

# INTERPLAY OF LIGHT, PHOTOPERIODISM AND CIRCADIAN CLOCK FUNCTION IN PLANT DEVELOPMENT

May, 4<sup>th</sup>-6<sup>th</sup>, 2011

ORGANIZERS



**International Center  
for Scientific Debate**

Initiative fostered by:



**Welfare Projects**  
"la Caixa" Foundation



# INTERPLAY OF LIGHT, PHOTOPERIODISM AND CIRCADIAN CLOCK FUNCTION IN PLANT DEVELOPMENT

**May, 4<sup>th</sup>-6<sup>th</sup>, 2011**

**Museu Colet, c/ Buenos Aires, 56  
Barcelona - Spain**

## Introduction

The “**Interplay of Light, Photoperiodism and Circadian Clock Function in Plant Development**” is a workshop dedicated to explore the mechanistic insights and connections among three main aspects of plant biology: circadian clock function, photoperiodism and light signalling pathways. Relevant discoveries on these topics, the cellular and molecular nodes of interaction as well as future research directions on plant development will be discussed by the most prominent experts in each field.

The workshop to be held at the Museu Colet, Barcelona, on May 4<sup>th</sup> to 6<sup>th</sup> 2011, will be organized around seminars and round table sessions in which we will discuss and explore the recently emerged research in plants and its connection with the environment. The interest of the proposed workshop will be bolstered by exploring the biological implications and the intricate links of light, photoperiodism and circadian function in plants. Addressing the molecular events and mechanisms that translate ambient changes into proper adjustments of plant growth and physiological responses might also be particularly helpful to understand how plants cope with new environmental challenges such as those caused by global warming.

The meeting is organized by the International Centre for Scientific Debate (ISCD), an initiative of Biocat, with the support of Welfare Projects “la Caixa” Foundation, and the Centre for Research in Agricultural Genomics (CRAG) and supported by the European Molecular Biology Organization (EMBO), Fundación Genoma España and the Spanish Ministry of Science and Innovation (MICINN).

# Program

Wednesday, May 4<sup>th</sup>

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- 8:45** Registration and posters
- 9:30** Presentation
- 10:00** Lecture: “*Phytochrome Photosensory Signaling and Transcriptional Networks*”  
Peter H. Quail. University of California. USA
- 10:45** Lecture: “*Regulation of elongation growth by a network of bHLH class transcription factors*”  
Christian Fankhauser. University of Lausanne, Switzerland
- 11:15** Coffee break
- 11:45** Lecture: “*Shining light on temperature signaling*”  
Karen Halliday. University of Edinburgh, UK
- 12:15** Lecture: “*SVP and BZR1 integrate brassinosteroid signaling and circadian clock to regulate flowering time and body size in Arabidopsis*”  
Tsuyoshi Mizoguchi. University of Tsukuba, Japan
- 1:00** Lunch
- 2:30** Lecture: “*Structure and function of rice Hd3a florigen*”  
Ko Shimamoto. Nara Institute of Science and Technology, Japan
- 3:15** Lecture: “*Photoperiodic regulation of flowering and life cycle adaptation in sugar beet*”  
Ove Nilsson. Swedish University of Agricultural Sciences, Sweden
- 3:45** Lecture: “*Transcriptional regulation of CONSTANS in photoperiodic flowering*”  
Takato Imaizumi. University of Washington, Seattle, USA
- 4:15** Coffee break
- 4:45** Lecture: “*Photoperiodic regulation of tuber induction by potato CONSTANS*”  
Paula Suárez-López. Center for Research in Agricultural Genomics. Barcelona, Spain
- 5:15** Round table  
Moderators: Peter H. Quail and Ko Shimamoto
- 6:15** Bus to CosmoCaixa
- 6:45** Visit to CosmoCaixa
- 7:45** Cocktail at CosmoCaixa
- 9:00** Bus to the Hotel Balmoral

Thursday, May 5<sup>th</sup>

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**9:00 Poster session**

**9:30 Lecture: “Large Scale Discovery Approaches for Plant Circadian Networks”**  
Steve A. Kay. University of California San Diego. La Jolla, USA

**10:15 Lecture: “A role for PRMT5 in the post-transcriptional regulation of the Arabidopsis circadian network”**  
Marcelo J. Yanovsky. Universidad de Buenos Aires. Buenos Aires, Argentina

**10:45 Coffee break**

**11:15 Lecture: “The circadian clock heats up in Arabidopsis”**  
Paloma Más. Center for Research in Agricultural Genomics”. Barcelona, Spain

**11:45 Lecture: “Effector binding to a co-repressor complex sustains the plant circadian oscillator as a light-responsive process”**  
Seth Davis. Max Planck Institute for Plant Breeding Research. Colony, Germany

**12:15 Lecture: “Multiple signaling pathways associated with correct biological timing in Arabidopsis”**  
Alex A. Webb. Univesity of Cambridge. Cambridge, UK

**1:00 Lunch**

**2:30 Lecture: “Spatio-temporal regulatory network controlling stem elongation under the shade”**  
Akira Nagatani. Kyoto University. Kyoto, Japan

**3:15 Lecture: “Intra- and intercellular features of phytochrome-A mediated signaling”**  
Ferenc Nagy. Biological Research Centre. Szeged, Hungary

**3:45 Lecture: “PIF4 integrates light and temperature signals during plant development”**  
Keara Franklin. University of Leicester. Leicester, UK

**4:15 Coffee break**

**4:45 Lecture: “PIF3 regulates rhythmic growth in Arabidopsis”**  
Elena Monte. Center for Research in Agricultural Genomics”. Barcelona, Spain

**5:15 Round table**  
**Moderators: Steve A Kay and Akira Nagatani**

**6:15 Free time**

## Friday, May 6<sup>th</sup>

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### 9:00 Poster session

**9:30 Lecture: “Genetic Architecture of Circadian Clock Function In *Brassica rapa*”**  
Robert McClung. Dartmouth College. Hanover, USA

**10:15 Lecture: “Using functional genomics to identify new clock genes”**  
Stacey Harmer. University of California. Davis, USA

### 10:45 Coffee break

**11:15 Lecture: “The PIF transcription factors: key integrators of light, gibberellin (GA) and brassinosteroid (BRs) signals”**  
Salomé Prat. Centro Nacional de Biotecnología – CSIC. Madrid, Spain

**11:45 Lecture: “Interactions of light, photoperiodism, and the clock in shade avoidance and rhythmic growth”**  
Julin Maloof. University of California. Davis, USA

### 12:15 Concluding remarks

**1:15 Bus to Center for Research in Agricultural Genomics (CRAG)**

**2:00 Lunch at CRAG**

**3:00 Presentation and visit to the CRAG by Pere Puigdomènech**

**4:30 Bus to the Hotel Balmoral (Via Augusta, 5. Barcelona)**

	Wednesday, May 4 <sup>th</sup>		Thursday, May 5 <sup>th</sup>		Friday, April 6 <sup>th</sup>
<b>9:00</b>	Registration and posters	<b>9:00</b>	Poster session	<b>9:00</b>	Posters session
<b>9:30</b>	Welcome	<b>9:30</b>	Steve A. Kay	<b>9:30</b>	Robert McClung
<b>10:00</b>	Peter H Quail	<b>10:15</b>	Marcelo J. Yanovsky	<b>10:15</b>	Stacey Harmer
<b>10:45</b>	Christian Fankhauser	<b>10:45</b>	Coffee break	<b>10:45</b>	Coffee break
<b>11:15</b>	Coffee break	<b>11:15</b>	Paloma Mas	<b>11:15</b>	Salomé Prat
<b>11:45</b>	Karen Halliday	<b>11:45</b>	Seth Davis	<b>11:45</b>	Julin Maloof
<b>12:15</b>	Tsuyoshi Mizoguchi	<b>12:15</b>	Alex A. Webb	<b>12:15</b>	Concluding remarks
<b>1:00</b>	Lunch and Poster session	<b>1:00</b>	Lunch and Poster session	<b>1:15</b>	Bus to CRAG
<b>2:30</b>	Ko Shimamoto	<b>2:30</b>	Akira Nagatani	<b>2:00</b>	Lunch at CRAG
<b>3:15</b>	Ove Nilsson	<b>3:15</b>	Ferenc Nagy	<b>3:00</b>	Visit to the CRAG
<b>3:45</b>	Takato Imaizumi	<b>3:45</b>	Keara Franklin		
<b>4:15</b>	Coffee break	<b>4:15</b>	Coffee break		
<b>4:45</b>	Paula Suárez-López	<b>4:45</b>	Elena Monte		
<b>5:15</b>	Round table. Moderators: Peter H Quail and Ko Shimamoto	<b>5:15</b>	Round table. Moderators: Steve A. Kay and Akira Nagatani		
<b>6:15</b>	Bus to CosmoCaixa				
<b>6:45</b>	Visit to CosmoCaixa				
<b>7:45</b>	Cocktail at CosmoCaixa				
<b>9:00</b>	Bus to the Hotel				

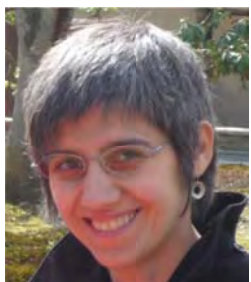


## Scientific organizers



**Paloma Más** is Associate Professor at the **Centre for Research in Agricultural Genomics (CRAG)**. She qualified in Biochemistry and Molecular Biology at the University of Valencia and obtained a PhD degree in Biology at the University of Murcia (CEBAS-CSIC, Murcia) in 1997. During a first post-doctoral period (1997-1999) in the laboratory of Prof. Roger N. Beachy at The Scripps Research Institute (La Jolla, California, USA), her studies focused on the essential mechanisms regulating the viral infectious cycle. In a second post-doctoral stage (2000-2003) in the laboratory of Prof. Steve A. Kay at the Institute of Childhood and Neglected Diseases (ICND, La Jolla, CA, USA), her research focused on the study of the circadian system and the importance of circadian clock function in plant physiology

and metabolism. She was appointed as an Assistant Professor in 2004 at the Institute of Molecular Biology of Barcelona (IBMB-CSIC) and was later promoted to Associate Professor within the area of Biology and Biomedicine, rated number 1 of the promotion. Current studies in Dr. Más laboratory focus on signalling pathways and mechanisms of clock progression using *Arabidopsis thaliana* as a model system. She has published a number of high-impact reviews and research articles in relevant journals such as Nature and Science. She has also been awarded as principal investigator in a number of research grants from different funding agencies, including the highly prestigious EURI Award. She is also member of the EMBO YIP program since 2007.



