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The Physics of Cellular Decision Making During Epithelial–Mesenchymal Transition

Shubham Tripathi,[1,2,3] Herbert Levine,[2,3] and Mohit Kumar Jolly[4]

[1]PhD Program in Systems, Synthetic, and Physical Biology, Rice University, Houston, Texas 77005, USA

[2]Center for Theoretical Biological Physics, Rice University, Houston, Texas 77005, USA; email: h.levine@northeastern.edu

[3]Department of Physics, Northeastern University, Boston, Massachusetts 02115, USA

[4]Centre for BioSystems Science and Engineering, Indian Institute of Science, Bangalore, Karnataka 560012, India; email: mkjolly@iisc.ac.in

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Abstract

The epithelial–mesenchymal transition (EMT) is a process by which cells lose epithelial traits, such as cell–cell adhesion and apico-basal polarity, and acquire migratory and invasive traits. EMT is crucial to embryonic development and wound healing. Misregulated EMT has been implicated in processes associated with cancer aggressiveness, including metastasis. Recent experimental advances such as single-cell analysis and temporal phenotypic characterization have established that EMT is a multistable process wherein cells exhibit and switch among multiple phenotypic states. This is in contrast to the classical perception of EMT as leading to a binary choice. Mathematical modeling has been at the forefront of this transformation for the field, not only providing a conceptual framework to integrate and analyze experimental data, but also making testable predictions. In this article, we review the key features and characteristics of EMT dynamics, with a focus on the mathematical modeling approaches that have been instrumental to obtaining various useful insights.

Contents

1. INTRODUCTION

How an individual cell responds to a given stimulus is decided by a complex interplay among both intrinsic factors [genetic makeup of the cell, cell-to-cell variability in biochemical processes, history of the cell (16)] and extrinsic factors [biochemical (66) and biomechanical (26) parameters of the environment, cell–cell communication (40)]. This complex interplay gives rise to a highly nonlinear process facilitating different spatiotemporal dynamics within a cell and in a population of cells (9, 84). Decoding and predicting such systems-level dynamics of cellular response remains a daunting task.

Recent progress in using mathematical models to integrate the information painstakingly collected via decades of molecular and cell biology experiments has led to a better understanding of the dynamics of underlying biochemical regulatory networks (74). These advances have uncovered fundamental design principles of these networks both in microorganisms such as bacteria and yeast (4, 72) and in eukaryotes, including humans (87). Mathematical modeling approaches have begun to show promise in elucidating the dynamics of (*a*) embryonic development (70), (*b*) cell differentiation and reprogramming (54), and (*c*) diseases such as HIV (62) and cancer (45). An iterative interplay between mathematical models and experiments has been the hallmark of these success stories.

In this article, we review the current state of understanding of epithelial–mesenchymal transition (EMT) and the corresponding reverse process, mesenchymal–epithelial transition (MET). EMT and MET are cell biological processes involved in embryonic development and cellular reprogramming. Both EMT and MET are also involved in driving cancer metastasis and therapy resistance—the two grand clinical challenges in cancer treatment. EMT and MET involve multiple morphological and biochemical changes within a cell, including changes in cell shape, cell–cell adhesion, cell polarity, and cell migration and invasion. EMT and MET are also associated with other cellular traits that are crucial during embryonic development and/or cancer metastasis, such as stemness or pluripotency (56). Recent years have witnessed a surge in efforts by the experimental and theoretical biophysics communities to investigate EMT and/or MET dynamics at multiple scales (**Figure 1**).

2. DYNAMICS OF EPITHELIAL–MESENCHYMAL TRANSITION

EMT for an individual cell proceeds via partial or complete loss of canonical epithelial characteristics, such as cell–cell adhesion and apico–basal polarity, and acquisition of migratory abilities.

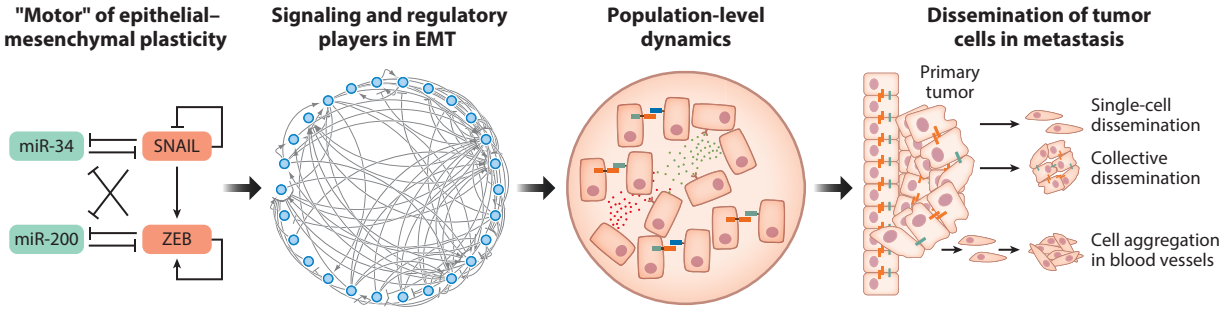

Figure 1

Different scales of dynamics of epithelial–mesenchymal transition (EMT). A small, core circuit driving EMT and mesenchymal–epithelial transition (MET) operates within a large network, which includes scores of molecular species involved in the control of EMT. At the population level, EMT dynamics is determined by intercellular communication, which can be mediated via multiple pathways. Finally, EMT- and MET-associated heterogeneity and plasticity determine the mechanism of cancer cell dissemination from the primary tumor.

