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Accessory Publication

Fig. S1. Phylogenic diagram demonstrating that the different peptide classes are not related. This Radial Cobalt Tree was produced using NCBI (National Centre for Biotechnology Information) COBALT multiple alignment tool. All 126 available Arabidopsis amino acids sequences of the different peptide classes (CEP (5 sequences), CLE (31), EPF (7), IDA (6), PROPEP (7), PNP (2), PSK (7), PSY (1), RALF (34), SCRL (26) and TPD1 (1)) as well as the tomato prosystemin sequence (in brown) segregated into the different peptide classes. Refer to Table 1 for abbreviations and links to a description of each class. Closer examination shows that some of the peptide families are clustered on particular chromosomes as discussed below. Two groups of three PROPEP encoding genes are found on chromosome 5, PROPEP 7, 4, 5 (At5g09978, At5g09980 and At5g09990) and PROPEP2, 1, 3 (At5g64890.1, At5g64900 and At5g64905) as well as a single gene (PROPEP6) on chromosome 2. It is unlikely that PROPEP7, 4, 5 and PROPEP 2, 1, 3 are the result of a single duplication event as PROPEP3 and PROPEP6 are also similar to each other and more similar to PROPEP1 and 2 than PROPEP7, 4 or 5. In fact amino acid sequence similarity suggests that PROPEP5 diverged first, then PROPEP7 followed by PROPEP4 before PROPEP3 and PROPEP6 diverged forming their own clade from the PROPEP1 and PROPEP2 clade. Similarly the more expanded signalling peptide groups such as RALFL, SCRL and CLE also have clustered encoding genes. In the case of RALFL several encoding gene groups are clustered and most similar in sequence suggesting recent duplication such as RALFL2 (At1g23145) with RALFL3 (At1g23147) and RALFL8 (At1g61563) with RALFL9 (At1g61566). Although close proximity of encoding genes does not necessarily mean there is a high level of sequence similarity as is the case with RALFL25 (At3g25165) and RALF26 (At3g25170) or the cluster of SCRL encoding genes SCRL8 (At1g60983), SCRL6 (At1g60985), SCRL5 (At1g60987) and SCRL7 (At1g60989) of which SCRL7 and SCRL8 are most similar but these are in the same clade as SCRL3 (At1g0695.1).

