Family-Wide Evaluation of Arabidopsis thaliana RAPID ALKALINIZATION FACTOR (RALF) Peptides and Their Implication in Immunity

Dissertation

zur

Erlangung der naturwissenschaftlichen Doktorwürde (Dr. sc. nat.)

vorgelegt der

Mathematisch-naturwissenschaftlichen Fakultät

der

Universität Zürich

von

Alicia Abarca Cifuentes

aus

Spanien

Promotionskommission

Prof. Dr. Cyril Zipfel (Leitung der Dissertation)

Prof. Dr. Ueli Grossniklaus

Prof. Dr. Clara Sanchez

Zürich, 2023

Table of content

Acknowledg	gments	v
Agradecimi	entosv	ii
Abstract	i	x
Table of co	ntentx	ci
List of Figu	resX	V
List of Supp	plementary Figuresxvi	ii
List of Table	esxiz	ĸ
List of Supp	lementary Tablesxi	K
Chapter 1.	Introduction20)
1.1 Sec	creted signaling peptides in plants22	2
1.1.1	Role of plant signaling and cell-to-cell communication	2
1.1.2	Structure and classification of secreted signaling peptides24	1
1.1.3	Identification approaches of small secreted signaling peptides in plants 25	5
1.1.4	Receptors of secreted signaling peptides in plants	3
1.1.5	Signaling axis and specificity)
1.1.6	Evolution of secreted signaling peptide families	3
1.2 RA	PID ALKALINIZATION FACTORS (RALFs)42	2
1.2.1	Discovery and structure of RALFs 42	2
1.2.2	Evolution of RALFs	1
1.2.3	Functions of RALFs	3
1.3 RAI	LF peptide signaling pathways52	2
1.3.1	The RALF perception axis: CrRLK1Ls/LLGs/LRXs	2
1.3.2	CrRLK1Ls and related proteins in growth	5

1.3	3.3 CrRLK1Ls and related proteins in reproduction	57
1.3	CrRLK1L and related proteins in stress response	58
1.4	Regulation of pattern-triggered immunity (PTI) by peptides	67
1.4	Phytocytokines vs damaged-associated molecular patterns (DAMPs).	3 7
1.4	2 Examples of phytocytokine signaling	3 9
1.5	Aim of the thesis	73
Chapter during in	r 2. Investigating the regulation of S1P-mediated PRORALF23 cleavage	је 76
2 1	Abstract	78
2.2	Introduction	79
2.3	Materials and methods	36
2.4	Results	90
2.4 act	.1 Does PAMP perception induce the cleavage of S1P for proteas	;e 90
2.4 inh	.2 Does PAMP perception facilitate the dissociation of S1P and it ibitor?	ts 2
2.4	.3 Does PAMP perception induce the phosphorylation of S1P?9	5
2.4 PR	.4 Does PAMP perception affect the subcellular localization of S1P an ORALF23?	d 7
2.5	Discussion	13
2.6	Supplemental information	8
Chapter peptide	r 3. Family-wide evaluation of RAPID ALKALINIZATION FACTO s 111	R
Chapter	r 4. In search of RALFs that play a positive role in immunity	3
4.1	Abstract14	5
4.2	Introduction14	6
4.3	Materials and methods15	i0
4.4	Results	56

4.4	4.1	Identification of RALFs as putative PROFITs156
4.4	4.2	The effect of PROFIT RALF candidates is LLG1- and LRX-dependent 158
4.4 co	4.3 mple	Treatment with RALF PROFIT candidates does not alter FLS2-BAK1 x formation
4.4 p r e	4.4 oduci	ralf24ralf32 mutant plants tend to have reduced PAMP-induced ROS tion
4.4 D0	4.5 C300	<i>ralf24ralf32</i> mutant plants tend to have increased susceptibility to <i>Pto</i> 0
4.4	4.6	RALF23 might be dependent on ANX1/ANX2 167
4.5	Disc	cussion
4.6	Sup	plemental information
Chapte screen	er 5. in se	DEDUCEing RALF-CrRLK1L receptor pairs: Dependency mutant redlings
5.1	Abs	stract
5.2	Intro	oduction
5. 3	Mat	erials and methods203
5.4	Res	sults
5.4	4.1	Seedling and root growth inhibition in herk1 anjea mutant plants 206
5.4	4.2	Seedling and root growth inhibition in anxur1 anxur2 mutant plants 210
5.4	4.3	Seedling and root growth inhibition in erulus mutant plants
5.4	4.4	Seedling and root growth inhibition in letum 1letum2 mutant plants 215
5.4	4.5	Seedling and root growth inhibition in medos1/2/3/4 mutant plants 217
5.4	1.6	Seedling and root growth inhibition in theseus1 mutant plants
5.4	1.7	Seedling and root growth inhibition in <i>curvy1</i> mutant plants
5.4	4.8	Next steps in the identification of receptor-RALF pairs
5.4	1.9	Using DEDUCE beyond CrRLK1Ls
5.5	Disc	cussion

