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29 September 2005

Yves Brun
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To the Search Committee,

I am delighted to recommend Dr. Nicolas Sokol with greatest enthusiasm for a faculty position in your department. Nick was a wonderful graduate student in my lab who used his considerable powers of deductive reasoning and hard work to produce a superb thesis, easily among the best at Yale. He showed keen foresight by choosing what was then the nearly brand-new and exciting field of microRNAs for postdoctoral training. He brought his extensive skills working with flies to one of the foremost worm labs studying miRNAs. He has been extraordinarily successful as a postdoc, and is now ready to launch his own lab and tackle the biology of miRNAs. His work promises to advance the miRNA field toward a detailed understanding of how miRNAs contribute to development and disease.

Nick started out in my lab with a project to clone the *cheerio* gene in *Drosophila*. We thought this would be relatively straightforward since we had collected several P element insertion alleles from various sources, which should have made cloning a snap. However, after recovering genomic DNA flanking the P elements, Nick realized that they were not all in the same place. Worse yet, none of the insertions was actually resident at the *cheerio* locus. Apparently, this gene is an attractive target for insertions, but equally good at expelling the transposons with a lesion left behind. After this disappointment, Nick quickly rallied and went to the literature to figure out a new strategy. He decided to use a classic genetic mapping technique that ultimately led to identifying the gene. I still remember his excitement when he came into my office and announce that *cheerio* encodes filamin and how full of ideas he was about what to do next. It was like releasing someone from cloning purgatory. Nick proceeded to raise useful antibodies to filamin and show that it is highly enriched at ring canals. This is consistent with the ring canal phenotype he had observed in mutant egg chambers. Unfortunately, writing the manuscript describing this work had to be done under difficult circumstances because we learned that a competitor was a few weeks ahead of us. Nick really paid his dues with this part of his thesis work and ended up producing a beautiful article that was almost immediately accepted at Current Biology.

Nick pursued several projects after the cloning of *cheerio*. One of these involved characterizing an unusual allele of *cheerio* that is caused by the insertion of an EP element that miraculously stayed put near the 5' end of the transcription unit. Rather than affecting germline ring canals as other *cheerio* alleles do, the EP insertion specifically affected follicle cell expression of filamin and caused aberrant

follicle cell migrations. This provided strong evidence for a role of filamin in cell migration. An additional insight from Nick's work is that alleles that result in filamin truncations do not have follicle cell defects. In other words, Nick discovered that the N-terminal half of filamin is sufficient for follicle cell migration. Making transgenes that encoded truncated filamin and testing for rescue of the EP allele confirmed this. A paper describing this work was published in *Developmental Biology*. I should say that while this is now a clear story and beautifully documented, it was not easy to sort out in part because of interallelic complementation among *cheerio* alleles. Nick's talents as an insightful geneticist and his persistence in pursuing the project are what made the difference.

Throughout his work in my lab, Nick was fearless about venturing into new territory. He became an expert in molecular and classical genetics in flies. One remarkable trait of Nick's is his ability to carefully interpret fragments of data and synthesize a testable hypothesis. He is very intuitive about his results. This has come in quite handy for figuring out what is going on with a fairly complex gene and navigating the web of potential filamin interactions.

Nick is a joy to interact with and contributed considerably to overall lab life. His research presentations were always clear and very enjoyable, reflecting his ability to weave together an interesting story. He always has lots of good ideas and is eager to pursue them. Nick has the skill, creativity, motivation and intellectual curiosity necessary to build a successful career as a scientist. He will be a stimulating and helpful faculty colleague, and I strongly urge you to consider him for your department.

Sincerely yours,

Lynn Cooley

Lynn Cooley
Professor

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09/14/05

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Dear Dr. Brun;

This is a letter of enthusiastic support of Nicholas Sokol's application for a faculty position in the Indiana University Department of Biology.