Conversely, MET proceeds via increased expression of junctional proteins and gain of cell–cell adhesion, and involves loss of abilities to migrate and invade. EMT and MET have largely been considered to be binary processes, bringing about transitions between two specialized phenotypes—epithelial (high cell–cell adhesion, cobblestone shape, low cell migration and invasion) and mesenchymal (low cell–cell adhesion, spindle shape, high cell migration and invasion) (56). This concept largely stemmed from the measurement of a few markers at a small number of time points (typically two). These measurements were mostly carried out at a population level, obfuscating the nonlinear dynamics of EMT and MET and the heterogeneity in the time-dependent and/or dosage-dependent response of cells to various EMT- and MET-inducing signals. Recent experimental advances, including increased resolution in the dynamic measurement of multiple biomarkers at a single-cell level, have revealed widespread heterogeneity in the response of isogenic cells to identical treatment with inducers of EMT (38, 44). A fundamental observation has been that cells do not just transition between discrete epithelial and mesenchymal states. Rather, they can exhibit a spectrum of phenotypes differing in the relative presentation of epithelial (E-cadherin and EpCAM) and mesenchymal (VIM and N-cadherin) markers. Predictive mechanistic mathematical models have been at the forefront in furthering our understanding of EMT (41). Mathematical modeling studies were among the first to suggest that a hybrid epithelial–mesenchymal (hybrid E/M) state, with coexpression of epithelial and mesenchymal markers, is not just a transitional cellular state but a stable phenotypic state that cells can exhibit (49, 81). Together, in vitro and in vivo experiments and mathematical modeling approaches have transformed the perception of EMT from a binary process to one of a multistable process wherein cells can exist in and spontaneously switch among multiple phenotypic states (56).

2.1. Experimental Characterization of Epithelial–Mesenchymal Transition

Multiple experimental methodologies have played key roles in unraveling the dynamics of EMT and MET and in elucidating the functional role played by these two processes during development and cancer progression.

2.1.1. Single-cell versus population-level characterization. Most experimental studies in vitro have focused on population-level, or bulk, measurements in terms of assessing changes in the presentation of epithelial and mesenchymal markers (29, 57, 68). More recently, single-cell

studies using flow cytometry have revealed that cells with the same genetic background may exhibit different EMT-associated phenotypes (6, 28, 30), revealing nongenetic heterogeneity in EMT dynamics. The multimodal distribution of E-cadherin observed across multiple cell lines further exemplifies this observation (14). Single-cell immunofluorescence analysis of populations characterized as hybrid E/M on the basis of the population average (68) has been helpful in distinguishing between populations wherein the cells predominantly exhibit the hybrid E/M phenotype and mixed populations of epithelial and mesenchymal cells with few hybrid E/M cells (36, 42). In addition, single-cell measurements of multiple markers in cells undergoing EMT and then reverting to an epithelial state have revealed the diverse hybrid E/M phenotypic states that cells can attain and the different paths that they can take while traversing the multidimensional landscape of EMT and MET (44). Single-cell RNA-seq analysis of patient samples has further contributed to our knowledge regarding the existence, spatial localization, and clinical implications of tumor cells exhibiting the hybrid E/M phenotype(s) (61).

2.1.2. Static versus dynamic characterization. Besides investigating EMT at a single-cell level, recent studies have also focused on characterizing the expression of epithelial and mesenchymal markers at various time points during the transition. This trend represents a definite improvement over a large majority of previous in vitro studies, which focused on two endpoint measurements—epithelial and mesenchymal. Identification of genes that vary together dynamically has unraveled the dynamics of coupling between EMT and other altered cellular traits (89). Moreover, live-cell imaging using dual fluorescent reporter systems can detect relative levels of epithelial and mesenchymal markers and consequently track dynamical switching between different cell states (82). Similar reporter and genetic labeling systems used in vivo have revealed unprecedented insights into how cells undergoing EMT and MET to varying extents contribute to metastasis (76). The choice and fidelity of the marker(s) used to examine EMT and MET in these systems remains a question of intense debate (43), highlighting the dire need to better elucidate the multidimensional landscape of EMT and MET in a quantitative and predictive way.

2.2. Mathematical Modeling of Epithelial–Mesenchymal Transition

Mathematical models at the level of gene circuits and cellular populations have been helpful not only in analyzing the data generated from experiments, but also in making testable predictions and in designing new experiments to advance our understanding of the dynamics of EMT and MET.

2.2.1. A core regulatory circuit governing epithelial–mesenchymal transition and mesenchymal–epithelial transition. Over the many decades that EMT has been a topic of interest among developmental biologists and cancer biologists, multiple signaling pathways have been implicated in the control of EMT—HGF, TGF-β, p53, HIF-1α, EGF, FGF, Hedgehog, Wnt, and Notch signaling (56, 80). The activities of many of these diverse pathways, however, can be considered to converge onto a core regulatory circuit, which consists of two families of transcription factors, SNAIL and ZEB, and two families of micro-RNAs, miR-34 and miR-200 (**Figure 2*a***) (56). In this circuit, ZEB inhibits the expression of miR-200 by binding to conserved sites in the promoter region of miR-200. miR-200 can bind the 3′ untranslated region in ZEB mRNAs, an interaction that can mark both the micro-RNA and the mRNA for degradation, as well as possibly interfering with mRNA translation. ZEB and miR-200 thus form a mutual inhibitory feedback loop. SNAIL and miR-34 also form a similar mutual inhibitory feedback loop, which interfaces with that of ZEB and miR-34. Both SNAIL and ZEB can regulate their own transcription, and their expression drives EMT, promoting the expression of the mesenchymal markers N-cadherin and Vimentin while inhibiting the expression of the epithelial marker E-cadherin. In