**Paula Suárez-López** is a group leader at the **Centre for Research in Agricultural Genomics (CRAG)**, CSIC-IRTA-UAB. She obtained her PhD in Biological Sciences at the "Severo Ochoa" Molecular Biology Centre in Madrid (Spain) in 1993 working on the variability of flu viruses. After a postdoctoral transition period working on plant viruses, she moved to her main research interest, which is plant development, at the John Innes Centre in Norwich (UK), working in the laboratory of Dr. George Coupland. She joined the Department of Molecular Genetics of the Institute of Molecular Biology of Barcelona (which later became part of CRAG) in 2002. Her research focus is the photoperiodic regulation of plant developmental processes, as

well as the long-distance signaling mechanisms involved. She has made contributions to the understanding of flowering time control in the model plant *Arabidopsis*, in particular on the interaction between photoperiodic signals and the circadian clock. Her findings have also helped to understand how photoperiod leads to the production of graft-transmissible flowering signals in the leaf phloem. More recently, she has focused on the photoperiodic regulation of potato tuber induction, identifying the first microRNA involved in this process and proposing a genetic pathway for the control of tuberization.



**Elena Monte** is a CSIC (Spanish Research Council) researcher at the **Centre for Research in Agricultural Genomics (CRAG)**, in Barcelona, Spain. She received her Ph.D. in Biochemistry from the UAB University of Barcelona in 1998 for her work on the photoperiodic control of potato tuberization in the laboratory of Salomé Prat. She continued to pursue her interest in light signaling in the laboratory of Peter H. Quail at the Plant Gene Expression Center-University of California Berkeley. In 2005, she joined the Department of Molecular Genetics at the Molecular Biology Institute (IBMB) in Barcelona as group leader. The Department recently integrated as one of the founding members of CRAG. Her research interest focuses on understanding the light signaling mechanisms that regulate plant growth and development. Her work has contributed to the field of photoperiodic regulation of tuber formation in potato,

and more recently to the field of light perception and signaling by the phytochromes and the phytochrome-interacting factors PIFs in *Arabidopsis*. Her findings include identification of regulatory factors such as PHOR1 in potato or PIF7 in *Arabidopsis*, the characterization of phyC function in *Arabidopsis*, and the uncovering of the role of the PIFs in the implementation of the transcriptional network that orchestrates skoto- and early photo-morphogenesis in *Arabidopsis* seedlings. Her current research interest aims at further deciphering the function of PIF3 and its target genes in *Arabidopsis* seedling growth ([www.montelab.org](http://www.montelab.org)). Her research activity includes funding from the EU (Marie Curie Actions), the Spanish Ministry of Science and Innovation (MICINN), and the Catalan Agency for Management of University and Research Grants (AGAUR).

## Invited speakers

Wednesday, May 4<sup>th</sup>

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**Peter H. Quail**, B.Sc., Ph.D., is Professor of Plant and Microbial Biology, University of California, Berkeley, and Research Director, ARS/UCB Plant Gene Expression Center, Albany, California. He is a recipient of the American Society of Photobiologists Research Award, the LI-COR Award for Distinguished Contributions to Photochemistry/Photobiology, Corresponding Membership of the Australian Society of Plant Physiologists, recipient of ISI award for top 15 most highly cited authors in the Plant & Animal Science discipline, Fellow of the American Association for the Advancement of Science, recipient of the Stephen Hales Award from the American Society of Plant Biologists and a Member of the U.S. National Academy of Sciences.

His research is focused on defining the molecular mechanism by which the phytochrome (phy) family of plant sensory photoreceptors controls gene expression, and thereby plant growth and development, in response to informational light-signals from the environment. His laboratory has provided evidence that the signalling mechanism involves rapid, direct, intranuclear interaction of the light-activated photoreceptor molecule with a sub-family of basic helix-loop-helix (bHLH) transcription factors (called PIFs), with resultant induction of phosphorylation of the PIF proteins, as a prelude to degradation of the bHLH factors and consequent gene-expression changes.

### **Phytochrome Photosensory Signaling and Transcriptional Networks. - Peter Quail**

Current evidence indicates that the phytochrome (phy) signaling process involves rapid translocation of the light-activated photoreceptor into the nucleus, where it interacts with specific bHLH transcription factors, termed **Phytochrome-Interacting Factors (PIFs)**, inducing transcriptional responses in target genes. We have shown that a quadruple *pif* mutant (*pif1pif3pif4pif5* (*pifq*)) exhibits a *cop*-like phenotype in dark-grown seedlings, indicating that these transcription factors collectively repress photomorphogenesis in post-germinative darkness, and that photoactivated phy reverses this repression by inducing rapid phosphorylation and subsequent degradation of the PIFs upon initial light exposure. Previously, using targeted and random mutagenesis, we identified binding sites on phyB and PIF3 necessary for productive signaling interactions in vivo. Recently, we have defined residues within the PIF3 protein that are targets of light-induced phosphorylation. Using genome-wide transcriptome analysis of wild-type and *pifq* seedlings, we have identified PIF-regulated genes that respond rapidly and reciprocally, in a PIF-dependent manner, to phy photoactivation and deactivation upon initial light-exposure and vegetative-shade exposure, respectively. Coupled with ChIP-seq analysis, these data have permitted identification of potential direct targets of the phy-PIF signaling system. Interestingly, increasing evidence indicates that the PIFs have a role in multiple signaling pathways, in addition to light, thereby suggesting that they function as components of a central cellular signaling hub.

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**Christian Fankhauser** received a Bachelor in Biology from the University of Lausanne and a Master degree in Molecular Biology from the University of Geneva (1990). He obtained his PhD from the University of Lausanne in 1994 after carrying out his thesis on cytokinesis in fission yeast at the Swiss Institute for Experimental Cancer Research (ISREC) in the laboratory of Viesturs Simanis. He performed postdoctoral studies with Marty Yanofsky at UCSD then with Joanne Chory at The Salk Institute for Biological Studies in San Diego. He became a Swiss National Science Foundation Assistant Professor at the Department of Molecular Biology of the University of Geneva in 2000. In January 2005 he was appointed Associate Professor at the Center for Integrative Genomics from the University of Lausanne. In January 2011 he was promoted to full Professor. His scientific research is in the area of plant

molecular genetics concentrating on the effect of light on *Arabidopsis* growth and development.

## Regulation of elongation growth by a network of bHLH class transcription factors

Christian Fankhauser, Séverine Lorrain, Patricia Hornitschek, Markus Kohnen and Tino Dornbusch.

In plants the shape and nature of developing organs is influenced by environmental conditions. In shade intolerant plants such as *Arabidopsis* the shade avoidance response (SAR) is a good illustration of this concept. Light filtered by a layer of vegetation is depleted in the red (R) and blue portion of the spectrum while far-red light (FR) is readily transmitted. Phytochromes measure the change in R/FR ratio indicative of vegetational shading. In response to this signal phytochromes control stem elongation, leaf development and the transition to flowering. Collectively these responses allow the plant to grow out of the shade or reproduce. Light-activated phytochromes enter the nucleus where they interact with members of the PIF (Phytochrome Interacting Factor) family of bHLH class transcription factors. This interaction triggers the degradation of those transcriptional regulators. We have shown that PIF4 and PIF5 are two important regulators of the SAR. Their activity is inhibited by phytochrome in sunny environments while in the shade the drop in R/FR allows PIF4 and PIF5 to promote elongation growth typical of the SAR. HFR1 a related bHLH factor prevents excessive PIF activity in the shade by forming non-DNA binding heteromers with PIF4 and PIF5. Interestingly this network of bHLH factors is also implicated in plant growth responses to elevated temperature, which triggers very similar changes to plant architecture as shading. How PIF4 and PIF5 control plant growth remains largely unknown. We are analysing PIF-mediated gene expression and the PIF5 binding sites genome wide to address this question.

