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Dear Dr. Brun,

Please consider my application for a faculty position in Systems Biology. I have included my C.V., a brief research statement, a description of my teaching interests and philosophy, and copies of four publications. Four letters of recommendation will be sent directly to you. The names and addresses of my referees are listed in my C.V.

At the present time, I am working with Dr. Elliot Meyerowitz at the California Institute of Technology. My current research focuses on the analysis of the developmental mechanisms underlying organogenesis in plants. In particular, I am interested in the regulation of flower development in the model plant *Arabidopsis thaliana*. I have chosen a systems biology approach to obtain novel insights into this important biological process and have made extensive use of gene expression profiling by microarray analysis to characterize the gene regulatory network underlying flower development. I now plan to conduct genome-wide localization studies and targeted network perturbations to identify the binding sites and target genes of key floral regulators.

I look forward to hearing from you.

Sincerely,

Frank Wellmer

Frank Wellmer - Research Statement

1. Research Interests

Fundamental questions in developmental biology are how the body plan of an organism is established and how different types of organs are formed from small sets of undifferentiated precursor cells. It is known that such developmental processes are largely dependent on the cellular capacity for differential gene expression. That capacity is hardwired and encoded in the genome, in the form of *cis*-regulatory elements that determine when, where, and how genes are expressed, of transcription factors and transcriptional co-regulators that act on those sequences, and of other types of regulatory proteins or RNAs that modify or modulate the activities of other proteins or genes. However, our current understanding of how the genome is brought to play throughout development is in the best of cases still rudimentary, and in plants, vastly lacking.

In order to obtain a more detailed understanding of the molecular mechanisms underlying development in plants, I study the regulation of gene expression during flower development in the model plant *Arabidopsis thaliana*. The large number of well-characterized developmental mutants, the ease with which forward and reverse genetics can be conducted, and the availability of the genome sequence make *Arabidopsis* an ideal system for studying the basic concepts of developmental biology.

Flower development has been intensively studied by genetic analysis and many key regulators of this essential process have been identified, most of which encode transcription factors. However, the target genes of these factors, the *cis*-regulatory elements to which they bind, and their regulatory interactions are still largely unknown. Furthermore, many important floral regulators have likely been missed in genetic screens because their role in flower development is masked by functional redundancy or because their loss-of-function phenotypes are subtle.

The development of genomic technologies such as microarray analysis has opened the possibility to study the gene regulatory networks that control development at a global scale. In addition, public databases for different types of biological information, from transcription factor binding sites to gene ontology predictions, or the development of software tools for the prediction of *cis*-regulatory elements, aid tremendously in the analysis of the large datasets obtained by genomic technologies. By combining such genomic and computational approaches with classic developmental genetics, I hope to decipher the hierarchical organization of the flowering gene network.

2. Background

Arabidopsis flowers are initiated at the shoot apex as floral primordia, which are composed of a small number of undifferentiated cells. These primordia are programmed to undergo flower development by the activity of the so-called floral meristem identity genes such as *LEAFY*, *APETALA1*, or *CAULIFLOWER*, all of which encode transcription factors. These genes are involved in activating the expression of the floral homeotic genes that are required for the specification of the different types of floral organs (1). The floral homeotic genes encode transcription factors that interact in certain combinations to control the developmental programs required for floral organ formation (2). However, the target genes of these factors and their role in flower development are largely unknown.

3. Research Accomplishments

Genome-wide identification of network components

Known floral regulators are often expressed in a dynamic fashion, i.e. they are expressed only in certain regions of the flower or during certain developmental stages. A possible strategy for identifying novel network components is therefore to analyze gene expression during flower development on a genome-wide scale and to identify genes with organ or stage-specific expression. To this end, I applied global expression profiling using whole-genome DNA microarrays for a detailed analysis of spatial gene expression during flower development by comparing the gene expression profiles of inflorescences of wild-type plants to those of the floral homeotic mutants lacking different types of floral organs (3). By combining the data sets from these experiments, I was able to identify genes expressed specifically (or predominantly) in distinct floral organs.
For the analysis of temporal gene expression during flower development, I developed a floral induction system based on the specific activation of the floral meristem identity factor APETALA1 (AP1) in an *ap1 cauliflower* (*cal*) double mutant. In *ap1 cal* plants, flower formation is delayed leading to a massive accumulation of meristems at the shoot apex (4). The specific activation of AP1 in this background causes the transition of these meristems to floral primordia, which subsequently develop in a synchronized manner. Thus, hundreds of same-stage floral buds can be collected from a single plant, which allowed, for the first time, a stage-specific dissection of gene expression during early flower development (5). Among the genes with stage or organ-specific expression identified in these experiments, many encode putative regulatory proteins and thus might be involved in controlling important steps of flower development.

Identification of genes downstream of key floral regulators

Most of the previously characterized floral regulatory genes encode transcription factors (2). In order to understand their role in flower development in more detail, I have analyzed some of these factors with respect to their downstream target genes. To this end, I utilized inducible systems that allow monitoring changes in gene expression in response to an activation of these factors. That this type of experimental approach in conjunction with microarray analysis is suitable for the identification of novel target genes of floral regulators was first demonstrated for the floral homeotic factor AGAMOUS (AG). In collaboration with Dr. Toshiro Ito, I identified *NOZZLE/SPOROCYTELESS*, a key regulator of sporogenesis, as a direct target gene of AG (7). However, a common problem with this type of analysis is that the transcription factors are usually expressed only in a very limited number of cells making tissue collection for microarray experiments extremely challenging. A good example is the floral meristem identity factor LEAFY (LFY), which is active only in a few cells at the shoot apex (1,8). In order to enrich for LFY-responsive cells, I have used (in collaboration with Dr. Doris Wagner) a tissue culture system that responds to specific LFY activation with an induction of known LFY target genes, including *AP1* and *CAL* (9). Since *AP1* and *CAL* expression is rapidly induced by LFY, some of the novel LFY response genes identified in these experiments might actually be under the direct control of these factors and not of LFY. To distinguish between these possibilities, I have analyzed gene expression changes that occur shortly after AP1 activation using the system described above, as well as the effect of LFY activation in an *ap1 cal* mutant background (unpublished results).

