Integrative models for TGF-β signaling and extracellular matrix

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Abstract

The extracellular matrix (ECM) is the most important regulator of cell-cell communication within tissues. ECM is a complex structure, made up of a wide variety of molecules including proteins, proteglycans and glycoaminoglycans. It contributes to cell signaling through the action of both its constituents and their proteolytic cleaved fragments called matricryptins [Hynes and Naba, 2012, Ricard-Blum and Vallet, 2019]. In addition, ECM acts as a "reservoir" of growth factors and cytokines and regulates their bioavailability at the cell surface. By controlling cell signaling inputs, ECM plays a key role in regulating cell phenotype (differentiation, proliferation, migration, etc.).
In this context, signaling networks associated with the polypeptide transforming growth factor TGF-β are unique since their activation are controlled by ECM and TGF-β is a major regulator of ECM remodeling in return.

1 TGF-β and Extracellular matrix, a win-win relationship

TGF-β is a prototype of a large family of growth factors that play an essential role in essential biological processes, such as tissue morphogenesis and homeostasis, but also in numerous diseases such as fibrosis and cancer[Tian et al., 2011]. Initially identified as a promoter of fibroblast growth and transformer of cell phenotype, TGF-β has been rapidly designed as a *bifunctional regulator of cellular growth* [Roberts et al., 1985] depending on the environmental context. Beside its role in cell proliferation, TGF-β is implicated in numerous biological functions including cell differentiation, migration, chemotaxis and ECM production and remodeling.

TGF-β is synthesized as an inactive homodimeric large precursor molecule consisting of a self-inhibiting propeptide, the latency-associated protein (LAP), in addition to the covalently linked active form of TGF-β. Pro-TGF-β is then intracellularly cleaved by furin-type enzymes to generate mature TGF-β, which remains non-covalently associated with LAP as the small latent complex (SLC) and the LAP dimer is covalently bound by a latent TGF-β-binding protein (LTBPs) to form the large latent complex (LLC). Complexes are sequestered in the extracellular matrix (ECM) where LTBP interacts with several components, including fibronectin and fibrilin. The activation process of TGF-β requires dissociation of TGF-β from the ECM-bound LLC and implicates protease — and/or non protease — dependent mechanisms, which differ according to the cell microenvironment [Lodyga and Hinz, 2019, Robertson and Rifkin, 2016] (see Figure 1). Mechanisms of activation mainly include mechanical interactions [Hinz, 2015] involving integrins such as αvβ6 and αvβ8 integrins [Brown and Marshall, 2019], chemical interaction involving proteases, such as matrix metalloproteases MMP-2 and MMP-9, and thrombospondin-1 [Murphy-Ullrich and Suto, 2018] and physical stress such as heat or reactive oxidative species [Annes et al., 2003]. Together, all molecules involved in the dynamic storage and destocking of TGF-β form a protein network in which the role of each one in TGF-β signaling is obviously part of the sum.

When activated, TGF-β binds to specific receptors to induce a variety of signaling pathways depending on the cell and the microenvironmental context. TGF-β receptors are transmembrane serine/threonine kinases, that include type I (TGFBR1) and type II (TGFBR2) receptors. The canonical pathway involves a Smad-dependent cascade which induces nuclear signalling to regulate transcription of target genes. Receptor regulated Smads (or R-Smads) are transcription factors initially anchored to the cell membrane by SARA proteins. Following their phosphorylation by TGFBR1, Smads are detached and shuttled to the nucleus, where they activate gene transcription. Numerous Smad-binding partners and transcriptional coactivators and cosrepressors for Smads have been reported as leading to a wide variety of TGF-β-dependent transcriptional signatures [Feng and Derynck, 2005]. Additionally, cross talk between the TGF-β/Smad pathway and other signaling pathways such as Wnt