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**Karen Halliday** is a reader at Edinburgh University, with expertise in environmental signal integration, molecular genetics and dynamical mathematical modelling in the model plant *Arabidopsis*. Career highlights include the characterisation of nuclear localised, PIF3, the first known interacting partner for the light receptor phytochrome. These findings contributed to a sizeable shift in our understanding of light signal transduction which was previously thought to be triggered in the cytosol. For this and follow-on work Karen was awarded a Research Fellowship by Bristol University in 2003. Karen was the first to demonstrate that phytochrome signalling was temperature-dependent. She has identified PIFs and PIF-like genes as central integrators of light and temperature signals. This mechanism enables light to buffer the otherwise deleterious effect of warm temperature on plant biomass and viability. A collaborative project with Graham (York) has identified a molecular motif that ensures molecular responses and growth-control are maintained through wide fluctuations in light quality. Current interests include the application of Systems Biology approaches to elucidate the impact of temperature change on plant molecular signalling, physiology and development. As the Earth surface temperature is predicted to rise, the challenge is to identify protective molecular mechanisms that prevent signalling functions from breaking down as temperature increases. Karen directs ROBuST, a large interdisciplinary combining experimentation, computational modelling and mathematical methodologies to address this problem.

### Shining light on temperature signaling.- Karen J. Halliday et al.

In the natural environment, plants experience wide daily and seasonal variations in temperature. We want to understand how plants maintain control of growth and development even when exposed to large temperature fluctuations. Our work has shown that the light pathways have an unexpected role in protecting the molecular network from the potentially harmful effects of temperature. For instance, removal of specific light receptors leaves the plant more vulnerable to temperature change, which can significantly reduce fitness. Our goal is to establish how light protects or “buffers” the molecular circuitry from temperature. Work to date has shown that light counters the impact of warm temperatures on the circadian oscillator and clock regulated components that modulate growth. For instance, under blue light, loss of *cry1* and *cry2* obliterates the transcriptional oscillator at warm temperatures and prohibits the accumulation of the clock regulated growth regulator, HFR1. Thus, CRYs appear to operate by countering the impact of warm temperature on internal and external coincidence mechanisms. To improve our understanding of how CRYs protect the transcriptional clockwork we have combined experimental and theoretical approaches which provide a more rigorous framework for formulating and testing predictions. We will present data that shows levels of HFR1 protein and its target PIF4 are determined by the combined effects of light and temperature. We will also demonstrate that this molecular circuitry ensures that growth remains under close control even when temperatures soar.





**Tsuyoshi Mizoguchi** Associate Professor & Deputy Director at the Gene Research Center, University of Tsukuba, Japan. He obtained a PhD degree in Plant Molecular Biology at University of Tsukuba, Japan in 1995. During a post-doctoral period (1995-1997) in the laboratory of Prof. Kazuo Shinozaki at The Institute of Physical and Chemical Research (RIKEN, Tsukuba, Japan), his studies focused on the stress-responses of protein kinase genes in *Arabidopsis thaliana*. In a research associate stage (1997-2002) in the laboratory of Prof. Kazuo Shinozaki at RIKEN (Tsukuba, Japan), his research focused on the study of the roles of mitogen-activated protein kinase (MAPK) cascades in stress-responses in *Arabidopsis*. During this stage, he stayed in the laboratory of Dr. George Coupland at John Innes Centre (JIC, UK) as a long-term fellow of the Human Frontier Science Program (HFSP) and his studies focused on roles of circadian clock proteins, LHY and CCA1, in maintaining circadian rhythms and controlling photoperiodic flowering under long-days and short-days. He was appointed as an Assistant Professor in 2002 at the Gene Research Center, University of Tsukuba, Japan and was later promoted to Associate Professor within the area of Molecular Genetics. Current studies in Dr. Mizoguchi laboratory focus on interactions between internal (endogenous) and external rhythms for the control of development and photosynthesis in plants using *Arabidopsis thaliana* and tomato as model systems.

### **SVP and BZR1 integrate brassinosteroid signaling and circadian clock to regulate flowering time and body size in *Arabidopsis***

Kanae Niinuma, Kana Miyata<sup>a</sup>, Riichiro Yoshida, Masahide Takase, Takeshi Nakano, Ayumi Yamagami, Fuminori Takahashi, Hirokazu Tsukaya, Atsushi Oda, Tadao Asami, Kazuo Shinozaki, George Coupland, Tsuyoshi Mizoguchi

Brassinosteroid (BR) signaling has been proposed as a mechanism for controlling the size of plants by regulating hypocotyl and petiole elongation that is reduced in light and promoted in dark. Recently, an important role of BR in flowering time regulation has also been realized again. However, the molecular mechanism underlying such processes and a potential crosstalk between these has not been elucidated. We have found that a diurnal accumulation of SVP protein is controlled by circadian clock in *Arabidopsis*. Here we demonstrate that plants with mutations in clock genes LHY and CCA1 exhibit not only late flowering, but also semi-dwarf phenotypes, under continuous light. We also show these phenotypes of *lhy;cca1* are suppressed by *svp* and SVP interacts with BZR1, providing a molecular mechanism that SVP plays a key role as a negative regulator of BR signaling during the light periods without affecting the *FLC* mRNA level.



**Ko Shimamoto** BS Genetics Kyoto University, Japan, Ph.D. Genetics University of Wisconsin-Madison, USA. In 1980, he became a postdoctoral fellow at the Friedrich Miescher Institut in Basel, Switzerland. In 1983 he joined a newly founded Plantech Research Institute in Mitsubishi Chemical Corporation. In 1994 Professor, Plant molecular Genetics Nara Institute of Science and Technology, Japan, working on regulation of flowering and signaling in innate immunity in rice. Dr. Shimamoto has served as an Editor for "Plant Cell Reports" (1992-1995) and "The Plant Journal" (1995-1998) and is currently an Editor of "Plant and Cell Physiology" (2000-) and "Plant Physiology" (2000-) and a member of the Advisory Editorial Board of "Trends in Plant Sciences". Dr. Shimamoto received the Distinguished Research Award from the Genetics Society of Japan (1990), the Society Award from Japanese Society of Breeding (1993), and Kihara Memorial Foundation Prize (2000).

### **Structure and function of rice Hd3a florigen**

Ko Shimamoto, Ken-ichiro Taoka, Hiroyuki Tsuji, Shojiro Tamaki, Izuru Ohki, Chojiro Kojima

We have previously demonstrated that Hd3s protein is a rice florigen based on the analysis of transgenic rice plants expressing *Hd3a pro::Hd3a-GFP*. Since then we found that rice has another florigen RFT1, encoded

by a gene highly homologous to *Hd3a*, and that it mainly functions under LD conditions. We also showed that rice does not flower when both florigen genes are downregulated, suggesting that rice absolutely requires florigen for flower induction. The main focus of our recent research is to study how rice *Hd3a* florigen functions to induce flowering. One approach is to understand the localization of rice *Hd3a* florigen in the apical meristem after transition to the flowering stage. We study this by using transgenic rice expressing *Hd3a pro:: Hd3a-GFP*. The second approach is to identify interactors of *Hd3a* and analyze their functions during floral induction. We found several interactors by yeast two hybrid screens and are now studying their functions and interactions with *Hd3a*. Recent results will be presented.

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**Ove Nilsson** received his Ph.D. From the Swedish University of Agricultural Sciences in 1995. His thesis work studied the effects of the *rol* genes from *Agrobacterium rhizogenes* on plant growth and development. From 1995-1997 he worked as a post-doctoral research fellow in the group of Detlef Weigel at the Salk Institute for Biological Studies in La Jolla, USA. During this time he initiated his work on the regulation of flowering time and flower meristem identity in *Arabidopsis* as well as in trees. He then returned to the Swedish University of Agricultural Sciences where he took part in the formation of the Umeå Plant Science Centre (UPSC) and since 2002 he is a full professor at the Department of Forest Genetics and Plant Physiology within this centre. Since 2005 Ove Nilsson has been the Chairman of the UPSC Board and has been the director of first a Centre of Excellence in Plant Developmental Biology, and later a Centre for Forest Biotechnology research at UPSC. In 1999 Ove Nilsson participated in the formation of the spin-off company "SweTree Technologies" which is now one of the world-leading companies in the commercialisation of forest biotechnology. Ove Nilsson served as the first CSO of this company and is now serving on the company's Board of Directors. In 2007 Ove Nilsson received the international Marcus Wallenberg Prize for his work on the regulation of tree flowering which was considered as breakthrough research in areas relevant for the forest industry.

### Photoperiodic regulation of flowering and life cycle adaptation in sugar beet

Pierre A. Pin, Reyes Benlloch, Dominique Bonnet, Elisabeth Wremmerth-Weich, Thomas Kraft, Jan J.L. Gielen, and Ove Nilsson

Cultivated beets (*Beta vulgaris* ssp. *vulgaris*), such as many other biennial flowering plants, are not able to form reproductive shoots during the first year of their life cycle. Transition only occurs if plants pass through the winter and are subsequently exposed to the increasing photoperiod gradually taking place in spring. The most important determinant of the difference between annual and biennial beet varieties is the B locus, where the dominant B allele is associated with the annual growth behaviour. We will describe the cloning and characterization of the B gene from beet. We will also show how the B gene controls flowering time in sugar beet through regulation of an antagonistic pair of *Arabidopsis* FLOWERING LOCUS T (*FT*) homologs. One of the genes (*Bv FT2*) is functionally conserved with *FT* and is necessary for the flowering of annual sugar beet plants. In contrast, the other *FT*-like gene (*Bv FT1*), is preferentially expressed in juvenile biennial plants and is down-regulated in response to vernalization. The *Bv FT2* gene is positively regulated by increasing photoperiod, while *Bv FT1* is negatively regulated. *Bv FT1*-overexpressing annual and biennial plants are non-flowering and do not respond to vernalization suggesting that *Bv FT1* is indeed acting as a repressor of bolting and flowering. We conclude that the regulation of flowering time in beets is controlled by the interplay between two *FT*-like gene paralogs that have evolved antagonistic functions and that the down-regulation of the *Bv FT1* repressor is crucial for the vernalization response in beet.

