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PLANT MOLECULAR GENETICS

The aim of the Plant Molecular Genetics Department is the study of the regulatory mechanism and pathways controlling plant development, adaptation to the environment, and defense responses to biotic and abiotic stresses.

Research lines focused on developmental processes include the study of root architecture, shoot branching, photomorphogenesis and photoperiodism. Plant adaptive responses to nutrient starvation, toxic concentrations of metals or defensive responses to pests and pathogens are also subject to intense research efforts. In addition to the basic interest of the key biological questions that underlie these processes, our work aims at generating new tools and knowledge for improving crop production. For this ultimate goal, we exploit natural diversity resources as well as genetic engineering, including CRISPR/Cas9 technology for precise genome editing, as promising tools and methods. Direct biotechnological applications of plants are also addressed, such as their use as biopharmaceutical factories or as tools for alleviating metal pollution and related environmental conditions. The model species *Arabidopsis thaliana* is the routine system of choice for our research, with much experimental work also carried out in *Nicotiana benthamiana*. Substantial effort has recently been devoted to the development of novel, more amenable model species for plant research, such as the duckweed Lemna spp or the liverwort *Marchantia polymorpha*, in which our Department has already made significant contributions. Crops such as tomato, potato and *Prunus* are also major subjects of our studies, to which knowledge generated in the model species is applied.

HEAD OF DEPARTMENT

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Aerial tubers formed in the axis of leaves of potato plants lacking the BRANCHED1B function (From Pilar Cubas' lab).

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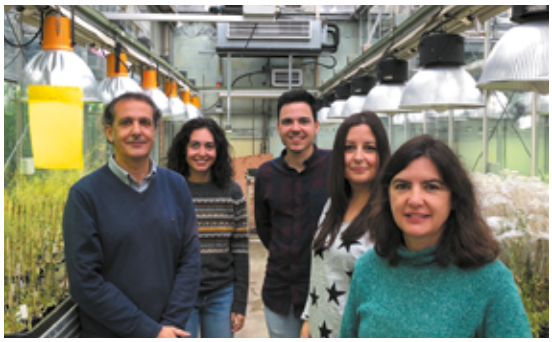

Natural variation of plant development

The main goal of our laboratory is to understand the genetic, molecular and evolutionary mechanisms involved in plant adaptation. In particular, we are interested in understanding how developmental traits, such as flowering time, vegetative growth, or trichome patterning, enable plant adaptation. To address this question we are exploiting the genetic variation that exists in nature within the wild, annual, and model plant *Arabidopsis thaliana*.

Given the relevance of climate change, our research is currently focused in identifying new genes and natural alleles that are involved in the adaptation to different climates. To this end, we are exploiting an *A. thaliana* regional collection of more than 400 wild accessions collected in the Iberian Peninsula (Montes *et al*., 2019; Castilla *et al*., 2020). The analysis of this collection for plant growth has identified an accession from Doñana National Park (Don-0) that is not able to grow at high temperature (Figure 1). Further genetic and molecular analyses identified *ICARUS2* as a new gene involved in adaptation to temperature seasonality. In addition, we are studying *A. thaliana* natural populations for other relevant traits, such as stomata density (Delgado *et al*., 2019), or microbiome composition (Thiergart *et al*., 2020).

Finally, in collaboration with Antonio Leyva's laboratory from the CNB, we are also studying the application of natural varieties of duckweed aquatic plants (*Spirodela polyrhiza* and *Lemna sp*) for water phytoremediation. In particular, our lab is currently involved in the project "Duckweed technology for improving nutrient management and resource efficiency in pig".

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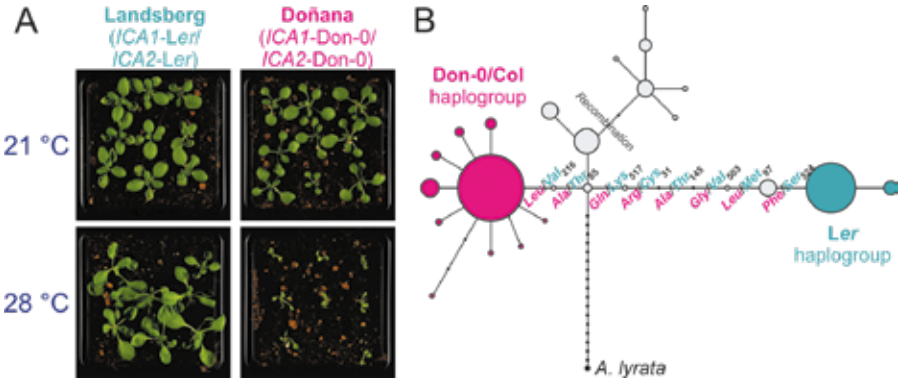

1 *ICARUS2 gene is essential for plant growth at high temperature. A) Growth phenotype of two* Arabidopsis *accessions, Landsberg and Doñana, at 21 and 28 °C. B) Genetic diversity (as haplotype network) of ICA2 proteins from worldwide accessions of* Arabidopsis thaliana *and* A. lyrata. *Each node corresponds to an amino acid substitution. Areas of nodes are proportional to frequency.*

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Thiergart T, Durán P, Ellis T, Vannier N, Garrido-Oter R, *et al.* Root microbiota assembly and adaptive differentiation among European Arabidopsis populations. Nat Ecol Evol 2020; 4: 122-131.