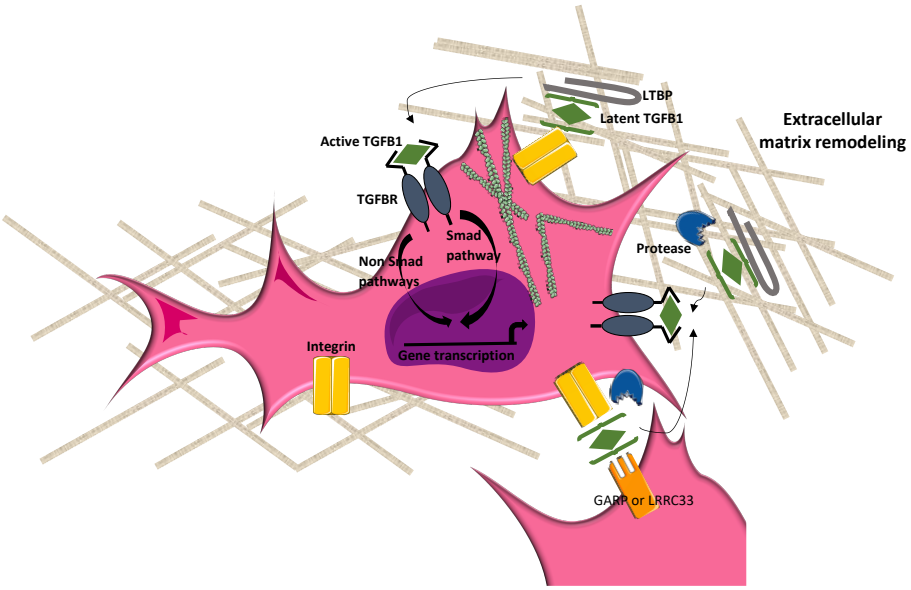

Figure 1: Schematic representation of TGF-β activation and signaling (adapted from [Lodyga and Hinz, 2019]). Latent form of TGF-β is sequestered within ECM as a large latent complex associated with LTBP that binds to ECM. Release of the active peptide of TGF-β from this large latent complex involves mechanical and non-mechanical mechanisms depending or not upon protease activities. Integrins or other cell surface receptors such as GARP and LRRC33 bind latent-TGF-β. Strength constraints between cytoskeleton-linked integrins and LTBP-linked ECM induce release of active TGF-β peptide. Protease activities are involved in activation of latent-TGF-β bound to cell surface receptors and contribute to release of TGF-β during extracellular matrix remodeling. Active TGF-β signals through TGF-β receptors (TGFBR) and activation of smad- and non smad-dependent pathways leading to the transcriptional regulation of TGF-β target genes. Most of them are genes coding for ECM compounds and proteases that contribute to ECM remodeling and regulation of TGF-β signal in return.

and Hyppo [Luo, 2017, Piersma et al., 2015] complicates the Smad-dependent signature. Otherwise, TGF-β induces non-Smad pathways through binding to TGFBR2, leading to activation of mitogen-activated protein kinase (MAPK), Rho-like GTPase signaling pathways and phosphatidylinositol-3-kinase/AKT pathways [Zhang, 2017]. Because all of these pathways are also activated by many other extracellular factors and matrix components, the expression of TGF-β-target genes is highly modulated by the cell environment.
Together the TGF-β-related signal behaves as a system with numerous competitive pathways and regulatory loops, allowing a fine tuning of cell response to various conditions. Understanding how ECM and TGF-β work together to maintain tissue homeostasis, and how alteration of this equilibrium is affected in various pathologies, requires integrative and modeling approaches. Such models aim to predict cell responses to a "TGF-β dependent signal" and, ultimately, identify putative targets suitable for future therapy.

