Dynamic Intercellular Communication Within Pluripotency Networks

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***Short Abstract* — Gap junctions, formed from connexin hexamers, act as unique conduits for regulation cellular functions by mediating intercellular molecular transport. We observe connexin 43 (Cx43) to be largely distributed at the edges of embryonic stem cell colonies and more sparsely present at the colony interior. GAP-Fluorescence Recovery After Photobleaching (FRAP) was performed on cells with varying degrees of colony connectivity to characterize spatial features of inter-cellular communication. A preliminary computational agent-based model that incorporates gap junction communication as a function of differentiation produces spatial patterns being used to how direct intercellular molecular exchange may regulate differentiation and loss of pluripotency.**

***Keywords* — Gap junctions, connexins, embryonic stem cells, spatial differentiation.**

I. Introduction

Gap junctions are composed of two hexamers of connexins located in the plasma membrane of two neighboring cells, allowing diffusion of ions and small molecules below 1 kDa [1]. In cell colonies, the concurrent transfer through gap junctions creates an intercellular communication network for small molecules.

Multiple cellular functions are associated with gap junctions and connexins, such as modulation of cell signaling [2]. Specifically, inhibition of gap junctions results in abrogation of spatial patterns during differentiation, suggesting gap junctions provide a novel mechanism of regulating spatial differentiation within cell populations [3].

This work interrogates the intercellular network within ESC colonies and its effect on differentiation using convergent experimental and computational modeling techniques. Implementation of experimentally-determined bimodal transport rates associated with cell phenotypic state into an agent-based model of ESC colony growth and proliferation [4] allows us to examine propagation of spatial patterns within colonies.

II. Results

Mouse ESC cells were fixed, permeabilized, stained with Cx43 antibody, counterstained with Hoechst to identify mitotic cells, and analyzed by confocal microscopy. Cx43 was noticeably expressed by colony edge cells, and displayed distinct membrane localization in cells undergoing mitosis.

A. Intercellular Transport rates

GAP-FRAP is the measurement of fluorescence recovery from the diffusion of calcein, a low molecular weight molecule, into a previously bleached cell from neighboring cells. We observed a bimodal distribution of transport rates within ESC colonies with positional dependence. Cells undergoing mitosis yielded lower calcein intensity at recovery, however the kinetics matched that of non-dividing cells. This finding suggests that the two transport rates reflect changes in gap junction permeability.

B. Modeling Transport on Differentiation

We hypothesize that dynamic connexin expression as a function of colony position will result in different kinetics. Separate transport rates were assigned to differentiated and undifferentiated cells for a generic low molecular-weight molecule. Differentiation was predominantly a function of concentration thresholds of the molecule. Using these rules, spatial patterns of the molecular distribution across the colonies formed and preceded differentiation.

III. Conclusion

Interrogation of intercellular molecular mobility as a dynamic characteristic in ESC colonies via computational modeling provides hypothetical mechanisms of network communication which yield collective behavior of differentiation patterning. Our preliminary experimental and computational results suggest that differentiated cells have faster intercellular transport kinetics, thereby allowing regulatory molecules to propagate spatial differentiation within ESC colonies.

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