5.6	Supplemental information	240
Chapter	6. General conclusions and future perspectives	255
Reference	ices	265

List of Figures

Figure 1.1 The functional diversity of plant secreted signaling peptides	23
Figure 1.2 Schematic representation of the structure and classification of pre-p	ro-
peptides	24
Figure 1.3 Schematic representation of the classification of secreted signali	ing
peptides	25
Figure 1.4 Acquisition of signal specificity.	31
Figure 1.5 Model for embryonic cuticle integrity monitoring.	33
Figure 1.6 EPF1/EPF2 and EPF9 antagonistically regulate stomatal patterning	34
Figure 1.7 Schematic representation of a conserved regulatory signaling module	36
Figure 1.8 Apolar SGN1 leads to ectopic lignin accumulation in endodermal cells	39
Figure 1.9 Scheme of nematode and fungal infections in Arabidopsis roots	40
Figure 1.10 Growth retardation caused by overexpression of RALF23 depends	on
S1P	44
Figure 1.11 Chromosomal locations of AtRALFs.	45
Figure 1.12 Main functions of RALFs.	47
Figure 1.13 CrRLK1L-RALF receptor complexes identified	52
Figure 1.14 Evolutionary history of CrRLK1Ls, RALFs, LLGs, and LRXs	54
Figure 1.15 PRR and NLR signaling co-regulate immune responses	61
Figure 1.16 Regulation of immunity by CrRLK1Ls.	64
Figure 1.17 Difference between DAMPs and phytocytokines	67
Figure 1.18 Metacaspases activate defense responses upon wounding	68
Figure 1.19 Model of phytocytokine-mediated regulation of plant immunity	71
Figure 2.1 Phylogenetic tree of the 56 SBTs in Arabidopsis.	81
Figure 2.2 AtRALF23 is a S1P substrate	82
Figure 2.3 S1P cleavage increased upon PAMP treatment.	83
Figure 2.4 Schematic representation of S1P structure	90
Figure 2.5 Processing of S1P is not dependent on PAMP treatment.	91
Figure 2.6 The S1P cleavage site is found within the RCL of Serpin1	93
Figure 2.7 Hypothetical model for the involvement of Serpin1 in PTI.	94
Figure 2.8 Serpin1 is not involved in PAMP-induced ROS production.	94
	•

Figure 2.9 S1P Phosphorylation is not detected using α -P-thre and α -P-ser
antibodies
Figure 2.10 S1P and RALF23 localize in cytoplasmic dots
Figure 2.11 The fluorescence intensity of 35::RALF23-GFP plants is unaffected by
PAMP treatment
Figure 2.12 Apoplastic extraction of 5-week-old Arabidopsis plants overexpressing
RALF23-GFP
Figure 2.13 RALF23 cleavage does not increase after PAMP or bacteria perception.
Figure 2.14 Schematic representation of how fluorescence acts as an indicator of
cleavage
Figure 4.1 Targeted approaches to identify PROFIT RALF candidates
Figure 4.2 Unbiased approach to identify PROFIT RALF candidates
Figure 4.3 The effect of PROFIT RALF candidates in ROS production is LLG1
dependent
Figure 4.4 The effect of LRX3/4/5/ on candidate PROFIT RALF-induced ROS
production
Figure 4.5 PROFIT RALF candidates do not alter ligand-induced FLS2-BAK1
complex formation
Figure 4.6 Generation of ralf24ralf32 double mutant
Figure 4.7 ralf24ralf32 mutant plants tend to have slightly reduced PAMP-induced
ROS responses
Figure 4.8 Exogenous RALF24 or RALF32 pre-treatment does not induce resistance
to <i>Pto</i> DC3000
Figure 4.9 ralf24ralf32 mutant plants seem more susceptible to Pto DC3000
Figure 4.10 RALF23 responses might be ANX1/ANX2 dependent
Figure 4.11 Working model illustrating how RALFs regulate immune responses 172
Figure 5.1 Seedling growth inhibition of synthetic RALFs in Col-0 and herk1 anj
mutant seedlings
Figure 5.2 Root growth inhibition of synthetic RALFs in Col-0 and herk1 anj mutant
seedlings
Figure 5.3 Seedling growth inhibition of synthetic RALFs in Col-0 and anx1 anx2
mutant seedlings

