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Dear Colleagues,

I am writing to give my most enthusiastic support for Mara Duncan. She is one of the smartest people I have met and combines her intellect with immense ingenuity, commitment, and perseverance. I am confident that she will be a terrific asset to your program.

Mara joined my lab in 2001 following her Ph.D. research in David Drubin's lab at UC Berkeley. In the Drubin lab, Mara demonstrated that a component of the endocytic machinery in yeast, the Pan1 protein, can stimulate actin polymerization by directly activating the Arp2/3 complex. This was a landmark study in the field, providing the first direct mechanistic link between the process of endocytosis and actin filament assembly.

Mara's PhD project stimulated her interest in protein traffic and she came to my lab to study clathrin-mediated transport between the TGN and endosomes. We had recently characterized a role for the Gga protein adaptors in TGN/endosome transport. Gga proteins share a C-terminal domain with a subunit of another TGN/endosome clathrin adaptor, the AP-1 complex. Mara decided to search for binding partners of this domain using a 2-hybrid screen. She was immediately successful, identifying two interacting partners, Ent3p and Ent5p. These proteins contain domains homologous to the phosphoinositide-binding ENTH/ANTH domains of mammalian endocytic adaptors. Very quickly she obtained evidence that the two proteins contained novel, conserved motifa responsible for interaction with Gga proteins and the AP-1 complex. Together with another postdoc in the lab, Mara demonstrated co-localization with TGN/endosome clathrin coats and characterized a transport defect in cells lacking both Ent3p and Ent5p. This study extended roles for ENTH/ANTH domain proteins from endocytosis to TGN/endosome trafficking pathways, thereby establishing this family of proteins as a fundamental, conserved element of clathrin-coated vesicle formation.

Since publication of this work, Mara has pursued two related but distinct projects. The first is a direct extension of her initial studies of the Ent proteins. Like many clathrin adaptors, Ent5p contains a lipid-binding domain and motifs for interaction with clathrin and other clathrin adaptors (in this case Gga proteins and AP-1). This type of modular organization is postulated to provide multiple low-affinity contacts with other clathrin coat components, allowing cooperative assembly of a functional coat. Although there have been some tests of this paradigm *in vitro*,

Mara recognized that she was in a position to address the model in an *in vivo* setting. Accordingly, she has generated different strains with mutations in each binding module, or combinations of those mutations, and used these strains to characterize protein transport and association with clathrin coats *in vivo*. She has uncovered a couple of surprises. First, her results indicate that the Ent5p lipid-binding domain plays a role in clathrin-mediated transport beyond the expected function in localizing Ent5p to nascent clathrin coats. Second, Mara observed that clathrin binding by Ent5p strongly depends on Ent5p association with other adaptors. This result suggests the important concept that cooperative interactions between different adaptors allows a type of internal quality control: full clathrin coat assembly occurs only when the complete complement of adaptors is present.

While Mara's first project falls well within the normal approaches used in my lab, Mara's second project illustrates her independence and initiative. On her own, Mara devised and carried out a clever screen for small molecule inhibitors of TGN/endosome traffic. We have often struggled with the dynamic and malleable state of TGN/endosome trafficking pathways. Early studies in my lab indicated that cells can adapt to a loss of clathrin function and regain the ability to sort proteins from the TGN through endosomes to the vacuole. Furthermore, we frequently encounter minimal effects of single gene deletions on trafficking between the TGN and endosomes; we attribute these weak phenotypes to redundancy in protein components and the multiple routes available between the TGN and endosomes. Mara realized that a chemical inhibitor that acted rapidly and isothermally would be an invaluable aid in addressing some of these issues. Furthermore, given the conservation between yeast and mammalian trafficking pathways, any compound with effects in yeast has the potential to act in a similar way on mammalian cells. The clever part of the project is that Mara has adapted the method of synthetic genetic analysis (combinations of mutations that alone are innocuous but together cause severe defects) to screen for chemical inhibitors. This approach should be applicable to a wide variety of pathways and cell types. Mara's particular strategy was based on our observation that mutations in either Gga or AP-1 adaptors had, at best, marginal effects on cell growth but together caused a severe growth defect. So she decided to search for inhibitors of AP-1 mediated pathways by screening compounds for effects on the growth of a strain lacking Gga proteins.

Mara's screen has yielded several inhibitors of clathrin-mediated transport, three of which appear specific for AP-1-mediated traffic. This has not been easy - she has had to develop small volume methods for many of our standard trafficking assays and to devise procedures that maintain the activity of the compounds under the conditions required to assay trafficking. As one illustration of the utility of the compounds, Mara has already screened the 4800 viable yeast knock-out strains for those that are hypersensitive to one of the compounds and through this approach discovered a role for AP-1 in transport of a plasma membrane amino acid permease.

I stress that the chemical genetics project was entirely Mara's idea and she displayed an impressive level of initiative and determination in bringing the project to fruition. When she embarked on the project, UCLA did not have an established screening facility nor access to diverse chemical libraries. Undaunted, Mara took it upon herself to apply for and receive permission to use the Harvard ICCB chemical screening facility. She carried out her initial screens there and although she did not obtain any clear positives, she came back to UCLA with the experience to continue the screens here. At just this time, UCLA decided to establish a chemical screening facility and obtained a diverse chemical library. However, there was very little pertinent expertise or equipment here so Mara had to carry out her screens pretty much on her own. I believe she was the first person at UCLA to carry out a growth-based screen for small

molecule inhibitors. Because of her pioneer status, Mara has been intimately involved with the chemical screening facility from the beginning and has served as an adviser for several subsequent screens by other researchers here. She also initiated a collaboration with an organic chemistry lab and is working closely with a student to carry out structure-activity analysis.

I have elaborated on Mara's current projects to illustrate some of her strengths and also, in part, because they are not yet published. They will be submitted very soon, both are in the last phases of experiments, and I expect Mara to start writing in the next few months. Because she has been working simultaneously on two projects, almost entirely by herself, progress on each has been slower than if she focused only on one. Nevertheless, in a little over three years Mara has carried out three significant projects, one (chemical screening) begun completely from scratch.

Mara spends long hours in the lab, combining bench work with an avid and broad interest in the literature. But she is also very generous with her time, often providing advice to other students and postdocs in the lab. She now has taken on the responsibility of training a new lab assistant in yeast molecular genetics and I have been impressed by her ability to teach both concepts and techniques. In addition, I am frequently asked by other labs for help with their yeast projects and Mara has willingly helped guide students and postdocs from these groups. Based on observing these interactions I am certain she will be adept at establishing a productive and interactive research group.

Mara also has an active interest in teaching. She volunteered to co-organize a new graduate seminar course in the department that will involve presentations by invited outside speakers and class sessions discussing the seminar and relevant papers. Her research seminars are well-organized and clearly-presented, promising similar clarity in classroom lectures.

In summary, Mara's intelligence, creativity, and fearlessness place her among the top 5% of the postdocs I have known. Her quick and penetrating intellect gives her a productively critical perspective on her own research and that of others. She has several projects (e.g. adaptor characterization and small molecule inhibitors) that are strong foundations for an independent research program. Her friendly and interactive personality makes her highly collaborative. I am certain she will be a productive and collegial addition to your faculty and I give her my strongest endorsement . If I can be of any further assistance please do not hesitate to contact me.

Sincerely,

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