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HOWARD HUGHES MEDICAL INSTITUTE

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Yves Brun, Department of Biology
Systems Biology/Microbiology Faculty Search
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Dear Search Committee,

It is a pleasure to write in enthusiastic support of Dr. Kirst King-Jones' application for a faculty position in your department. Kirst has a deep commitment to understanding growth control and metabolism in higher organisms and has the right combination of intelligence, ambition, and technical ability that will allow him to be a leader in these areas of research. On top of this, he is an absolutely delightful person who will make an ideal faculty colleague.

Kirst was a graduate student with Prof. Günter Korge at the Freie Universität Berlin, where he studied *Drosophila Sgs-4* glue gene regulation. The *Sgs* genes were among the first eukaryotic genes cloned, in the mid-1970s. They received this distinction because of their precise stage- and tissue-specific expression, providing a model for coordinate gene circuits in other organisms. Kirst's studies of *Sgs-4*, together with those of his immediate mentor, Michael Lehmann, have provided a detailed understanding of how these genes are regulated. This work led to three publications for Kirst, defining roles for Fork head, dAP-4, and Daughterless in *Sgs-4* control. Kirst received excellent training as a graduate student in the Korge lab. He came to my lab with a fearless approach toward technology and a good understanding of *Drosophila* gene regulation. He also had a clearly stated interest in expanding his work into new areas, studying roles for nuclear hormone receptors in regulating growth and metabolism during development.

Kirst pursued several projects in my lab, focusing on two *Drosophila* nuclear receptors: DHR4 and DHR96. When he arrived, these genes were little more than names. *DHR4* had just been discovered as part of the genome sequencing effort, while *DHR96* remained unstudied in our lab since its discovery five years earlier. Neither gene had available mutants. Kirst decided to tackle these projects using gene targeting. At that time, this technology had only been attempted in a few labs and there was an ongoing debate as to its efficiency and ease. Kirst, however, showed no hesitancy in jumping on this method, reasoning that it provided an ideal means to advance his studies of both receptors. Kirst started by creating a general targeting vector that included a number of new features, along with a strong eye-specific GFP marker to follow the transgene. He then designed mutations in genomic copies of *DHR4* and *DHR96*, moved these sequences into his targeting vector, established transformants for each, and went through the crosses required to identify specific targeting events. He had the same success rate as other labs at that time – 50% – recovering a targeted mutation in *DHR96*.

As this work was underway, Kirst received a *DHR4* mutant from another source, our collaborator Jean-Philippe Charles in France. Kirst created transformants to disrupt *DHR4* function by RNAi and to rescue the mutant with wild type *DHR4* expression. He also raised antibodies against DHR4 and, together with a technician in our lab, Geanette Lam, determined the temporal and spatial patterns of DHR4 expression. Taken together, this work demonstrated a central role for the receptor in growth and maturation. It was published earlier this year in Cell, with Kirst as first author. This paper provides an excellent representation of Kirst's abilities. He did everything that you see in that paper, including the extensive supplemental material. The only exceptions are the isolation of the *DHR4* mutant and its transgenic rescue by Jean-Philippe (depicted in Supplemental Fig. 1), and the antibody stains in Fig. 3, which were done by Kirst and Geanette. This study represents a massive amount of work and is a testimony to Kirst's ability to correctly

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identify the next key experiment to pursue, as well as his technical abilities and diligence. In addition, Kirst was the first person in our lab to use microarrays. Although this method is relatively straightforward, analyzing the data is not. It takes a special person to undertake data analysis on this magnitude, particularly in the absence of local expertise. Kirst read extensively, contacted a number of people, and set up appointments with faculty and biostatisticians on campus. The result was an extensive analysis of both gain-of-function and loss-of-function *DHR4* mutants by microarray, which revealed roles for this receptor in the repression of hormone-regulated genes and starvation. These transcriptional phenotypes fit with the developmental defects in the mutants and gave Kirst a complete story for publication. In addition, Kirst has been the guiding force behind subsequent microarray studies in our lab. He is our expert on the benefits of dChip versus gcRMA, how to use SAM to eliminate false negatives/positives, and how to analyze array data with Microsoft Access. The spreadsheets that he sets up for his data are truly impressive, with built-in links to access gene ontology listings and FlyBase data files for each gene. Our ability to analyze microarray data will suffer when Kirst leaves the lab.