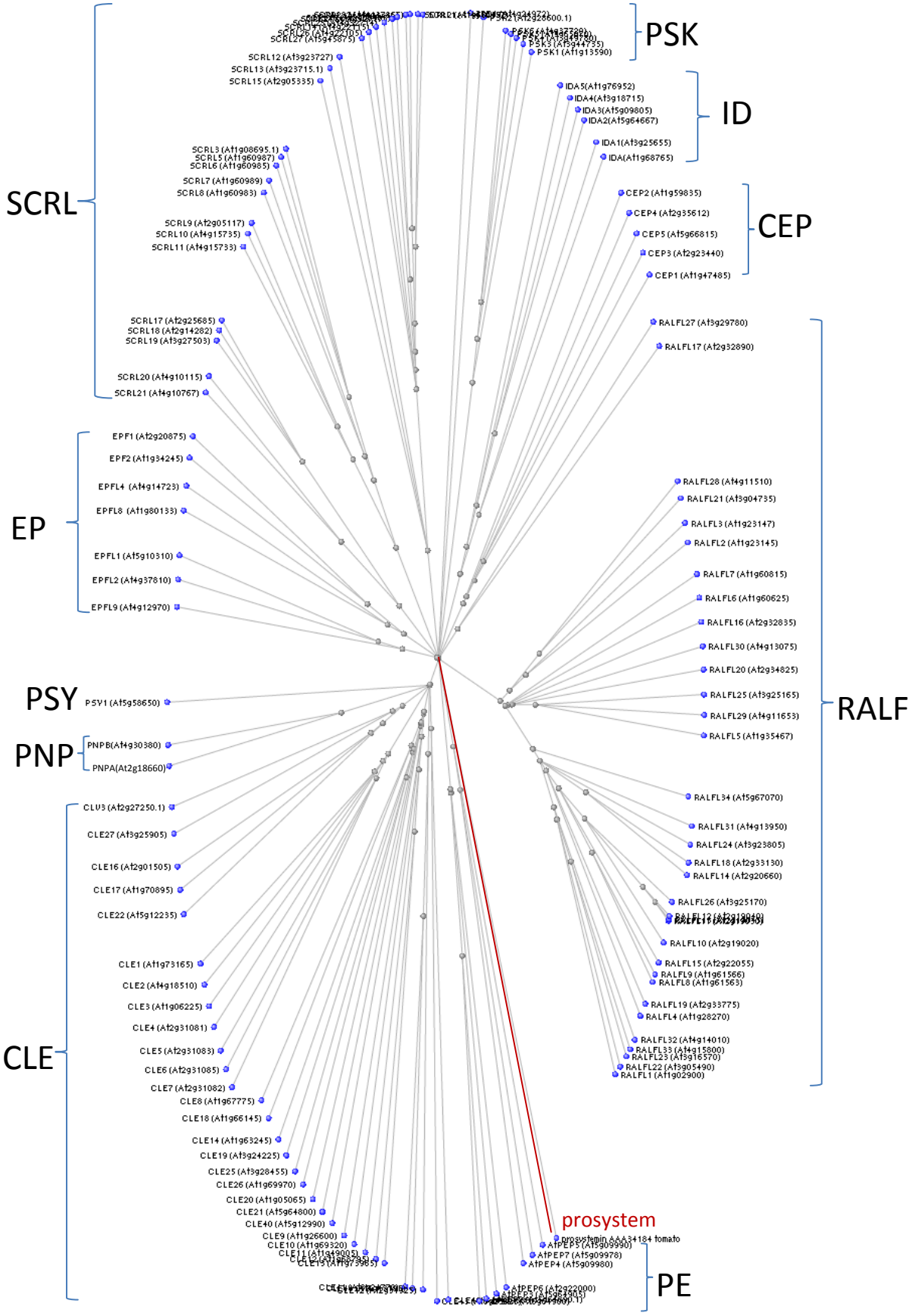
PSK
PSK3 (At5g44735)
PSK1 (At1g13590)
SCRL12 (At3g23727)
SCRL13 (At3g23715.1)
SCRL15 (At2g05335)
ID
IDA5(At1g76952)
IDA4(At3g18715)
IDA3(At5g09805)
IDA2(At5g64667)
IDA1(At3g25655)
IDA(At1g68765)
SCRL
SCRL3 (At1g08695.1)
SCRL5 (At1g60987)
SCRL6 (At1g60985)
SCRL7 (At1g60989)
SCRL8 (At1g60983)
SCRL9 (At2g05117)
SCRL10 (At4g15735)
SCRL11 (At4g15733)
CEP
CEP2 (At1g59835)
CEP4 (At2g35612)
CEP5 (At5g66815)
CEP3 (At2g23440)
CEP1 (At1g47485)
SCRL17 (At2g25685)
SCRL18 (At2g14282)
SCRL19 (At3g27503)
SCRL20 (At4g10115)
SCRL21 (At4g10767)
RALFL27 (At3g29780)
RALFL17 (At2g32890)
EP
EPF1 (At2g20875)
EPF2 (At1g34245)
EPFL4 (At4g14723)
EPFL8 (At1g80133)
EPFL1 (At5g10310)
EPFL2 (At4g37810)
EPFL9 (At4g12970)
RALFL28 (At4g11510)
RALFL21 (At3g04735)
RALFL3 (At1g23147)
RALFL2 (At1g23145)
RALFL7 (At1g60815)
RALFL6 (At1g60625)
RALFL16 (At2g32835)
RALFL30 (At4g13075)
RALFL20 (At2g34825)
RALFL25 (At3g25165)
RALFL29 (At4g11653)
RALFL5 (At1g35467)
PSY
PSY1 (At5g58650)
PNP
PNPB(At4g30380)
PNPA(At2g18660)
RALF
RALFL34 (At5g67070)
RALFL31 (At4g13950)
RALFL24 (At3g23805)
RALFL18 (At2g33130)
RALFL14 (At2g20660)
RALFL26 (At3g25170)
RALFL10 (At2g19020)
RALFL15 (At2g22055)
RALFL9 (At1g61566)
RALFL8 (At1g61563)
RALFL19 (At2g33775)
RALFL4 (At1g28270)
RALFL32 (At4g14010)
RALFL33 (At4g15800)
RALFL23 (At3g16570)
RALFL22 (At3g05490)
RALFL1 (At1g02900)
CLE
CLV3 (At2g27250.1)
CLE27 (At3g25905)
CLE16 (At2g01505)
CLE17 (At1g70895)
CLE22 (At5g12235)
CLE1 (At1g73165)
CLE2 (At4g18510)
CLE3 (At1g06225)
CLE4 (At2g31081)
CLE5 (At2g31083)
CLE6 (At2g31085)
CLE7 (At2g31082)
CLE8 (At1g67775)
CLE18 (At1g66145)
CLE14 (At1g63245)
CLE19 (At3g24225)
CLE25 (At3g28455)
CLE26 (At1g69970)
CLE20 (At1g05065)
CLE21 (At5g64800)
CLE40 (At5g12990)
CLE9 (At1g26600)
CLE10 (At1g69320)
prosystem
prosystemin AAA34184 tomato
AtPEP5 (At5g09990)
AtPEP7 (At5g09978)
AtPEP4 (At5g09980)
PE
AtPEP6 (At2g22000)

