Mary Teruel - Research, Teaching, Service

Introduction

The overarching goal of my research is to understand the feedback, noise, and the dynamic control principles that underlie cell differentiation. In particular I want to understand how organisms control the rate of cell differentiation since the answer to this question is of fundamental importance to all multicellular organisms that have to create, maintain, and repair tissues and organs. My laboratory studies adipocyte (fat cell) differentiation since it is arguably one of the most accessible experimental systems for investigating terminal cell differentiation in mammals. Understanding adipogenesis also has great medical relevance since defects in adipogenesis and adipocyte function underlie the current worldwide epidemics in obesity, insulin resistance, diabetes, and cardiovascular disease. We focus on adipogenesis and cell differentiation (Research Focus 1), but also have a broad interest in understanding principles of how noise and feedback control cell signaling and cell decision processes (Research Focus 2).

Research Focus 1: Dynamic Understanding of the Regulatory Network Controlling Adipogenesis

Progenitor and differentiated cells are very different from each other with distinct gene expression, morphology, and function. In the case of adipogenesis, preadipocytes are proliferating, fibroblast-like cells. Adipocytes no longer divide and have the unique capacity to store and release exceedingly large amounts of lipid. When I started my lab, it was unknown how the transition between these two very different cell types dynamically occurs. Is it a gradual process that proceeds over the entire multi-day differentiation process? Or does an irreversible bistable switch from an undifferentiated to differentiated state occur, and if so, how and when is this switch triggered? Understanding the dynamics of differentiation is essential for therapeutically controlling the process since, for example, an irreversible switch mechanism can be controlled by temporal fluctuating regiments while graded processes cannot be.

Hundreds of proteins have been implicated as regulators of adipogenesis, making it challenging to uncover the core machinery. See for example our work in which we identified 45 human regulators downstream of PIP3, a main lipid second messenger involved in adipogenesis **(Park *et al*, Molecular Cell 2008)**. In addition, a major bottleneck in understanding the dynamics of how state changes are made is that measurements need to be made at the single-cell level and the tools to do so were missing. To overcome this bottleneck, my laboratory developed and applied single-cell imaging, targeted proteomics, and computational modeling tools that allow us - for the first time – to perturb and monitor the dynamics of a terminal differentiation process in order to identify the critical components, timing, and molecular control principles. Below I describe our main results so far.

In our study (**Park *et al*, Cell Reports 2012)**, we used single-cell imaging and identified the core architecture of the regulatory circuit that converts preadipocytes into adipocytes. We showed that adipogenesis occurs through a positive-feedback driven bistable switch that separates an undifferentiated from a differentiated state, and we identified core molecular feedbacks that control this dynamic transition. Using our experimental data, we developed the first quantitative molecular model of adipogenesis. Our finding of how differentiation can be made an irreversible process provides a general conceptual model that likely applies to many, if not most, cell differentiation decisions.

In our study **(Ahrends *et al*, Science 2014)**, we answered the fundamental question of how organisms can reliably control the low rates of cell differentiation needed to maintain tissue size and health. Since differentiation occurs through a bistable switch, low rates of differentiation should not be possible since all progenitor cells exposed to the same differentiation stimulus should switch to the differentiated state at the same time or all should remain undifferentiated. Our theoretical analysis demonstrated that protein expression noise (cell-to-cell variability) combined with a highly-connected multi-feedback system is sufficient to resolve the challenge of triggering differentiation in only a small fraction of the progenitor population while at the same time preventing differentiated cells from dedifferentiating. Using targeted, quantitative proteomics, we showed that adipogenesis is driven by at least 7 interconnected positive feedback loops that make the decision robust. Together, our results provide a conceptual framework of how organisms use noise and multi-feedback architectures to effectively control low rates of cell differentiation without sacrificing the robustness of the differentiated state.

In Ahrends *et al*, we had uncovered the systems architecture that enables control of low rates of cell differentiation for basal, homeostatic conditions. However, cells *in vivo* are subjected daily to strong fluctuating differentiation-inducing hormone signals. In particular, glucocorticoid and other adipogenic hormones are secreted in mammals in circadian oscillations. Loss of this circadian oscillation pattern during stress and disease correlates with increased fat mass and obesity in humans, raising the intriguing question of how hormone secretion dynamics affect the process of adipocyte differentiation. In our study **(Bahrami-Nejad *et al*, Cell Metabolism 2018)**, we used live, single-cell imaging of the key adipogenic transcription factors CEBPB and PPARG, endogenously tagged with fluorescent proteins and discovered that pulsatile circadian hormone stimuli are rejected by the adipocyte differentiation control system, leading to very low adipocyte differentiation rates. In striking contrast, equally strong persistent signals trigger

maximal differentiation. We discovered that the mechanism of how hormone oscillations are filtered is by a combination of slow and fast positive feedbacks centered on PPARG. Our study is to our knowledge the first to investigate differentiation in response to periodic stimulus inputs, and our discovery of a temporal control mechanism for differentiation is relevant to understand the regulation of fat mass in humans, suggesting that therapeutic regiments that reduce the daily duration of high hormones or increase the daily trough period during which hormones are low are beneficial to reduce fat mass. Given that oscillating hormones are ubiquitous in mammals, the temporal filtering mechanism we discovered likely represents a general principle for the control of cell differentiation.

