Computational study of adhesion receptor clustering at cellular interfaces

A PROJECT REPORT

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DECLARATION

I certify that

a) the work contained in this report is original and has been done by me under theguidance of my supervisor(s).

b) I have followed the guidelines provided by the Department in preparing the report.

c) I have conformed to the norms and guidelines given in the Honor Code of Conduct of the Institute.

d) whenever I have used materials (data, theoretical analysis, figures, and text) from other sources, I have given due credit to them by citing them in the text ofthe report and giving their details in the references. Further, I have taken permission from the copyright owners of the sources, whenever necessary.

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CERTIFICATE

It is certified that the work contained in this report titled "**Computational study of adhesion receptor clustering at cellular interfaces**" is the original work done by **Vaibhav Agarwal** and has been carried out under my supervision.

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ABSTRACT

Adhesion receptors, such as E-Cadherin, are crucial proteins found on the cell surface that facilitate cell adhesion by mediating physical attractions between the cell and its surrounding environment, specifically the extracellular matrix (ECM). E-Cadherin plays a pivotal role in various biological functions, including cell attachment, mobility, and signal communication. A notable feature of E-Cadherin is its ability to form mechanical links between neighbouring cells in epithelial tissues, which underpin many multicellular processes. These molecules tend to cluster, providing stability to the mechanical links between cells. This paper delves into the mechanisms controlling E-Cadherin's recycling and eventual endocytosis. Using the Drosophila follicular epithelium as a model, this study uses computational modelling to delve into the physical processes behind E-Cadherin recycling and endocytosis. We utilize Langevin dynamics simulations to observe E-Cadherin molecules on a plasma membrane. Furthermore, we simulate the interactions among the proteins using conventional pair potentials and examine how E-Cadherin endocytosis influences the clustering mechanism.

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LIST OF ABBREVIATIONS

Abbreviation	Description
ECM	Extracellular Matrix
PM	Plasma Membrane
ZA	Zonula Adherens
Rab11, RabX1, Rab5	Member of Rab family of GTPases
DE-cad	Drosophilla E-cadherin

INTRODUCTION

Cells utilize adhesion molecules to interact with their surroundings. These structural proteins emerge from non-covalent bonds between adhesion molecules on one cell surface and receptors on adjacent cells or mediator molecules within the Extracellular Matrix (ECM). Cell adhesions are pivotal for maintaining tissue cohesion and facilitating cell communication. Through these adhesions, cells can transmit forces, enabling interaction and sensing.

E-cadherin, a transmembrane protein, is paramount in orchestrating cell attachment in epithelial cells. The accumulation of E-cadherin at intracellular junctions is vital for preserving tissue integrity and modulating epithelial tissue activity. Cell-cell adhesion is fortified when E-cadherin receptors on neighbouring cells interact and cluster at the cellular membrane. The integrity of tissues and the regulation of epithelial tissue activities hinge on the aggregation of E-cadherin at these intracellular boundaries and the maturation of these cell-cell adhesions.

E-cadherin facilitates cell-cell adhesion by establishing homophilic contacts with E-cadherin molecules on adjacent cells. The intracellular domain of E-cadherin interacts with several cytoplasmic proteins, including beta-catenin, alpha-catenin, and p120 catenin. These interactions are essential for linking the E-cadherin adhesion complex to the cell's actin cytoskeleton. The formation of E-cadherin clusters at cellular interfaces is a pivotal step in the evolution of cell-cell adhesion. The lateral association of E-cadherin molecules, facilitated by calcium-dependent interactions and connections between the cytoplasmic domains of E-cadherin and catenin, leads to the clustering of E-cadherin.

Associations between E-cadherin and F-actin are mediated by beta-catenin, alpha-catenin, and other F-actin-binding proteins like Vinculin and EPLIN. As epithelia develop, significant restructuring of cell junctions occurs. This necessitates the regulation of cell-cell adhesion, such as by modulating cadherin levels or renewing them through endocytic recycling. A recycling mechanism redistributes E-cadherin from the lateral PM to the apicolateral PM, leading to its accumulation at the ZA. Recycling involves molecular interactions between Rab11, the exocyst complex, and beta-catenin. The study identifies RabX1 as a critical new component for DE-cadherin recycling, placing its function between the early and the

recycling endosome. In RabX1 mutants, endocytosed DE-cadherin protein is not properly recycled but accumulates together with Rab5 and Rab11 in a large compartment. This targeted recycling is essential for the maintenance of the ZA and cell shape.