Table S1. N-S scores of individual signalling peptides

The nitrogen (N) score was assessed for prepropeptides using the formula $\Sigma(n_i \times p_i)$ devised by Acquisti *et al.* (2009) where n_i is the number of N atoms in the side chain and p_i is the proportion of these in the final prepropeptide. The amino acids with nitrogen rich side chains were scored: $n = 1$ for asparagine, glutamine, lysine and tryptophan; $n = 2$ for histidine; $n = 3$ for arginine; and $n = 0$ for the remainder. The use of sulphur containing amino acids was assessed in addition to the nitrogen rich amino acids where $\Sigma(s_i \times p_i)$ with $s = 1$ for cysteine and methionine; and $n = 0$ for the remainder. The calculated number was added to the N score to form the N-S score

Peptide	At gene family	At code	Molecular weight	Amino acid #	pI	N score	S score	N+S
Prosystemin		na	22999	200		0.320	0.060	0.380
CEP1	5	At1g47485	10080	91	9.09	0.440	0.044	0.484
CEP2		At1g59835	14118	126	9.66	0.381	0.032	0.413
CEP3		At2g23440	8693	82	6.50	0.256	0.024	0.280
CEP4		At2g35612	9755	86	10.37	0.465	0.047	0.512
CEP5		At5g66815	11585	105	7.67	0.314	0.190	0.505
CLV3	31	At2g27250	10870	96	7.82	0.438	0.146	0.583
CLE1		At1g73165	8338	74	9.59	0.432	0.243	0.676
CLE2		At4g18510	7829	75	7.62	0.307	0.107	0.413
CLE3		At1g06225	9481	83	9.50	0.494	0.048	0.542
CLE4		At2g31081	9046	80	9.50	0.463	0.100	0.563
CLE5		At2g31083	9132	81	10.61	0.444	0.074	0.519
CLE6		At2g31085	9192	81	9.10	0.420	0.074	0.494
CLE7		At2g31082	9738	86	10.10	0.430	0.070	0.500
CLE8		At1g67775	9703	86	11.09	0.442	0.093	0.535
CLE9		At1g26600	13966	120	11.57	0.600	0.083	0.683
CLE10		At1g69320	12553	107	11.35	0.617	0.075	0.692
CLE11		At1g49005	11414	99	10.66	0.424	0.040	0.465
CLE12		At1g68795	14044	118	10.55	0.576	0.034	0.610
CLE13		At1g73965	11952	107	11.54	0.421	0.019	0.439
CLE14		At1g63245	9037	80	10.95	0.425	0.075	0.500
CLE16		At2601505	11756	103	10.15	0.524	0.078	0.602
CLE17		At1g70895	11365	99	11.19	0.495	0.081	0.576
CLE18		At1g66145	11363	101	6.68	0.426	0.020	0.446
CLE19		At3g24225	8149	74	7.69	0.324	0.135	0.459
CLE20		At1g03065	9538	83	11.85	0.554	0.072	0.627
CLE21		At5g64800	12377	106	11.69	0.594	0.075	0.670
CLE22		At5g12235	11510	103	10.45	0.485	0.019	0.505
CLE25		At3g28455	8625	81	12.16	0.444	0.025	0.469
CLE26		At1g69970	13631	118	7.61	0.432	0.068	0.500
CLE27		At3g25905	10286	91	9.66	0.363	0.132	0.495
CLE40		At5g12990	8856	80	9.76	0.300	0.075	0.375
CLE41		At3g24770	11018	99	12.16	0.465	0.081	0.545
CLE42		At2g34925	10106	88	12.22	0.568	0.091	0.659
CLE43		At1g23425	11306	96	10.63	0.573	0.063	0.635
CLE44		At4g13195	12522	112	12.35	0.500	0.089	0.589
CLE45		At1g69588	14475	124	10.48	0.548	0.081	0.629
EPF1	7	At2g20875	11445	104	8.79	0.394	0.250	0.644
EPF2		At1g34245	13317	120	8.70	0.358	0.250	0.608
EPFL1		At5g10310	13652	122	9.40	0.361	0.148	0.508
EPFL2		At4g37810	14318	128	8.38	0.391	0.203	0.594