4. Research Plan

Functional characterization of putative floral regulators

The results of the microarray experiments described above led to the identification of many genes that encode putative regulatory proteins and that have distinct patterns of expression during flower development. In particular, I observed a significant enrichment for genes encoding transcription factors in the microarray data sets. These genes are good candidates for novel components of the regulatory network underlying flower development. I now plan to study the function of some of these genes by reverse genetics focusing especially on closely related genes that have similar gene expression profiles and thus might control flower development in a redundant manner. To this end, I will first verify their expression patterns by *in situ* hybridization. Subsequently, I will test whether a loss of gene activity results in a mutant floral phenotype by analyzing either T-DNA insertion or RNA interference lines for these genes. Newly identified

regulatory genes will then be studied with respect to their hierarchical relationship to known floral regulators by genetic and molecular analysis.

Identification of transcription factor target genes

An essential step in unraveling the regulatory network underlying flower development is the identification of target genes of transcription factors and of the *cis*-regulatory elements to which these factors bind. However, a major obstacle in the functional characterization of transcription factors during flower development is the collection of sufficient plant material for approaches such as DNA microarray analysis or chromatin immunoprecipitation (ChIP) experiments. This problem is especially pronounced for the study of early flower development because young floral buds are very small.

The AP1-GR *ap1 cal* floral induction system described above circumvents these problems, as it leads to a massive accumulation of young, synchronized floral buds. Thus, I plan to use this system to study the function of selected transcription factors during early flower development.

I am especially interested in understanding how the floral homeotic factors mediate organ specification and differentiation. As mentioned above, the target genes of these factors are largely unknown. In order to identify genes that are under their control I will introduce inducible RNA interference (RNAi) constructs (10) for the floral homeotic genes into the AP1-GR *ap1 cal* floral induction system. After an activation of AP1 (which induces flower formation, see above), silencing of the floral homeotic genes by RNAi can be induced in a stage-specific manner. Because silencing by RNAi will disrupt the gene regulatory network at specific points, a comparison between the gene expression profiles of flowers in which the floral homeotic genes were silenced, or not, will then lead to the identification of genes whose expression depends on these factors.

To test whether the promoters of these genes are bound directly by the floral homeotic factors, I plan to apply ChIP in conjunction with microarray analysis using whole-genome tiling-arrays. As specific antibodies for the floral homeotic factors are not readily available, I will generate epitope-tagged versions of the factors, which will be expressed under the control of their endogenous promoters in the AP1-GR *ap1 cal* background. Activation of AP1 will then lead to the expression of the tagged proteins in their normal domains. Subsequently, ChIP can be performed in a stage-specific manner using epitope-tag specific antibodies. The results of the ChIP experiments will then be combined with those of the RNAi experiments described above to identify genes that are bound by the factors and whose expression depends on their activities. The pro-

moters of the target genes will be screened for common sequence motifs using existing promoter analysis software (11). Putative regulatory elements will then be mutated and the effect of the disruption on the expression of these genes will be studied using *in vitro* binding assays as well as by generating transgenic plants expressing reporter genes under the control of wild-type and mutated promoters, respectively.

5. Conclusions

The experiments described above should identify novel genes and pathways that are involved in controlling different aspects of *Arabidopsis* flower development. By combining global expression analysis and approaches for a genome-wide localization of transcription factor binding sites with computational methods, new insights into the gene regulatory network underlying flower development should be obtained.

6. References

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Frank Wellmer – Teaching Interests

My primary teaching interests are plant molecular biology, developmental biology, and systems biology. During my time as a graduate student and as a postdoctoral scholar I had already the opportunity to participate in the training of students in all of these areas (please see my C.V. for details) and I found this experience in general very rewarding. I am therefore very motivated to establish my own teaching program, to implement my teaching philosophy, and to further develop my skills as a teacher and mentor.

During the course of my own education, I have observed several teaching strategies that I found particularly effective and I hope to be able to implement these strategies in the classes that I teach. For example, I strongly believe that the development of critical thinking skills is the most far-reaching part of the curriculum, in that these skills will be necessary for the success of students in any future scientific endeavor. I would therefore like to teach a class in which selected papers are dissected with respect to their strengths and weaknesses. In this context, the ethical obligations scientists are bound to could be discussed as well.

Another important aspect of my teaching philosophy is to communicate that the invention of new methods is crucial for the advancement of science. I believe that this can be best demonstrated by describing the influence that breakthrough technologies, such as the polymerase chain reaction or DNA sequencing, had on biology. Thus, in addition to teaching the principles of these methods, certain historical aspects of biology should be discussed as well.

Finally, I would like to encourage students to learn about the different experimental approaches that modern biology has to offer and not to focus solely on their favorite subjects. I have learnt during my own academic education that this type of diversified training is very rewarding because it allows addressing biological questions from different perspectives. To go even further, I would strongly encourage students to develop their skills in other disciplines such as mathematics, physics, chemistry, or informatics, as it is clear that major advances in biology will depend more and more on multi-disciplinary approaches.