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**Takato Imaizumi** Assistant Professor of the Department of Biology, University of Washington. While pursuing his Ph.D., Takato worked on the identification and functional characterization of blue-light photoreceptor cryptochromes in the fern *Adiantum capillus-veneris* and the moss *Physcomitrella patens* with Dr. Masamitsu Wada at the National Institute for Basic Biology (NIBB) in Okazaki, Japan. After receiving his Ph.D., Takato joined Dr. Steve Kay's lab at the Scripps Research Institute (TSRI), where he worked on elucidating the molecular mechanisms underlying seasonal (especially day-length dependent) flowering response in *Arabidopsis thaliana*. Takato joined the faculty of the University of Washington in 2008. At UW, Takato's group continues to work on elucidating the molecular mechanisms of seasonal flowering in *Arabidopsis*.

#### **Transcriptional regulation of *CONSTANS* in photoperiodic flowering.** - Takato Imaizumi

The precise alignment of the timing of flowering with the proper season is crucial for reproductive success. Many plants monitor day-length changes and utilize the information to regulate seasonal flowering. In *Arabidopsis thaliana*, the transcriptional regulation of the *CONSTANS* (CO) gene and the posttranslational regulation of CO protein are crucial mechanisms for day-length sensing. Currently, the CYCLING DOF FACTOR (CDF) family is the only known transcription factor family that directly regulates the expression of CO. However, it is improbable that CDFs are the only transcription factors that regulate CO expression. Therefore, we performed yeast one-hybrid screening to search for additional transcriptional regulators of CO and isolated a bHLH transcription factor that binds to the CO promoter in yeast. We anticipated that if bHLH regulates CO transcription *in vivo*, overexpression of the transcription factor likely induces altered flowering time due to changes in CO expression. Overexpressors of bHLH caused early flowering under short day conditions. We named the transcription factor FLOWERING BHLH 1 (FBH1). *FBH1* has a close homolog in the *Arabidopsis* genome, and overexpression of the homolog (named *FBH2*) also caused the same flowering phenotype. When we analyzed the expression of CO in those overexpressors, CO expression levels were elevated. This result suggests that both FBH1 and FBH2 may induce the expression of CO. In addition, we demonstrated that FBH1 associated with the CO chromatin *in vivo*, indicating that FBH1 directly regulates CO transcription. At this meeting, I will present our current understanding of the molecular function of FBHs.

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**Paula Suárez-López** (see her CV in "Scientific Organizers" section)

#### **Photoperiodic regulation of tuber induction by potato *CONSTANS***

Paula Suárez-López, Nahuel D. González-Schain, Mercedes Díaz-Mendoza\* and Marek Żurczak

The CO/FT (CONSTANS/FLOWERING LOCUS T) regulatory module is involved in the photoperiodic control of plant developmental processes, including flowering in several species and seasonal growth cessation and bud set in trees. In potato (*Solanum tuberosum*) tuber formation is induced by short days (SDs) in all species and varieties, whereas the response to long days (LDs) is highly variable. Tuberization of *S. tuberosum* ssp. *andigena* is strongly induced by SDs, moderately induced by SDs plus night break (SD+NB) and completely inhibited by LDs. We have cloned a potato CO-like gene, which we have named *StCO*, and have tested whether it is involved in the photoperiodic regulation of tuberization. Silencing of *StCO* accelerates tuberization under SD+NB and induces it under LDs, but does not have any effect under strongly inductive SDs, indicating that *StCO* represses tuberization in a photoperiod-dependent manner. This is further confirmed by the delay in tuberization caused by *StCO* overexpression. In addition, potato plants with increased or reduced levels of *StCO* show early flowering. The effect of this gene on tuber induction is transmitted across a graft union, suggesting that *StCO* acts upstream of long-distance signalling molecules. This is supported by the fact that *StCO* affects the level of *StBEL5* mRNA, which was previously shown to be mobile. Finally, *StCO* regulates the expression of *StFT*, a potato FT homologue, strongly suggesting that the CO/FT module has been recruited for the photoperiodic control of tuberization in potato plants.

Thursday, May 5<sup>th</sup>

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**Steve Kay** has been the Dean of the Division of Biological Sciences and holds the Richard C. Atkinson Chair in Biological Sciences at the University of California, San Diego since 2007. He is also a UCSD Distinguished Professor of Cell and Developmental Biology. Dr. Kay is trained in genetics and genomics and received his Ph.D. from the University of Bristol, UK. He has held faculty positions at The Rockefeller University, University of Virginia and The Scripps Research Institute prior to joining UCSD. He has also served as the Director of Discovery Research at the Genomics Institute of the Novartis Research Foundation (GNF), where he built a large Department applying human genome science to biomedical research and drug discovery. Dr. Kay was recently the founding Director of the San Diego Center for Algae Biotechnology (SD-CAB) which is a research hub dedicated to the development of large scale algal platforms for bioenergy, production of green

chemicals and therapeutic proteins. His research involves the large scale application of genomics technologies and systems approaches to understand complex regulatory networks in plant and animal cells. He has published over 200 papers and is named by ISI as a highly cited scientist. His recent interests lie at the interface between food and energy security with basic plant and microbial science, as well as continuing to apply genomics to the discovery of therapeutics. Dr. Kay has founded several start-up biotechnology companies. His work has been cited in *Science* magazine's "Breakthroughs of the Year" consecutively in 1997, 1998 and again in 2002. In 2008 he was elected a Member of the National Academy of Sciences USA, in 2009 elected a AAAS Fellow, and in 2010 awarded the UCSD Chancellor's Associates Faculty Award for Excellence in Research.

### **Large Scale Discovery Approaches for Plant Circadian Networks**

Steve A. Kay, Katia Bonaldi, Marcela Carvallo-Pinto, Brenda Chow, Ben Cole, Colleen Doherty, Joshua Gendron, Elisabeth Hamilton, Anne Helfer, Jasmine King, Elsebeth Kolmos, Dawn Nagel, Jeffrey Nelson, Dmitri Nusinow, Jose Pruneda-Paz, Mariko Sawa

Our laboratory is undertaking systems level approaches to understanding circadian clock function in plants. The long-term goal is to understand the circuitry required to generate robust, physiologically relevant rhythms, as well as using a comparative approach to understand the evolution of circadian clocks and the underlying design principles. We combine forward genetics with cell-based assays and whole-genome transcriptome approaches in an attempt to understand the network of circuits that are required for the core clock, and how the clock exerts its outputs upon the cell. These outputs include the rhythmic control of a substantial proportion of the transcriptome, and thus understanding the hierarchy of factors that must be required to achieve phase-specific expression of large numbers of genes is also of interest to us. We are beginning to discover that circadian clocks of plants, as in animals, are composed of complex hierarchical circuits acting at the transcriptional and post-transcriptional level. We are developing an homogenous gold standard GATEWAY<sup>TM</sup> compatible collection of Arabidopsis transcription factors to generate application-ready collections for functional genomics. We have performed the first genome wide screens with early versions of the library and have identified several novel circadian transcriptional regulators. In addition we are deploying tandem mass spectrometry with protein tagging to identify the composition and dynamics of protein complexes essential for the clock to gate environmental responses. The first such complex we have identified is the ELF4-ELF3-LUX Evening Complex (EC) that links the circadian clock to diurnal control of hypocotyl growth.

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**Marcelo J. Yanovsky** gained his PhD in Biological Sciences in 1999 from the University of Buenos Aires, Argentina, in the lab of Dr. Jorge Casal, before completing his postdoctoral studies in the lab of Dr. Steve Kay, at The Scripps Research Institute, USA in 2003. He is currently PI at Fundación Instituto Leloir-IIBBA (CONICET), and Associate Professor at the University of Buenos Aires. Dr. Yanovsky has received the Howard Hughes Medical Institute International Scholar award (2007), the Bunge y Born young investigator award in Plant Biology (2006) and the Bernardo Houssay young investigator award in Biomedicine (2005). Dr. Yanovsky has always been interested in understanding how plants flower at particular times of the year, studying the action of photoreceptors that discriminate day from nights, biological clocks that measure the passage of time, and mechanisms integrating these processes. His work in the lab of Dr. Casal contributed to characterize the signaling pathways through which the photoreceptor phytochrome A controls developmental processes in *Arabidopsis thaliana* and potato plants, focusing on its role adjusting the circadian clock to external light/dark cycles. As a post-doc in the lab of Dr. Steve Kay, Dr. Yanovsky collaborated in developing the first model explaining at the molecular level how the plant circadian clock operates, and, how the activity of the circadian clock interacts with that of specific photoreceptors to regulate the expression of key flowering time genes, allowing *Arabidopsis* plants to flower more rapidly under long photoperiods. More recently, Dr. Yanovsky's work has focused on the use of forward genetic approaches to dissect regulatory mechanisms controlling light and circadian signalling pathways. This allowed his group to identify a role for Protein Arginine Methyltransferase 5 (PRMT5) in the modulation of circadian rhythms in plants and flies, at least in part, through effects on alternative splicing, a process whose importance in the regulation of gene expression and protein diversity is being increasingly recognized.