2 Modeling approaches for TGF-β signaling

Numerous models have been developed to describe the behaviour of the canonical Smad-pathway [Clarke et al., 2006b, Zi et al., 2012]. These models, using chemical reaction networks (CRN) and ordinary differential equations (ODEs) focused on Smad phosphorylation [Clarke et al., 2006b]; receptor trafficking [Vilar et al., 2006b]; Smad nucleocytoplasmic shuttling [Melke et al., 2006b, Schmierer et al., 2008a]; and Smad oligodimerization [Nakabayashi and Sasaki, 2009a] that allow an understanding of the dynamics and flexibility of the Smad-dependent pathway.
Importantly, models for receptor trafficking that control the transient or permanent TGF-β-dependent response, enriched the behaviours of TGF-β dependent phenotypes [Vilar et al., 2006b] and integrative models have now coupled receptor trafficking to Smad pathways [Chung et al., 2009, Zi et al., 2011a, Wegner et al., 2012, Nicklas and Saiz, 2013, Shankaran and Wiley, 2008]. The general picture is that the interaction between various Smad channels is a major determinant in shaping the distinct responses to single and multiple ligand stimulation for different cell types [Nicklas and Saiz, 2013]. The amount of Smad shuttled to the nucleus seems, for most of these models, to depend in a graded, linear manner on the concentration of ligands, while remaining able to be temporally modulated in a transient or oscillatory manner [Cellière et al., 2011]. The Smad pathway is also able to encode the speed of variation of the input signal into the shape, transient or permanent [Vilar et al., 2006a], or the amplitude [Sorre et al., 2014] of the output signal, with possible important consequences for morphogen readout and patterning in developmental biology [Sorre et al., 2014]. Parametric sensitivity analysis of these models emphasized the importance of various processes for the Smad response [Clarke et al., 2006b]. Recently, we have developed an alternative analysis method, based on tropical geometry, that extends steady state calculation to calculation of metastable (long live transient) states [Samal et al., 2016]. This method de-

tected two classes of metastable states with antagonistic low and high TGFR1 and TGFR2 values, suggesting that important signal processing leading to flexible response is already performed at the level of the receptor system. These states were given phenotypic interpretations. Using this approach, analysis of proteomic data from NCI-60 cancer cell lines associated non-aggressive and agressive lines to low and high expression TGFBR2 states, respectively [Samal et al., 2016].

Taking advantages of such ODE-based models, we have developed our own models to study the role of the tumor biomarker ADAM12 [Gruel et al., 2009] and the tumor suppressor TIF1γ [Andrieux et al., 2012], thereby demonstrating that small numerical differential models may be useful tools to investigate the role of new regulatory components of the canonical TGF-β signaling pathway. Some of these models are available on the BioModels database [Malik-Sheriff et al., 2019], see Table 1. Although most of these models did not integrate the events that take place within the extracellular space and only consider cell surface receptors and free ligands as inputs, a few models include ECM variables providing crude descriptions of the coupling interactions between ECM and TGF-β signalling.

Combinatorial explosion of variables and parameters prevents the use of ODE approaches to integrate all TGF-β dependent pathways including Smad, non Smad-dependent pathways and cross-talk with other pathways. To overcome this limitation, we previously developed a discrete formalism for a large-scale model for TGF-β dependent signaling [Andrieux et al., 2014]. In this formalism molecular species and complexes are represented as boolean variables placed in the nodes of a network and connected by "guarded transitions", i.e. monomolecular transformations taking place if logical conditions on regulators and events defining the order of firing are satisfied. Due to the events, both synchronous and asynchronous network dynamics can be simulated. In this case, a trajectory of the network represents a sequence of such transitions. Based on this formalism, we generated a TGF-β network composed of more than 9000 nodes extracted from the Pathway Interaction Database (now available at https://www.pathwaycommons.org), including ECM biochemical interactions, and that allowed us to explore 15934 trajectories involving 145 TGF-β-target genes [Andrieux et al., 2014]. A guarded transition-based model, however, is not appropriate to describe the dynamics of extracellular networks that regulate TGF-β activation. A discrete dynamic modeling approach was also used to model TGF-β-driven epithelial-mesenchymal transition in hepatocellular carcinoma [Steinway et al., 2014, Steinway et al., 2015]. The authors focused on the dynamics of cross-talks between TGF-β signaling and other signaling pathways but did not integrate extracellular matrix regulation. To take into account the complexity of extracellular matrix dynamics regulating TGF-β signaling, we develop new approaches that are described in the two next parts. The first one uses the rule based formalism Kappa [Danos and Laneve, 2004] that allows us to describe the extracellular interaction networks. The second uses mesoscopic PDEs over time, space and structure dimension to integrate multi-scale and multi-physical parameters.