Nick is superb. He is one of the most accomplished postdoc that I have every had, and I can say that I have never known a postdoc as independent and resourceful as Nick. Nick joined my lab having already established himself as an *Drosophila* developmental geneticist during his Ph.D. work with Lynn Cooley. He approached me with already well-formulated plans for exploring the roles of microRNA pathways in animal development. Nick chose for his focus a set of microRNA genes that are highly conserved between flies and humans, with the aim of utilizing the superb tools of *Drosophila* genetics to determine the functions of these conserved microRNAs, and hence provide a basis for understanding their roles in vertebrate development and disease. Nick established the scope and focus of the project entirely by himself, designed the overall strategy, and fleshed out the experimental details entirely on his own. I was essentially a spectator in this process. Nick then proceeded, entirely by himself, to set up *Drosophila* as an experimental system in my lab. I want to emphasize that neither I nor anyone else in the lab had any experience with *Drosophila* prior to Nick's arrival. Nick and I benefited immensely from the use of Yashi Ahmed and Claudio Pikielney's fly facility, but I am sure that in exchange, Nick has provided advice and mentorship for Yashi and Claudio's students and postdocs.

Nick used the Golick homologous gene replacement method to generate deletion mutations of two *Drosophila* microRNA loci, *mir-1* and the *let-7* cluster (which contains *let-7*, *mir-125* and *mir-100*). This was an especially remarkable feat, because at the time the Golick method had been successfully used by only a handful of labs, and certainly none of these was at Dartmouth. The fact that Nick was able to make the method work so effectively *entirely on his own* is a testament to his absolutely remarkable independence, guts and fortitude.

From Nick's postdoctoral work, I anticipate at least two more papers in addition to the collaborative Sempere et al 2003 paper, and the Sokol and Ambros, 2005 G & D paper on *mir-1* loss-of-function phenotype. Nick has performed an initial phenotypic characterization of his *let-7* cluster knockout, and he plans a manuscript describing the

phenotype, and at least one manuscript following that on more detailed analysis of the functional relationships among the three genes of the *let-7* cluster. This gene cluster is fascinating; Nick has found that all three microRNAs are coordinately up-regulated at the time of pupariation in the fly, and that they seem to play a critical function during metamorphosis. Nick's detailed genetic and functional analysis of the let-7 cluster, particularly the evolutionarily conserved members let-7 and mir-125 (the fly lin-4 ortholog), will almost certainly be of general significance, as these two microRNAs are known to control developmental timing in nematodes, and to be associated with certain cancers in humans.

In the course of his analysis of the mir-1 mutant phenotype Nick has discovered an unanticipated function for microRNAs. To Nick's credit, the mir-1 phenotype is sufficiently novel and that a less astute experimentalist or a less rigorous geneticist could easily have missed or misinterpreted the phenotype. In this case, *mir-1* is expressed in muscle and is required for the integrity and survival of muscle cells during rapid postembryonic cell growth. Remarkably, *mir-1* mutant larvae are viable and motile as long as one does not feed them. However, once fed, all larvae rapidly become immobile and die! Nick has proposed that *mir-1* functions to repress the activity of muscle-toxic genes that become expressed during rapid cell growth. He has suggested that perhaps nuclei of rapidly dividing and/or growing cells are prone to an inevitable level of promiscuous transcription, and microRNAs (in this case *mir-1*) serve to inhibit translation from these cell type-inappropriate transcripts. This is an provocative idea of Nick's that I'm sure will strongly influence the thinking of others in the field over the coming years.

Nick will have a vigorous research plan in a field that promises to be eminently fundable for many years. He will not compete with my lab in the least, because he will take the entire *mir-1* and *let-7* cluster projects with him. MicroRNAs is a very competitive field, filled with bright workers. But I think Nick stands out as exceptional: First, Nick is deeply committed to rigorous genetic approaches to understand the function of genetic pathways, and so his work will stand the test of time. Second, Nick is personally amazingly productive, so I expect that his research group will likewise be dynamic. Finally, Nick is creative, but also careful and meticulously critical, in his thinking.

In summary, I strenuously urge you to interview Nick Sokol for a faculty position. He is truly exceptional, and among the best postdocs I have seen anywhere. He will be an exciting and stimulating colleague for a department that appreciates genetics, developmental biology and/or microRNA regulatory pathways. Please give me a call if you wish to hear more.

Sincerely,

Victor Ambros

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September 30, 2005

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Dear Members of the Search Committee:

I am very pleased to provide this letter of recommendation for **Dr. Nicholas Sokol**, who has applied for a junior faculty position in your department.