#### **A role for PRMT5 in the post-transcriptional regulation of the *Arabidopsis* circadian network**

Sabrina E. Sanchez, Ezequiel Petrillo, Xu Zhang, Craig G. Simpson, John W.S. Brown, Justin O. Borevitz, Fernanda Ceriani, Paloma Mas, Alberto R. Kornblihtt & Marcelo J. Yanovsky

Circadian clocks allow plants and animals to adjust physiological and developmental processes in anticipation of daily and seasonal changes in the environment. Epigenetic mechanisms and alternative splicing (AS) are increasingly being linked to the regulation of circadian rhythms across eukaryotic organisms, but the players linking these processes are largely unknown. We have recently found that *Protein Arginine Methyl Transferase 5* (PRMT5), which transfers methyl groups to arginine residues in histones and spliceosomal proteins has an important role modulating circadian networks in plants and flies. Mutations in *prmt5* impair multiple circadian rhythms in *Arabidopsis* and this phenotype is caused, at least in part, by a strong alteration in alternative splicing of the core-clock gene *PSEUDO RESPONSE REGULATOR 9* (PRR9). In addition, PRMT5 expression shows daily and circadian oscillations, and this contributes to mediate the circadian regulation of expression and alternative splicing of a subset of genes. Circadian rhythms in locomotor activity are also disrupted in a mutant affected in the *Drosophila melanogaster* PRMT5 homolog, and this is also linked to alterations in splicing of several clock associated genes. Thus, our results point to PRMT5 as a key player that helps different organisms to synchronize physiological processes with daily changes in environmental conditions, through effects on AS and gene expression. There are multiple mechanisms through which PRMT5 could affect AS, and approaches to dissect them will be discussed at the meeting.

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**Paloma Más** (see her CV in “Scientific Organizers” section)

#### **The circadian clock heats up in Arabidopsis-** Sergi Portolés and Paloma Más

The protein kinase CK2 is one of the few clock components that is evolutionary conserved among different taxonomic groups. CK2 regulates the stability and nuclear localization of essential clock proteins in mammals, fungi, and insects. Two CK2 regulatory subunits, CKB3 and CKB4, have been also linked with the *Arabidopsis thaliana* circadian system. However, the biological relevance and the precise mechanisms of CK2 function within the plant clockwork are not known. In our studies, we were able to identify a temperature-dependent function for CK2 modulating circadian period length. We found that CK2 antagonizes the key clock regulator CIRCADIAN CLOCK-ASSOCIATED 1 (CCA1). CK2 activity does not alter protein accumulation or subcellular localization but interferes with CCA1 binding affinity to the promoters of the oscillator genes. High temperatures enhance the CCA1 binding activity, which is precisely counterbalanced by the CK2 opposing function. Altering this balance by over-expression, mutation or pharmacological inhibition affects the temperature compensation profile, providing a mechanism by which plants regulate circadian periods at changing temperatures. Therefore, we establish a new model demonstrating that two opposing activities (CCA1-CK2) are essential for clock temperature compensation in Arabidopsis. Despite the conserved function with the Neurospora circadian system, our study demonstrates a divergent mechanism by which CK2 regulates circadian temperature responses in Arabidopsis.

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**Seth Davis** is, since 2002, Research Group Leader (BAT 1A / TVöD 15) of the Department of Plant Developmental Biology, Max Planck Institute for Plant Breeding Research, Köln, Germany (Associate Professor equivalent). 2000-2002: Research Fellow, Department of Biological Sciences, University of Warwick, Coventry, UK. 2001: Visiting Scientist, Institute of Plant Biology, Szeged, Hungary. 2000: Research Associate with Dr. Richard D. Vierstra, Program in Cellular and Molecular Biology, University of Wisconsin-Madison, Madison WI, USA. 1994-2000: Research Assistant, University of Wisconsin-Madison, Laboratory of Genetics, Madison WI, USA. 2008-2009: University of Bonn, Bonn, Germany (Habilitation in Genetics). 1994-2000: University of Wisconsin-Madison, Madison, WI (Ph.D. in Genetics). Dr. Seth Davis studied (1991-1994) in the University of Central Florida, Orlando, FL (B.S. in Molecular Biology and Microbiology, Minor in Chemistry), Graduated *Magna cum Laude*.

#### **Effector binding to a co-repressor complex sustains the plant circadian oscillator as a light-responsive process. - Seth J. Davis**

The plant circadian clock is proposed as a network of three interconnected feedback loops and loss of any component leads to an acceleration of oscillator speed. Our group previously reported that ELF4 is required to sustain this oscillator and that the *elf4* mutant is arrhythmic. This phenotype is shared with *elf3* and *lux*. Here I will discuss how ELF4 functions as an effector that restricts ELF3 to the nucleus. Expression data was used to direct a mathematical position of *ELF3* in the clock network. This predicted direct effects on the morning clock gene *PRR9*, and ELF3 was found to associate to a phylogenetically conserved region of the *PRR9* promoter. A conserved cis-element in this region suggested ELF3 recruitment by the GARP-type DNA-binding protein LUX. Consistent with this, both *ELF3* and *LUX* act genetically downstream of *ELF4*. From this, by forward genetics and QTL cloning, respectively, two reduced-function alleles of *ELF3* were isolated that fail to properly process light input to the oscillator. Their phenotypes in phytochrome-signalling will be discussed. Taken together, a co-repressor complex pivotal to sustain the plant circadian oscillator serves as one input point for light perception to this clock.

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**Alex Webb** is Senior Lecturer, University of Cambridge. Uses systems approaches to identify the signalling network by which the circadian oscillator regulates cell physiology. He has demonstrated that the circadian clock increases assimilation, growth and survival of plants (Dodd et al., 2005 *Science* 309, 630 – 633), there are circadian and diurnal  $\text{Ca}^{2+}$  oscillations that encode photoperiodic information in *Arabidopsis* (Love et al., 2004 *Plant Cell* 16, 956 – 966) and these circadian oscillations of  $[\text{Ca}^{2+}]_c$  are driven by oscillations of cyclic ADP ribose forming a cytosolic loop of the clock (Dodd et al., 2007 *Science* 318, 1789 -1792). I have also shown that there is circadian modulation of low temperature-induced increases in  $[\text{Ca}^{2+}]_c$  in plant cells (Dodd et al., 2006 *Plant J.* 48, 962 – 973; Hotta et al., 2007 *PCE* 30, 333-349). They found that there are cell-specific circadian oscillators (Dodd et al., 2004 *New Phyt.* 162, 63-70; Xu et al., (2007) *Plant Cell* 19, 3474-3490; Somers et al., 1998 *Development*. 125, 485-494). I have collaborated with Dr Gonçalves to develop systems approaches to understand circadian signalling, achievements include providing a global understanding of the circadian control of biological timing. and GIGANTEA is required for the response of the plant circadian oscillator to sucrose, providing a mechanism for metabolic input to the circadian clock (Dalchau et al 2011 *PNAS* 108, 5104 -5109). As well as his studies of circadian function, He continues also to make advances in basic  $\text{Ca}^{2+}$  signalling research. His previous research focused on  $\text{Ca}^{2+}$  signalling in stomatal guard cells.

### **Multiple signaling pathways associated with correct biological timing in *Arabidopsis*. -Alex A.R. Webb**

To understand the biology of plants it is essential to understand how cells cope with the stresses caused by the rotation of the Earth and seasonal changes. Our goal is to identify hitherto unconsidered mechanisms and provide new understanding that can be used by plant breeders to develop crops to alleviate the major problems facing the planet, food shortage, energy production and water use. We are particularly interested in how plants measure time and how this information is integrated with stress signalling. Stress signalling is tightly linked to the daily rhythms of the plants so that the plant can make the appropriate responses to a stress signal, such as cold, dependent on the time of day the signal is perceived. The research in my lab is focused on how interactions between the internal circadian clock and physiological and environmental signals provide physiological benefits to the plant (Dodd *et al.* 2005, *Science* **309**, 630 – 633). We particularly focus on the signalling mechanisms associated with cell physiology and the association of these signalling networks with the molecular mechanisms of the circadian clock (e.g. Dodd *et al.*, 2007 *Science* **318**, 1789 -179). I will describe physiological and systems approaches that provide new insight in to how environmental signals (Dalchau *et al.*, 2010 *PNAS* **107**, 13171-13176) and cellular energy status (Dalchau *et al.*, 2011 *PNAS* **108**, 5104 -5109) regulate circadian timing in plants. I will also provide new data demonstrating that the circadian oscillator is regulated by  $\text{Ca}^{2+}$  signalling networks.



**Akira Nagatani** is Professor at the Graduate School of Science, Kyoto University, Japan. He qualified in Biochemistry and obtained a PhD degree in Biochemistry at the University of Tokyo, Japan, in 1984. As a post-doctoral fellow, he worked in Prof. Masaki Furuya's laboratory first at National Institute for Basic Biology, Okazaki, Japan (1984-1987) and later at RIKEN, Wako, Japan (1987-1992). From 1992 to 1995, he continued as a deputy head of the research team of Prof. Richard Kendrick at RIKEN, Wako, Japan. During this period, he analyzed photomorphogenic and phytochrome mutants in cucumber, tomato, pea and later *Arabidopsis*. As a main achievement in this period, he isolated the phyA deficient mutants simultaneously with a few other groups (1993). He also conducted the structure/function study of phytochrome first using tobacco and then using *Arabidopsis*.