Ref.	Pubmed/Biomodels Id	# vars	Method	ECM
[Andrieux et al., 2012]	22461896	21	ODE	no
[Andrieux et al., 2014]	24618419	9000	BAN	yes
[Ascolani and Liò, 2014]	24586338	13	DDE	no
[Cellière et al., 2011]	22051045/BIOMD0000000600	18	ODE	no
[Chung et al., 2009]	19254534	17	ODE	no
[Clarke et al., 2006a]	17186703/BIOMD0000000112	10	ODE	no
[Khatibi et al., 2017]	28407804	11	DDE	no
[Li et al., 2017]	29322934	14	ODE	yes
[Lucarelli et al., 2018]	29248373	13	ODE	no
[Melke et al., 2006a]	17012329	17	ODE	no
[Musters and van Riel, 2004]	17270884	5	PL	yes
[Nakabayashi and Sasaki, 2009b]	19358856	7	ODE	no
[Nicklas and Saiz, 2013]	23804438	38	ODE	no
[Proctor and Gartland, 2016]	27379013/BIOMD0000000612	37	ODE	no
[Schmierer et al., 2008b]	18443295/BIOMD0000000173	26	ODE	no
[Shankaran and Wiley, 2008]	18780891	∼15	ODE	no
[Steinway et al., 2014]	25189528	65	BAN	no
[Steinway et al., 2015]	28725463	69	BAN	no
[Strasen et al., 2018]	29371237	26	ODE	no
[Tortolina et al., 2015]	25671297/MODEL1601250000	460	ODE	no
[Venkatraman et al., 2012]	23009856/BIOMD0000000447	13	ODE	yes
[Vilar et al., 2006a]	16446785/BIOMD0000000101	6	ODE	no
[Vilar and Saiz, 2011]	22098729	12	ODE	no
[Vizán et al., 2013]	24327760/BIOMD0000000499	26	ODE	no
[Wang et al., 2014]	24901250	27	ODE	no
[Warsinske et al., 2015]	26384829	10	ODE	yes
[Wegner et al., 2012]	22284904/BIOMD0000000410	53	ODE	no
[Zhang et al., 2018]	29872541	7	ODE	no
[Zi and Klipp, 2007]	17895977/BIOMD0000000163	16	ODE	no
[Zi et al., 2011b]	21613981/BIOMD0000000342	21	ODE	no

Table 1: Models of TGF-β signaling. ODE = ordinary differential equations; DDE = delay differential equations; BAN = Boolean automaton networks. PL=hybrid, piecewise linear ODEs.

3 Kappa, a formalism adapted to model the biological component networks of the extracellular matrix

Modeling the ECM can hardly be done by traditional techniques, because it involves the formation of large compounds of proteins. We use the Kappa modeling environment [Boutillier et al., 2018b] to summarize the knowledge that is available in the literature about the molecular interactions surrounding the activation of TGF-β in the ECM.

Complex systems of interaction between molecules are difficult to model for several reasons. Firstly, due to many potential bindings between proteins and numerous potential post-translational changes of conformation, there exists a large (if not infinite, in the case of polymers) number of different kinds of molecular complexes. It is often even impossible to enumerate them. Secondly, the dynamics of these systems is usually triggered by concentration- and time-scale separation; competition against shared-resources; complex causality chains; and non linear feedback loops. As a consequence, taking a biochemical approach, which consists in summarising reactions between molecules as generic, local patterns of interactions, seems to be the only viable alternative for modeling these systems and understanding how the dynamics of their populations of molecules may emerge from individual interactions at the microscopic level.

