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Professor

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Yves Brun
Systems Biology/Microbiology Faculty Search
Department of Biology and Biocomplexity Institute
Indiana University
Jordan Hall 142
1001 E. 3rd Street
Bloomington IN 47405-7005

Re: Joseph Pomerening

Dear Dr. Brun:

I am writing in enthusiastic support of Joseph Pomerening's application for a faculty position in your department. In my view, Joe's studies of the systems-level biochemistry of the mitotic oscillator is absolutely stellar. He is poised to become a leader in the quantitative analysis of cell cycle regulation.

As a postdoc Joe set out to functionally dissect the Cdc2/APC network and determine what enables the network to function as a robust, reliable oscillator. Experimental work from many labs has shown that the Cdc2/APC network includes a negative feedback loop (Cdc2 activates the APC, which inactivates Cdc2) and a number of positive feedback loops and double-negative feedback loops (Cdc2 activates Cdc25, which activates Cdc2; Cdc2 inactivates Wee1, which inactivates Cdc2...). The purpose of the negative feedback loop is clear; without it the system would get stuck in M-phase, and indeed when *Xenopus* egg extracts are treated with APC-resistant, non-destructible cyclin proteins, this is exactly what happens. Goldbeter and others have correctly pointed out that the negative feedback loop by itself could, in principle, support sustained oscillations in the activities of Cdc2 and the APC.

However, as Tyson, Novak, Thron, and others have noted, the positive feedback loops could be quite important as well. Positive feedback can, under the proper circumstances, result in bistability, so that mitotic oscillations could be thought of as beginning with a positive feedback-mediated "toggling" between discrete interphase and M-phase states, followed by a negative feedback-mediated restoration of the initial interphase state. Joe has focused on the question of what the contribution of positive feedback is to the cell cycle. He has addressed this question by experimental studies of *Xenopus* extracts, complemented by computational studies of realistic models of the Cdc2/APC system.

Joe's first important finding was that the positive feedback loops do generate a bistable response. That is, for certain cyclin concentrations (~40-80 nM, enough to bind to ~20-40% of the Cdc2 in an extract), there are two possible stable steady states: one with Cdc2 on (and Wee1 off) and one with Wee1 on (and Cdc2 off). Extracts in the Cdc2-on state are arrested in mitosis, with condensed chromatin, broken-down nuclear envelopes, and hyperphosphorylated Cdc25, Wee1, and p42 MAPK, whereas extracts in the Cdc2-off state are arrested in interphase, with diffuse chromatin, intact nuclear envelopes, and hypophosphorylated Cdc25, Wee1, and p42 MAPK. This work was published in *Nature Cell Biology* in 2003, and it was featured (together with a *PNAS* paper on the same subject by the Sible and Tyson groups) in a *PNAS* commentary by Mark Solomon.

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A second important finding came out of the computations. Joe noted that it is not possible to obtain limit cycle oscillations from a simple two-component negative feedback loop, and it is not easy to obtain them with a three-component loop either; the parameters have to be chosen just right, or the oscillations will ultimately damp to a stable steady state. However, adding positive feedback tended to make it much easier to obtain oscillations. Joe hypothesized that the oscillations of the Cdc2/APC may be more robust in the presence of positive feedback than in the absence of it.

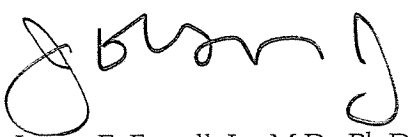
Building upon this idea, he carried out a series of beautiful experimental studies where he manipulated the strength of the Cdc2/Wee1/Cdc25 positive feedback loops in *Xenopus* extracts. He showed that eliminating this positive feedback makes Cdc2 activation become much less temporally abrupt—activity increases linearly with time, rather than an increasing at an accelerating pace as it normally does. This argues that the translational positive feedback loop (active Cdc2 promotes cyclin B1 translation) discovered by Richter and colleagues is unlikely to be of much significance. Joe also discovered that the activation of the APC is compromised when positive feedback is eliminated. Without a spike of Cdc2 activation driving the APC, the APC turns on only partially, and the result is that the extract carries out a normal S-phase, followed by a sluggish M-phase, followed by a protracted intermediate state that is not quite M phase and not quite interphase. These findings establish the importance of positive feedback for keeping the phases of the cycle discrete. This work was published in *Cell* this summer, and was featured in a nice preview in *Dev Cell* written by Fred Cross and Eric Siggia. I think Joe's work is an important step forward in our understanding of the systems-level logic of biological oscillators.

Joe and I have submitted a third paper, on amplitude and frequency control in biological oscillators, and he has a fourth paper on positive feedback and oscillations in HeLa cells that is almost ready to be submitted. This work will round out a very productive postdoctoral career.

In addition to being a first rate experimentalist with computational modeling skills, Joe is one of the most talented teachers I have ever seen. He is an incredibly engaging speaker and is devoted to education. He received teaching awards as a graduate student, has published a paper on science education, and loves teaching both in a formal classroom setting and in a one-on-one basis.

I have very high expectations of Joe. He has really blossomed during his time as a postdoctoral fellow, and I recommend him with the highest enthusiasm.

Sincerely,



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