10/20/05

SALK INSTITUTE
FOR BIOLOGICAL STUDIES

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To Dr. Brun and Members of the Search Committee:

I am delighted to write in enthusiastic support of Len Duncan as a faculty candidate.

I have known Len since he joined Dr. David Kirk's laboratory as a postdoctoral fellow in 1997, during which time I was a postdoctoral fellow in Dr. Ursula Goodenough's laboratory. Besides being in close physical proximity, the Kirk and Goodenough laboratories held regular joint group meetings, so I interacted with Len on a daily or weekly basis throughout the time he was at Washington University. Since Len left the Kirk lab and I started my own laboratory at the Salk Institute, we have been in less frequent contact, but I have kept up with his progress and encouraged him to get back into an academic setting where he so clearly belongs.

Embryogenesis in *Volvox* involves a highly stereotyped set of cell divisions, and Len's first project in the Kirk laboratory involved an important screen to look for transposon-tagged *pcd* (pre-mature cessation of division) mutants that halt cell division prematurely. Such mutants would be expected to reveal the molecular machinery that tells an embryo when it has divided the appropriate number of times, and *Volvox* is arguably one of the best, if not the best, system in which to address this fundamental question in developmental biology. Although chemically induced *pcd* mutants were known to exist, no previously isolated *pcd* mutation had been cloned. Len isolated a number of tagged *pcd* lines, and the next step was to identify the mutant loci. At the time he was carrying out the *pcd* screen the method for cloning transposon-tagged mutants was to identify lines that had reverted to a wild type phenotype, and to correlate the reversion event to an RFLP of the transposable element, *Jordan*. Because *Jordan* is present in multiple copies and is relatively mobile, finding such RFLPs and correlating them with reversion is non-trivial and requires some luck. It is unfortunate that Len was unable to identify a good *Jordan* RFLP for any of his *pcd* mutant lines, but it was certainly not because of lack of skill or determination. I know it was difficult for Len to drop this project because he had the mutants in-hand, but it was probably wise for him to cut-bait and move on.

In the mean time, Len had been looking for other mobile elements that might be harnessed as an alternative to *Jordan*. In doing so, he came across and characterized a new type of

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element, *Kangaroo*, which is related to retrotransposons, but which belongs to a distinct and poorly-understood subfamily that is found in many eukaryotes. Len was able to characterize *Kangaroo* and infer the existence of a novel circular transposition intermediate. While this work was somewhat of a diversion from Len's real interests and from the forte of *Volvox* as a developmental system, it was a significant contribution that yielded insight into a transposon family that is widespread among eukaryotes. This study also showed that *Kangaroo* expression is developmentally regulated, a finding that is likely to have significance for understanding how genomes interact with potentially deleterious genetic elements.

As Len was finishing up his work on *Kangaroo*, he made an important observation related to the *Volvox regA* gene. As described in his Research Interests, RegA is one of the critical regulators of cell type specification in *Volvox carteri*, but its biochemical function is still an enigma. Len cloned the *regA* locus from a closely related species, *Volvox kawasakiensis*, and found that the gene had diverged considerably. Moreover, he found evidence for a *regA* paralog just upstream of the *regA* gene in both species. With the very recent availability of a draft genome for *V. carteri* it became apparent that *regA* lies in a cluster of four paralogs that were likely formed from tandem duplications, and that a RegA family of proteins with a conserved putative DNA binding domain called GAR exists in the Volvocacean algae. Importantly, the GAR domain is related to a more widespread DNA binding motif called the SAND domain, so Len's future research program will be likely to impact a much wider area than just the green algae.

Although Len's work in *Volvox* took some unexpected turns, like all good scientists he has made the most of his opportunities. Importantly, Len's future research program is focused on some key questions in evolution and development. For those who have followed *Volvox* as a model organism, RegA has been the holy grail for understanding cell-type specification, and now that the *regA* locus has been cloned, the time is ripe to test hypotheses for how it evolved to become a developmental regulator. As described in Len's Research Statement, the newly available genome sequences of *Chlamydomonas* and *Volvox* make this a particularly exciting time to be able to ask questions regarding the function and evolution of RegA. Moreover, because David Kirk no longer has an active laboratory, the field is wide open. I can't think of anyone better qualified than Len to carry the torch and take this classic problem in development to the next level of molecular analyses.

Len's Research Statement speaks for itself as a model of clarity and depth, and it gives a very good indication of how he thinks and communicates. His presentations at lab meetings and other functions are equally clear and thoughtful, and they indicate his rare ability to synthesize and communicate data in a way that is characteristic of the very best scientists. I have worked at three top-level institutions--UCSF, Washington University and the Salk Institute-- and without a doubt Len is on par with the best post-docs I've known; he is easily in the top 5%. Had it not been for the unfortunate timing of funding cycles and the pressure to support his family, Len would probably already be a PI. His eagerness to get back into academia is evident from his continued work on his *regA* story, even while employed in industry and working on unrelated projects.