EPFL4		At4g14723	12057	109	11.05	0.459	0.165	0.624
EPFL8		At1g80133	11321	99	7.99	0.434	0.222	0.657
EPFL9		At4g12970	11940	102	9.39	0.549	0.216	0.765
IDA	6	At1g68765	8433	77	11.09	0.364	0.208	0.571
IDL1		At3g25655	9782	86	11.63	0.442	0.093	0.535
IDL2		At5g64667	10712	95	12.52	0.516	0.042	0.558
IDL3		At5g09805	11047	99	11.33	0.515	0.061	0.576
IDL4		At3g18715	10899	93	11.61	0.613	0.065	0.677
IDL5		At1g76952	13060	111	11.70	0.613	0.126	0.739
ProPep1	7	At5g64900	10388	92	9.36	0.467	0.087	0.554
ProPep2		At5g64890	12343	109	5.28	0.367	0.055	0.422
ProPep3		At5g64905	10399	96	4.19	0.250	0.083	0.333
ProPep4		At5g09980	9077	81	10.37	0.370	0.025	0.395
ProPep5		At5g09990	9944	86	10.71	0.593	0.186	0.779
ProPep6		At5g22000	11633	104	4.50	0.385	0.096	0.481
ProPep7		At5g09978	9286	85	4.76	0.388	0.071	0.459
PNP-A	2	At2g18660	14032	126	9.50	0.444	0.127	0.571
PNP-B		At4g30380	13261	123	8.37	0.236	0.146	0.382
proPSK1	5 (6)	At1g13590	9653	87	5.08	0.241	0.575	0.816
proPSK2		At2g28600	9627	87	5.03	0.287	0.253	0.540
proPSK3		At3g44735	9292	81	5.55	0.395	0.198	0.593
proPSK4		At3g49780	8897	79	5.05	0.304	0.127	0.430
proPSK5		At5g65870	8749	77	5.55	0.312	0.130	0.442
proPSK6 *		At4g37720	9701	87	4.75	0.287	0.161	0.448
PSY1		At5g58650	7928	75	8.53	0.320	0.080	0.400
RALF1	34	At1g02900	12967	120	8.11	0.350	0.133	0.483
RALFL2		At1g23145	11111	97	10.57	0.691	0.186	0.877
RALFL3		At1g23147	10240	90	9.73	0.467	0.133	0.600
RALFL4		At1g28270	12662	110	10.23	0.573	0.182	0.755
RALFL5		At1g35467	10414	89	9.89	0.461	0.202	0.663
RALFL6		At1g60625	9035	81	9.62	0.395	0.173	0.568
RALFL7		At1g60815	9093	81	10.04	0.457	0.148	0.605
RALFL8		At1g61563	8906	82	9.93	0.415	0.146	0.561
RALFL9		At1g61566	8260	75	9.93	0.440	0.160	0.600
RALFL10		At2g19020	7980	73	10.01	0.178	0.164	0.342
RALFL11		At2g19030	7877	72	8.37	0.403	0.194	0.597
RALFL12		At2g19040	7880	72	8.49	0.403	0.167	0.570
RALFL13		At2g19045	7881	72	8.37	0.417	0.194	0.611
RALFL14		At2g20660	11719	101	9.62	0.515	0.139	0.654
RALFL15		At2g22055	8830	79	10.59	0.544	0.177	0.721
RALFL16		At2g32835	10668	95	7.86	0.368	0.189	0.557
RALFL17		At2g32890	8377	77	3.99	0.273	0.208	0.481
RALFL18		At2g33130	11390	103	9.60	0.369	0.214	0.583
RALFL19		At2g33775	12397	110	10.04	0.518	0.145	0.663
RALFL20		At2g34825	7717	72	9.03	0.278	0.278	0.556
RALFL21		At3g04735	11951	105	10.06	0.457	0.210	0.667
RALFL22		At3g05490	13029	119	8.36	0.403	0.151	0.554
RALFL23		At3g16570	15049	138	8.60	0.449	0.130	0.579
RALFL24		At3g23805	13243	118	8.20	0.407	0.220	0.627
RALFL25		At3g25165	8523	74	10.63	0.554	0.189	0.743
RALFL26		At3g25170	8610	76	10.85	0.658	0.184	0.842
RALFL27		At3g29780	12698	117	8.10	0.299	0.120	0.419
RALFL28		At4g11510	9390	85	9.09	0.447	0.259	0.706

