BIOGRAPHICAL SKETCH

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NAME: Skotheim, Jan M.

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POSITION TITLE: Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Massachusetts Institute of Technology, Cambridge, MA, USA	B.S.	06/1999	Physics
Massachusetts Institute of Technology, Cambridge, MA, USA	B.S.	06/1999	Mathematics
University of Cambridge, Cambridge, UK	CASM	05/2001	Mathematics
University of Cambridge, Cambridge, UK	Ph.D.	10/2004	Mathematics

A. Personal Statement

My laboratory is well positioned to address the long-standing question of cell size control in budding yeast both conceptually and technically. Conceptually, the question of how budding yeast regulate their size was the key question that drew me into molecular and cellular biology from the field of soft matter physics 10 years ago and one which I have been studying and thinking about ever since. Technically, my laboratory has built up the expertise in live-cell imaging and, now, biochemistry, which I believe is required to break through and achieve a more fundamental understanding of how yeast control their size.

Cell size is important because it determines what functions a cell can perform and what niches a single-celled organism can fill. Moreover, cell size determines the size of many organelles including the nucleus, spindle and centrosome (reviewed in Turner et al 2012). Although many key regulatory proteins affecting cell size were known, the molecular mechanisms by which cell size triggers cell proliferation were unclear when we began our studies.

Cell size control in budding yeast occurs in G1 and is sensitive to the level of the G1 cyclin Cln3, whose activity is thought to trigger cell division (Di Talia et al., 2007). However, Cln3 concentration is nearly constant in G1 so the mechanism through which it would trigger division was unclear. *When we began our work, the central question in the field was what biochemical mechanism converts the constant Cln3 concentration to a size-dependent signal triggering division?* We now show that this was the wrong question. Rather than cell size increasing Cln3 activity, we see cell size inhibiting the activity of Whi5, a rate-limiting transcriptional inhibitor of cell division. Whi5 is a stable protein synthesized only outside G1 phase so that cells are born with a fixed amount. As cells grow during G1, Whi5 is continually diluted until it drops below a threshold where it is no longer able to restrain the G1/S transition. Size control is due to all cells receiving the same total dose of Whi5. Therefore, smaller cells are born with a higher Whi5 concentration that requires them to grow more than a larger cell to reach the same Whi5 threshold concentration. In contrast, *CLN3* synthesis scales with cell size. *Our results completely invert the previous model. Whereas it was thought size activated Cln3, and Whi5 set the threshold requirement, we now show that Whi5 is inhibited by cell size through dilution, while Cln3 sets the Whi5-concentration threshold determining how much dilution is required to trigger division* (Schmoller et al., 2015).

This result breaks open the problem of how to understand and study the link between growth and proliferation. We are extending these methods to examine this link in mammalian cells and have discovered that the famous tumor suppressor and cell cycle inhibitor Rb is diluted by growth during the G1 phase of the cell cycle. In addition, we just published a manuscript on BioRχiv showing that the mammalian cell cycle inhibitor, the retinoblastoma protein Rb is diluted by cell growth in mouse and human cells (Zatulovskiy et al 2018)

- DiTalia, S., Skotheim, J.M., Bean, J.M., Siggia, E.D., and Cross, F.R. (2007) "The effects of molecular noise and size control on variability in the budding yeast cell cycle", *Nature* 448, 947-951.
- Turner, J., Ewald, J., and Skotheim, J.M. (2012) "Cell size control in yeast" *Current Biology*, **22**, R350-9.
- Schmoller, K., Turner, J.J., Kõivomägi, M., and Skotheim, J.M. (2015). Dilution of the cell cycle inhibitor Whi5 controls budding yeast cell size. Nature doi:10.1038/nature14908.
- Zatulovskiy, E., Berensen, D., Topacio, BR, and Skotheim, J.M. (2018), "Cell growth dilutes the cell cycle inhibitor Rb to trigger cell division, bioRxiv 470013; doi: https://doi.org/10.1101/470013.