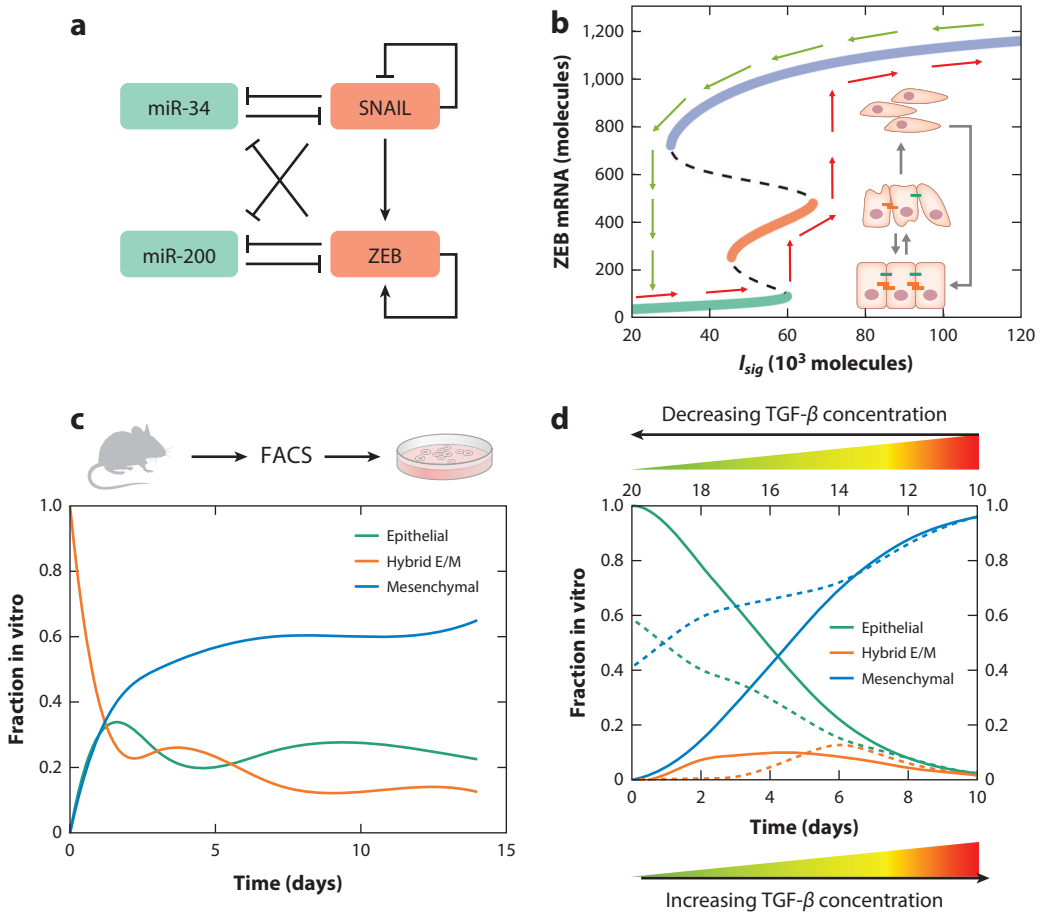

Figure 2

Dynamics of epithelial–mesenchymal transition (EMT) control at the cellular and population levels. (*a*) A core regulatory network consisting of two coupled mutual inhibitory feedback loops that controls EMT and mesenchymal–epithelial transition (MET) dynamics. (*b*) Steady states of the regulatory network in panel *a* correspond to different EMT-associated phenotypes—epithelial, hybrid epithelial/mesenchymal (E/M), and mesenchymal. The multistability of the dynamics leads to hysteresis in the dynamics of EMT. Red arrows indicate a possible trajectory, in the phenotypic space, of a cell undergoing EMT, while green arrows indicate a trajectory that may be adopted by a cell undergoing MET. (*c*) Spontaneous phenotypic plasticity in a population of mouse prostate cancer cells. The behavior shown is representative of the observations of Ruscetti et al. (64). (*d*) Phenotypic plasticity in a population exposed to different concentrations of an EMT inducer (in this case, TGF-β). Solid lines indicate the behavior from day 0 to day 10 (increasing TGF-β concentrations), while dotted lines indicate the behavior from day 11 to day 20 (decreasing TGF-β concentrations). Some cells seem to undergo a permanent EMT, retaining the mesenchymal phenotype even after TGF-β has been withdrawn. The behavior shown is representative of that described by Tripathi et al. (83). Additional abbreviation: FACS, fluorescence-activated cell sorting.

contrast, high levels of miR-34 and miR-200 tend to drive an epithelial phenotype (56). MicroRNA-mediated regulation is expected to increase the robustness of regulatory networks by decreasing the magnitude of fluctuations in the expression levels of proteins arising from intrinsic or extrinsic noise (24), thereby potentially preventing the aberrant triggering of EMT and MET.

Two mathematical models were initially proposed to describe the dynamics of this regulatory circuit. Both studies showed that the regulatory circuit can allow for the existence of a stable hybrid E/M phenotype. Lu et al. (49) used ordinary differential equations (ODEs) with detailed

characterization of micro-RNA-mediated post-transcriptional regulation to characterize the dynamical behavior of the core EMT regulatory circuit described above. This model proposed that the SNAIL/miR-34 loop exhibits monostable dynamics and acts as an integrator of the multiple inputs to the regulatory circuit, whereas the ZEB/miR-200 feedback loop, with self-activation of ZEB, exhibits switch-like dynamical behavior and can exhibit three distinct types of steady states—epithelial (low ZEB, high miR-200), mesenchymal (high ZEB, low miR-200), and hybrid E/M (coexpression of intermediate levels of ZEB and miR-200) (**Figure 2*b***). The model thus predicted that the ZEB/miR-200 feedback loop can act as a ternary switch instead of a binary switch, and the self-activation of ZEB plays a key role in the existence of the hybrid E/M phenotypic state.

Tian et al. (81) proposed an alternative mechanism by which the abovementioned core regulatory circuit can control cell fate switching between epithelial, mesenchymal, and hybrid E/M phenotypes. In this ODE-based model, both SNAIL/miR-34 and ZEB/miR-200 feedback loops act as bistable switches—flipping between low SNAIL (ZEB), high miR-34 (miR-200) and high SNAIL (ZEB), low miR-34 (miR-200) states. A cell switches from an epithelial to a hybrid E/M phenotypic state when the SNAIL/miR-34 feedback loop flips to a high SNAIL, low miR-34 state while the ZEB/miR-200 feedback loop stays in the low ZEB, high miR-200 state. Once again, the hybrid E/M phenotype is a stable phenotype and not a transitional state. In this model, the hybrid E/M phenotype arises due to the early switching of the SNAIL/miR-34 feedback loop in response to the EMT inducer, which is a direct input to SNAIL. The ZEB/miR-200 feedback loop flips only at a later stage, once the concentration of SNAIL has crossed a threshold.