RALFL29		At4g11653	10308	90	10.52	0.444	0.156	0.600
RALFL30		At4g13075	8217	76	8.79	0.329	0.237	0.566
RALFL31		At4g13950	12676	113	4.77	0.363	0.159	0.522
RALFL32		At4g14010	12919	117	8.05	0.368	0.188	0.556
RALFL33		At4g15800	12806	116	9.36	0.440	0.138	0.578
RALFL34		At5g67070	14729	129	6.76	0.434	0.078	0.512
*RALFL		At1g60913	9062	79	9.26	0.430	0.152	0.582
*RALFL		At2g32785	8016	71	9.68	0.366	0.169	0.535
*RALFL		At2g32788	7210	64	9.74	0.266	0.156	0.422
*RALFL		At2g32885	7414	72	9.04	0.264	0.222	0.486
*RALFL		At4g14020	12790	111	6.68	0.477	0.090	0.567
SCRL1	27	At4g10457	10283	92	8.40	0.380	0.304	0.685
SCRL2		At1g65113	10470	92	8.57	0.359	0.304	0.663
SCRL3		At1g08695	9892	88	8.50	0.386	0.318	0.705
SCRL4		At1g60986	10528	97	7.59	0.320	0.247	0.567
SCRL5		At1g60987	11119	97	6.95	0.340	0.309	0.649
SCRL6		At1g60985	10561	95	7.91	0.274	0.295	0.568
SCRL7		At1g60989	10308	92	8.41	0.413	0.217	0.630
SCRL8		At1g60983	10183	89	7.85	0.360	0.247	0.607
SCRL9		At2g05117	10273	90	6.47	0.367	0.289	0.656
SCRL10		At4g15735	11901	107	7.54	0.355	0.262	0.617
SCRL11		At4g15733	9391	86	8.14	0.256	0.279	0.535
SCRL12		At2g23727	10876	92	9.44	0.511	0.283	0.793
SCRL13		At2g23715	10795	95	9.30	0.453	0.274	0.726
SCRL14		At4g22115	9207	86	6.95	0.256	0.256	0.512
SCRL15		At2g05335	11553	101	7.90	0.356	0.238	0.594
SCRL16		At2g06983	9887	87	9.80	0.414	0.322	0.736
SCRL17		At2g25685	10853	98	7.91	0.337	0.224	0.561
SCRL18		At2g14282	11115	97	8.49	0.443	0.206	0.649
SCRL19		At3g27503	11097	98	9.04	0.429	0.204	0.633
SCRL20		At4g10115	10983	97	9.48	0.443	0.268	0.711
SCRL21		At4g10767	12301	109	8.85	0.385	0.165	0.550
SCRL22		At4g33465	11179	98	7.84	0.347	0.306	0.653
SCRL23		At4g14785	10865	95	7.57	0.347	0.274	0.621
SCRL24		At4g32717	10277	89	8.40	0.360	0.292	0.652
SCRL25		At4g32714	10132	87	8.26	0.379	0.299	0.678
SCRL26		At4g22105	9641	87	8.29	0.356	0.276	0.632
SCRL27		At5g45875	10736	93	9.05	0.409	0.258	0.667
TPD1	1	At4g24972	19457	176	9.04	0.352	0.136	0.489

