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MEETING REPORT

Meeting report – INDEPTH kick-off meeting

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ABSTRACT

The precise location of chromatin domains within the cell nucleus has seen growing recognition in the past decade as an additional mechanism of controlling gene expression in both plants and animals (Dekker et al., 2017). Consequently, international efforts are devoted to understanding the organising principle of this organelle in plants, and notably the nature and the role of functional compartments on gene expression (Graumann et al., 2013; Sotelo-Silveira et al., 2018). The European cooperation 'Impact of Nuclear Domains on Gene Expression and Plant Traits' (INDEPTH) brings together molecular cell biologists, plant physiologists, bioinformaticians, image analysts and computer scientists. They aim to address the question of how nuclear architecture, chromatin organisation and gene expression are connected in plants, particularly in relation to traits of interest such as biomass, reproduction and resistance to pathogens (https://www.brookes.ac.uk/indepth/). The kick-off meeting of the INDEPTH consortium took place in Clermont-Ferrand, France, on 12–14th March 2018, where more than 80 researchers set the agenda for the coming four years of research and collaboration.

The kick-off meeting of the INDEPTH consortium revolved around an exciting overall theme of an increased appreciation about how gene expression can be affected by the sequestering of loci within different nuclear compartments. The meeting showed promising technological advances that will overcome challenges particular to plant tissues both in the detection of single-locus positions and in dedicated image analysis. Another emerging theme is the development of new approaches that will combine information gained from microscopic images that provide single-cell resolution with the information that is gained from conformation-capture techniques that represent an average view of multiple cells in a tissue. Together with the characterisation of nuclear structures that are specific to plants, these techniques will allow this community to gain insight into the plant-specific mechanisms of gene expression control and to compare and contrast these with the mechanisms identified in yeast and mammalian model organisms.

Organisation of the INDEPTH consortium

A key component of the INDEPTH COST Action (Box 1) is its organisation into five complementary workgroups (Fig. 1), whose activities were introduced at the kick-off meeting. Three of the workgroups focus on evaluating plant chromatin domains at different scales, from imaging nuclear domains (workgroup 1) through analysis of the function of chromatin domains in controlling gene expression (workgroup 2) to assessing their effect on plant phenotypes and their dynamics during stresses (workgroup 3). Workgroup 4 is involved in tackling the challenge of storage and sharing of 'omics'-based and image data, something that has relevance to a much wider community of researchers beyond the INDEPTH consortium, and workgroup 5 organises training and dissemination of INDEPTH outputs from each of the other four workgroups.

The origins and aims of INDEPTH

The INDEPTH grant officially started in December 2017 but its origin can be traced back to the formation of the International Plant Nucleus Consortium (IPNC) at Oxford Brookes University in 2011 (https://www.brookes.ac.uk/bms/research/groups/molecular-cell-and-developmental-biology/plant-cell-biology/plant-nuclear-envelope/ipnc/). David Evans (Oxford Brookes University, UK) was a driving force in the establishment of the IPNC, which comprised many members of the current INDEPTH consortium, including the chair Christophe Tatout (Université Clermont Auvergne, France). David Evans provided a keynote plenary at this meeting and highlighted the accomplishments of the IPNC, which included the characterisation of protein components of the plant nuclear envelope, including the components of the linkers of the nucleoskeleton to the cytoskeleton (LINC) complex (Meier et al., 2017). These proteins span the nuclear envelope, thereby acting as a potential signalling module between the nucleus and the cytoplasm. Although these proteins have been extensively studied in animals, most had not been characterised in plants. David Evans ended his talk with a provocative set of questions in order to challenge delegates to tackle important areas that have not yet been addressed in plants: currently, the community is only starting to establish a 3D-atlas of chromatin domains and has little understanding of the proteins that take on the equivalent function to that of the animal lamina, which is a protein meshwork underneath the nuclear membrane that interacts with chromatin. In addition, how does nuclear shape affect gene expression, and how does signalling occur across the nuclear envelope? How does the nuclear periphery interact with chromatin domains to influence gene expression in plants?