To better understand different regulatory inputs controlling adipogenesis, we carried out a study **(Ota *et al*, Journal of Lipid Research 2015)** in which we expanded upon the absolute protein expression quantification method we had developed in Ahrends *et al* (2014), and made a methodological advance by showing that we can sensitively quantify large numbers of low-abundant proteins in primary adipose tissue isolated from mice. We compared for the first time the endogenous abundance of more than 40 adipogenic transcription factors, chromatin-remodeling, and other key nuclear proteins in different adipose depots from two mouse models of insulin-resistance. Our method allowed us to uncover new candidate proteins involved in insulin-resistance that are thus potential drug targets, as well as to understand fundamental differences between insulin-resistant and insulin-sensitive adipocytes *in vivo* at the protein level. This method has broad utility, and we are continuing to use it to understand the origin of adipocyte dysfunction in other mouse models of insulin resistance, as well as in humans, with the goal to identify key players and molecular mechanisms in the adipocytes that can be therapeutically targeted to treat obesity and metabolic disease.

On-going work: The process of differentiation is defective in many human diseases. Our work described above has shown for the first time that dynamic regiments such as restoring oscillations in the input stimuli can be used to effectively control the fraction of cells that differentiate. This led to the question how the also ongoing cell cycle and circadian oscillations of progenitor cells control differentiation and dedifferentiation. By combining our endogenously-tagged PPARG cell lines with live-cell readouts of the cell cycle and live reporters of circadian rhythms, we have recently discovered that there are dynamic "windows-of-opportunities" during these cycles in which adipogenesis is permitted or suppressed, arguing that therapeutic oscillations of differentiation stimuli have to be coordinated with the time of day and potentially by transiently arresting the normal cell cycle with drugs such as CDK4 inhibitors.

Our current and future work is focused on validating *in vivo* the control mechanisms we discovered *in vitro*. We have developed approaches to very sensitively monitor protein networks in primary adipose and other tissues from mice and rats using SRM mass spectrometry (Ota *et al*, 2015; Khor *et al*, 2015). Building on work by Mandrup *et al* (PNAS, 1997), we also established a protocol to carry out CRISPR-based knockout of genes of interest *in vitro* in adipocyte precursor cells, implant these cells into mice, and test *in vivo* for differentiation and fat pad formation using histology. We successfully used these protocols to demonstrate that our finding that adipogenesis occurs through a bistable switch in PPARG expression (Park *et al*, 2012) does indeed occur also *in vivo* in mice. We have also used our fat pad formation method to validate that FABP4 has a critical role *in vivo* as a key feedback partner of PPARG (Bahrami-Nejad *et al*, under review), and we are currently completing a study using a mouse model we generated with endogenously-tagged PPARG to validate *in vivo* the timing and order of the feedback loops found in Ahrends *et al* (2014). We are also excited about a new strategy we developed using implanted micro pumps to oscillate hormone concentrations in mice, and we are using this strategy to determine how different oscillating stimuli selectively control adipogenesis *in vivo*. Our long-term goal is to develop a comprehensive research program in which we carry out our assays in parallel *in vitro* and *in vivo* to more readily translate our discoveries of dynamic molecular control mechanisms into optimal temporal regiments for treating adipocyte-related human diseases.

Research Focus 2: Exploring the logic of how noise and feedback influence cell decision processes

The advent of single cell approaches has made it clear that noise (cell-to-cell variability) is inherent in all cell populations, but how noise originates, propagates, and affects cell signaling outcome has been largely unexplored. Our goal is to identify and understand the different mechanisms that can be used to stabilize noisy signaling systems and how noise can be modulated to resolve the conflict that noise is harmful for analog signaling but at the same time is needed for robust control of binary cell-fate decision signaling. Below I describe our recent findings.

In our study **(Abell *et al*, PNAS 2011)**, we applied a novel combined mass spectrometry and modeling strategy to the calcium signaling system, a system important in nearly all eukaryotic cells, to understand how cells can prevent signaling failure despite the inherent noise in expression of individual signaling components. Instead of relying on accurate co-expression or a single homeostatic feedback, our experiments and model calculations showed that parallel adaptive feedback is a powerful means to stabilize basal cytosolic and endoplasmic reticulum calcium levels and also to reduce the variability in the signaling response between cells. The control principle we uncovered likely extends to other signaling systems and provides a general explanation of how cells reliably connect receptor inputs to cell function, despite large cell-to-cell variations in the expression of individual signaling components.