B. Positions and Honors.

Experience

2005-2008	Postdoctoral Fellow, The Rockefeller University
2008-2015	Assistant Professor in Biological Sciences and by courtesy in Chemical and Systems Biology, Stanford University
2015-present	Associate Professor in Biological Sciences and by courtesy in Chemical and Systems Biology, Stanford University

Professional Activities and Honors

1999-Present	Member of the Phi Betta Kappa, Sigma Xi and Sigma Pi Sigma honor societies
1999-2000	Fulbright Grant to study in Spain
2001	Foundation Scholarship and Bachelor Scholarship from Queens' College, University of Cambridge
2003	Rayleigh-Knight Prize, University of Cambridge
2000-2003	National Science Foundation Graduate Fellowship
2006	The François Naftali Frenkiel Award from the American Physical Society for a significant contribution to Fluid Mechanics by a researcher under 40.
2006-2008	National Institutes of Health – Ruth L. Kirschstein National Research Service Award (Individual Fellowship)
2008-present	Recipient of the Burroughs Wellcome Fund Career Award at the Scientific Interface
2008-present	Member of The Stanford Biophysics Program
2009-present	Member of The Stanford Bio-X Program for interdisciplinary research
2011	Recipient of an NSF CAREER Award
2012	Named David Hunington Dean's Faculty Scholar
2016	Trends in Cell Biology, Young and Trending
2016	Named HHMI, Gates Foundation & Simons Foundation Faculty Scholar

C. Contribution to Science

For a full list of publications see:
http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/40631145/?sort=date&direction=descending

The central aim of my laboratory is to understand the regulatory principles underlying the control of cell proliferation. A cell's decision to divide is based on information of extra- and intra-cellular origins that is transmitted through specific signaling pathways to activate downstream molecules. These downstream signaling pathway molecules then serve as inputs for the core cell cycle network that determines when a cell should proliferate. Importantly, downstream regulator activity is not exclusively determined by the current activity of upstream pathway components, such as the number of currently bound receptors, but rather reflects the time-dependent history of upstream activity. *I.e.*, the signaling pathway must *process* a dynamic input signal to determine downstream regulator activity. Currently, signaling pathways are most often described as static schematics based on a combination of genetic dependencies and biochemical interactions. While a good first step, such a characterization can neither describe nor predict the pathway dynamics that process input signals

to determine cellular response. My research has aimed to determine the dynamic regulatory mechanisms underlying cellular signal processing and computation mostly in relation to the decision to divide. Our most important achievements to date include understanding:

1. How cell size triggers division in budding yeast
2. How the frog mid-blastula transition is triggered by a cytoplasm-to-DNA ratio
3. How dynamic differentiation signals control cell division
4. Determining the mechanisms and function of the timing of transcriptional activation in the cell cycle
5. Evolution of cell size in response to changing environments

1. Cell size control in budding yeast

This work is described in the personal statement as it is most directly related to this proposal.

2. Histone-to-DNA ratio controls the Mid-Blastula Transition in *Xenopus*

A DNA-titration mechanism was proposed to regulate the critical cell size at which zygotic transcription is initiated in frog embryos. Prior to this maternal-to-zygotic transition, many species execute rapid and synchronous cell divisions without growth phases or cell cycle checkpoints. The coordinate onset of transcription, cell cycle lengthening, and cell cycle checkpoints comprise the Mid-Blastula Transition (MBT). For *Xenopus laevis*, a long-standing model proposed by Newport and Kirschner posits that MBT timing is controlled by a maternally loaded inhibitory factor that is titrated against the exponentially increasing amount of DNA. Although several potential regulators have been proposed, the identity of the titrated factor(s) remained unclear. To shed light on this question, we developed an assay using *Xenopus* egg extract that recapitulates the activation of transcription only above the DNA-to-cytoplasm ratio found in embryos at the MBT. We used this system to biochemically purify factors responsible for inhibiting transcription below the threshold DNA-to-cytoplasm ratio. This unbiased approach identified histones H3 and H4 as concentration dependent inhibitory factors, whose reduction in embryos induced premature transcriptional activation and cell cycle lengthening. Our observations support a model for MBT regulation by DNA-based titration and suggest that depletion of free histones regulates the MBT (Amodeo et al., 2015). More broadly, our work demonstrates how a constant concentration DNA binding molecule can effectively measure the amount of cytoplasm per genome (cell size) to coordinate division, growth, and development.