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CEP1 (C-terminally encoded peptide 1)

A database search was used to identify novel secreted proteins that contain conserved C terminal ends which revealed the CEP family containing five members in Arabidopsis (Ohyama *et al.* 2008). A secreted and processed 14–15 amino acid CEP1 peptide containing two hydroxyproline residues derived from the conserved C terminal domain was detected in whole plant submerged cultures of CEP1-overexpressing plants (Ohyama *et al.* 2008). ProCEP1:GUS assays indicate that CEP1 is mainly in the lateral root primordia while synthetic CEP1 (and CEP1 overexpressing plants) inhibits root growth (Ohyama *et al.* 2008) indicating that CEP1 has a localised role in root development.

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CLE family

The CLE family is named after *CLAVATA3* (*CLV3*) from Arabidopsis (Fletcher *et al.* 1999) and *EMBRYO SURROUNDING REGION* (*ESR*) from maize (Opshal-Ferstad *et al.* 1997) and forms one of the largest families of plant peptide signalling molecules that is present throughout the plant kingdom (Cock and McCormack 2001; Oelkers *et al.* 2008) containing over 30 annotated genes in Arabidopsis. Since there are several excellent recent reviews on this family (see Butenko *et al.* 2009; Fukuda *et al.* 2007; Jun *et al.* 2008; Miwa *et al.* 2009), we will only briefly review it here. CLE family members are relatively small secretory peptides (<15 kDa) that contain the CLE domain (14 amino acids) near the C terminal (Cock and McCormack 2001). CLV3 is the best characterized CLE member with a major role in meristem development which was revealed from the study of loss of function mutants. The *clv3* mutant contains excess stem cells in shoot apical and floral meristems that continue to enlarge over time (Clark *et al.* 1995). CLV3 is secreted into the meristematic apoplast (Rojo *et al.* 2002) where it binds to its receptor CLV1 which is a member of the leucine rich repeat receptor like kinase (LRR-RLK) family (Clark *et al.* 1997). Both the *clv1* and *clv2* mutants exhibit a similar phenotype to the *clv3* mutant (Clark *et al.* 1993; Kayes and Clark 1998) which helped identify them as potential receptors for CLV3. While CLV1 is a full length active LRR-RLK, CLV2 is a receptor like molecule similar to CLV1 but lacking any kinase domain. CLV2 is thought to form a dimer with CLV1 linked by disulphide bond to stablise CLV1 in the inactive form (Jeong *et al.* 1999). However, there is a growing amount of evidence that CLV1 can form dimers with other closely related LRR-RLKs such as BARELY ANY MERISTEM (BAM) to bind CLV3 (DeYoung and Clark 2008); and that CLV2 associates with other LRR-RLKs such as CORYNE (CRN) rather than CLV1 (Müller *et al.* 2008; reviewed in Butenko *et al.* 2009). The CLE motif of CLV3 is all that is required for its activity (Fiers *et al.* 2006) and a modified 12 amino acid peptide containing two hydroxyproline residues derived from the CLE region of CLV3 has been

identified in Arabidopsis tissues (Kondo *et al.* 2006) and CLV3 has been shown to be processed by meristematic tissue (Ni and Clark 2006). More recently, direct evidence has been obtained of the interaction of modified CLV3 with the external domain of the LRR-RLK CLV1 (Ogawa *et al.* 2008).