Kappa [Danos and Laneve, 2004] is a site-graph rewriting formalism, that is freely inspired by reaction schema encountered in organic chemistry. The main idea is to describe each instance of protein as a node in a graph. Each kind of protein has some interaction sites which can bind pair-wise. The interactions between molecules are formalised by the means of rewrite rules. The rules either stand for interactions that are detailed in the literature or for some fictitious interactions that exist to make assumptions about the information that is missing or to roughly simplify some parts that we do not want to detail too much. The use of rules eases frequent updates of the models, which enables the modeler to test numerous scenarios or to modify the environment of the model. A set of rules can be interpreted as a dynamical system which describes the evolution of a soup of molecules. There are several choices: when the number of different kinds of molecular complexes is not too great, the set of rules may be translated into ODEs [Camporesi et al., 2017]. Each set of rules also induces a continuous time Markov chain the execution traces of which can be sampled by simulation [Danos et al., 2007b]. Thanks to the use of specific data-structures [Danos et al., 2007b, Boutillier et al., 2017], the computation cost of such simulation does not depend on the number of kinds of molecular complexes, which may even be infinite.

Kappa ecosystem [Boutillier et al., 2018b] offers several tools to assist the modeler during her task. Static analysis [Danos et al., 2008, Feret and Lý, 2018, Boutillier et al., 2018a] may be used to curate models. Canonical and secondary pathways may be extracted thanks to causality analysis [Danos et al.,

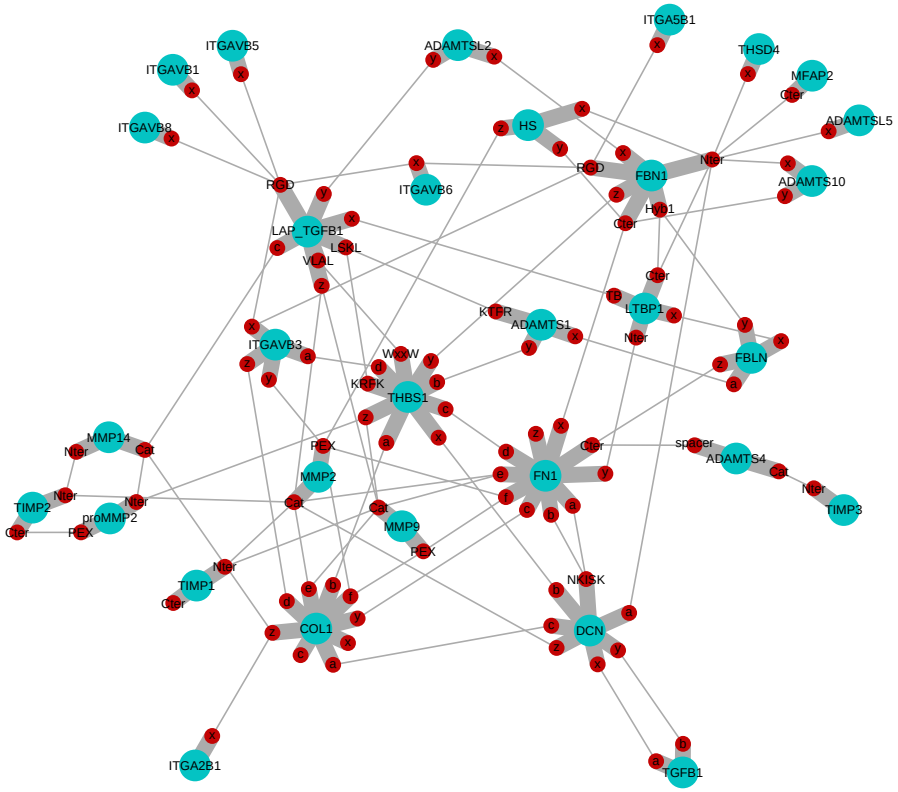

Figure 2: Contact map of the Kappa model for TGF-β activation: Projection of model describing the molecule interaction networks. Proteins and glycosaminoglycans are represented by turquoise nodes, binding sites are represented by red nodes (if the sequence involved is not known, the site is designated by a letter, x, y, z etc). The lines between the sites illustrate potential links involving these binding sites. ITGA-x-B-y, Integrin alpha-x Beta-y ; LAP-TGFB1, latent TGFB1 ; THBS1, Thrombospondin ; HS, Heparan Sulfate ; FBN1, Fibrillin 1 ; FN1, Fibronectin, FBLN, Fibulin ; THSD4, ADAMTSL-6 ; MFAP2, Microfibril Associated Protein 2 ; LTBP1, Latent Transforming Growth Factor Beta Binding Protein 1 ; MMP, Matrix Metalloprotease ; TIMP, Tissue inhibitor of MMP ; COL1, Type 1 collagen ; DCN, Decorin.