In 1995, he was appointed as an Associate Professor in the University of Tokyo, Tokyo, Japan, and headed his own laboratory until 1998. In this period, he discovered the phytochrome nuclear localization first using GUS (1996) and then using GFP (1999) as tags. He was appointed as Professor at the Graduate School of Science, Kyoto University, Kyoto, Japan in 1998. Currently, he focuses on three research topics; 1) structure/function analysis of phyA specific functions, 2) inter-tissue/organ communication in photomorphogenesis, and 3) signal transduction mechanism of phototropin responses. From 2010 to 2014, he heads a major group research project (Grant-in-Aid for Scientific Research on Innovative Areas from Japanese Ministry of Education, Culture, Sports, Science and Technology) on plant sensing of environmental stimuli.

## Spatio-temporal regulatory network controlling stem elongation under the shade.

Akira Nagatani, Junko Kobayashi, Kazumi Watahiki, Toshiaki Kozuka, Yoichiro Hosokawa and Nobuyoshi Mochizuki,

Phytochromes (phys) regulate growth responses under the shade. Phys are expressed in almost all the organs/tissues in the seedling. Hence, light stimuli perceived in different parts of the seedling should be properly integrated to trigger unified whole-plant responses. To understand such a complex phenomena, we need to elucidate how the light signals are propagated in time and space in the plant body. Experimentally, the end-of-day FR (EOD-FR) treatment, which is given at the end of the light period in light/dark cycles, elicits the shade avoidance responses such as promotion of hypocotyls elongation. We first confirmed that cotyledons but not the hypocotyl were the sites of EOD-FR perception. Next, we examined the role of cotyledons by irradiation with a delayed red light pulse and surgical removal of cotyledons. Consequently, the cotyledons were suggested to complete the light signal processing in the early phase (4-6 hrs) of the dark period, whereas the major hypocotyl growth was observed towards the end of dark period. We then tested the involvement of shoot apex in the FR-promotion of hypocotyls elongation by disconnecting the apex from other parts by laser ablation. Although such a treatment abolished the FR-promotion, exogenously applied auxin fully restored the response in the laser-ablated plants. Hence, the hypocotyl appeared to change the responsiveness to auxin rather than change the level of auxin to promote the hypocotyls elongation. Currently, expression of typical shade-responsive genes such as *AtHB2*, *HFR1*, *GH3* and *IAA19* in cotyledons and the hypocotyl is under examination to link those genes to above processes.

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**Ferenc Nagy** is a plant molecular biologist. He obtained his Ph.D. in genetics with Prof. Pal Maliga in the Plant Physiology Institute of the Biological Research Centre in Szeged, Hungary in 1981. In 1983 he moved to New York and worked as a postdoctoral fellow in the laboratory of Prof. Nam-Hai Chua at the Rockefeller University until 1988. During these years, whilst he contributed as a tissue culture expert to regenerate the first transgenic plants expressing in vitro assembled chimeric genes, he got interested in photo- and circadian biology. In 1988-1996 he worked in Basel, Switzerland, established his own research group in the Friedrich-Miescher Institute and continued his work in photo- and circadian biology. In 1996 he returned to Hungary as a Howard Hughes Medical Institute International Scholar and set up his

research group in the Biological Research Centre. Between 2000-2006 he held the position of General Director of the Agricultural Biotechnology Centre in Godollo and since 2009 he has been Deputy Director of the Plant Biology Institute of the Biological Research Centre in Szeged. Since 2004 he has been an Honorary Professor at the University of Freiburg, and from 2008 he holds the Chair of Plant Cell and System Biology at the University of Edinburgh, UK. His main research interest is the molecular characterisation of cellular processes and components that mediate very early steps of phytochrome-controlled signalling.

## Intra- and intercellular features of phytochrome-A mediated signalling

Daniel Kirchenbauer, Eva Adam, Stefan Kircher, Laszlo Kozma-Bognar, Eberhard Schafer and Ferenc Nagy

Immunohistochemical studies and analysis of promoter-GUS and GFP fusion proteins in transgenic plants demonstrated that the red/far-red sensing phytochrome-A (phyA) photoreceptor is ubiquitously expressed at all developmental stage in *Arabidopsis thaliana*. The ubiquitous expression pattern of phyA and the fact that far-red light, in contrast to red light, readily penetrates plant tissues suggested that the majority of phyA-controlled responses occurs in cell autonomous fashion. To validate this hypothesis we produced a series of transgenic lines in the *phyA-201* mutant that expressed the PHYA:PHYA:YFP, ML1:PHYA-YFP and SUC2:PHYA:YFP transgenes. We analysed a number of phyA-controlled responses including hypocotyl growth inhibition, cotyledon size, inhibition of greening, anthocyanin accumulation and stomatal development at seedling stage and shortening of flowering time by day length extension with far-red light in plants. Finally, we performed whole-genome transcriptome analysis on WT (Laer), *phyA-201*, ML1:PHYA:YFP/*phyA-201* and SUC2:YFP/*phyA-201* seedlings and identified those genes whose expression is controlled by phyA HIR and VLFR. Our data suggest that the majority of the analysed responses, at least at the physiological level, are manifested in a cell autonomous fashion, yet cell to cell signal transduction may play an unexpected role in regulating phyA-dependent responses.





**Kerry Franklin** is a Royal Society Research Fellow at the University of Bristol, UK. Prior to this she worked as a postdoctoral researcher with Professor Garry Whitelam at the University of Leicester. Her work aims to understand the molecular mechanisms through which plants integrate multiple environmental signals to regulate development. Initial research at the University of Leicester focussed on elucidating individual phytochrome functions and plant shade avoidance signalling. More recently, her work has investigated crosstalk between light and temperature signalling pathways. Research highlights include the discovery that light quality signals can regulate cold acclimation and the identification of PIF4 as a central component of plant high temperature signalling. Current work focuses on identifying the signalling processes controlling plant architectural adaptations to light and temperature stimuli and investigating the adaptive significance of different developmental strategies. In 2010 Kerry was awarded both the Society of Experimental Biology (SEB) President's medal in Plant Science and the Federation of European Societies of Plant Biology (FESPB) young scientist award.

#### **PIF4 integrates light and temperature signals during plant development. - Kerry Franklin**

The PHYTOCHROME INTERACTING FACTOR (PIF) subfamily of bHLH transcription factors are emerging as cellular hubs of environmental signal integration<sup>1</sup>. PIF4 and PIF5 drive elongation growth in de-etiolated plants. PIF4 acts redundantly with its homologue, PIF5, to regulate diurnal growth rhythms and elongation responses to the perceived threat of vegetational shade<sup>2,3</sup>. In contrast to light signalling, PIF4 performs a dominant role in mediating elongation responses to high temperature<sup>4,5</sup>. It has previously been reported that PIF4 activity is limited, in part, by binding to both the bHLH protein LONG HYPOCOTYL In FAR RED 1 (HFR1) and the DELLA family of growth-repressing proteins<sup>6,7</sup>. Despite the importance of PIF4 in integrating multiple environmental signals, the mechanisms through which PIF4 controls growth in de-etiolated plants remain unknown. We have used high temperature treatment to elucidate a novel function for PIF4 in driving plant elongation, via the hormone auxin. Upstream and downstream targets in this signalling pathway will be discussed and parallels with shade avoidance explored.

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**Elena Monte** (see her CV in "Scientific Organizers" section)

#### **PIF3 regulates rhythmic growth in Arabidopsis. - Elena Monte**

In diurnal conditions, plant growth rate varies during the day to coordinate with the availability of energy and water. Under short days, seedling growth rate is maximal at the end of the night. Regulation of this precise timing of growth has been shown to involve integration of the external light cue with internal circadian rhythms. Recent studies have started to explain how light and clock action might coordinate to precisely time hypocotyl growth. These studies reported that the phytochrome-interacting factor 4 (PIF4) and PIF5 function as positive regulators of diurnal growth in Arabidopsis. The abundance of PIF4 and PIF5 proteins is regulated by light through phytochrome action to keep their levels low during the day. In turn, the clock regulates their transcript levels, and coincidence of high transcript levels and protein accumulation at the end of the dark period allows for growth promotion to peak at the end of night. In our studies, we have obtained evidence that PIF3 is necessary to regulate rhythmic growth under short-day cycles in Arabidopsis. We have found that PIF3 accumulates during the night under these conditions and reaches a maximum at the end of the dark period. Mutant combinations show that PIF3 function is additive to PIF4 and PIF5. Interestingly, unlike PIF4 and PIF5, PIF3 transcript levels do not oscillate in diurnal conditions. These data suggest that PIF3 action is integrated with the internal circadian rhythms to fine tune the timing of growth promotion through a different mechanism than the previously described for PIF4 and PIF5. Mechanistic alternatives for PIF3 and clock interaction will be discussed.



**Robertson McClung** is Professor of Biological Sciences at Dartmouth College. He earned his bachelor's degree in biology at Queen's University in 1976 and his M.Sc. in biology from Dalhousie University in 1979. His Ph.D. (1986) is from Michigan State University where he studied the symbiosis between nitrogen-fixing bacteria and soybeans. This interaction allows the plants to grow in the absence of nitrogenous fertilizer and is of considerable agronomic significance, especially in the developing world. McClung came to Dartmouth in 1986 to conduct postdoctoral research with Jay C. Dunlap in the Biochemistry Department of Dartmouth Medical School. There he studied circadian rhythms in the model fungus, *Neurospora crassa*, using a combination of genetic and molecular biological techniques. In 1988 took a faculty position in the Dep. of Biological Sciences, where he has moved through the ranks, becoming full professor in 2001 and, in 2010, the Patricia F. and William B. Hale 1944 Professor in the Arts and Sciences. He served as Associate Dean for the Sciences. His research focuses on the basis of endogenous biological clocks, now emphasizing the model plant, *Arabidopsis thaliana*. Because plants are closely related, it is quite likely that understanding derived from *Arabidopsis* studies will be readily transferred to agronomically important species. In the context of climate change and the need to exploit increasingly marginal habitats, fuller understanding of clock mechanism may offer strategies to improve crop productivity. He has been awarded grants from the National Science Foundation to expand his research into the crop plant, *Brassica rapa*.