Thus, mathematical modeling of the proposed core regulatory circuit controlling EMT provided a strong indication that cells can exhibit a stable phenotype that is characterized by the coexpression of epithelial and mesenchymal markers at intermediate levels. This prediction was confirmed experimentally, both in vitro (34, 68) and in vivo (1, 58, 61). One reasonable hypothesis is that both mechanisms [proposed by Lu et al. (49) and Tian et al. (81)] are possible and are differentially present in different cell lines. Furthermore, these models have been extended to include other modulators of EMT and MET and to identify factors that can stabilize one or more hybrid E/M phenotypes. The ability of such factors to stabilize hybrid E/M phenotypes was later experimentally validated (32, 42).

Both of the mathematical models of dynamics of the core EMT regulatory circuit highlight the role of a toggle switch motif in determining EMT dynamics. This network motif consists of two regulatory factors, *A* and *B*, which inhibit each other's expression and can exhibit two stable steady states—(high *A*, low *B*) and (low *A*, high *B*) (37). Thus, if *A* and *B* are master regulators of distinct cell fates, a toggle switch motif can act as a binary cell-fate decision-making circuit and has been shown to recur in regulatory networks involved in cellular decision-making processes (91). If either *A* or *B* (or both) activates its own expression, then the toggle switch can exhibit a third stable steady state with coexpression of *A* and *B*, thereby acting as a ternary switch (37, 91). Such a ternary switch formed by miR-200 and ZEB plays a key role in the model proposed by Lu et al. (49), while the model proposed by Tian et al. (81) relies on the activity of two coupled toggle switches—ZEB/miR-200 and SNAIL/miR-34.

2.2.2. Modeling larger networks driving epithelial–mesenchymal transition and mesenchymal–epithelial transition. Developmental pathways that affect EMT are crucial during embryonic development and wound healing, and their dysregulation is key to cancer progression. Integration of the dynamics of these pathways with the dynamics of EMT regulation can help make useful predictions regarding the effect of signaling disruptions and perturbations on EMT dynamics. These signaling-cum-regulatory networks, however, involve a very large number of molecular species and regulatory relationships. For example, dbEMT (90) lists 377

genes whose role in EMT has been experimentally identified. Given the difficulty of inferring or even adequately estimating the large number of kinetic parameters that govern these regulatory relationships, it is hard to characterize the dynamics of these networks using traditional ODE-based models. Instead, Boolean modeling approaches (2) that rely only on the knowledge of the qualitative nature of regulatory relationships (i.e., whether a regulatory link is activating or inhibiting) have been used to analyze the dynamics of these large networks.

Steinway et al. (77) used Boolean logic-based rules to describe the 135 regulatory relationships among 70 molecular species involved in EMT signaling and regulation in hepatocellular carcinoma. Using asynchronous node updates to model the discrete time dynamics of this model, the study identified two stable fixed points corresponding to the epithelial and mesenchymal phenotypes. The epithelial fixed point was acquired by the network nodes when all EMT-inducing pathways were fixed in the OFF state. Sustained TGF-β signaling, modeled by fixing the TGF-β node in the ON state, activated SMAD complex formation followed by the induction of MAPK and AKT signaling, finally leading to the network exhibiting the mesenchymal fixed point, which is characterized by the loss of E-cadherin expression. The study further identified eight feedback loops that stabilize the mesenchymal state, revealing certain key drivers of EMT in hepatocellular carcinoma. A follow-up study described the emergence of new fixed points that exhibit features of both epithelial and mesenchymal fixed points. These fixed points thus represent putative hybrid E/M phenotypes (78).

Font-Clos et al. (27) expanded the regulatory network constructed by Steinway et al. (77) and, modeling the network as a spin glass, showed that multiple hybrid E/M fixed points separate the epithelial and mesenchymal fixed points. These hybrid E/M fixed points are less stable and are less likely to be reached when starting from any initial state as compared to the epithelial and mesenchymal fixed points. This observation is reminiscent of the free energy landscape observed in glassy systems and disordered solids (52).

Another approach to probing the dynamical behavior of large EMT regulatory circuits is the RACIPE (Random Circuit Perturbation) method (33). In this method, kinetic parameters governing network dynamics are varied to create an ensemble of ODE-based models; this reflects the assumption that additional modulators of EMT or exogenous signaling inputs will vary significantly from cell to cell, and thus robust features of the network will be those that are common to significant subpopulations in this ensemble. Analysis of the dynamics of a 26-node circuit using this method revealed that the core EMT regulatory mechanism is preserved upon the inclusion of additional factors, and one can still identify the characteristic three classes of EMT-associated phenotypes—epithelial, mesenchymal, and hybrid E/M—from among the stable steady states of this expanded regulatory topology (36). A recent comparison of Boolean- and RACIPE-based approaches for modeling EMT network dynamics has identified the design principles of these networks (31a).

2.2.3. Population-level dynamics of epithelial–mesenchymal transition. Recent studies have drawn attention to the population-level dynamics exhibited by cells undergoing EMT and MET. Ruscetti et al. (64) used fluorescence-activated cell sorting to monitor the expression of EpCAM and Vimentin in mouse prostate cancer cells at different time points. Using the expression of these canonical EMT markers to classify cells as epithelial, mesenchymal, and hybrid E/M, they observed that the fraction of different phenotypes in a population can change spontaneously over time. A remarkable observation was the capability of a population of hybrid E/M cells to quickly generate a mixture of epithelial and mesenchymal cells (**Figure 2*c***). Furthermore, Risom et al. (63) have shown that the transitions between epithelial-like and mesenchymal-like states in breast cancer cell lines can be altered by targeting specific cellular pathways. More recently, Bhatia et al. (7), using EpCAM expression to classify cells from the PMC42-LA breast cancer