2007a, Danos et al., 2012]. Formal methods can also be used to identify the key elements in information propagation. The result is a model reduction which never loses any information about the quantities that are observed in the model [Feret et al., 2009, Danos et al., 2010, Camporesi et al., 2013].
We wrote a model for the influence of the ECM on TGF-β signal, including numerous extracellular interactions that are documented in the literature, and some fictitious rules to stub gene activity and its interaction with TGF-β. The model is made of around 300 interaction rules which are freely available on the web [Théret et al., 2020b]. Each rule is parameterised by a kinetic rate. Some of the rates are deduced from precise information about the concentration of proteins at stationary distribution and their half-time periods. Some others are chosen approximately, in order to best model what is known about the time scales of each interaction. Our model comprises around 30 kinds of proteins. The potential bonds between these proteins are summarised in Fig. 2, which provides a convenient snapshot of the model, while not detailing every rule. Selected portions of the models are depicted and explained intuitively in [Théret et al., 2020a].
Our goal, when designing this model, is three-folds. Firstly, the interaction rules are written to organize what is known in the literature and to let us make some assumptions about what is not known. It is a way to make knowledge about the models and its different variants navigable. Secondly, the different semantics of Kappa allow the execution of the rules. This makes knowledge executable. The last, longer term objective, is to understand how the macroscopic behaviour may emerge from the interactions between the individual instances of proteins. In the end, the semantics of Kappa make it possible to better approach the dynamics of the multiple molecular interactions that contribute to the activation of TGF-β. Using parameters specific to the pathological context, this model can allow us to identify the key events that regulate this activation and consequently potential therapeutic targets.

4 Mesoscale and multi-scale tissue models integrating TGF-β signaling and its interaction with the ECM

The coupling of TGF-β with the ECM is a multi-scale and multi-physical problem. It involves chemical kinetics of intracellular signaling and of ECM biochemical processes, but also more complex physico-chemical processes such as polymerisation and viscoelastic dynamics of collagen and fibrin fibres of the ECM, as well as population dynamics of various cell types.
A simple, but rather limited, solution to modelling such a complex situation is model merging. Models representing several levels of organisation can be merged together to cope with the coupling between scales. Merging, however, is not straightforward even when models of different levels are of the same type (PDEs, ODEs or Markov processes). For instance, it is relatively easy to

