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Changing SERKs and priorities during plant life

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SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASES (SERKs) are coreceptors for diverse extracellular signals. SERKs are involved in a wide array of developmental and immune related processes first discovered in *Arabidopsis*. Recent work demonstrates the evolutionary conservation of *SERKs* in all multicellular plants, and highlights their functional conservation in monocots and dicots.

Role of SERKs in signaling events at the plant plasma membrane

SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASES (SERKs) are plasma membrane coreceptors involved in plant growth, development, cell death, and immunity [1]. The extracellular domain comprises 4.5–5 leucine rich repeats (LRR domain) and a small membrane-proximal serine-proline rich domain (SPP). The single span transmembrane domain is followed by an intracellular juxtamembrane domain, a catalytic kinase domain, and a C-terminal tail. SERK proteins, defined originally through the founding member AtSERK1 with roles in the developing seed and somatic embryogenesis, belong to LRR kinase subfamily II and are identified as SERKs by the presence of the unique SPP domain (Figure 1A) [2]. SERK proteins are best studied in *Arabidopsis thaliana*, which contains five family members. AtSERK1 and 2 play roles in sporogenesis, floral organ separation, and immunity to aphids. AtSERK3 and 4 are involved in receptor kinase (RK)-mediated immune signaling and cell death. AtSERK1, AtSERK3, and AtSERK4 regulate plant growth through participation in receptor complexes for the phytohormone brassinosteroid [1]. No role for *AtSERK5* has been identified and it may be a pseudogene [3]. SERK proteins rose to prominence with the identification of AtSERK3 (also known as BAK1, for BRI1-ASSOCIATED KINASE) as a common partner for receptors that control brassinosteroid and plant immune signaling. In each case, AtSERK3 forms a complex with a primary receptor protein; BRASSINOSTEROID-INSENSITIVE 1 (AtBRI1) for brassinosteroids, or FLAGELLIN-INSENSITIVE 2 (AtFLS2) for bacterial flagellin, immediately after ligand binding. AtSERK3 interacts with the external LRR domains of both receptors and also forms important contacts with each ligand; the ligands thus function as 'molecular glue' stabilizing the receptor–coreceptor interface (Figure 1A). In turn, establishment of the receptor complex through interactions between the extracellular domains leads to auto- and transphosphorylation events between the respective intracellular kinase domains, activating downstream signaling [4].

Evolutionary conservation of SERKs

SERK proteins have conserved roles in plant development and immunity across evolution. Misregulation of *SERK* expression alters immune responses and/or development in many plant species including dicots such as tomato (*Solanum lycopersicum)*, lettuce (*Lactuca sativa*), *Nicotiana benthamiana* and cotton (*Gossypium hirsutum*), and monocots such as rice (*Oryza sativa*) [5,6]. The recent paper by de Vries and colleagues builds on these phenotypic studies to perform a phylogenetic analysis of SERK proteins from 28 diverse plant species [3]. This includes 24 seed setting plants [spermatophytes] (16 eudicots and 8 monocots) and four non-seed setting plants (two bryophytes, one lycopodiophyte, and one alga). It appears that SERKs arose with the onset of multicellularity in plants. All multicellular plants contain SERK proteins belonging to the AtSERK1/2 clade (Figure 1B). SERK homologs are also present in bryophytes and lycopodiophytes, which lack the important ligand binding receptors FLS2 and BRI1 known to interact with SERK proteins [3,5]. This suggests that these evolutionarily rather recent signaling pathways co-opted SERKs from more ancient pathways already present in multicellular non-seed setting plants, for example reproductive development controlled by homologues of the LRR-RK EXTRA SPOROGENOUS CELLS (EXS).

In dicots, the SERK family is expanded with new members belonging to the AtSERK3/4 clade (Figure 1B). The main divergence between these two clades resides in the SPP domain and the C-terminal tail (Figure 1A). In effect, there are two clades within the SERK family, the largest apparently ancestral containing dicot SERK1/2, monocot SERKs and non-vascular plant SERKs, and a more recent derivative clade containing dicot SERK3/4 (Figure 1B). This can make it difficult to identify true functional orthologs of Arabidopsis SERKs based on primary sequence analysis. For example, monocotyledonous rice has two SERK homologs belonging to the AtSERK1/2 clade. Both SERKs contribute to BR signaling, whereas only OsSERK2 is required for RK-mediated defense signaling and immunity against bacterial pathogens [7]. In dicots, both of these functions are associated with SERKs belonging to the AtSERK3/4 clade, raising several intriguing questions. How did the newly divergent SERKs (that arose probably by gene duplication events) become major players in evolutionarily conserved signaling pathways? Do the divergent SPP domains and C-terminal tails have specific roles in receptor–coreceptor interactions and/or signaling?