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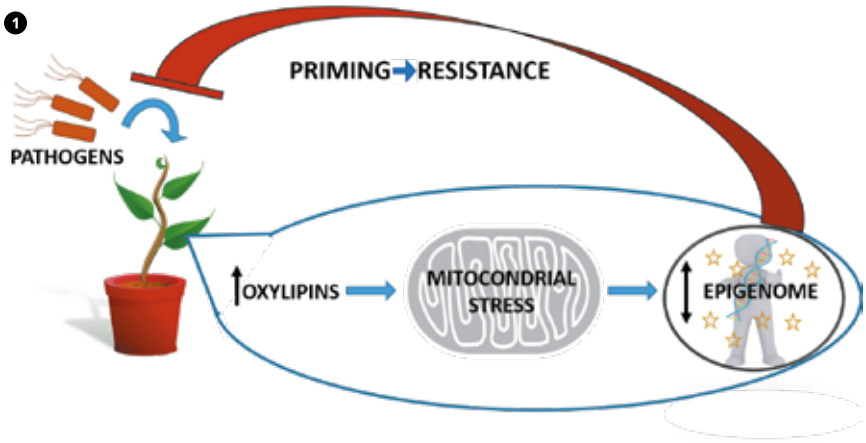
Plant immunity strategies against microbial pathogen infection

Plant pathogens cause diseases in many economically important crop plants, leading to severe losses in food production that are also of fundamental importance for forestry, other plant-derived products and for the sustainability of natural environments. This circumstance, together with an increasing world population, poses a severe threat to agriculture and plant sustainability. An important requirement for the development of successful plant disease control strategies is the understanding of host-pathogen interactions and, in particular, of the molecular mechanisms evolved in plants to avoid pathogen infection. This knowledge will be critical to devise effective approaches to minimise plant losses due to infection by microbes.

To this end, we focus our research on exploring the activities of oxylipins, a family of lipid derivatives activating immune responses in plants. Over the last years, our research has revealed that oxylipins, produced by the biosynthetic pathways initiated by fatty acid alpha-dioxygenases (alpha-DOXs) and 9-lipoxygenases (9-LOXs), contribute to the activation of local and systemic defence. In our studies, we showed that cellular organelles such as lipid droplets and mitochondria are important players during the response to pathogen infection and that global translational reprogramming contributes to activation of plant immunity. Moreover, we found that mitochondrial stress signals trigger the induction of epigenetic changes causing a primed state in which plants activate more effective immune responses leading into long-lasting resistance against different types of pathogens (a schematic representation of our working model is shown in Figure 1). Presently, we focus our research in examining these defence mechanisms and defining the signalling processes activating plant defence responses to control pathogen infection. The characterisation of the mentioned processes will contribute to define new mechanisms, signals, pathways, and genes involved in controlling plant immunity.



1 *Working model. Intracellular signaling process leading to plant defence and priming of immune responses. After a pathogen attack there is an intracellular increment in oxylipin production followed by disruption of mitochondrial function. The induced mitochondrial stress leads into epigenetic changes, which modify the inducibility of defence related genes, mediating a long-lasting resistance phenotype.*

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Genetic control of shoot branching patterns in plants

The control of branch outgrowth is critical for plant fitness, stress resilience and crop yield. We are studying the genetic basis of the control of axillary bud activity and dormancy in the model system Arabidopsis, and in the crop species tomato and potato in which the control of lateral shoot branching is of great agronomical interest. The *Arabidopsis thaliana* transcription factor BRANCHED1 (BRC1) plays a pivotal role in this process as it is a potent growth inhibitor that prevents axillary bud outgrowth in response to environmental conditions. We have combined ChIP-seq, transcriptomic and systems biology approaches to characterise the BRC1-regulated gene network. We have identified a group of BRC1 direct target genes encoding transcription factors (BTFs) that orchestrate, together with BRC1, an intricate transcriptional network enriched in abscisic acid signalling components.

We have also been studying a novel role of a potato *BRC1* gene. The control of carbon allocation, storage and usage is critical for plant growth and development and is exploited for both crop food production and CO_2 capture. Potato tubers are natural carbon reserves in the form of starch that have evolved to allow propagation and survival over winter. They form from stolons, below ground, where they are protected from cold temperatures and animal foraging. We have shown that *BRANCHED1b (BRC1b)* acts as a tuberisation repressor in aerial axillary buds, which prevents buds from competing in sink strength with stolons. *BRC1b* loss of function leads to ectopic production of aerial tubers and reduced underground tuberisation. In buds, *BRC1b* promotes dormancy, ABA signalling and downregulation of plasmodesmata gene expression. This limits sucrose unloading and access of the tuberigen factor SP6A to axillary buds. Moreover, BRC1b directly interacts with SP6A and blocks its tuber-forming activity in aerial nodes. Altogether these actions help promote tuberisation underground.