#### **Genetic Architecture of Circadian Clock Function In *Brassica rapa*. - C. Robertson McClung et al.**

We have used the circadian rhythm in cotyledon movement to extend the study of the plant circadian clock mechanism to *Brassica rapa*. We have identified Quantitative Trait Loci (QTL) for period, amplitude and temperature compensation of circadian clock, as well as for a number of morphometric parameters, including flowering time, size of floral organs, and hypocotyl length, in a set of Recombinant Inbred Lines (RILs) derived from a cross between a yellow sarson (R500; ssp. *trilocularis*) and a rapid cycling Chinese cabbage (IMB211; ssp. *pekinensis*). We have emphasized a QTL for period length on Chr. A9. One candidate, *GIGANTEA* (*GI*), identified as a clock component in *Arabidopsis*, maps to that region. *GI* is polymorphic between R500 and IMB211 and the inheritance of the two alleles is consistent with *GI*<sup>R500</sup> conferring shorter period than *GI*<sup>IMB211</sup>. We have introduced the two *B. rapa* *GI* alleles into the *Arabidopsis gi-201* null mutant. *B. rapa* *GI*<sup>IMB211</sup> rescues the short period phenotype of *gi-201* whereas the *GI*<sup>R500</sup> allele does not, which is consistent with their relative effects on period in *B. rapa*. Both alleles rescue the late flowering phenotype of *gi-201*. We have identified several putative null *gi* alleles in the *B. rapa* TILLING collection at the John Innes Center. Loss of *gi* function in *B. rapa* shortens the period in circadian cotyledon movement, consistent with the *Arabidopsis gi-201* phenotype. We will use the *B. rapa gi* mutants to test the effects of the two *B. rapa GI* alleles on period length in *B. rapa*.



**Stacey Harmer** is Associate Professor in the Department of Plant Biology, University of California, Davis. She earned her bachelor's degree at the University of California, Berkeley, graduating with Highest Honors and Highest Distinction in General Scholarship from the Department of Biochemistry. She went on to obtain a PhD at the University of California, San Francisco, in 1998. While at UCSF, she was a Howard Hughes Predoctoral Fellow in the laboratory of Dr. Tony DeFranco and explored mechanisms underlying B cell receptor signal transduction. For her post-doctoral studies, she switched from immunology to plant biology to work with Dr. Steve Kay at the Scripps Research Institute as a National Research Service Award post-doctoral fellow (1998 – 2002). In the Kay lab, she studied the molecular basis of circadian rhythms in *Arabidopsis thaliana*. After starting her own lab at UC Davis, she has continued to use genetic and functional genomic approaches to investigate mechanisms underlying the plant clock and its role in the coordination of plant physiology. Her work has been funded by the National Science Foundation and the National Institutes of Health and is published in top journals. In addition to her productive research program, she is also an active member of the broader plant biology community, serving on the Board of Directors of the iPlant Collaborative, on the *Plant Physiology* Editorial Board, and acting as lead instructor for the Cold Spring Harbor Laboratory Plant Course.

## Using functional genomics to identify new clock genes

Stacey L. Harmer, Matthew A. Jones, Nozomu Takahashi, Polly Hsu, Reetika Rawat, Michael F. Covington<sup>1</sup>, Luciano DiTacchio, Christopher Vollmers, Satchidananda Panda, Jacob Schwartz, Michelle R. Salemi, Brett S. Phinney

Circadian rhythms are found in most eukaryotes and some prokaryotes and are generated by an endogenous oscillator or clock. A functional circadian clock provides an adaptive advantage, presumably by allowing organisms to anticipate regular changes in the environment. Basic features of the circadian system are shared across eukaryotes, but most clock components are not conserved across higher taxa. A combination of genetic, genomic, and modeling approaches has led to rapid progress in determining the molecular nature of the circadian oscillator in the model plant *Arabidopsis thaliana*. As in metazoa, multiple interlinked transcriptional feedback loops influenced by a variety of post-translational regulatory mechanisms comprise the plant circadian clock. But despite these advances, there are conspicuous gaps in our understanding of the molecular clockwork in higher plants. To help fill these gaps, we have taken functional genomic approaches to identify new clock genes and place them within the clock mechanism. Using mass spectrometry, we identified a novel transcription factor that binds specifically to a circadian cis-regulatory motif. We show that this factor, RVE8, forms a negative feedback loop with another clock protein, PRR5. We have also used a data mining approach to identify genes co-regulated with the well-known clock gene *TOC1*. We found that one such gene, *JMJD5*, acts in parallel with *TOC1* to promote expression of morning-phased clock genes. Surprisingly, we found that the human ortholog of *JMJD5* acts within the human circadian clock and that the plant and human genes have conserved cellular functions.

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**Salomé Prat** Professor at the Centro Nacional de Biotecnología (CNB-CSIC) in Madrid. She graduated in Pharmacy by the University of Barcelona and obtained a PhD degree in Plant Genetics by the same University. She worked as a post-doctoral fellow and associate researcher in the group of L. Willmitzer at the Institut für Genbiologische Forschung in Berlin (1987-92) where she was engaged in two projects aimed to the study of tissue-specific transcriptional control in potato and JA-mediated activation of defense responses against insect attack. In 1993 she was appointed as Associate Professor at the Institute of Molecular Biology of Barcelona (IBMB-CSIC) and moved to the CNB in Madrid in 2003, being promoted to Professor by 2005. As an independent group, her research has focused in photoperiodic control of storage organ formation in potato and hormonal control of cell elongation in *Arabidopsis*. She was awarded with the Ciutat of Barcelona Scientific Research award in 2002 and the Olchemim Scientific award in 2010. Since 2007, she is director of the Department of Plant Molecular Genetics of the CNB and was elected EMBO member in 2008.

## The PIF transcription factors: key integrators of light, gibberellin (GA) and brassinosteroid (BRs) signals.

Stella Bernardo, Miguel de Lucas, Jean-Michel Davière and Salomé Prat.

Light is crucial to plants as it provides the energy for photosynthesis besides of serving as an informational cue of the growing environment or the seasonal changes in a year. Well characterized light-regulated responses are those leading to seedling de-etiolation after emergence from soil or the response to *shade* in adult plants, aimed to cope with competition by other plants. These two developmental responses, triggered by dark to light transition or by changes in the R/FR ratio of light ratio are to a large extent regulated by the transcription factor PIF4 (PHYTOCHROME INTERACTING FACTOR 4), which is destabilized in the light by the PHYB photoreceptor. The plant hormones gibberellins (GA) and brassinosteroids (BRs) play a central role in transducing the light signal as judged from the dark de-etiolated phenotype of mutants with a block in the synthesis or response to these hormones. As for GA mutants, this phenotype is caused by an accumulation of the DELLA repressors, a family of nuclear proteins that repress GA-regulated gene expression and are rapidly destabilized by GAs. Evidence provided by our group showed that DELLAs bind the bHLH DNA recognition domain of the PIF factors and block DNA binding ability of these transcriptional regulators. PIF4 and its close homologue PIF5 regulate the expression of several genes driving cell

elongation by recognizing a G-box element in their promoters, growth restraint imposed by DELLAs then being mediated through a block in PIF4/PIF5-mediated activation of these genes. Interestingly, BR-deficient/-response mutants do not respond to GAs. Of note, this impaired response does not correlate with an increased stability of DELLAs, which suggests that a regulatory step downstream of these repressors mediates GA-/BR- cross-talk. BRs were actually found to stabilize the PIF4/PIF5 factors in the light, these results identifying these factors as key master regulators with a central role in cell elongation control. Evidence will be provided showing a function of these TFs not only as integrators of light- (by PHYB-mediated destabilization) and GA- signals (by inactive complex formation with DELLAs) but also in BR-signaling, this family of transcriptional regulators thus serving as a node of interaction between the signaling cascades controlling plant growth and the exterior.

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**Julin N. Maloof** is Associate Professor in the Department of Plant Biology, University of California, Davis. Dr. Maloof earned his bachelor's degree at Haverford College, graduating with High Departmental Honors in Biology. As a Howard Hughes Predoctoral Fellow he studied *C. elegans* development in Dr. Cynthia Kenyon's lab at the University of California, San Francisco, earning his PhD in 1998. Dr. Maloof switched his focus to plants at the start of his postdoc in order to better study interactions between the environment and development. He was co-mentored by Drs. Joanne Chory and Detlef Weigel at The Salk Institute and was funded by a Helen Hay Whitney Fellowship while there. At The Salk, Dr. Maloof pioneered the use of quantitative genetics and the study of natural variation in the Chory and Weigel labs. Dr. Maloof started as an Assistant Professor at UC Davis in 2002 and was promoted

to Associate Professor in 2009. The Maloof Lab studies the regulation of plant development by light, using a wide-range of techniques including time-lapse photography, forward and quantitative genetics, and genomics. Research organisms include *Arabidopsis*, tomato, and *Brassica rapa*. In addition to a highly active research program, Dr. Maloof has served as the chair of the Multi-National *Arabidopsis* Steering Committee Subcommittee on Natural Variation and Comparative Genomics (2007-2010) and is a co-instructor for the Cold Spring Harbor Laboratory course "Frontiers and Techniques in Plant Science". Work in Dr. Maloof's lab is funded by the United States National Science Foundation; past funding has also come from the Human Frontier Science Program.