cell line as epithelial or mesenchymal, demonstrated that a population of only epithelial or only mesenchymal cells eventually reacquired the phenotypic composition of the parental population (80% epithelial, 20% mesenchymal). Populations derived from single cells exhibited phenotypic compositions that were different from one another and different from the phenotypic composition of the parent population. This behavior could not be attributed to the chromosomal instability of the population, indicating that a nongenetic mechanism must be at play. Other studies have used cell morphology to characterize the temporal dynamics of EMT. Mandal et al. (50) observed a continuum of morphological changes in a human keratinocyte cell line exposed to TGF-β to induce EMT and used a Markov model-based approach to characterize the phenotypic plasticity exhibited by these cells. Leggett et al. (47) used a Gaussian mixture model to classify cells in a population as epithelial or mesenchymal on the basis of morphological features including radius of the nucleus, Vimentin area, and cytoplasm form factor. The mixture model was then used to characterize the fraction of epithelial and mesenchymal cells in populations over time. The analysis revealed that SNAIL induction drives rapid EMT, while TGF-β induction drives gradual EMT.

Most mathematical modeling efforts to describe the behavior of populations undergoing EMT and MET have been phenomenological. A popular approach has been to use Markov models wherein the phenotypic composition of a population at a future point in time depends only on its phenotypic composition at the current time point (17, 31, 44, 50, 63). Such models are described by specifying a state transition matrix with probabilities of transition of a cell between different phenotypic states. These have been useful as conceptual frameworks for describing the experimental data regarding the population-level dynamics of EMT. However, their utility is limited by the lack of molecular mechanistic bases. Mathematical models that link the well-characterized dynamics of EMT regulatory networks to the population-level behavior of cells are needed to describe the behavior of populations and to determine molecular targets for modulating the behavior of cancer cell populations in a useful way. These models must incorporate key features of population-level behavior such as stochastic transitions among various phenotypic states (83) and interaction between heterogeneous subpopulations (54a).

One such model, proposed by Tripathi et al. (83), considered the effect of noise in the partitioning of parent cell molecules among the daughter cells at the time of cell division. The study showed that this noise, coupled with the multistability of EMT regulation, can lead to daughter cells acquiring a phenotype (epithelial, mesenchymal, or hybrid E/M) distinct from that of the parent cell. While the dependence of the phenotypic state of a daughter cell on the phenotypic state of its parent cell remains to be experimentally characterized, the abovementioned mechanism can result in the spontaneous emergence of epithelial–mesenchymal heterogeneity in an isogenic cancer cell population, even if the population is phenotypically homogeneous to begin with. The model was able to recapitulate the high phenotypic plasticity of hybrid E/M populations and the ability of such populations to quickly generate a mixture of epithelial and mesenchymal cells, as shown experimentally by Ruscetti et al. (64) for mouse prostate cancer cells.

2.2.4. Hysteresis in the dynamics of epithelial–mesenchymal transition. Systems exhibiting multistable behavior can exhibit hysteresis, wherein the steady state exhibited by the system depends not only on the present input to the system but also on the history of inputs to the system (16). Given the multistability exhibited by the EMT and MET regulatory circuits, hysteresis in the dynamics of EMT is expected. As shown in **Figure 2*b***, starting from an epithelial state, if the concentration of the EMT inducer is increased, then the cell will transition to a hybrid E/M state once the concentration of the EMT inducer exceeds a threshold. Upon further increase in the concentration of the EMT inducer, the cell will acquire a mesenchymal phenotype. If the concentration of the EMT inducer is now progressively decreased, then the cell will retain the

mesenchymal state before directly transitioning to an epithelial state once the EMT inducer concentration becomes very low. Thus, while EMT proceeds via the hybrid E/M state, the bifurcation diagram suggests that the hybrid E/M phenotype will not be encountered during MET. When hybrid E/M phenotype-stabilizing factors such as GRHL2 or OVOL2 are included, the hybrid E/M phenotype may be encountered during both EMT and MET. However, the trajectories via which the two processes proceed still remain distinct (42). These observations regarding the distinct trajectories encountered during EMT and MET have been made on the basis of the dynamics of a small, core EMT regulatory circuit. Upon considering a larger set of EMT-modulating factors, these trajectories are likely to become complex and multidimensional. However, the distinctiveness of EMT and MET trajectories is expected to be preserved (44).

Recently, Celià-Terrassa et al. (14) showed experimentally that hysteretic dynamics are exhibited by multiple normal and cancer cell lines derived from humans and mice. The study further demonstrated that the hysteresis arises from the multistable dynamics exhibited by the ZEB/miR-200 mutual inhibition feedback loop. When the ability of ZEB to repress miR-200 was abrogated using CRISPR/Cas9, the cells still underwent EMT upon treatment with TGF-β, an EMT inducer. However, bimodality in the expression of E-cadherin during TGF-β treatment was no longer observed. This change happens because, upon loss of inhibition of miR-200 by ZEB, the ZEB/miR-200 feedback loop is no longer operational and cannot exhibit multistable dynamics. As a result, a cell is likely to go through the same spectrum of phenotypic states during both EMT and MET.

Karacosta et al. (44) exposed HCC827, a non–small cell lung cancer cell line, to increasing concentrations of TGF-β (to induce EMT), followed by a progressive decrease in the concentrations of TGF-β (to induce MET). Mass cytometry was used to determine the expression of 28 markers at a single-cell level, and the expression levels were obtained at multiple time points (and, thus, at multiple TGF-β concentrations). When the mass cytometry data was used to assign one of eight EMT-associated phenotypes (three epithelial, three hybrid E/M, and two mesenchymal) to cells, it was observed that the cells went through different sequences of phenotypic states when undergoing EMT than when undergoing MET. Furthermore, some cells seemed to have undergone a permanent EMT, retaining the mesenchymal phenotype even when TGF-β had been withdrawn (**Figure 2*d***). The study used a Markov model-based approach, with transition probabilities inferred from experimental data, to describe this behavior of cells. Distinct transition probabilities during EMT and MET accounted for the differing phenotypic trajectories observed during EMT and MET. These transition probabilities likely reflect the topology of some high-dimensional bifurcation describing EMT control.