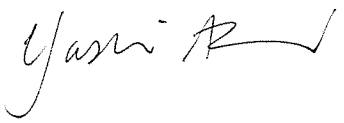
I know Nick quite well, as we have interacted almost daily for the past three years, since I joined the faculty in the Department of Genetics at Dartmouth Medical School. Nick shares a "fly pushing" room with myself, and the other people in my lab. As such, I have had frequent interactions and scientific discussions with Nick. I have been extremely impressed with Nick during this period of time.

The long-term goal of Nick's work is to utilize the classical tools of Drosophila genetics to gain insights into microRNA function, in the anticipation that these insights can be extrapolated to microRNA function in mammals, and its misregulation in disease. His research has now established the basic genetics of two evolutionarily conserved Drosophila microRNAs, and for one, he has identified a number of modifier loci that will provide additional handles on its cellular function. He is an excellent scientist with strong research experience in molecular biology and the genetics of model organisms. He would, therefore, be a superb colleague.

In his Ph.D. research, Nick characterized the function of Filamin in ovarian ring canals. This work resulted in solid publications in Genetics, Current Biology, and Developmental Biology, and provided him with a strong experimental background in Drosophila genetics and modern molecular techniques. When he arrived in Victor Ambros' lab at Dartmouth, Nick headed Victor's lab's efforts to extend their studies of microRNA function in C. elegans to microRNA function in Drosophila. In his initial experiments, Nick determined the temporal expression pattern and regulation of many Drosophila microRNAs. He then focused on the function and regulation of two evolutionarily conserved microRNAs, miR-1, and the miR-100, let 7, and miR-125 cluster, for further analysis. These are among the first microRNAs in Drosophila that have been subjected to rigorous genetic analysis. He has made great progress on both of these projects, which has resulted in a recent major publication in Genes and Development, with another one soon to follow.

As an experimentalist, Nick is remarkable, displaying great insight and intuition for complex biological systems. Nick has demonstrated a high level of independence in his post-doctoral work with Victor Ambros, being the only person in Victor's lab who is studying the function of Drosophila microRNAs. He is a sharp, quick thinker, with a high level of energy and enthusiasm. He recognizes the right experiment and then gets it done. He is very clearly an excellent young scientist with tremendous potential for success. He has been an extraordinary teacher for the people in my lab, and is well-liked and respected by all of them. I have no doubt that his work will have broad and lasting impact. I am thus delighted to extend my highest recommendation of him to you.

Sincerely yours,



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European Molecular Biology Laboratory
Europäisches Laboratorium für Molekularbiologie
Laboratoire Européen de Biologie Moléculaire

EMBL

September 29, 2005

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Dear Dr. Brun,

It is a pleasure to write in support of Dr Nicholas Sokol's application for a faculty position in your Department. This letter will be very brief because I don't know Nick personally, but I have followed his work on microRNAs in Drosophila.

Nick is doing superb work on the functions of microRNA genes in fly development, including miR-1. miR-1 is among the most highly conserved miRNAs and is found in nematodes, flies and vertebrates, where it is specifically expressed in muscle tissue. On this basis, it was expected that miR-1 mutants would play a major role in muscle patterning or differentiation. Yet surprisingly, Nick's miR-1 mutants develop normal functional muscles and show muscle defects only later in development. I thought this paper was really excellent - not just because his work is carefully done and rigorous - but because he pushed beyond the first level of analysis to find out what the problem is with the mutant animals.

This is truly first rate work and should have been published in a top tier journal like Nature or Science. Unfortunately, a paper appeared in Nature on miR1 in mouse and so Nick chose to send his paper to Genes and Development. I usually don't like to write mini-reviews on recent papers, but agreed to do so in this case because I felt that Nick's work was by far the better of the two papers and wanted to try to highlight its qualities.

Although this is a 'hot' field currently - and one can publish high profile papers that take short-cuts - Nick hasn't tried to take this path. I admire him for choosing to take the long view and make miRNA mutants, analyze them carefully to understand what they really do and then to dig deeper into the biology of the system. He is getting it right. As indicated in his research proposal he has a set of well though-out, exciting plans on which to build his independent research program.

Nick is off to a great start in this field and I am confident that he will continue to be very successful. Victor Ambros can give you a better, in depth assessment of Nick's qualities, but I will close by saying that I would be more than happy to hire him into our Developmental Biology Unit at EMBL if he were to apply for a faculty position here.

Sincerely

Stephen Cohen

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