SELECTED PUBLICATIONS

Rameau C, Goormachtig S, Cardinale F, Bennett T, Cubas P (2019). Strigolactones as plant hormones. In Koltai H, Prandi C (eds) Strigolactones Biology and Applications. Springer, Cham.

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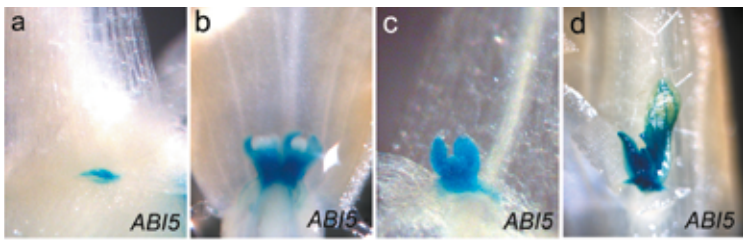

1 *Motifs overrepresented in the BRC1 network. Simplified representation of the BRC1 network, that exemplifies overrepresented motifs (multi-output Feed Forward Loops, regulated Feedback Loops and multi-ouput Feedback Loops) using specific cases. Orange and green circles represent genes encoding BRC1-dependent Transcription factors (BTFs, ATAF1, ABI5, ABF3, GBF2); blue circles, other bona fide BRC1 targets; grey circles, BRC1-dependent genes bound by BTFs but not by BRC1. Green and orange arrows indicate direct binding of the BTFs; red arrows, direct binding of BRC1.*

2 *The BTFs (BRC1-dependent Transcription factors) are expressed in axillary buds. ABI5 is a direct BRC1 target that, consistently, is expressed in axillary buds. GUS activity in axillary buds of transgenic lines carrying the construct ABI5pro:BETA-GLUCURONIDASE*

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Plant-pathogen-host interaction in viral infections

Plants are frequently infected in nature by viruses. Most of these infections are symptomless, or even give rise to mutualist associations, but plant viruses can also cause severe diseases. Breeding for resistance has been useful to fight some viral diseases, however, natural sources of resistance are scarce. The development of genetic engineering has expanded the available arsenal to generate virus-resistant plants. Understanding natural resistance mechanisms and viral amplification processes is essential to find appropriate targets for biotechnological antiviral strategies. Our research aims to contribute to meet this need. We are mainly interested in the family *Potyviridae*, especially in *Plum pox virus*, which causes sharka, a devasting disease of trees of the genus *Prunus*. In these two years we have paid attention to two viral functions that still have not been intensively studied, the proteolytic processing of viral polyproteins and the post-translational modifications (PTMs) of viral proteins. We have shown that the efficiency of the potyviral leader protease may be restricted to avoid that the uncontrolled release of the silencing suppressor HCpro triggers antiviral defences through complex hormonal and transcriptomic changes. We have also obtained data suggesting that alteration of the proteolytic cleavage between NIapro and VPg proteins is involved in the unique known escape of PPV from the HR-like resistance of some *Prunus domestica* cultivars. Regarding PTMs, our results have led us to propose that, whereas joint and opposite action of O-GlcNAcylation and phosphorylation at the N-terminal protrusion of the PPV capsid protein regulates the stability of this factor, phosphorylation at its core region controls assembly and disassembly of viral particles. Other remarkable results have been the finding that the sterol isomerase HYDRA1 restricts PPV infection and the demonstration that the viral genomic sequence is shaped by specific dinucleotide restrictions, so that an increase in UpA frequency causes a strong reduction of virus accumulation. *[Research supported by grants of the Spanish government BIO2016-80572-R and PID2019-109380RB-100 (IPs J.A. García and Carmen Simón) BIO2017-92613-EXP (IP C. Simón), and BIO2015-73900-JIN and PID2019-110979RB-100 (IP A. Valli)]*

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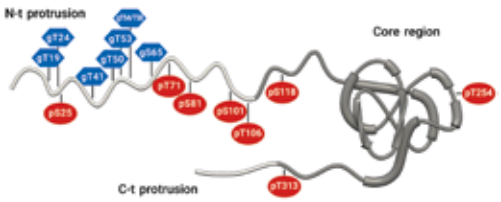

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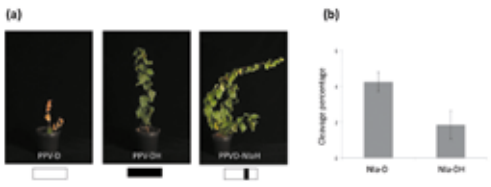