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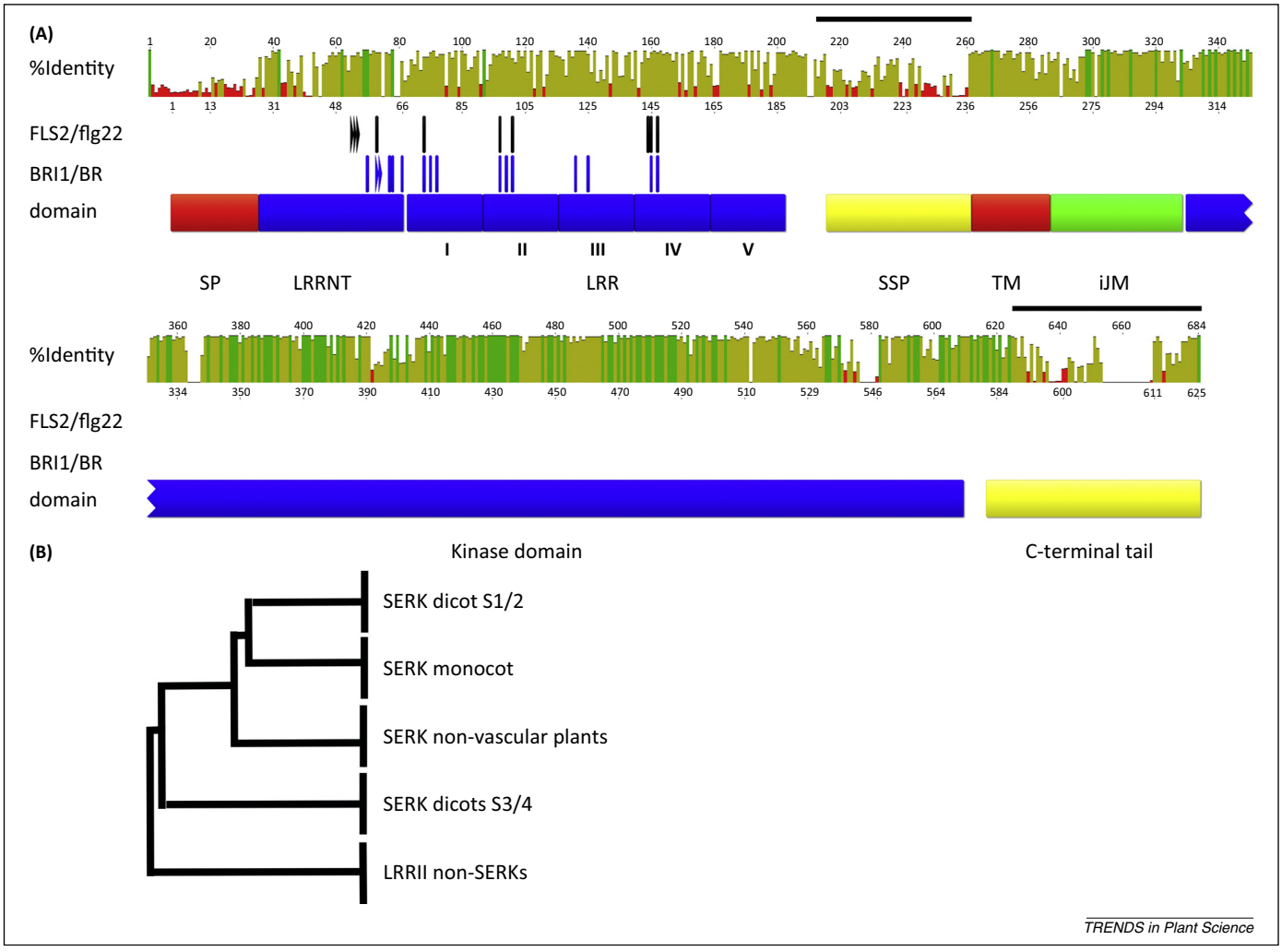

Figure 1. Evolutionary conservation of SERK proteins. **(A)** The overall SERK topology is conserved during evolution. %Identity is derived from a multiple sequence alignment of all 59 true SERKs [2]. Horizontal bars indicate domains with highest amino acid variability within the SERK family. Vertical bars and arrows indicate the SERK residues (AtSERK1 numbering) that interact with the primary receptor or the ligand, respectively. Abbreviations: SP, signal peptide; LRR, leucine rich repeat; LRRNT, LRR capping domain; SPP, serine-proline rich domain; TM, transmembrane domain; iJM, intracellular juxtamembrane domain. Alignment and visualization was done using *Geneious*. **(B)** Simplified Neighbor-Joining tree of SERKs from multicellular plants showing the relationship between clades [2]. Not to scale.