Kirst has two other papers in the works – both of a magnitude comparable to his studies of *DHR4* – both dealing with the DHR96 nuclear receptor. The first study, which is currently being prepared for submission, addresses roles for DHR96 in *Drosophila* xenobiotic responses. All organisms have to deal with toxic compounds in their environment, with the continual task of detoxification and excretion. From a human perspective, these pathways are key to our ability to cope with pollutants in the environment. In addition, drug-drug interactions are a major problem in the development of effective new pharmaceuticals. Kirst's study of DHR96 tested the hypothesis that this receptor, like its vertebrate orthologs CAR and PXR/SXR, is a key regulator of xenobiotic responses. Together with a technician in our lab, Mike Horner, he identified conditions in which the *DHR96* mutant is more sensitive to a toxic compound than wild type animals, using the cytochrome P450 agonist phenobarbital. He showed that this drug has a dramatic effect on wild type gene expression – resulting in ~1000 genes changing their expression, some by as much as 100-fold. Gain-of-function and loss-of-function *DHR96* mutants created by Kirst show changes in the expression of a significant number of these xenobiotic response genes, positioning this receptor as a central mediator of detoxification responses in insects. This paper will provide the first genomic analysis of a xenobiotic response in an invertebrate model organism and sets the stage for understanding how these detoxification pathways are coordinated by the animal.

Kirst's final study also concerns DHR96, this time as a cholesterol receptor. Again, its vertebrate ortholog, SXR/PXR, has been described as a sterol sensor and is activated by a wide range of sterols; however, we have no understanding of how this receptor might function in sterol metabolic pathways other than detoxification. Kirst discovered that his *DHR96* mutants cannot grow on minimal medium without added cholesterol. In addition, our collaborator at the University of Toronto, Henry Krause, has discovered that full-length DHR96 expressed in insect cells co-purifies with cholesterol, suggesting that this compound acts as a ligand for the receptor. We are currently pursuing more detailed phenotypic characterization of the *DHR96* mutant. Our recent microarray data has shown that this mutant (maintained on minimal medium) has specific defects in the regulation of lipid metabolic genes, and initial metabolic profiles indicate selective reductions in fatty acid and cholesterol levels. This project provides Kirst with the ability to exploit *Drosophila*, for the first time, to define cholesterol metabolic pathways.

Kirst has recently co-authored three review articles with me for Nature Reviews Genetics, The Handbook of Cell Signaling, and Science. He was the primary motivating force behind each of these papers. He did the literature research, composed the review, and wrote the manuscript. My role was as a sounding board for his ideas, and as an editor. The Nature Reviews Genetics paper in particular can give you a good feeling for the breadth of Kirst's knowledge and his focus on interpreting results with fly nuclear receptors in the context of their mammalian counterparts.

Kirst has also been gaining recognition this past year for his presentations at meetings. He applied to present a poster at two of these: a Gordon Conference on Hormone Action and a FASEB meeting on "Nutrient Control of Gene Expression". His work was selected at both meetings for an oral presentation,

and he later received awards from both meetings to cover his travel expenses. This was not surprising to me since Kirst works hard on his talks and is an excellent speaker. He has a knack for drawing the audience into his talks and engaging their interest. I heard good things back from all three meetings he attended this year (including one in Tübingen Germany). For example, the organizer of the Gordon Conference, Darcy Kelley (Columbia), told me that Kirst gave an excellent talk and that he got very good feedback from his presentation. He returned from these meetings literally brimming with ideas and filled with enthusiasm as to how to channel his efforts in profitable directions. I have no doubt that he will be successful in his endeavors.

Only recently have studies of growth and metabolism become a focus of research in simple genetic systems. Very little, however, is understood about how lipid metabolism is regulated in *Drosophila*, and no effort has been made (outside our lab) to integrate nuclear receptor signaling into these pathways. Kirst's work is the first in this area, positioning him to be a leader in this new field of research. If you have an interest in gene regulation, growth, or metabolism, I urge you to seriously consider Kirst's application for a faculty position in your department. If nothing else, you should have him out for a seminar. He is an absolutely delightful person who is a pleasure to spend time with and talk science. He is exactly the kind of person you would like to have working down the hall as a colleague. Please feel free to contact me if you have questions or require additional information.