In our study **(Kovary *et al*, Molecular Systems Biology 2018)**, we investigated a fundamental problem in mammalian signal transduction that the accuracy of signal transmission has been reported to be severely impaired by high variation in regulatory protein expression. When simulated in a typical signaling pathway, such high degree of noise prevents signaling systems from accurately transmitting even large input stimuli to cellular outputs. However, cells need very high signaling noise to control binary cell functions at the population-level, raising the fundamental question of how cells can achieve the right amount of noise for different signaling situations. Here we developed quantitative mass spectrometry and single cell imaging strategies to more accurately measure the variation of proteins between individual cells and discovered actual protein variations in the range of 5-15%, compatible with analog single-cell signal transmission. Furthermore, we discovered for one of the most important signaling pathways, the RAS-MAPK pathway, that the protein levels of MEK and ERK covary with each other, raising the question of the benefit of covariation for signaling. We discovered that covariation provides a key mechanism for fractional activation of population-level binary signaling outputs. Finally, our results allowed us to generate a first model of how cells use covariance to balance opposing needs for noise in accurate single-cell analog signaling and accurate population-level binary signaling.

On-going work: Our long-term goal is to understand broadly how noise and feedback are used in both analog signaling processes, as well as in binary decisions such as cell differentiation, to develop accurate computational models as we have done in initial models in Abell *et al* (2011), Park *et al* (2012), Ahrends *et al* (2014), Bahrami-Nejad *et al* (2018), Kovary *et al* (2018). Much evidence now suggests that future therapies will employ synergies and timing in signaling networks to increase on-target and reduce off-target effects. Accurate computational dynamic models are needed to develop such timed combination therapies, and we have been attacking this problem by developing quantitative analysis methods and multi-reporter live-cell imaging to measure absolute expression levels, posttranslational modifications, feedback connections, and cell-to-cell variation in endogenous protein expression over time. The methods we have been developing for this goal are broadly useful and we have used them, for example, to quantify protein expression levels and posttranslational modifications in collaborative discoveries of heterogeneous ribosome composition controlling specific mRNA pools **(Shi *et al*, Molecular Cell 2017)** and specific multisite phosphorylation of the cilia and hedgehog-regulated Gli transcription factors **(Niewiadomski *et al*, Cell Reports 2014)**. We are particularly excited about our recent success in endogenously tagging two positive feedback partners (for example FABP4 and PPARG) in the same cell which now allows us to understand when and how different feedbacks engage to drive differentiation, providing intriguing opportunities for targeted timed therapies.

Recognition: I serve as an ad-hoc reviewer for *Science*, *Cell Metabolism, Cell Systems, Molecular Systems Biology, Nature Biotechnology, Nature Structural Biology* and other journals. Over the past six years, I have been invited to give numerous domestic and international presentations. My research has been awarded a Stanford BioX Seed Grant, a Stanford Diabetes Center Seed Grant, two NIH RO1 grants on which I am the sole PI, a third NIH RO1 on which I am Co-Investigator, as well as funding from the NIH-funded (P50) Stanford Center for Systems Biology for which I serve as Co-Investigator and Co-Director of the Technology Core.

Teaching and Mentoring: I am currently training four PhD students and five post-doctoral fellows in my lab. I have developed and taught three graduate-level courses here at Stanford: An intensive, hands-on 6-week graduate student level course entitled "Biological Light Microscopy" which I currently direct and teach every year. With my colleague Josh Elias, I developed and taught a 10-week course on Proteomics, and with my colleagues Tobias Meyer and Dan Jarosz, I developed and taught for 3 years the Chemical and Systems Biology intensive 1-week bootcamp course for incoming graduate students. I also organize and teach each year 10 to 12 pre-seminar discussion classes for the first and second year Chemical and Systems Biology PhD students. I have also served as a Stanford Pre-Major Faculty Advisor for 3 years, advising 6 Stanford science or engineering undergraduates each year.

Service: I spearheaded, organized, and wrote the bulk of a $10.5 million, 14 Co-Investigator, NIH P50 application to start the Stanford Center for Systems Biology in July 2013 (Dr. James Ferrell is the PI of the center). I am actively involved in running the day-to-day operations of the center together with James Ferrell and Tobias Meyer, and also serve as Co-Investigator and Co-Director of the Technology Core. The center provides a hub for the Stanford Systems Biology community; tuition and stipends for 4 Stanford PhD students per year for the last 5 years; as well as 4 x $25,000 seed grants every year to enable Stanford students and postdocs to explore their own ideas and carry out Systems Biology research between labs. The center also runs a weekly Quantitative Biology meeting by student/postdocs and faculty. The Center has greatly added to the systems biology intellectual environment at Stanford. I also organize and run an endowed Chemical and Systems Biology departmental seminar series with 10-12 invited outside speakers each year which adds greatly to the Stanford intellectual environment.

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