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Chemical Genomics: A Fruitful Strategy to Unravel Root System Architecture Plasticity

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Plant roots are crucial for nutrient and water uptake, which are usually a limiting factor in many lands, becoming critical components of the overall plant productivity. Controlling root system architecture (RSA) is fundamental to respond to changing environmental conditions and therefore critical for plant survival. The RSA is determined by endogenous factors as well as soil conditions, rhizosphere microorganisms and many environmental stresses like drought, flooding, heat and low nutrient availability. Root branching through lateral root formation increases the total surface area allowing the plant to explore the soil environment. Thus, root branching plays a major contribution to the adaptability. Therefore, the study and further understanding of the complex mechanisms regulating root development and modifications of the RSA is essential for improvement of crop yields.

The study of root development has progressed tremendously in recent years through research in the model organism *Arabidopsis thaliana* [1]. The critical advantages of using Arabidopsis as a plant genetic model system include the established pools of T-DNA or transposon insertions mutants and rapid generation of transgenic lines enabling forward and reverse genetic approaches. These studies have revealed an tight crosstalk among plant hormone signaling, including auxin, brassinosteroid, cytokinin, gibberellic acid, strigolactone, abscisic acid and salicylic acid to regulate cell division, elongation, differentiation and adaptive processes of the RSA [2]. These multiple hormonal inputs ultimately converge on the modulation of auxin levels that acts as a common integrator to many endogenous and environmental signals regulating overall RSA [3]. Nevertheless, and despite these recent advances, additional regulatory mechanisms and components regulating adaptive modification of the RSA remain to be identified and characterized. A great body of evidence indicates that auxin predominantly controls root branching however, as this hormone has an effect on the entire plant the mutant phenotypes are often pleiotropic or lethal [4]. On the other hand due to plant gene redundancy no obvious phenotypes are observed [5]. Furthermore, it is difficult to use conventional genetics to observe the direct or immediate effects of a gene product in the adaptive processes [6]. Several strategies have been developed to overcome the deficiencies of conventional genetic of loss or gain of function mutations. One of these approaches, named "chemical genetics", utilizes small molecules (chemicals) to interfere with the function of a specific gene product rendering a specific phenotypic outcome [3]. These small molecules are organic compounds (<500 Da), cell-permeable selective, fast and most of the times reversible biomodulators that can be locally administered with temporal and quantitative control. The systematic high-throughput search for selective small molecule modulators has been defined as forward chemical genomics [6]. This strategy can be performed at the level of molecules, cells and also in whole organisms looking for the induction of specific phenotypes. Diverse forward chemical genomics screens have been focused in finding small molecules capable of modulate root development [7-10]. These screenings have selected a number of small molecules that alter root development by targeting auxin transporters as Gravacin or altering auxin responses, like Rootin [7,8]. Interestingly, the small molecule Naxillin was selected for inducing lateral root development [11]. Naxillin was found to target the conversion of the auxin precursor Indol butyric acid (IBA) into the auxin Indol acetic acid (IAA) [11]. Further genetic and molecular studies on Naxillin mode of action revealed that IBA was specifically produced at the root cap inducing IAA and subsequently lateral root development [11]. Therefore the discover of the biomodulator Naxillin allowed to describe a new mechanism by which a local release of the IAA precursor is sufficient to modify the RSA by stimulating root branching with a minimal effect on primary root elongation.

Although auxin and its receptor SCF^{TIR} are pointed as primary responsible by the molecular signaling root branching, an alternative lateral root development pathway has been described by means of the biomodulator Sortin2. Sortin2 inhibits primary root elongation and triggers lateral root formation [12,13]. Genetic and molecular analysis of the effect of this small molecule allowed to describe a lateral root formation mechanism independent of SCF^{TIR} [13]. Therefore plants have at least two different mechanisms for lateral root remodeling, one dependent and one independent of the SCF^{TIR} auxin signaling. Interestingly, Sortin2 targeted the endocytic pathway by accelerating protein trafficking from late endosomal compartments to the vacuole in Arabidopsis. Thus Sortin2-induced root branching depends on Sortin2 effect on protein trafficking, which is distinctive of the SCF^{TIR}-dependent mechanism. Further research will unravel the role of this novel mechanism of root branching. Since endosomal trafficking has been shown to be a signaling strategy is possible that certain stimuli could differentially activate endocytic trafficking leading to an increase in lateral root occurrence as an alternative RSA adaptation strategy. In conclusion Chemical genomics approaches provided evidence that support the existence of pathways that could allow the plant to specifically alter the RSA without affecting general auxin related developmental processes. We believe that these critical examples illustrate the impact of chemical genomics on the knowledge of root development. Clearly this approach is powerful, and when combined with genetic and diverse biochemical and molecular information gives a more complete understanding of adaptive changes in RSA that

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otherwise will remain unnoticed.

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