This work identifies histones as the candidate protein controlling the MBT in response to the DNA-to-cytoplasm ratio. Future work in my laboratory aims to determine the mechanism in which transcriptional activation and cell cycle lengthening are controlled by the histone-DNA ratio.

- Amodeo, A.A., Jukam, D., Straight, A.F., and Skotheim, J.M. (2015). Histone titration against the genome sets the DNA-to-cytoplasm threshold for the Xenopus midblastula transition. Proc Natl Acad Sci *112*, E1086-1095.
- Jukam, D., Shariati, A., and Skotheim, J.M. (2017), “Zygotic genome activation in vertebrates”, *Developmental Cell* **42,** 316–332.

3. Control of cell division by dynamic differentiation signals

I have led several studies revealing the importance of single-cell analysis for understanding the G1/S transition, which is where cells make the decision to commit to division. Beyond the G1/S transition, know as the restriction point in mammalian cells and *Start* in yeast, cells proceed through the division cycle despite loss of mitogenic signaling. Using a combination of microfluidics, quantitative imaging, and single cell analysis, we showed that commitment to cell division in yeast is directly determined by the activation of a positive feedback loop of G1 cyclins (Doncic et al., 2011). In another study, we show how the decision to commit to cell division in yeast depends not only on current input MAPK pathway activities, but also on its history of activity (Doncic and Skotheim, 2013). Moreover, we showed that this memory can be enhanced by the compartmentalization of the cell cycle inhibitor Far1 (Doncic et al., 2015). This demonstrates that the proliferation decision in yeast depends on the dynamics of the input signals. Taken together, the yeast studies attest to the power of single-cell analysis and microfluidics technology to identify regulatory principles that led to a qualitatively new understanding of cell cycle commitment that places a central emphasis on the dynamics of signaling pathways. Taken together, this work demonstrates that we have an extensive experience successfully applying single cell and systems biological approaches to the study of cell fate decision processes. Our work has recently been extended in the lab to the study of animal cell cycle control (Schwarz et al 2018).

- Doncic, A., Falleur-Fettig, M., and Skotheim, J.M. (2011). Distinct Interactions Select and Maintain a Specific

Cell Fate. Mol Cell *43*, 528–539.
- Doncic, A., and Skotheim, J.M. (2013). Feedforward regulation ensures stability and rapid reversibility of a cellular state. Mol Cell *50*, 856–868.
- Doncic, A., Atay, O., Valk, E., Grande, A., Bush, A., Vasen, G., Colman-Lerner, A., Loog, M., and Skotheim, J.M. (2015). Compartmentalization of a bistable switch enables memory to cross a feedback-driven transition. Cell *160*, 1182–1195.
- C Schwarz, A Johnson, M Koivomagi, E Zatulovskiy, C Kravitz, A Doncic & JM Skotheim, "A precise Cdk2 threshold determines passage through the restriction point" *Molecular Cell*, 69 (2), 253-264, e5 (2018).

4. Mechanisms and function of the timing of transcriptional activation in the cell cycle
The timing of transcription is a rapidly evolving feature of cell cycle regulation (Bertoli et al., 2013). We showed that the G1 cyclins *CLN1* and *CLN2* are expressed prior to other genes co-regulated by the SBF and MBF transcription factors including the negative feedback element *NRM1*. Temporal order of the transition is shown from left to right. We named this type of regulation 'feedback-first', and found it in other yeasts as well as human cells, where the functionally orthologous positive feedback components Cyclin E, E2F1 and Skp2 are activated prior to other cell cycle regulated targets of the cell cycle regulated E2F transcription factor. *Thus, feedback-first regulation ensures that the G1/S transition is a two-step process in which a cell first decides its fate by activating positive feedback and then activates the genome-wide transcription program to change its state* (Eser et al., 2011).