1 *Scheme of Plum pox virus CP and its post-translational modifications. Phosphorylated and O-GlcNAcylated residues are represented as red ellipses and blue hexagons, respectively.*

2 *Unraveling the mechanism of induction of hypersensitive response associated to resistance to Plum pox virus in European plums. (a) Resistant* Prunus domestica *trees inoculated with standard PPV-D isolate, the resistance-escaping isolate PPV-DH or a chimeric virus with the NIa sequence of PPV-DH in a PPV-D backbone. Schematic representation of each virus can be seen on the bottom of each picture. (b) In planta expression of NIa proteins from PPV-D and PPV-DH with a Myc tag to detect self-cleavage activity by western blot. Quantification of the cleavage percentage observed for each protease is shown in the panel.*

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Regulation of gene expression in plants

Plant plasticity during adaptation to the environment involves specific transcriptional signal-response networks that allow them to reprogram their growth and development. Regulation of these networks relies on sequence-specific transcription factors (TFs), regulatory proteins responsible for the transcriptional activation or repression of target genes.

Research in our group is focused in the study of the components that determine specific recognition of TF target genes and which may influence in the levels of gene expression. During the last few years we have contributed to the characterisation of one of these components, such as the short DNA sequences bound by TFs, known as TF-binding sites (TFBS). Despite TFBS sequence is the major factor determining target recognition, during the last two years we have explored the role of some other components involved in this process. With this regard, we have demonstrated that binding of some TFs extends beyond the TFBS core sequence, as some distant nucleotides, likely determining DNA-shape, are necessary for protein binding. We are also studying the role of the cytosine methylation epigenetic mark in the TFBS region during TF-target recognition, as well as its genetic control, what will allow adding a new layer of regulation of gene expression

In parallel to the experimental approaches, we are developing some easy-to-use bioinformatic tools useful for the interpretation of transcriptional data and for the prediction of TFBS involved in the regulation of biological processes. These tools would contribute to a better and faster interpretation of biological data for the plant biology community, particularly in the case of non-expert researchers in bioinformatics or in the study of non-model species.

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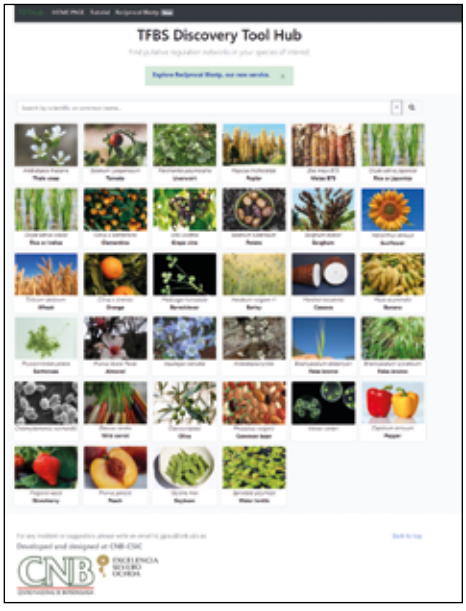

❶ *Selection of bHLH transcription factors target genes in plants. Specific binding of MYC bHLH to targets depends on the recognition of the G-box and of some nucleotides distantly located contributing to confer a particular shape to DNA. This 'double check' mechanism is conserved throughout the plant phylogeny and determines the specification of targets.*

❷ *A web-based tool for the identification of transcription factor binding sites in plants.*

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Mechanisms underlying nutrient uptake and phytoremediation

SELECTED PUBLICATIONS

Mateo C, Navarro M, Navarro C, Leyva A. Arsenic phytoremediation: Finally, a feasible approach in the near future (2019). In Environmental Chemistry and Recent Pollution Control Approaches, ed. Hugo Saldarriaga-Noreña. IntechOpen, London.

Plants have an extraordinary capacity to capture large quantities of nutrients and toxic compounds including heavy metals and arsenic. Arsenic can enter into the food chain through water consumption or crops (particularly rice) and therefore is considered a silent threat to public health.

For the last two years we kept working on the characterisation of the molecular mechanisms involved in arsenic perception and detoxification. Recently we finished the characterisation of a ubiquitination complex involved in the degradation of the transcriptional activator of the arsenate/phosphate transporter (*Navarro et al., under review in Molecular Plant;* Figure 1). We also followed different approaches to identify the key transcriptional activator of the arsenic responses using genetic and in silico strategies. In this context, we identified several transcription factors involved in the regulation of the arsenic response (Figure 2). In parallel we screened an Arabidopsis collection of Iberian natural accessions for arsenic tolerance and performed a Genome-wide association study, identifying several candidate genes.

In the last two years, we also performed a study of the natural variation of arsenic accumulation in duckweed, a hyperaccumulator aquatic plant with tremendous phytoremediation potential. To this end we obtained a new collection of duckweed natural accessions in collaboration with Carlos Alonso-Blanco at the CNB. Furthermore, we just finished a European project funded by the LIFE programme that aimed to use duckweed to extract nitrogen and phosphate form pig slurry in order to be used as a fertilizer (LIFE 15 ENV/ES/000382).