Daniel Schubert (Freie Universität Berlin, Germany) opened the kick-off meeting and, as if to foreshadow David Evans' challenges, his laboratory is beginning to investigate the relationship between the nuclear periphery and chromatin domains, through an analysis of Polycomb group proteins (PcG). They have demonstrated that the PWWP-DOMAIN INTERACTOR OF POLYCOMBS1 (PWO1) protein interacts with the Polycomb Repressive Complex 2 (PRC2) (Hohenstatt et al., 2018). They have followed up this work by showing that PWO1 localises to nuclear speckles and interacts with several nuclear periphery-localised proteins, including members of the CROWDED NUCLEUS (CRWN) family that might perform a similar role to that of lamins in metazoa. The findings from the Schubert laboratory provide a potential mechanism that links the

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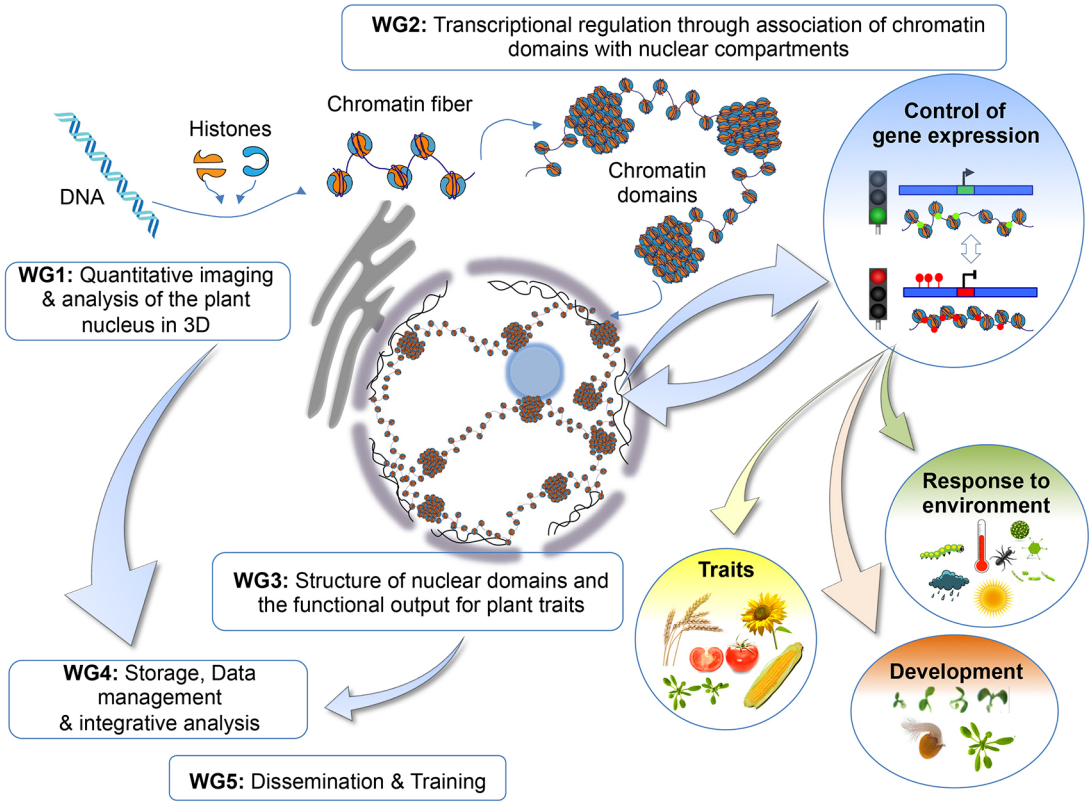

Fig. 1. The INDEPTH workgroups and their meeting topics. DNA in eukaryotic organisms is organised into chromatin, which plays a critical role in regulating genome function by packaging and compartmentalising DNA, and controlling access of the cellular machinery to the DNA. Within the 3D nuclear space, chromatin fibres form higher-order chromatin domains and adopt specific positions relative to different subnuclear compartments, such as the nuclear envelope and its nuclear pore complexes, Polycomb bodies and the nucleolus. However, little is known how nuclear architecture contributes to regulating gene function in plants and ultimately how it influences different traits, development and the response to the environment. The INDEPTH consortium is chaired by Christophe Tatout (Université Clermont Auvergne, France) and Célia Baroux (University of Zürich, Switzerland) and is organised in five complementary workgroups (WGs), led, respectively, by Katja Graumann (Oxford Brookes University, UK) and Dimiter Prodanov (University of Leuven, Belgium) (WG1), Stefanie Rosa (Swedish University of Agricultural Sciences, Sweden) and Sara Farrona (University of Galway, Ireland) (WG2), Ales Pecinka (Institute of Experimental Botany, Czech Republic) and Monica Pradillo (Complutense University of Madrid, Spain) (WG3), Björn Grüning (University of Freiburg, Germany) and Stefan Grob (University of Zürich, Switzerland) (WG4), and Geraint Parry (Cardiff University, UK) and Aline Probst (Université Clermont Auvergne, France) (WG5).

nuclear periphery with epigenetic control of certain loci (Mikulski et al., 2017 preprint).