MOTIVATION

The cellular landscape is a complex web of interactions, processes, and dynamics. Within this intricate framework, the recycling of molecules, particularly e-cadherin, stands out as a pivotal process. E-cadherin, a cornerstone of cell-cell adhesion, plays a significant role in maintaining tissue integrity and orchestrating various cellular activities. Its recycling, specifically through endocytosis, is a managed interaction that influences its organization on the cell surface of numerous metazoan organisms.

Given the importance of e-cadherin and its recycling, understanding the nuances of this process is crucial. Dysregulation of E-cadherin endocytosis has been linked to various diseases, including cancer and developmental disorders. For instance, rapid e-cad acquisition by endocytosis and a significant reduction in E-cadherin concentrations are associated with the collapse of the columnar epithelial structure during epithelial-mesenchymal transition (EMT), a critical phase in cancer development. Variations in the appearance of adhesion molecules have also been identified as a cause of the cell-sorting process in tissue culture.

However, despite its significance, several aspects of e-cadherin recycling remain enigmatic. The nature of specific interactions leading to e-cadherin clustering, the dynamics of its endocytosis, and its movement on the cell surface are areas that require deeper exploration. Traditional experimental methods, while invaluable, may not capture the full spectrum of molecular dynamics and interactions at play.

This is where computational modelling steps in. Computational modelling offers a powerful tool to simulate, analyse, and predict the behaviour of molecules in various scenarios. By leveraging computational tools, we can delve into the microscopic world of e-cadherin recycling, exploring the nature of interactions between E-cadherins and understanding the coupling between the dynamics of endocytosis and the movement of E-cadherin on the cell surface. Such models can provide insights that are challenging to obtain through experimental means alone.

Furthermore, the research paper highlighted the role of Rab11 in controlling the transport of newly synthesized E-cadherin from the Golgi to the plasma membrane, emphasizing the significance of recycling pathways in maintaining E-cadherin's presence at the ZA. By integrating these insights into computational models, we can gain a holistic understanding of the recycling process, its regulatory mechanisms, and its implications in health and disease.

LITERATURE SURVEY

Endocytosis, a key cellular process, plays a pivotal role in modulating the amount of E-cadherin on cellular interfaces. This recycling mechanism not only regulates the distribution of E-cadherin but also selectively targets and reduces the formation of large E-cadherin clusters. The underlying hypothesis suggests that larger clusters might be more susceptible to endocytosis due to their propensity to facilitate the assembly of endocytic machinery. Such macroscopic clusters, if unchecked, could potentially disrupt the actomyosin system, leading to a cessation of tissue movements. By regulating the size and distribution of these clusters through recycling, endocytosis ensures the smooth functioning of cellular processes.

While E-cadherin can aggregate independently of actin, experimental data underscores the importance of E-cadherin's associations with actin for maintaining cluster stability in live epithelia. Recent computational models have proposed that E-cadherin might spontaneously aggregate under the influence of lateral forces. Actin-based control plays a crucial role in preventing the disintegration of cadherin complexes. Despite evidence suggesting that E-cadherin mediates force transmission between the cytoskeleton and the cellular environment, the capacity of E-cadherin clustering to regulate this force transfer remains an area of active research. The recycling of E-cadherin, especially through endocytosis, is thought to play a role in stabilizing these clusters and maintaining cellular adhesion.

E-cadherin's role in cellular motility is multifaceted. While it restricts cell movement on matrices, its influence on cell movement through cell-rich tissues remains ambiguous. In-depth studies using in vivo mechanical stress sensors and other advanced techniques have revealed that E-cadherin-mediated adhesion between border cells and nurse cells stabilizes forward-directed protrusion, ensuring consistent mobility. This adhesion mechanism also

facilitates cellular communication, with leading cells providing directional cues to follower cells.

RAB5A, an essential endocytic protein, has been observed to stimulate the formation of distinct actin-based protrusions. These protrusions generate traction forces that are transmitted over extended distances through junctional contacts. This intricate interplay between mechanical coupling and polarity establishes a feedback loop, enabling cells to receive directional cues from neighboring cells. Such interactions enhance the dynamism of multicellular organisms, optimizing junctional E-cadherin dynamics to accommodate changes in cellular proximity, volume, density, and stress. The recycling of E-cadherin, especially its redistribution from lateral to apicolateral regions, plays a crucial role in this dynamic process.

Metastasis is a leading cause of cancer-related deaths. A notable observation is the inverse relationship between in vitro movement and E-cadherin concentrations. Despite the majority of breast cancers being invasive ductal carcinomas that express E-cadherin, its depletion has been linked to increased invasion. However, this also results in reduced tumor growth, survival, and metastatic spread. Strategies targeting E-cadherin-mediated survival pathways could offer potential therapeutic avenues for metastatic breast cancer.