In the near future we aim to study Arabidopsis natural variation of the ionome in relation with arsenic response to understand the interconnected regulatory networks between arsenic and mineral nutrients. The idea will be to identify new mechanisms underlying metal and arsenic extraction in order to improve bio-fortification and phytoremediation capacity in plants.

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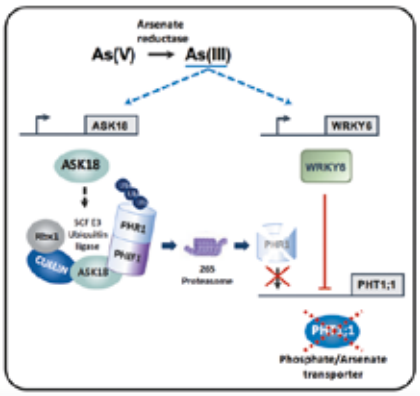

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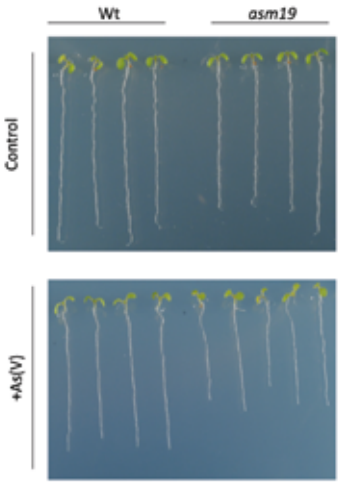

1 *Proposed model for the control of As(V) uptake in* Arabidopsis *roots. As(V) is transported inside the cell by the Pi transporter PHT1;1. As(V) is then rapidly reduced to As(III) by the action of the arsenate reductase ARQ1. As(III) signalling modulates the major regulators of PHT1;1 by inducing the transcription of WRKY6 (PHT1;1 repressor) and ASK18 a component of the SCF complex that interacts with the F-box protein PHIF1. PHIF1 targets the PHR1 (the PHT1;1 activator) for protein degradation. As a result of these coordinated events, PHT1;1 expression is repressed and As(V) uptake is reduced.*

2 *As(V) sensitive phenotype of wild-type and asm19 mutant. Plants were grown in horizontal plates containing 10 µM Pi alone (control) or in combination with 15 µM As(V) for 8 days.*

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Regulation of gene activity in plants. The Phosphate starvation rescue system

We focus our study on the plant phosphate (Pi) starvation rescue system, which consists of an array of developmental, physiological and molecular responses that allow plants to cope with growth under Pi limiting conditions. This rescue system is a suitable model for studies on regulation of gene activity, and in addition, recently it has attracted considerable interest due to its potential to help design plants with increased Pi acquisition and use efficiency, a necessary requirement to implement low-input sustainable agricultural practices. In the past two years, our main activity has been to exploit natural variation to identify QTLs controlling transcription of Pi starvation genes and affecting Pi acquisition and use efficiency. Our transcriptomic analysis of recombinant inbred lines and natural accessions allowed the identification of a large set of transcription factors controlling expression of Pi starvation responsive genes (Figure 1). And the use of GWAS approaches have uncovered candidate genes affecting growth under Pi limiting conditions (Figure 2), whose characterisation is underway.

Additionally, we have examined the dynamics of interchromatin interactions in response to Pi starvation using Hi-C related approaches. We found no large effects of Pi starvation on chromatin interactions, but observed that genes induced by Pi starvation (PSI) tend to display increased chromatin interconnections among themselves, indicating a constitutive predisposition for coordinated PSI gene expression

Finally, we have also initiated an study of extrachromosomal circular DNA formation in response to Pi starvation. It is presently well established that eccDNA formation is a widespread characteristic of eukaryotes, where eccDNAs are originated from thousands of locations of their genomes. We have examined eccDNA formation during Pi starvation and in line with expectations more than 1500 eccDNA have been identified, out of which a 3% appear to be Pi starvation specific. We are presently studying their biogenesis and their potential functional significance

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Arabidopsis ALIX regulates stomatal aperture and turnover of abscisic acid receptors. García-León M, Cuyas L, El-Moneim DA, Rodriguez L, Belda-Palazón B, *et al.* Plant Cell 2019; 10: 2411-2429.