Unfortunately, insight into these questions from recent structural studies is missing, because the SPP domains and C-terminal tails are absent from the available crystal structures [4]. In the case of dicotyledonous immune RKs it appears that features of the SPP domains and C-terminal tails of AtSERK3/4 clade members are dispensable in heterologous expression systems, because monocot AtSERK1/2 clade members can apparently complement dicot immune RK function. Specifically, expression of the brassicaceous receptor AtEFR (EF-Tu RECEPTOR) which normally requires AtSERK3 confers EF-Tu perception and bacterial immunity in transgenic wheat (*Triticum aestivum*) and rice [8,9]. By contrast, the C-terminal tail of AtSERK3 is differentially involved in BR versus immune signaling in *Arabidopsis*.

The modular structure of SERK proteins

The extracellular LRR and intracellular kinase domains are highly conserved among 57 analyzed SERK proteins (Figure 1A). Analysis of the available sequence variation suggests that SERKs are predominantly under purifying selection to limit diversity. This makes sense because structural integrity is required to maintain effective co-receptor function of the LRR domain, specifically on the concave side that interacts both with ligand and the primary receptor molecule (Figure 1A) [3,4]. Similarly, the kinase domain is highly conserved due to structural restraints imposed by catalytic requirements, with variant residues mapping away from the catalytic cleft [3]. Despite their structural conservation, the LRR and kinase domains are not generally interchangeable between different SERKs. For example in *Arabidopsis* the kinase domains but not the extracellular domains of SERK1/2 and SERK3 are interchangeable for function in male sporogenesis. In contrast, neither domain of SERK1/2 was able be substituted for the respective SERK3 domain to support flagellin sensing by AtFLS2 in *Arabidopsis* [3]. This is at odds with the finding that rice SERK2 and a wheat SERK1/2 protein supported ligand perception by AtEFR, and may imply that the hybrid proteins are non-functional within receptor complexes [8,9]. Intriguingly, kinase domains can be interchangeable in some cases, which implies the existence of further rules for the specific activation of different intracellular signalling pathways that are currently undefined.

Much remains to be learned about this intriguing family of proteins. Some of the questions arising stem from the SERK3/4 clade that has evolved in *Arabidopsis* and other dicots (Figure 1B). What are the novel functions associated with this lineage, and why was it necessary to evolve it given that the known functions of immunity and brassinosteroid perception are conserved with monocots that possess only SERK1/2s? Are the molecular mechanisms for brassinosteroid and pathogen ligand binding conserved in monocots and dicots? Limitation of the size of the SERK family, and purifying selection acting on its members, makes sense in terms of the functionality that must be preserved particularly in concert with diverse primary receptor partners, but AtSERK3 at least is a target of pathogen virulence effector proteins which would be expected to drive diversification but clearly has not happened [3,10]. The current paper by de Vries and colleagues is an important platform to answer these and future questions with fundamental implications for our knowledge of plant biology.

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A new chromosome was born: comparative chromosome painting in *Boechera*

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Comparative chromosome painting is a powerful tool to study the evolution of chromosomes and genomes. Analyzing karyotype evolution in cruciferous plants highlights the origin of aberrant chromosomes in apomictic *Boechera* and further establishes the cruciferous plants as important model system for our understanding of plant chromosome and genome evolution.

Comparative chromosome painting: a powerful tool to bridge cytogenetics, genomics and evolutionary biology

Chromosome painting was introduced more than 25 years ago [1] as method to visualize chromosomes or larger chromosome fragments using fluorescent-labeled chromosome-specific homologous DNA probes. However, repetitive sequences in the target as well as the probe DNA often resulted in non-specific binding and hybridization signals. Therefore extensive blocking with excess of unlabeled total genomic DNA was required, ideally enriched for repetitive fraction, and the term chromosomal *in situ* suppression was introduced as synonym for chromosome painting [2]. Comparative chromosome painting (CCP) uses cross-specific hybridization of fluorescent-labeled probes of different size. Given a defined reference genome as source for these probes (e.g. *Arabidopsis thaliana*) and assuming chromosome collinearity among the species to be compared, CCP provides a powerful tool box to reconstruct chromosome and genome structure, as well as the evolutionary history of chromosomes, karyotypes, and whole genomes [3]. Despite more than 30 million years of Brassicaceae crown group evolutionary history the Brassicaceae genomes show very high levels of collinearity among all evolutionary lineages, which allows for the reliable cross-species identification of large chromosome homologues via CCP using *Arabidopsis thaliana* chromosome-specific BAC (Bacterial Artificial Chromosome) contigs representing 24 genomic blocks of the entire ACK (Ancestral Crucifer Karyotype).

Boechera: not only a model system for the evolution of apomictic reproduction

The genus *Boechera* (Böcher's rock cress) from the crucifer family is one of the few model systems available to study the evolution of apomixis in plants, the production

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