The model proposed by Tripathi et al. (83), which is built on the multistability of EMT regulation and includes random partitioning of the EMT inducer in the parent cell during cell division, can exhibit hysteretic dynamics when cells are exposed to progressively increasing concentrations of TGF-β followed by progressively decreasing concentrations of TGF-β, as demonstrated experimentally by Karacosta et al. (44). In this model, hysteresis arises from the nature of the bifurcation diagram of the EMT regulatory circuit (**Figure 2*a*,*b***). In this bifurcation diagram, while the ranges of EMT inducer concentrations for which epithelial and hybrid E/M phenotypes can exist are bounded, the range for which there exists a mesenchymal phenotype is theoretically infinite. The noise added to EMT inducer concentration during each cell division event can cause some cells in the population to acquire very high concentrations of the inducer. Thus, it becomes unlikely that such cells would acquire an inducer concentration low enough to revert back to an epithelial state within the time scale over which most experimental studies are carried out. As a result, these cells seem to have undergone a permanent EMT. The temporal behavior of fractions of different phenotypes in the population is thus effectively different during EMT (increasing

TGF-β) and during MET (decreasing TGF-β). This effective hysteresis can be observed even if the long-time population dynamics would lead to a uniquely determined phenotypic composition.

3. DETERMINANTS OF EPITHELIAL–MESENCHYMAL TRANSITION DYNAMICS

The dynamics of EMT is governed by factors acting at multiple levels—from regulatory networks in individual cells to cell–cell and cell–microenvironment signaling at a population level. Mathematical modeling studies have been helpful in developing new hypotheses for describing observed experimental behavior of cells undergoing EMT and in identifying certain fundamental mechanisms underlying EMT dynamics.

3.1. Topology of the Regulatory Network

The architecture of regulatory interactions between various molecular species within a cell is a key determinant of the dynamics of the regulatory network (3). Certain topological patterns, called network motifs, have been shown to recur in regulatory networks across biological processes and life forms (4). We describe above how the toggle switch motif plays a key role in EMT regulation. The importance of network topology in determining EMT and MET dynamics can further be seen in the topologies of the coupling between phenotypic stability factors (PSFs) and the ZEB/miR-200 feedback loop. PSFs promote the existence of cells in the hybrid E/M state. Various factors have been identified as PSFs—GRHL2, OVOL2 (32, 42), ΔNP63α (39), and NRF2 (10). Mathematical models have predicted that coupling between PSFs and the ZEB/miR-200 feedback loop increases the dynamic range of EMT inducer concentrations for which ZEB and miR-200 can be coexpressed and therefore for which the hybrid E/M state can stably exist. Moreover, in the presence of PSFs, there can exist ranges of EMT inducer concentrations for which the hybrid E/M state is the only stable state. In other words, for these concentrations of the EMT inducer, the hybrid E/M state can exist alone instead of coexisting with epithelial and/or mesenchymal states, as is the case in the absence of any PSFs. PSFs also increase the concentration of the EMT inducer required for the cell to exit an epithelial state. Both GRHL2 (42) and OVOL (32, 85) have been shown to act as critical molecular brakes on EMT.

Mathematical modeling has shown that any factor whose topology of interaction with the EMT circuit exhibits certain general features—a factor that forms a mutual inhibitory loop with ZEB or one that represses the expression of both ZEB and miR-200—can act as a PSF (42). Later studies revealed other network motifs that can stabilize the hybrid E/M phenotype, including those involving ΔNP63α (39) and NRF2 (10). Previous studies have similarly identified motifs that promote the maintenance of epithelial and mesenchymal phenotypes (77, 78).

Thus, whether a regulatory factor is a PSF or not can be predicted by comparing the topology of its interaction with the EMT regulatory circuit with the topology of circuits involving known PSFs. These predictions can then be tested experimentally to weed out any false positives. Given the huge number of genes that play a role in modulating EMT (90), insights gained from mathematical modeling can be immensely helpful in identifying promising candidates to target for attenuating hybrid E/M phenotype–associated metastatic aggressiveness. To date, PSF-associated topologies have mostly been explored in the context of small regulatory circuits. While such analysis has provided some very useful insights, all EMT modulating factors act as part of a complex regulatory network with numerous regulatory elements and widespread cross-talk. Therefore, approaches to identifying PSFs and associated motifs in more complicated contexts and in a more systematic manner are needed to improve the utility of mathematical models for developing therapies that can target the hybrid E/M phenotype in cancer.

3.2. Epigenetic Regulation

Epigenetic mechanisms of gene expression control involve DNA methylation and multiple modes of post-translational modification of nucleosomes, including histone methylation, acetylation, ubiquitination, and phosphorylation. These act by altering the accessibility of transcription factors to the cellular DNA and by recruiting regulatory factors (which may promote or inhibit transcription) to specific parts of the genome (18). Both DNA methylation and histone modification profiles can be inherited by daughter cells from the parent cell (35). Epigenetic processes are thus much slower compared to processes involved in transcriptional and post-transcriptional control, and epigenetic regulation can operate on much longer time scales (67). The dynamics of epigenetic regulation of EMT can thus differ remarkably from the dynamics of EMT regulation via other modes. This separation of time scales between transcriptional and epigenetic regulation can lead to the emergence of novel steady states in biological systems (5).

