario might not yet have been investigated experimentally, mathematical modelling is also helpful in that it can guide experimentalists in their experimental protocol designs. Models also help to identify the major

contributors to the process under investigation. Ultimately, mathematical modelling results in a theorization of the observed problem. As such, mathematical modelling combined with experimental data is a powerful tool of investigation in cell biology that can be used both for observation purposes and for generating novel theoretical hypotheses regarding the functioning of biological systems.

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Insights is edited by Barbara Graziosi, IAS Director and Professor of Classics.
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