Although I have no direct experience with Len's teaching style, based on the clarity of his research presentations I anticipate that he will excel in this area. I know that he had

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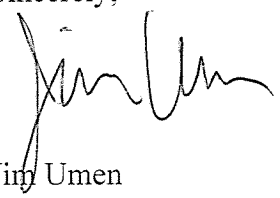
extensive experience as a graduate teaching assistant and was exposed to the highest standards of undergraduate instruction as a student at the University of Cambridge. Moreover, there is something about Len's enthusiastic personality and his ability to explain complex concepts in simple, concrete terms that suggests he will be a gifted and highly sought-after lecturer and mentor.

On a personal level Len is a fantastic colleague. He is easy-going, thoughtful, and has broad interests and thinking. From the moment he joined the Kirk laboratory he was an asset to our joint research group, and I greatly valued his contributions to lab meetings and other scientific discussions. In short, Len Duncan is probably one of the finest scientists I know, and would be a stellar addition to any department. I strongly and unreservedly urge you to interview him for this position.

Sincerely,



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Washington
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Arts and Sciences

October 15, 2005

Dr. Yves Brun
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Dear Colleagues,

Dr. Leonard Duncan is one of the brightest postdocs I have had the pleasure of working with over the past four decades, and I think that he has considerable promise as an independent investigator. Nevertheless, I doubt whether he is right for you – or you for him – at this stage of his career.

Len joined my research group with glowing recommendations and considerable enthusiasm. However, not long after he arrived in St. Louis his mother-in-law (who lived alone in rural Iowa, about 4-5 hours from here) began an agonizingly long and painful fight against terminal cancer. Without any doubt, the many weekends and holidays that Len spent driving his family to and from Iowa over subsequent years sapped his energy and blurred his focus. That, coupled with the fact that the first project he tackled here ended up in a blind alley, meant that he got off to a very, very slow start. Then when he was just beginning to really hit his stride, his predoctoral mentor, Rich Losick, made him a "now or never" offer to join his startup biotech company. After a bit of agonizing, Len took the job – leaving both of his main projects here unfinished. Since then, despite the very different demands of the industrial environment, he quickly brought one of those projects (the discovery of the *Kangaroo* retrotransposon family) to publication, and now has finally brought the other (on regA orthologs and paralogs) to the point where it is now under review. And he has become genuinely enthused about returning to a study of the molecular genetic basis for the evolution of multicellularity and cellular differentiation in the volvocine algae. Naturally, I am anxious to do all I can to support him in this goal.

In my opinion the projects Len has proposed for the near future are conceptually rich, but also both feasible and fundable. I think Len will flourish and become a highly productive evolutionary developmental biologist in the next few years if he finds the kind of fostering environment in which he can develop the grant proposals, lab facilities and other resources needed to build a strong and productive research group from scratch. But I would be surprised if he is ready at this time to hit the ground running with the speed that I suspect you expect of the person you hire as a result of this search. I would be thrilled if you proved me wrong in that assumption!

Collegially,

David L. Kirk
Professor of Biology Emeritus

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October 12, 2005

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Dear Dr. Brun,

I write in support of the application of Dr. Leonard Duncan for a position in the Department of Biology at Indiana University. Len was among the better graduate students I have had. In fact, I regarded his thesis work so highly that I nominated him for, and he co-won, the Nate Sternberg Prize of Cold Spring Harbor for the best Ph.D. thesis in microbial biology in the year he graduated. I recommend Len on two counts. First, he was productive and effective. He simply got a lot done, and he had numerous papers to his credit in the *J. Molec Biol.*, *PNAS*, *Cell*, and *Science*. Particularly noteworthy is his *Science* paper in which he demonstrated biochemically that a particular, membrane-tethered sporulation protein in *B. subtilis* acts as a phosphatase to trigger the activation of a cell-specific transcription factor, σ^F. The second is that Len is very thoughtful. He was the intellectual leader of the σ^F problem in my lab, and he became a mentor to two younger students and conceived of a Ph.D. project for one of them. After receiving his Ph.D, Len did a postdoc with David Kirk on Volvox. Although things went slowly for him at Washington University, he did end up with two manuscripts, one on a novel transposon and one on the nature of a key regulatory gene that governs the somatic state in Volvox. After Washington University, Len took a position at a small biotech company, but he is an academic at heart, and he now seeks both to teach and run a lab. Based on my very positive opinion of him from the days he was in my lab, I believe that he will effective in running his own lab and serving as a mentor and teacher to students. I am therefore pleased to recommend him highly.

Sincerely,

Richard Losick