Epigenetic mechanisms are involved in the control of EMT, acting both upstream and downstream from the core EMT regulatory circuit (56). Both histone modifications and DNA methylation modulate the expression of transcription factors and micro-RNAs involved in EMT control (88). The molecular processes involved in epigenetic regulation are complex, brought about via the activity of multiple molecular actors. This makes mathematical modeling of epigenetic regulation at a mechanistic level very difficult (75). Miyamoto et al. (53) proposed a phenomenological model wherein epigenetic regulation is incorporated by making the threshold for the activation of a gene into a dynamic variable. If the expression of a regulator gene is high, then the threshold for the activation of the regulated gene decreases, making it easier for the gene to turn on. In contrast, if the expression of the regulator gene is low, then the threshold for the activation of the regulated gene increases, making it harder for the gene to turn on. Thus, epigenetic regulation in this model acts to make it harder to change the state of a gene. Jia et al. (38) built on this model of epigenetic regulation to show that epigenetic feedback in the inhibition of miR-200 by ZEB can stabilize the mesenchymal phenotype. With some preliminary experimental validation, this study demonstrates that epigenetic feedback can also contribute to hysteresis in the dynamics of EMT. However, few studies have demonstrated a causal effect of epigenetic regulation on rates of transition between different phenotypic states. An interesting example is the study by Chaffer et al. (15), which showed that the bivalent or poised chromatin state carried by the ZEB1 promoter can facilitate rapid transitions between different phenotypic states. Further experimental studies are needed to fully characterize the role of epigenetic regulation in controlling the transition rates between different phenotypic states.

3.3. Cell–Cell Communication

In addition to gene regulation within a cell, non-cell-autonomous mechanisms can also govern the dynamics of EMT in cancer cells. Communication between cancer cells, mediated via different signaling pathways, can modulate the dynamics of EMT and the phenotypic composition of a cancer cell population.

Notch signaling is an evolutionarily conserved cell–cell communication pathway involving the binding of Delta or Jagged transmembrane ligands, expressed on the surface of cells, to the transmembrane receptor Notch expressed on the surface of a neighboring cell. The binding causes the cleavage of the intracellular domain of the Notch receptor (NICD), which is then released into the cytoplasm and eventually migrates into the nucleus. There, it acts as a transcriptional cofactor and can activate or inhibit the expression of multiple target genes (12, 73). Due to the juxtacrine nature of this cell–cell signaling mechanism, spatial patterns can arise in a population of cells. The nature of these patterns depends on the effect of NICD on the expression of Notch

ligands. NICD inhibits the expression of Delta ligands while promoting the expression of Notch receptors. As a result, in the presence of Notch–Delta signaling, neighboring cells acquire distinct phenotypes—one cell exhibits high Delta, low Notch expression (the sender cell), while a neighboring cell exhibits low Delta, high Notch expression (the receiver cell). This lateral inhibition mechanism leads to the emergence of salt-and-pepper patterning of phenotypes in a population of cells (71). In contrast, NICD promotes the expression of both Notch receptors and Jagged ligands. Neighboring cells communicating via Notch–Jagged signaling therefore tend to acquire the same phenotype, which is characterized by the coexpression of Notch and Jagged in each cell (the hybrid sender–receiver phenotype) (8). This lateral induction may enable spatial homogeneity in a population of cells to some extent.

Through the coupling of the Notch signaling circuit and EMT regulation, which has been experimentally identified, Notch–Delta–Jagged signaling is a key player in driving spatial patterning during EMT (**Figure 3*a***). NICD can drive EMT by promoting the expression of SNAIL (55, 65). miR-34 and miR-200, the two key micro-RNAs regulating EMT, inhibit the expression of Delta and Jagged ligands, respectively (11, 22). Furthermore, miR-34 also inhibits the expression of Notch receptors (13). Thus, Notch signaling–mediated spatial patterns can translate into spatial patterning of EMT-associated phenotypes. Since NICD promotes EMT by driving SNAIL expression, the receiver phenotype with high Notch receptor expression is associated with the expression of mesenchymal markers. Consequently, the salt-and-pepper patterning characteristic of Notch–Delta signaling translates into a spatial organization wherein hybrid E/M and mesenchymal cells are surrounded by epithelial cells. In contrast, due to the lateral induction mechanism in the presence of Notch–Jagged signaling, spatial clusters of hybrid E/M and mesenchymal cells can emerge in a population of cancer cells. More intricate spatial patterns can arise in the presence of diffusive signaling molecules such as TGF-β, which can drive EMT, and cytokines, which can modulate the expression of Notch ligands (**Figure 3*a***) (9).

3.4. Mechanosensing

Cells can sense mechanical properties of the extracellular matrix (ECM). The information conveyed across the cell membrane plays a central role during morphogenesis, regulating multiple processes including cell proliferation, cell differentiation, and cell death (69). ECM stiffness, which depends on its chemical composition and its organization, can be sensed by cells via integrins. Integrins are heterodimeric receptors present at cell–ECM adhesion sites that can transmit the mechanical stimulus from the ECM to downstream signaling pathways (86). ECM stiffness has been shown to modulate the migration and proliferation of cells, with high-stiffness environments promoting cell migration (60). Cancer cells grown on stiffer substrates become more mesenchymal-like, acquiring invasive capabilities (51). Kumar et al. (46) used ODEs to model the ECM/β-catenin/E-cadherin signaling nexus and showed that a tug of war between ECM density and β-catenin/E-cadherin complex formation governs the migratory capabilities of cells. Increased ECM density promotes FAK-mediated phosphorylation of β-catenin, which depletes the cell of nonphosphorylated β-catenin that forms the β-catenin/E-cadherin complex, thereby reducing cell–cell adhesion. In contrast, decreased matrix stiffness can promote apoptosis (48). Stiffness-mediated regulation of EMT can be mediated via multiple downstream signaling pathways, including YAP/TAZ (23, 79) and PI3K/Akt (48). EMT, in turn, can also alter the stiffness of the ECM via the production of lysyl oxidases (59). These enzymes cross-link collagen fibers in the ECM, which increases collagen density and fiber linearization, leading to an increase in matrix stiffness (20). This EMT–ECM stiffness feedback loop is further modulated by the dependence of cell migration modes on the stiffness of the matrix (25). Mechanosensing can thus lead to spatial