Sincerely,

Carl S. Thummel, Ph.D.
Professor, Human Genetics

P.S. Kirst's graduate advisor, Dr. Günter Korge is retired. I have thus offered to help him by including his letter in this mailing.

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Dear Members of the Search Committee:

It is my great pleasure to provide a letter of reference for Dr. Kirst King-Jones. I have known Kirst since 1991 when he joined my lab as an undergraduate student. From the very beginning it was evident that Kirst was among the brightest and most extraordinarily talented students I had come across in my 25 years as professor at the Free University of Berlin. Although I should mention that during his undergraduate and graduate career, his grades were always in the top 1% bracket of his cohort group, and his PhD thesis as well as his defense presentation were honored with the highest grade possible, "summa cum laude", I would also like to emphasize that it wasn't the grades that impressed me. Rather, Kirst is outstanding because he is gifted with a blend of talents you only find in the very best science students. The first thing that comes to my mind is his sparkling curiosity and excitement about science that makes him not only a wonderful colleague and energetic discussion partner, but also a skilled and enthusiastic teacher. Importantly, Kirst is a clear and critical thinker, he is exceptionally intelligent, possesses an impressive memory and a knack for mathematics, a great asset in the genomic era. His ability to consider the big picture, which is clearly linked to his broad interest in biology, is another great asset in a scientific world that has become more and more specialized.

Kirst started out in my lab with a strong interest in transcriptional regulation and developmental biology. He therefore decided to work on a steroid hormone-controlled gene, *Sgs-4*, in *Drosophila melanogaster*, in order to understand how the interplay of various transcription factors with a ubiquitous hormonal signal – the molting hormone ecdysone - achieves stage- and tissue-specific gene regulation. During his undergraduate studies, he isolated what today is known as the GAGA factor. His graduate work resulted in a thorough study that was published in JMB, where he describes the discovery of a new DNA regulatory element in the *Sgs-4* enhancer and the identification of three proteins – the Helix-Loop-Helix (HLH) factors dAP-4 and daughterless as well as an unidentified protein - that bind *in vitro* and *in vivo* to this gene in a temporally and spatially specific manner. He also collaborated with two colleagues on two other transcription factors, *broad* and *fork head*, that led to the novel finding that the ecdysone--controlled broad protein mediated repression of *Sgs-4* through downregulation of its activator, fork head, a story that was published in *Development*. Taken together, Kirst

greatly contributed to our understanding how a steroid-controlled target gene is regulated through a coordinate interplay of transcription factors. During his time in my lab he demonstrated expert skills in a broad array of molecular techniques, ranging from EMSAs, Far Westerns, One-hybrid screening as well as biochemical purification procedures like DNA affinity chromatography by FPLC, to name a few.

For his postdoctoral career Kirst moved to United States to join the lab of Dr. Carl Thummel. There he started to work on two virtually uncharacterized nuclear receptor genes, expanding his interest in the crosstalk between transcriptional regulation and endocrinology. Kirst made the exciting discovery that one of these genes, *DHR4*, regulates growth through a novel mechanism by promoting feeding behavior and relaying nutritional cues. He also showed that DHR4 plays a crucial role in transducing the ecdysone signal, thus revealing a link between growth control and a steroid hormone. He published this story recently as a first author in *Cell.* His second project is just as promising and will be split into two stories. The first story, were he is co-first author, describes how a mutation in the nuclear receptor gene *DHR96* affects detoxification responses in insects. I have seen the current manuscript, and have little doubt that it will be published soon in another top journal. The other story appears to be a major breakthrough and demonstrates that DHR96 is a sterol sensor which plays a critical role in cholesterol homeostasis. My lab has done some work on cholesterol metabolism but no work to date in *Drosophila* has come close to addressing the regulation of this metabolic pathway. In addition to these important scientific achievements, Kirst published three high-ranking reviews, two of them in top-rated journals, underlining his impact on the field.

In summary, I strongly support Kirst's application. He is a very bright and creative researcher, and it is my strong opinion that he will be up to any challenge. Additionally, Kirst gives great and clear talks, he is an accomplished writer and an excellent team player. You will also find Kirst to be a very likeable and honest guy with a great sense of humor, and I can assure you without hesitation that Kirst will be a joy to have as a colleague.

I therefore offer my highest recommendations for Dr. Kirst King-Jones to be strongly considered as a candidate for a faculty position in your department.

Berlin, 11. October 2005

(Prof. Dr. G. Korge)