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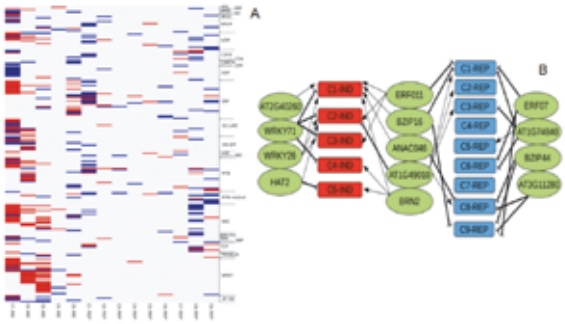

2

1 *The Pi starvation co-expression network in* Arabidopsis. *The network was constructed with the WGCNA program using transcriptomic data from a collection of 100 RIL lines. It consists of 5 and 9 co-expression clusters of Pi starvation induced (C-X-IND) and Pi starvation repressed clusters (C-Y-REP). A) Heat map of TFs whose targets are enriched and display positive (red) or negative (blue) correlation with genes in the indicated co-expression clusters. B) Image showing examples of TFs potentially upregulating (displaying positive correlation) and/or downregulating (displaying negative correlation) Pi starvation responsive clusters*

2 *GWAS for root and shoot growth. Images (A)-(D) are Manhattan plots for root (A,B) and shoot (C,D) biomass accumulation after growth for 12 days in low Pi (A, C) and control conditions (B,D). Significance thresholds of -log(P) = 4 or 8 (the first corresponding to that published for potential associations and the latter corresponding to 5% with Bonferroni correction for multiple testing) are shown as dashed horizontal lines.*

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Environmental control of plant growth

Progressive rise in temperature due to global warming negatively impacts on crops productivity and affects wild taxa phenology, interfering with adaptation to their local environment. In Arabidopsis, warm temperatures promote elongation of seedlings hypocotyl and petioles in a thermomorphogenic response addressed to cool the leaves and protect the shoot meristem from the warm soil. Phenotypic analyses of this output unveiled that the red/far red light phytochrome photoreceptors act as main thermosensors, increased temperatures being shown to accelerate bioactive Pfr reversion into the inactive Pr form. Downstream of phyB, the PIF4 factor modulates temperature induced cell elongation by activating auxin and brassinosteroid biosynthesis, and the expression of cell-wall loosening enzymes required for cell expansion. Elevated temperatures cause up-regulated PIF4 expression at night, by impairing function of the circadian clock "evening complex"(EC) loop, consisting of EARLY FLOWERING 3 (ELF3), ELF4 and the LUX ARRHYTMO (LUX) DNA-binding protein. They induce as well nuclear accumulation of the E3 ligase COP1, shown to promote seedlings etiolation by targeting proteasomal degradation of many PIF4-antagonising factors. However, how these signalling events converge to thermal elongation is not well understood. To gain insight on the thermal role of these main signalling hubs, we have measured hypocotyl lengths of different combinations of mutant/over-expression lines grown at 22ºC and 28ºC and variable day length conditions and fitted the hypocotyl growth data into a mathematical model build on the described interactions for these regulators. Notably, the adjusted model fully reproduced thermal elongation of the studied genetic backgrounds and correctly predicted the thermal response of novel genotypes, therefore showing that thermal regulation of phyB, ELF3/EC and COP1 is sufficient to fully explain thermormorphogenic growth of Arabidopsis seedlings. Moreover, the model underscored a main temperature signaling function of the E3 ligase COP1, that acted independently of its inactivation by phyB, and which we validated experimentally. COP1 was shown to act though this thermal signaling activity as a main input for temperature entrainment of the clock, our current research efforts being addressed to the molecular understanding of this entrainment mechanism. Main focus of research in our team is thus directed to:

- Study the role of BR signaling and the master regulator BIN2 kinase in the control of COP1 nuclear shuttling.
- Characterise the cellular mechanisms underlying temperature-induced nuclear COP1 accumulation.
- Test the possible role of ELF3-COP1 interaction in modulating each other's function.
- Gain a better understanding on how this temperature signaling network affects circadian clock function and response of Arabidopsis plants to combined heat and drought stresses.

Overall, results from this research will identify best loci for increased tolerance to heat and drought stress as influenced by day length, and therefore guide smart breeding of seasonal crops for increased resilience to climate change.

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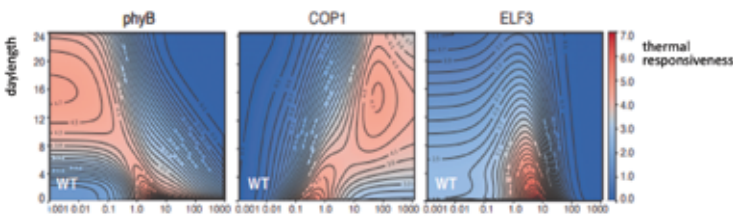

1 *Mathematical modelling estimation of the contribution of phyB, COP1, and ELF3/EC activities to termal elongation as a function of day length (collaboration with Saúl Ares and Pablo Catalán).*

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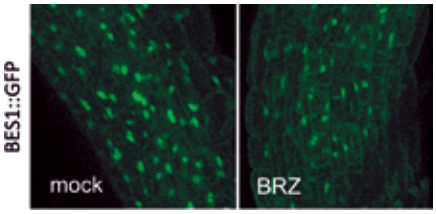

2 *Differential nucleocytoplasmic localisation of the BES1 factor in response to the BR biosynthesis inhibitor brassinazole.*

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Signalling networks in plant development and defense responses

Our group studies how plants adjust their growth and development to challenges from pests and pathogens.