### **Interactions of light, photoperiodism, and the clock in shade avoidance and rhythmic growth**

Julin N. Maloof, David Alabadí, María Verónica Arana, Stacey L. Harmer, Jose M. Jiménez-Gómez, Kazunari Nozue, Nora A. Marín-de la Rosa, Miguel A. Blázquez

The first part of this talk will focus on our efforts to understand the genetic mechanisms that underlie phenotypic variation in nature and how they interact with the environment. We focus on shade avoidance as a classic example of adaptive genetic variation in response to the environment. We used a segregating population to map QTL affecting acceleration of flowering in response to shade. We developed a network analysis method that combines genomic information from publicly available databases to identify the causative gene in the QTL interval as *ELF3*—a gene previously unknown to be involved in shade avoidance. Using genetic and transgenic methods we confirmed the effect of *ELF3* in the shade avoidance response, and showed that different alleles of this gene in natural populations of *Arabidopsis* result in different developmental times and circadian periodicity depending on the environmental conditions. The second subject of this talk will be control of rhythmic plant growth. We previously found that PIF4 and 5 integrate light and circadian clock signaling to generate rhythmic plant growth. Expression profiling and real-time growth assays have now been used to predict the growth networks functioning both downstream and independently of PIF4 and PIF5. We find that the auxin and GA pathways both contribute to diurnal growth control. Surprisingly, GA responsive genes are not enriched among genes regulated by PIF4 and PIF5, whereas auxin pathway and response genes are. GA signaling instead is gated through circadian regulation of the GA receptor; furthermore constitutive expression of the GA receptor expands the daily growth period in seedlings.



## Poster sessions *(in alphabetical order)*

<b>Name</b>	<b>Entity</b>	<b>City, Country</b>	<b>Title</b>
<b>David Alabadi</b>	Inst. Biología Molecular y Celular de Plantas (UPV-CSIC)	Valencia, Spain	<b>Hormonal regulation of rhythmic growth in Arabidopsis</b>
<b>Reyes Benlloch</b>	Center for Research in Agricultural Genomics	Barcelona, Spain	<b>Role of sumoylation on the regulation of the plant circadian system</b>
<b>Isabelle Carré</b>	University of Warwick	Coventry, UK	<b>Transcriptional networks regulating rhythmic transcription in plants</b>
<b>Jose A. Jarillo</b>	CBGP (INIA-UPM)	Madrid, Spain	<b>The E3 ubiquitin ligase HOS1 participates in the control of photoperiodic flowering in Arabidopsis negatively regulating CONSTANS abundance</b>
<b>Ake H. Johansson</b>	University of Edinburgh	UK	<b>Light receptor action is critical for maintaining plant biomass at warm ambient temperatures.</b>
<b>Laszlo Kozma-Bognar</b>	Biological Research Center of the Hungarian Ac.of Sciences	Szeged, Hungary	<b>Identification and characterization of a novel circadian clock mutant in Arabidopsis thaliana</b>
<b>Liron Krebs</b>	Max Planck Institute for Plant Breeding Research	Cologne, Germany	<b>The role of CONSTANS phosphorylation in flowering time regulation</b>
<b>Guiomar Martín</b>	Center for Research in Agricultural Genomics	Barcelona, Spain	<b>A novel mutant screen for suppressors of pifq to identify new regulators of seedling deetiolation acting downstream of the phytochrome-interacting factors (PIFs)</b>
<b>Jaime Martínez</b>	Center for Research in Agricultural Genomics	Barcelona, Spain	<b>Integration of shade perception and hormone-mediated growth in Arabidopsis thaliana by HD-ZIP II transcription factors</b>
<b>Kana Miyata</b>	University of Tsukuba	Japan	<b>Circadian clock proteins LHY and CCA1 regulate homeostasis of chlorophyll amount and a/b ratio under different photoperiods in Arabidopsis thaliana</b>

<b>Name</b>	<b>Entity</b>	<b>City, Country</b>	<b>Title</b>
<b>Michela Osnato</b>	Center for Research in Agricultural Genomics	Barcelona, Spain	<b>TEMPRANILLO integrates photoperiodic and GA signals to control flowering</b>
<b>Pablo Pérez</b>	Center for Research in Agricultural Genomics	Barcelona, Spain	<b>The Functional Interplay between Protein Kinase CK2 and CCA1 Transcriptional Activity Is Essential for Clock Temperature Compensation in Arabidopsis</b>
<b>Manuel Piñeiro</b>	CBGP (INIA-UPM)	Madrid, Spain	<b>The two Arabidopsis homologous proteins SHL and EBS are involved in the chromatin-mediated repression of flowering under non-inductive photoperiods</b>
<b>Alexandra Pokhilko</b>	University of Edinburgh	Edinburgh, UK	<b>Possible mechanisms of day length sensing by Arabidopsis</b>
<b>Maria Sentandreu</b>	Center for Research in Agricultural Genomics	Barcelona, Spain	<b>Branching of PIF3 signaling regulates seedling deetiolation</b>
<b>Samson Simon</b>	Max Planck Institute for Plant Breeding Research	Cologne, Germany	<b>Dissecting the <i>CONSTANS</i> promoter by Phylogenetic Shadowing and Reporter Lines</b>
<b>Jim Tepperman</b>	University of California	USA	<b>Transcriptome analysis of simulated-shade-induced changes in expression in wild-type (WT) and quadruple <i>pif1pif3pif4pif5</i> (<i>pifq</i>)-mutant seedlings</b>
<b>Gabriela Toledo</b>	Center for Research in Agricultural Genomics	Barcelona, Spain	<b>Identification of molecular components that regulate carotenoid biosynthesis in response to light signals.</b>
<b>Adrian Troncoso</b>	Center for Research in Agricultural Genomics	Barcelona, Spain	<b>Histone acetylation and the circadian clock: A role for the MYB transcription factor RVE8/LCL5</b>
<b>Federico Valverde</b>	Inst.Bioquímica Vegetal y Fotosíntesis, CSIC-Univ. Sevilla	Sevilla, Spain	<b>Regulation and evolution of photoperiodic flowering</b>

## Practical Information

### Venue

#### Museu Colet

c/ Buenos Aires, 56. Barcelona , Spain



#### Speakers hotel



#### Visit to CosmoCaixa (Wednesday, May 5<sup>th</sup>)

c/ Isaac Newton, 26. Barcelona, Spain



#### Visit to the Center for Research in Agricultural Genomics (CRAG) (Friday, May 7<sup>th</sup>)

Campus UAB. Bellaterra. Cerdanyola del Vallès. Barcelona, Spain



### Contact person during the event

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## Organizers



The **International Center for Scientific Debate** (ICSD) is an initiative of Biocat, fostered by Welfare Projects "la Caixa" Foundation, which aims to drive top-notch international scientific meetings promoting dialogue, collaboration and open exchange of knowledge among experts of renowned prestige and the Catalan Scientific community. The meetings are global, integrative and multidisciplinary focused helping to tackle social needs in the field of life sciences and health, taking into consideration the complexity and constantly changing conditions of the world. The ICSD also aims to collaborate in the dissemination of knowledge, approaching science to society and contributing to position Barcelona and Catalonia as a city and a country of scientific excellence.

More information: <http://www.biocat.cat/en/icsd>



The **Centre for Research in Agricultural Genomics** (CRAG) is a research institute focused on the molecular mechanisms underlying plant and farm animal biology and to the applications of molecular approaches to the breeding of species that are important for food production. CRAG is a joint initiative of CSIC, the main public institution for research of the central Spanish Government, IRTA, the institution for agricultural research of the Catalan Government and UAB, the Autonomous University of Barcelona. The University of Barcelona may soon join the centre that has the independent juridical structure of a public consortium. It has funding from the Catalan Government and the Consolidator program awarded by the Spanish Government. It is the first time that such a joint structure is created for a research centre in Spain.

More information: <http://www.cragenomica.es/>



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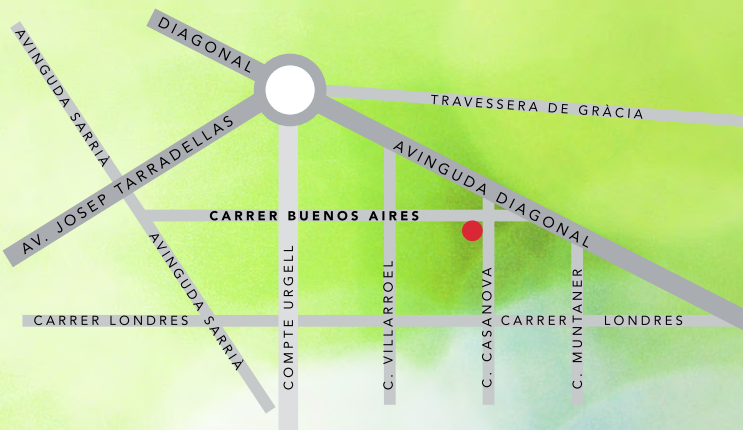
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## Venue



## Museu Colet

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