couple ODE models of intracellular pathways with models of the same type of the extracellular matrix, by using the standard technique of compartments [Venkatraman et al., 2012, Li et al., 2017]. It is much more difficult to couple single cell dynamics with the population dynamics because the variables of these models cannot be simply juxtaposed with different spatial locations.
Single scale, ODE population dynamics models were used to study the role of TGF-β in immunotherapy and wound healing [Waugh and Sherratt, 2006, Wilson and Levy, 2012, Hu et al., 2019, Arciero et al., 2004, Bianchi et al., 2015]. In these models the ECM variables are implicit in the cell-cell interactions but do not follow dynamical equations. These simplifications are extreme and could lead to inaccurate conclusions for processes depending critically on the dynamical structuring of the ECM, such as wound repair for instance.
Hybrid approaches combining discrete cell positions with continuous description of collagen matrix and other ECM components have been used to study the role of the TGF-β/ECM interactions in wound repair [Dallon et al., 2001, Wang et al., 2019, Cumming et al., 2009]. In these models, fibroblasts and immune cell motility and proliferation are affected by TGF-β, and cells interact one with another or with the collagen chemically or by direct, physical contact. A similar model was used to couple TGF-β signaling with the micro-environment in a preliminary study of tumor-stroma interactions [Morshed et al., 2018]. Furthermore, there is a need for models including mechanical stresses known to be generated in ECM by cell traction and vessel growth.
Agent-based modeling enabling cells to have individual behaviours including division and motility has been used for models of epidermis in the context of wound healing [Wang et al., 2009, Stern et al., 2012, Sun et al., 2009, Adra et al., 2010, Sun et al., 2009]. However, this solution is computationally expensive and has limitations in terms of biochemical details that can be used to model or parameterise the cell behaviour; its interaction with the micro-environment; or the number of cells and, moreover, is entirely based on numerical simulation.
Continuous modelling using partial differential equations (PDEs) can be justified by coarse graining (homogenisation) when the spatial scale of interest is much larger than the cell size and the typical dimensions of fibers. This modelling can take into account spatially inhomogeneous densities of various cell types and ligands, as well as collagen fibers and other ECM constituents. Directed, un-directed, and chemically mediated cell mobility are taken into account in Keller-Segel PDE systems or in similar systems used in oncology and likewise in the context of chondrogenesis or fibrosis [Bitsouni et al., 2017, Kim and Othmer, 2013, Friedman and Hao, 2017, Chen et al., 2018]. Although quite flexible, easy to simulate in 2D and 3D, and sometimes leading to analytic results, these models consider intracellular dynamics as instantaneous and do not handle mechanical interactions.
While informative, all these studies integrate the extracellular world as a very simplified input that did not capture the extracellular dynamics of TGF-β life in its full complexity. For instance, the kinetics of production and degradation of TGF-β including its latent form bound to ECM and its active soluble form

was considered in the study of TGF-β interaction with chondrocyte and mesenchymal stem cells [Chen et al., 2018]. The underlying biochemical networks that regulated such dynamics, however, remained unexplored. Importantly, the ECM is a complex molecular network combining proteins, proteolglycans and glycoaminoglycans that is constantly remodeled through modification of components' synthesis and their degradation by proteases. This specific tissue microenvironment is disrupted in pathological processes and directly affects the TGF-β-mediated signal by modifying its storage and release.

Because of slow intracellular dynamics, differences occur not only between cells of different types and genotypes but also between clonal cells. Non-genetic sources of variability are particularly important in the development of resistance to drug treatment of tumors or, more generally, in the adaptation of cell populations to stresses. We have recently introduced a new approach to cope with this variability while remaining in a continuous framework that is convenient for simulation and analysis. This approach uses mesoscopic PDEs over temporal, spatial, and structural dimensions [Hodgkinson et al., 2018, Hodgkinson et al., 2019]. Mesoscale models are obtained from the Liouville continuity equation. For illustration, let us consider that there are n types of cells. In this model cells are distinguished by two types of variables, a discrete one representing the type $i \in \{1,\dots,n\}$ and a continuous one $y \in \mathbb{R}^m$ representing the internal state (the vector of concentrations of m biochemical species). Then $c = (c_1,\dots,c_n)$ represents a vector of cell distributions satisfying the equation

$$\frac{\partial c(x,y,t)}{\partial t} = -\nabla_x F_x(c,x,y,t) - \nabla_y F_y(c,x,y,t) + S(c,x,y,t), \tag{1}$$

where x is the spatial position; y is the cell's internal state (structure variable); F_x is the spatial flux; F_y is the structural flux; and S is the source term. If the cell's internal state follows ODEs $\frac{dy}{dt} = \Phi(y)$, then the structural flux is advective $F_y = c\Phi$. The spatial flux function contains terms related to cell motility; undirected (diffusion) or directed (chemotaxis, haptotaxis). The source term integrates cell proliferation, death and transformation from one cell type to another.

The mesoscale formalism can also integrate mechanical stresses by addition of constitutive equations coupled to cell densities and biochemistry. Biochemistry has been coupled to stress by using microscopic Brownian dynamics of ECM remodeling [Malandrino et al., 2019] or Kramer's formula for chemical reaction rates with a free energy dependent on pulling force of actin filaments [Cockerill et al., 2015]. Another option would be to incorporate slower structural changes in ECM fibre density or mechanical stress into a temporally spatially, and structurally distributed ECM population, possessing its own dynamical PDE. Existing models are oversimplified and incomplete and there is strong need for a general continuous mechanical theory relating structure, deformation, cell population dynamics and biochemistry in the ECM.