Workgroup 1 – imaging the plant nucleus

Workgroup 1, is entitled 'Quantitative imaging and analysis of the plant nucleus in 3D' and its meeting session was split between cell biologists and image analysts. Tao Dumur, a PhD student from the laboratory of Ortrun Mittelsten Scheid (GMI, Vienna, Austria), presented his live-imaging setup in which he uses spinning-disc confocal microscopy to follow nuclear organisation in living root cells. He showed that nuclear shape and heterochromatin organisation in chromocentres can change independently of each other. Furthermore, Till Bey, who works with Paul Fransz at the University of Amsterdam, Netherlands, combined 4′,6-diamidino-2-phenylindole (DAPI) staining, to evaluate chromatin density, with immunolabelling to reveal RNA polymerase II localisation within the nucleus. The latter allowed a more precise assessment of functional compartmentalisation of both euchromatin and heterochromatin. Susan Duncan (Earlham Institute, Norwich, UK) discussed her recently published method of single-molecule RNA labelling (Rosa et al., 2016) and outlined how she intends to use it in the future in both *Arabidopsis* and wheat. Owing to the challenges of introducing probes into many plant tissues, the use of reliable and reproducible technologies that will allow visualisation of not only transcripts, but also the position of single-copy genes within the 3D space of the nucleus, is still missing from the field. Indeed, how these challenges might be solved was a reoccurring theme during the discussions.

Dimiter Prodanov (University of Leuven, Belgium), co-leader of workgroup 1 with expertise in image analysis, posed the important question as to whether the process of image segmentation is in fact art or science. To many of the biologists in attendance, the complexity of the analysis that is required to gain maximum insight from a set of images might have been somewhat surprising. Both Dimiter Prodanov and Zikrijia Avdagic (University of Sarajevo, Bosnia and Herzegovina) presented complex workflows that describe how machine learning facilitates the analysis of segmented 3D images.

This workshop also included a set of outstanding talks from early-career researchers. Masters student Zofia Parteka (University of Warsaw, Poland) described the construction of 3D models of chromatin loops from high-resolution microscopy images through photo-activated localisation microscopy (PALM). She then compared these models to those that are derived from chromosome conformation capture (Hi-C) and chromatin interaction analysis by paired-end tag sequencing (ChIA-PET) technologies that reveal chromatin contacts. Ultimately, modelling chromatin domains can lead to a fuller understanding of how they respond to changes in gene expression brought about by different environmental conditions.

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Box 1. EU COST Action and INDEPTH

The Cooperation in Science and Technology (COST; http://www.cost.eu/) association is Europe's longest-running intergovernmental framework; it promotes networking and provides collaborative grants for scientific training and meetings. A key component of COST Actions is to offer support for researchers from Inclusiveness Target Countries (ITCs) and Near Neighbour Countries (NNCs). INDEPTH is the first network that is devoted to investigating the functional role of nuclear compartments in plants. By using model and crop species, members of the INDEPTH consortium aim to decipher how the spatial (3D) organisation of the genome impacts gene expression, and ultimately plant development and traits, as well as the response of the plant to the environment (Fig. 1). To achieve these goals, members of the INDEPTH consortium employ a range of state-of-the art molecular, biochemical and microscopy imaging approaches. Within INDEPTH, researchers also have ambitions to move beyond a (necessary) description of plant nuclear organisation. A core motivation of the consortium is to elucidate how nuclear organisation dynamics are linked to cellular responses in plants following developmental and environmental cues. A key component of any COST programme includes provision for training of early career investigators (ECIs). INDEPTH supports short-term scientific missions (STSMs) that allow researchers to travel to laboratories in a different country for up to 3 months to learn a new experimental technique. INDEPTH has the ability to support up to 30 STSMs during the 4 years of the grant. COST actions are open projects. Any researcher interested in joining the INDEPTH COST Action can contact the chair at christophe.tatout@uca.fr.

Importantly, this type of modelling analysis is well suited for collaboration between researchers who work with different model systems. As Zofia Parteka is part of the multi-disciplinary laboratory of Dariusz Plewczynski, hopefully, the experimental approaches the group uses to analyse chromatin structure in other organisms and their computational tools for the analysis of next-generation sequencing data (Al Bkhetan and Plewczynski, 2018) will benefit INDEPTH plant scientists.