These are some of the questions we are currently addressing:

1) What mechanisms initiate stem cell differentiation in plants and how are they regulated by biotic stresses to modulate organ growth rates? Our working hypothesis is that nuclear migration of the Arabidopsis proteins IYO and RIMA functions as a switch to reprogram the transcriptome and trigger stem cell differentiation in plants. We are studying how IYO/RIMA nuclear localisation and activity is controlled by developmental and biotic cues to control plant growth.

2) What are the roles of vacuoles in plant development and defense? Through a genetic screen, we are characterising genes involved in transport to, and biogenesis of, plant vacuoles, and studying how interfering with their function affects growth and resistance to pests and pathogens.

3) Do non-vascular plants activate systemic defenses against herbivores? When herbivores damage tissues of higher plants, wound signals are transmitted through the vasculature to activate systemic defenses in undamaged tissues. Our studies could provide important clues on the development of systemic signalling systems during the evolution of land plants.

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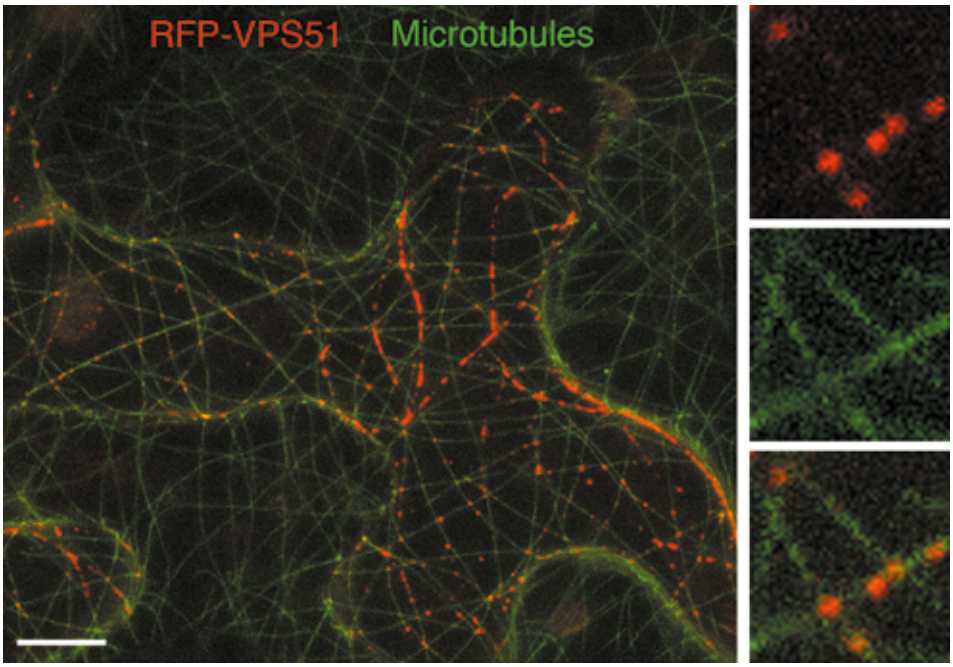

1 *VPS51 localises to Microtubule-Associated Compartments. Max. intensity projection of serial confocal images (depth: 10 µm) of Nicotiana benthamiana epidermal cells co-transformed with pUBI:RFP-VPS51 and the microtubule marker GFP-MAP4. Scale bar: 10 µm.*

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Role of ubiquitin in the control of plant growth and stress tolerance

The relevance of protein ubiquitination as an integral mechanism of many signaling pathways in plants has been demonstrated extensively. Ubiquitin (Ub) conjugation to proteins (i.e. ubiquitination) may trigger degradation of protein targets at the 26S proteasome or changes in their properties (e.g., protein activity, localisation, assembly and interaction ability), depending on the extent or specific Ub chain configurations. Protein ubiquitination is mediated by an enzymatic cascade in which different types of E3 Ub ligases provide the substrate specificity. Among them, Cullin4 RING E3 ubiquitin ligases (CRL4) have been involved in biological processes spanning the plant's whole life, including embryogenesis, seedling photomorphogenesis, circadian clock function, flowering and tolerance to different stresses (i.e. drought, high salinity, cold, osmotic stress) by promoting degradation of specific targets controlling those processes (Fig. 1). As an example, we have recently shown that DDA1, a substrate adaptor of CRL4-CDDD complexes, recognises abscisic acid (ABA) receptors, triggering their ubiquitination and proteasomal degradation (Irigoyen *et al*, The Plant Cell 2014). Therefore, CRL4-CDDD complexes act as repressors of ABA-mediated water stress responses under optimal growth conditions. Interestingly, CRL4-CDDD function is performed in close proximity to chromatin, which should enable rapid translation of environmental and stress signals into changes in gene expression. Indeed, recent results from our laboratory showed that CRL4-CDDD complexes are part of a molecular pathway controlling epigenetic homeostasis (including Histone2B ubiquitination) in response to external stimuli (i.e. light conditions; Nassrallah *et al*, eLife 2018). Our current objectives aim to identify and characterise additional mechanisms by which CRL4-CDDD controls the accumulation of specific epigenetic marks over the plant genome in response to environmental changes, to regulate expression of specific set of genes that lead to plant adaptation to changing climate conditions.