The CLE family has been divided into the A and B group based on the tissues that they affected; CLE B class molecules include CLE41-44 which affect vascular development while CLE A molecules form the remainder of the family that inhibit meristematic growth (Whitford *et al.* 2008). Combinations of the A and B peptides enhanced proliferation of vascular development in an auxin dependent manner (Whitford *et al.* 2008). These results indicate that receptors can either recognize different CLE ligands or alternatively that multiple CLE receptors are expressed in the developing vascular and meristematic regions.

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EPIDERMAL PATTERNING FACTOR (EPF)

In Arabidopsis there are seven genes in the EPF family and all have the predicted N terminal secretory domain and a conserved C terminal domain containing 6 cysteine residues (Hara ***et al.*** 2009). EPF1 and EPF2 are involved in determining epidermal cell division events that lead to stomatal formation in leaf and stem epidermis (Hara ***et al.*** 2007; Hara ***et al.*** 2009; Hunt and Gray 2009). Stomata form from asymmetric divisions of epidermal cells into the meristemoid mother cell that will eventually give rise to the guard cell and adjacent epidermal cells so that usually adjacent epidermal cells occur between stomata in dicotyledons (Sachs 1979). EPF1 was identified through an over-expressing screen of small peptide molecules and the plants contained significantly fewer stomata than wild type, while *epf1* mutants had increased stomatal density including overlapping guard cell pairs (Hara ***et al.*** 2007). Using double mutants, it was shown that EPF1 was likely to interact with TOO MANY MOUTHS (TMM) and ERECTA (ER) (Hara ***et al.*** 2007). The *tmm* mutant has a very similar phenotype to *epf1* (Yang and Sack 1995) and *TMM* encodes a leucine rich repeat receptor like protein (LRR RLP) that lacks a kinase domain (Nadeau and Sack 2002). ER and ERECTA-LIKE (ERL) 1 and ERL2 are important LRR-RLKs that control the number and placement of asymmetric divisions in the stomatal pathway in leaves although they seem to have an opposing effect in stems (Shpak ***et al.*** 2005) as well as many other functions (for a review see van Zanten ***et al.*** 2009). Thus it is plausible to speculate that EPF1 could be a ligand for these receptor complexes (containing combinations of TMM, ER, ERL1 and ERL2) and so enforce the epidermal one cell spacing that occurs between guard cells (see Bhave ***et al.*** 2009; Hara ***et al.*** 2007). However, both EPF1 and EPF2 do not require the subtilisin type protease STOMATAL DENISTY AND DISTRIBUTION (SDD) 1 for their function (Hara

et al. 2007; Hara *et al.* 2009). Since SDD1 is a subtilisin type serine protease involved in stomatal development (Berger and Altmann 2000) it suggests that EPF1 and EPF2 are not processed by this enzyme and that further secretory peptides and processing enzymes may be required. EPF2 appears to actually restrict the number of meristemoid cells that can develop into guard cells and so acts upstream of EPF1 (Hara *et al.* 2009; Hunt and Gray 2009). Like EPF1, EPF2 requires TMM and the ER receptor kinases (Hara *et al.* 2009; Hunt and Gray 2009). This suggests that part of the control is at the level of secretion as these receptor complexes are recognizing both EPF1 and EPF2 which have different actions.