Having established the function of feedback-first regulation, our current work aims to determine the molecular basis for timing variability among genes regulated by the same transcription factors. Preliminary data suggests that this is due to variations in the DNA sequence surrounding the core transcription factor binding motif.

- Eser, U., Falleur-Fettig, M., Johnson, A., and Skotheim, J.M. (2011). Commitment to a Cellular Transition Precedes Genome-wide Transcriptional Change. Mol Cell *43*, 515–527.

5. Evolution of cell size in response to changing environments
Environmental controls on cell size evolution are poorly understood. In collaboration with Dr. Jonathan Payne (Stanford, Geological Sciences), we investigated environmental control on cell size in *Foraminifera*, a marine protist. *Foraminifera* is an ideal study group or single-celled eukaryotes because they have been abundant in the oceans for the past 400 million years (My) and are represented by more than 50,000 fossil and 5,000 extant species. Working with a team of local high school students and Stanford undergraduates, we created a digital database comprising nearly the entire fossil record. The extensive fossil record and high modern diversity of *Foraminifera* provide an unparalleled system for studying environmental influences on cell size. Remarkably, we found that most variation in mean size correlates with atmospheric oxygen concentration. *Our work shows how environmental change exerts a selective pressure on cell size*(Caval-Holme et al., 2013; Payne et al., 2012; 2013).

- Payne, J.L., Groves, J.R., Jost, A.B., Nguyen, T., Moffitt, S.E., Hill, T.M., and Skotheim, J.M. (2012). Late paleozoic fusulinoidean gigantism driven by atmospheric hyperoxia. Evolution *66*, 2929–2939.
- Payne, J.L., Jost, A.B., Wang, S.C., and Skotheim, J.M. (2013). A shift in the long-term mode of foraminiferan size evolution caused by the end-Permian mass extinction. Evolution *67*, 816–827.
- Caval-Holme, F., Payne, J., and Skotheim, J.M. (2013). Constraints on the adult-offspring size relationship in protists. Evolution *67*, 3537–3544.

D. Additional Information: Research Support and/or Scholastic Performance

NIH RO1 GM092925-6 Skotheim (PI) 9/1/15 – 7/31/19
Mechanisms of restriction point response to dynamic growth factor signals
The major goal of this project is to examine how the G1/S regulatory network processes dynamic growth factor signals to determine whether or not to enter the cell division cycle.

NIH RO1 HD085135 Skotheim (co-PI) 4/1/16 – 1/31/21
Control and coordination of the maternal-to-zygotic transition
The major goal of this project is to determine the molecule mechanisms through which histone-DNA titration controls transcription and cell cycle duration through the maternal-to-zygotic transition. Co-PI Aaron Straight.

NIH RO1 GM115479 Skotheim (PI) 8/1/16 – 4/30/20
Determining the molecular mechanism of cell size control
The major goal of this project is to determine the molecular mechanism through which budding yeast measure and control their size.

HHMI, Simons and Gates Faculty Scholar Award Skotheim (PI) 11/1/16-10/31/21
Award to support continued development of creative early career faculty.

Completed Research Support (within 3 years)

NSF CAREER – 1054025 Skotheim (PI) 1/15/11 – 1/14/16
Cell Size Control

Burroughs Wellcome Fund (CASI) Skotheim (PI) 1/1/08 – 6/1/16
Career Award at the Scientific Interface

NIH P50 GM107615A (subaward) Skotheim (PI) 9/30/13 – 6/30/18
Systems Biology of Collective Cell Decisions

Bio-X Interdisciplinary Initiative Program Skotheim (PI) 10/1/16 – 9/30/18
Feedback mechanisms linking cell cycle control and stem cell pluripotency
(co-PIs Stanley Qi & Marius Wernig)