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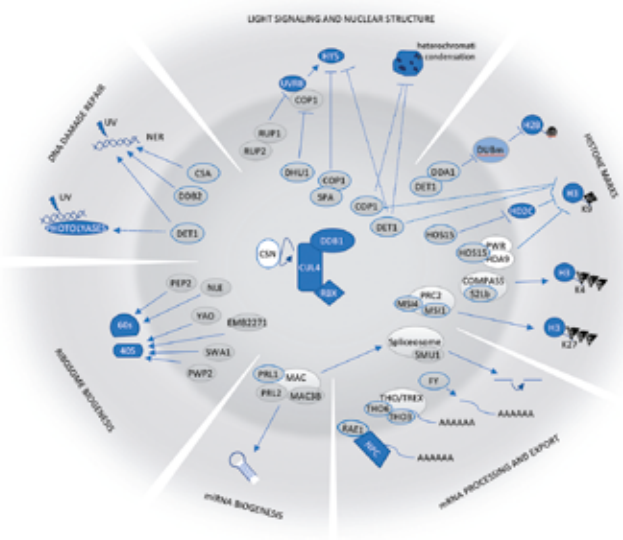

1 *Schematic representation of the chromatin functions in which* Arabidopsis *CRL4-CDDD and DWD-containing proteins play a role (adapted from Fonseca and Rubio, Front Plant Sci 2019)*

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Jasmonate signalling and plant defense

Jasmonates (JAs) are fatty acid-derived signalling molecules that are essential for the survival of plants in nature, since they are important activators of stress responses and developmental programs. The main focus of my lab is to understand the biological mechanims that govern the JA signalling pathway in plants; knowledge that is crucial to design biotech and agronomical applications that improve plant resistance to stresses and plant yield. We have traditionally worked in the model plant *Arabidopsis thaliana*, but have recently focused in the liverwort *Marchantia polymorpha* due to its remarkable genetic advantages, such as very low gene redundancy.

Our major achievements in the last two years are:

- Identification of a new pathway for thermotolerance in plants (Monte *et al.*, *Curr Biol*, 2020)
- Identification of CUL3^{BPM} E3 ubiquitin ligases that regulate MYC transcription factors stability and JA responses (Chico *et al.*, *PNAS*, 2020).
- Characterisation of conserved basal defence mechanisms in land plants (Gimenez-Ibanez *et al.*, *Curr Biol*, 2019).
- Design and obtention of a tomato resistant to bacterial speck by CRISPR/Cas9-based mutation of *SlJAZ2* (Ortigosa *et al.*, *Plant Biotechnology Journal*, 2019).
- Characterisation of MYC2 orthologs in *Marchantia polymorpha* (Peñuelas *et al.*, *The Plant Cell*, 2019).
- Characterisation of the single JAZ repressor in *Marchantia polymorpha* (Monte *et al., Mol. Plant,* 2019).
- Identification of a new function of MYCs in photomorphogenesis (Ortigosa *et al.*, *Plant J*, 2020).
- Collaborated in the characterisation of PIF transcription factors in reproductive development (Costa Galvão *et al., Nat Commun,* 2019).
- Discovery of bioactive hydroxylated derivatives of JA-Ile (Jimenez-Aleman *et al.*, *Biochim Biophys Acta Mol Cell Biol Lipids* 2019).
- Identified the DNA target sequence of many plant transcription factors using previously developed tools and in collaboration with several groups (Ramírez Gonzales *et al.*, *Plant J*, 2020).
- Collaborated in the integrated multi-omics analysis of the plant response to jasmonic acid (Zander *et al.*, *Nat Plants*, 2020)

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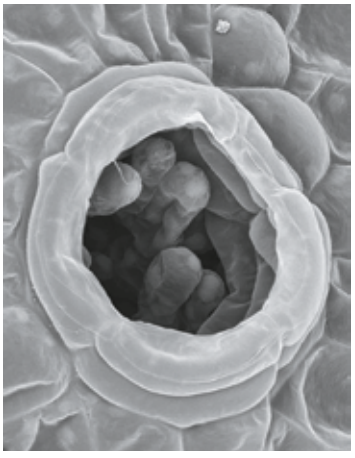

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❶ *SEM image of epidermal air pore of* Marchantia polymorpha

❷ *Archegoniophores of* Marchantia polymorpha