Christophe Tatout (Université Clermont Auvergne, France) gave the final talk in the workgroup 1 session and outlined his work that focuses on developing NucleusJ, an ImageJ plugin for 3D analysis of nucleus images; it is now being improved and adapted to perform high-throughput 3D image analysis (Poulet et al., 2017) of different nuclear parameters, such as size and shape of the nucleus, and the position of heterochromatin structures within in the nucleus in whole-mount tissues. Efficient phenotyping tools are indispensable assets to evaluate the impact of loss of specific nuclear components, such as components of the nuclear periphery, on, for example, nuclear organisation and function.

Workgroup 2 – investigating the role of chromatin domains in transcriptional regulation

Workgroup 2 ('Transcriptional regulation of chromatin domains through association with nuclear compartments') is arguably the workgroup with the greatest overlap of interest for the majority of meeting delegates. Chang Liu (University of Tübingen, Germany) showed how he compared results obtained with a restriction enzyme (RE)-mediated ChIP protocol and Hi-C data to obtain a better understanding of chromatin organisation at the nuclear periphery (Bi et al., 2017). He further showed unpublished data that was obtained using different fluorescence *in situ* hybridisation (FISH) probes to show altered chromatin organisation in plants lacking CRWN components of the nuclear lamina. Chang Liu also demonstrated that plant-specific non-CG DNA methylation, but not H3K9me2 epigenetic marks, are required for tethering chromatin at the lamina, which is different to the situation in metazoans. This confirms previous findings that the biology of the plant nuclear periphery is different from other systems, such as yeast and mammals. Specifically, his findings indicate that *crwn* mutant nuclei do not only have smaller nuclei, but that the overall distribution of chromatin is affected within these more tightly packed organelles. Fully understanding how the nuclear periphery influences gene expression through differential arrangement of chromatin domains is a key aim of INDEPTH-based collaborations.

Another functional compartment within the nucleus is the nucleolus, which has emerged as a central organiser of part of the genome. Frédéric Pontvianne (University of Perpignan, France) showed that genes associated with nucleolus-associated domains (NADs) have lower expression levels and, therefore, that the NAD region might act as a structural gene-silencing component (Pontvianne et al., 2016). As a prelude to the collaborations that might be fostered by INDEPTH, future work of the Pontvianne group in collaboration with Chang Liu will use Hi-C approaches with the aim to provide further insight into the functional relevance of these interactions.

The closing talk of the workgroup 2 session came from Stefan Grob (University of Zürich, Switzerland), who also used Hi-C to investigate interactions between *Arabidopsis* chromatin domains. He has serendipitously discovered a potential mechanism for the silencing of antibiotic resistance in T-DNA mutant collection insertion lines. Silencing of T-DNA antibiotic resistance has puzzled and frustrated *Arabidopsis* researchers for many years, and Stefan Grob has now discovered that T-DNA integrations can lead to perturbation of the 3D properties of the integration site, which is linked to a higher chance of silencing, and that this effect is trans-generational. By testing 100 lines with random T-DNA insertions, he showed that transgene insertion within a single chromosome – whether it is in euchromatin or heterochromatin – was not linked to the extent of silencing of antibiotic resistance genes. Previously, the location of transgene insertion was thought to be a primary determinant of silencing, so this exciting finding will be of interest to the entire plant science community.

Workgroup 3 – linking cell biology with phenotype

Workgroup 3 is called 'Structure of nuclear domains and the functional output for plant traits' and primarily aims to link cell biology to plant phenotype. Together, members of workgroup 3 use over ten different plant species that have diverse genome sizes and modes of chromatin organisation.

Endoreduplication commonly occurs in plants and is a key process that controls genome size and gene expression, although this latter response is less well characterised. Christian Chevalier (INRA Bordeaux, France) provided an overview of his work in analysing the role of endoreduplication in tomato fruit. They used FISH to develop a ploidy map of the tomato fruit where, extraordinarily, cells can undergo up to nine rounds of DNA replication without subsequent mitosis! Indeed, their recent work has demonstrated that cells with higher ploidy levels not only have increased overall gene expression, but that specific loci also show differential expression patterns that depend on ploidy level and location within the fruit (Pirrello et al., 2018).

An important theme within this workgroup includes analysis of the factors that control the mechanisms of meiosis and subsequent rates of recombination. Understanding these mechanisms is key for improved future breeding strategies, and so are relevant for both model and crops researchers. Nadia Fernandez works with Monica Pradillo at Universidad Complutense de Madrid, Spain, and

presented preliminary data from her PhD that makes use of *Arabidopsis* to investigate the function of nuclear pore proteins (NUP)160 and NUP96 during meiosis and DNA repair. Her work is already providing novel insights into how nuclear pore proteins might contribute to cellular processes other than nuclear transport.