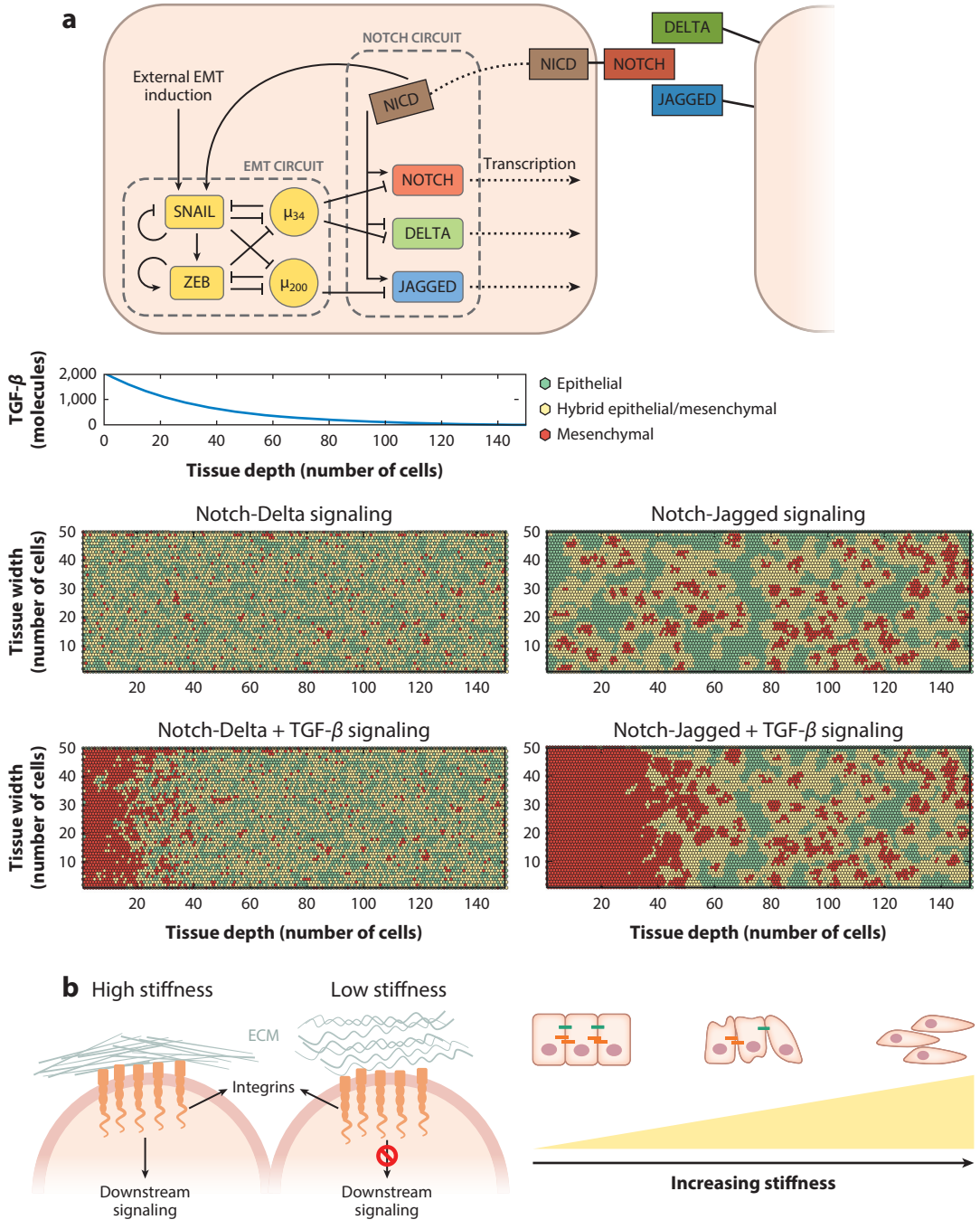

Figure 3

Key determinants of epithelial–mesenchymal heterogeneity in space. (*a*) Notch-Delta-Jagged signaling is a key juxtacrine signaling pathway that interfaces with the epithelial–mesenchymal transition (EMT) regulatory circuit. Different spatial patterns can arise depending on which of the signaling models is active and on exogenous concentrations of EMT inducers. Figure adapted from Bocci et al. (9). (*b*) Integrins are a key player in the mechanosensory control of EMT. High extracellular matrix (ECM) stiffness promotes EMT. Spatial differences in ECM stiffness may thus contribute to epithelial–mesenchymal heterogeneity.

heterogeneity in the expression of epithelial and mesenchymal markers if the ECM stiffness varies in space (**Figure 3*b***).

Physical and mathematical models of mechanosensing in cells (19), and those of cell migration (21), have provided useful insights into these critical biological processes. Multiscale models that connect the processes at the two levels will go a long way in furthering our understanding of the interplay between EMT and ECM stiffness and the role of this interplay in cancer metastasis.

SUMMARY POINTS

1. Mathematical modeling, along with recent advances in experimental techniques, has shown that cells undergoing EMT and MET can exhibit a spectrum of hybrid E/M states, with coexpression of epithelial and mesenchymal markers, in addition to the canonical epithelial and mesenchymal states. This discovery has transformed the perception of EMT from a binary decision-making process into a dynamic process with multistable behavior.
2. The dynamics of EMT and MET is driven by a core regulatory circuit consisting of two mutual inhibitory feedback loops—SNAIL/miR-34 and ZEB/miR-200—that operate within a larger network involving multiple signaling and regulatory factors.
3. Population-level EMT and MET dynamics is characterized by spontaneous changes in the phenotypic composition of the population, as well as by hysteresis, wherein EMT and MET proceed via distinct trajectories in the phenotypic space.
4. A key determinant of EMT and MET dynamics is the topology of the regulatory circuit controlling EMT and MET. Factors that can stabilize the hybrid E/M phenotypes couple with the core EMT regulatory circuit via specific topological motifs, such as by forming a mutual inhibitory feedback loop with ZEB.
5. Non-cell-autonomous mechanisms such as cell–cell communication, along with cell–ECM crosstalk, can drive spatial heterogeneity in the expression of epithelial and mesenchymal markers.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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