5 Conclusion

Modelling TGF-β signaling integrating extracellular activation processes and intracellular pathways raises several open challenges.

An important challenge is to simulate processes that occur at highly separated time-scales as well as the spatial organization of ECM interactions. Deriving a model that would scale up to the size of these systems, and to the long periods which have to be simulated to observe the phenomena of interest, requires precise abstractions of populations of cells. These abstractions consist in changing the grain of description of the behaviours of these cells and can be formalised in many ways thanks to mathematical tools such as closure operators, Galois connections, ideals, changes of variables [Cousot and Cousot, 1977]. Yet, it is important to keep the information that mainly drives the dynamics of the whole system. Indeed the diversity of behaviours — even among identical cells — may constitute an important part of the signal that is computed by the interactions between the proteins and that which controls the behaviour of the system at the macroscopic level. In our opinion, neither non-deterministic approximations nor homogeneous abstractions would likely offer satisfying solutions to solve this issue. Non-deterministic systems are systems in which, at each moment of the execution, the immediate future has to be chosen among the elements of a set of potential behaviours, but where the choice of element is not specified, as opposed to deterministic systems for which there always exists a unique potential future; reactive systems for which the choice of the next event is triggered by an interaction with an external environment; and stochastic systems for which a distribution of probabilities defines the likelihood of each potential immediate future behaviour. Non-deterministic abstractions flatly over-approximate all the potential behaviours of each cell without providing any information about their probability distribution. They can hardly be used in composite models, since many potential behaviours would have to be considered for each cell, and, thus, it would require the consideration of too many cases across the population of cells. Homogeneous abstractions consist in abstracting away the diversity of behaviours of the population of cells, and to replace them with several copies of a unique system, which behaves in the same way. Abstractions with too great a homogeneity would keep only most probable behaviours for the cells, whilst ignoring others. As a consequence, such a system would not faithfully model the diversity of behaviours among the population of cells, which may be a key ingredient in explaining the overall behaviour of the composite model. Stochastic models keep enough information about the distribution of the different behaviours within a population of cell. Moreover, they can be easily integrated within a multi-scale model. Nevertheless, they come with an important combinatorial cost and can hardly be simplified.

Mesoscale PDE models represent a promising direction for modeling the ECM. They are well suited for implementing middle-out modelling strategies, in which several levels of organisation are treated together, but with just enough details to render the essence of the overall organisation. Mesoscale

models can be built from scratch, but can also include already available models, or parts of them, after model reduction. The model reduction procedure, based on time-scale separation and singular perturbations, averaging or homogeneization, is not yet well established and is the subject of active research [Radulescu et al., 2012]. Although deterministic, these models can render the stochastic behaviour of "microscopic" variables, responsible, among other factors, for the heterogeneity of cellular decision. In this approach, stochastic simulations are replaced by calculation of probability distributions of microscopic variables, that follow PDEs in mesoscopic descriptions. In spite of recent progress, the important question of the coupling between mechanical stress, biochemistry and cell behaviour has been treated only superficially. The ECM is a complex medium, including insoluble fibers-forming molecules (collagen, fibronectin, elastin) and soluble molecules such as proteoglycans and glycoaminoglycans which constitute a hydrated gel, of relatively high viscosity, and confer elastic properties to the ECM and glycoproteins characterized by their adhesive properties (laminin, fibronectin, tenascin, etc.). The viscoelastic properties of this medium, in particular its capacity to transmit mechanical cues that further influence cellular processes, such as differentiation, proliferation, survival and migration, could be instrumental for tissue remodelling. Constitutive equations, eventually inspired from the physics of polymers and gels [Larson, 2013, Prost et al., 2015], should be able to provide the continuous mechanics theoretical framework needed for relating stress, strain, signaling and cell decisions in tissue models.

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