Figure 5.4 Root growth inhibition of synthetic RALFs in Col-0 and <i>anx1 anx2</i> mutant
Figure 5.5 Seedling growth inhibition of synthetic RALFs in Col-0 and <i>eru</i> mutant
Figure 5.6 Root growth inhibition of synthetic RALFs in Col-0 and <i>eru</i> mutant seedlings
Figure 5.7 Seedling growth inhibition of synthetic RALFs in Col-0 and <i>let1 let2</i> mutant seedlings
Figure 5.8 Root growth inhibition of synthetic RALFs in Col-0 and <i>let1 let2</i> mutant seedlings
Figure 5.9 Seedling growth inhibition of synthetic RALFs in Col-0 and <i>mds1/2/3/4</i> mutant seedlings
Figure 5.10 Root growth inhibition of synthetic RALFs in Col-0 and <i>mds1/2/3/4</i> mutant seedlings
Figure 5.11 Seedling growth inhibition of synthetic RALFs in Col-0 and <i>the1-6</i> mutant seedlings
Figure 5.12 Root growth inhibition of synthetic RALFs in Col-0 and <i>the1-6</i> mutant seedlings
Figure 5.13 Seedling growth inhibition of synthetic RALFs in Col-0 and <i>cvy1</i> mutant seedlings
Figure 5.14 Root growth inhibition of synthetic RALFs in Col-0 and <i>cvy1</i> mutant seedlings
Figure 5.15 H ⁺ flux changes in roots induced by some RALFs are FER-dependent. 226
Figure 5.16 RALF31-induced H ⁺ flux change is partially CVY1 dependent
Figure 5.18 Seedling growth inhibition of synthetic RALFs in Col-0 and <i>Ilg1-3</i> mutant seedlings
Figure 5.19 Seedling growth inhibition of synthetic RALFs in Col-0 and bak1-5 mutant seedlings
Figure 5.20 Root growth inhibition of synthetic RALFs in Col-0 and <i>bak1-5</i> mutant seedlings

List of Supplementary Figures

Supplementary Figure 2.1 S1P Phosphorylation is not detected in IP-MS analysis. Supplementary Figure 2.2 PRORALF23 cleavage is not increased after PAMP Supplementary Figure 4.1 The effect of PROFIT RALF candidates in ROS Supplementary Figure 4.2 The effect of multiple RALFs candidates is reduced in Supplementary Figure 4.3 The effect of some PROFIT RALF candidates in ROS Supplementary Figure 4.4 The effect of all PROFIT RALF candidates in ROS Supplementary Figure 4.5 RALF-mediated increase of elf18-induced ROS Supplementary Figure 4.6 RALF1 and RALF33 induce MAPK activation to a lesser Supplementary Figure 4.7 ralf24ralf32 mutant plants tend to have slightly reduced Supplementary Figure 4.8 ralf24ralf32 mutant plants tend to have slightly reduced Supplementary Figure 4.9 ralf22ralf31ralf34 mutant plants present PAMP-induced Supplementary Figure 4.11 Exogenous RALF24 or RALF32 pretreatment does not Supplementary Figure 5.1 Effect of RALF peptides at 10 µM on seedlings and root

Supplementary Figure 5.2 Seedling growth inhibition of synthetic RALFs in 0	Col-0 and
anj mutant seedlings.	241
Supplementary Figure 5.3 Seedling growth inhibition of synthetic RALFs in 0	Col-0 and
aggie101 anx2 mutant seedlings	
Supplementary Figure 5.4 FER-dependency of RALF23-induced H ⁺ fluxes.	242
Supplementary Figure 5.5 BAK1 dependency of RALFs in SGI and RGI	243

List of Tables

Table 4.1 Cloning strategy to generate ralf24ralf32 mutant plants	152
Table 4.2 Cloning strategy to generate ralf14ralf24ralf27ralf31ralf32	153
Table 4.3 Cloning strategy to generate ralf6ralf7ralf17ralf20ralf24ralf31ralf32	153
Table 5.1 AtCrRLK1L family members	198

List of Supplementary Tables

Supplementary Table 2.1 Lines used in this chapter	110
Supplementary Table 2.2 Antibodies and other reagents used in this chapter	110
Supplementary Table 2.3 Peptides used in this chapter.	110
Supplementary Table 4.1 Lines used in this chapter	183
Supplementary Table 4.2 Antibodies and other reagents used in this chapter	184
Supplementary Table 4.3 Peptides used in this chapter.	184
Supplementary Table 4.4 Primers used in this chapter	185
Supplementary Table 4.5 Plasmids used in this chapter	1 92
Supplementary Table 5.1 Lines used in this chapter	244
Supplementary Table 5.2 Peptides used in this chapter	245
Supplementary Table 5.3 RALF expression atlas cloning status	247
Supplementary Table 5.4 Plasmids used in this chapter	248
Supplementary Table 5.5 Putative receptor-ligand pairs deduced from SGI and I	RGI
assays comparing CrRL1KL mutants with Col-0 seedlings	249
Supplementary Table 5.6 Primers used in this chapter	251