Offering a crop perspective, Isabelle Colas (James Hutton Institute, Dundee, UK) showed how 3D structured illumination microscopy (SIM) imaging has revealed novel features of chromatin organisation in meiotic chromosomes in barley that do not occur in *Arabidopsis* (Colas et al., 2017). She is using barley mutants and temperature variations to define the genetic and environmental factors that control both crossover frequency and location during meiosis, with the ultimate aim of providing improved insights for barley breeders. This provided an excellent example of how complex microscopy imaging has the potential of a tangible impact for plant breeders and thus perfectly encapsulates the overall aim of workgroup 3.

Workgroup 4 – dealing with image and 'omics'-based data

Workgroup 4 is entitled 'Storage, data management and integrative analysis' and is the smallest workgroup of the consortium. The increasing size of data from imaging approaches presents a more significant challenge than the storage of other 'omics'-based data. Hence, this workgroup will investigate the possibility of how existing repositories can be used to store data from consortium participants as well as creating fully accessible and searchable metadata. The workgroup leader, Björn Grüning (University of Freiburg, Germany), introduced the Galaxy platform, an open web-based platform for accessible, reproducible and transparent computational research, and discussed how it facilitates data reproducibility, integration, management and visualisation, alongside the provision and development of tools for data analysis that can be used by both bioinformaticians and biologists (https://galaxyproject.org/). The Galaxy project also provides both extensive training, as well as a remote compute resource that users can access and utilise for the analysis of complex data sets. Whereas the pan-European Galaxy resource has been primarily set up for 'omics'-based datasets, the members of workgroup 4 will use their expertise to investigate similar methods for the sharing of imaging data. This will include establishing principles for submission of different image types and consistent metadata. One potential option for management of image data from the INDEPTH consortium is to interact with the open microscopy environment remote objects (OMERO) platform, which offers bespoke image storage solutions (Linkert et al., 2010). So far, OMERO is designed for storage, and, in the future, image analysis might be performed using Galaxy, as some of their servers already include ImageJ and Fiji tools, two popular image analysis tools. This opens the possibility to perform interdisciplinary analysis, such as 'omics'-based approaches and imaging, using the same platform. As a complementary avenue to the use of OMERO, Rémy Malgouyres (Université Clermont Auvergne, France) is developing an interoperability platform for image storage and accessibility through a bottom-up approach as a 'proof of concept' within the INDEPTH consortium. Furthermore, Giorgio Papadopoulos (University of Montpellier, France) discussed how he is using machine-learning modelling approaches to link epigenetic, transcriptional and architectural profiles during biological processes, such as erythroid lineage specification, to predict functional signatures (Papadopoulos et al., 2013). Finally, PhD student Michal Kadlof from Dariusz Plewczynski's laboratory in Warsaw, Poland, illustrated how he is translating Hi-C probabilities into distances by using molecular mechanics. This is a first step toward a 'magic box' that could turn genomic, epigenomic and microscope experimental data into functional models of single loops, domains and, eventually, the entire nucleus.

Conclusions

This kick-off meeting was a fantastic introduction to the research areas of the different workgroups, and was characterised by the sharing of unpublished information, the establishment of new collaborative prospects and the opportunity for younger researchers to experience an international meeting.

A key theme that emerged across the meeting was that some chromatin domains interact with the nuclear periphery and correspond to functional compartments with distinct transcriptional properties when compared to the rest of the genome. In animal models, sequestering genetic loci to the nuclear periphery is a well-established mode of both positive and negative gene regulation. However, the magnitude and significance of this effect in plants is mostly unknown. Arguably, the major challenge over the following four years for INDEPTH participants is to elucidate the causal relationship between functional chromatin compartments and gene expression: do chromatin domains – particularly those anchored at the periphery – regulate gene expression, or do they provide a nuclear environment securing a robust gene expression state that was previously established? At the level of the organism, we need to understand to what extent does the organisation into functional domains influence the state of the plant and its performance at the developmental and physiological level. Is this organisation acting as a lock, or is it flexible and operates in rewiring of the transcriptional programme of the plant cell in response to environmental cues? The collective efforts of the INDEPTH consortium will without doubt shed light on these exciting questions that open perspectives with regards to the understanding and beneficial manipulation of plant traits.

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Competing interests

The authors declare no competing or financial interests.

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