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INFLORESCENCE DEFICIENT IN ABSCISSION (IDA)

IDA was identified from a mutant *ida* that retains its floral organs indefinitely where it appears to regulate ethylene insensitive floral abscission (Butenko *et al.* 2003) which is supported by the observation that IDA overexpressing lines exhibited earlier floral abscission (Stenvik *et al.* 2006). proIDA::β-glucuronidase (GUS) reporter studies showed that IDA was restricted to the abscission zone at the bases of floral organs (Butenko *et al.* 2003). IDA encodes a protein containing a signal peptide of 77 amino acids that has been shown to be exported into surrounding apoplastic space using IDA:GFP fusion protein constructs expressed in transiently transfected onion cells (Butenko *et al.* 2003). The C-terminal tail sequence of 20 amino acids

was used to identify five other IDA-Like (IDL) genes in Arabidopsis and also in other plant species such as poplar and wheat that contained the extended PIP (EPIP) motif (Butenko *et al.* 2003), which is thought to be the active peptide. Consistent with this idea, it has recently been shown that IDA can be processed by cauliflower meristem extracts to yield peptide fractions (Stenvik *et al.* 2008) similar to the processing of CLV3. Arabidopsis IDL genes are expressed in different regions of the plant (e.g. AtIDL1 is found in roots while IDL3 is found in flowers and seedlings) (Butenko *et al.* 2003). This finding is suggestive of these proteins having defined roles in plant development, which may be constrained by their sites of expression. This idea is supported by the rescue of *ida* mutants by synthetic EPIP peptides of IDA and IDL1 and partial rescue by the other IDL peptides (Stenvik *et al.* 2008) indicating that a certain redundancy in the EPIP peptide. The phenotype of the double mutant of HAESA and HAESA-LIKE2 is similar to *ida* and as these are both leucine rich repeat receptor-like kinases (LRR-RLKs) it is likely that they represent the IDA receptor(s) (Stenvik *et al.* 2008).

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PEP1

AtPEP1 was originally isolated from Arabidopsis leaves as a 23-amino acid molecule where it was shown to induce synthesis of H_2O_2 and transcription of a plant defense gene, defensin (Huffaker *et al.* 2006) which are the hallmarks of plant innate immune responses. AtPEP1 is derived from the precursor protein PROPEP1 which is induced by wounding, methyl jasmonate and ethylene (Huffaker *et al.* 2006). PROPEP1 is a member of a seven gene family in Arabidopsis that vary at the N terminus but have similarities at the C terminus where the active peptide is present. Homologues have been found in both dicot and monocot economically important crop plants (Huffaker *et al.* 2006). The active region of the AtPEP1 resides in the 15 amino acid C terminus which is more highly conserved between species (Pearce *et al.* 2008). The AtPEP1 receptor (AtPEP1R) was isolated using a photoaffinity analogue of AtPEP1 and identified as a member of the leucine rich repeat receptor like kinase (LRR-RLK) family (Yamaguchi *et al.* 2006). The AtPEP1R contains an intracellular kinase domain and within that domain there is also a putative guanylate cyclase domain (Kwezi *et al.* 2007).

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Plant Natriuretic Peptides (PNPs)

PNPs were first identified using antibodies to mammalian atrial natriuretic peptide (ANP) in Florida beauty (*Dracena godseffiana*) and several other species (Vesely and Giordano 1991; Vesely *et al.* 1993). Subsequently, it was shown that synthetic ANP induced stomatal opening and bound to plant membranes (Gehring *et al.* 1996; Suwastika *et al.* 2000). Biologically active PNP immuno-analogues (irPNPs) were purified by immunoaffinity chromatography from several species including ivy and potato (Billington *et al.* 1997; Maryani *et al.* 2001; Pharmawati *et al.* 1998; Pharmawati *et al.* 2001). N and C terminal sequence data obtained from potato PNPs (Maryani *et al.* 2001) was used to identify two closely related homologous genes in Arabidopsis, *PNP-A* and *PNP-B* (Ludidi *et al.* 2002). PNP contains a predicted signal peptide which implies that it may function in the extracellular space (Ludidi *et al.* 2002) and this has been supported by proteomic studies showing that PNP-A is present in the apoplast (Boudart *et al.* 2005). Immunoreactive tissue printing studies showed that irPNP was localised in the vascular tissue (Maryani *et al.* 2003) and it has recently been shown to be expressed in the metaxylem and vascular parenchyma and phloem tissue in vascular bundles (Wenzel *et al.* 2008).

PNPs are small proteins (approximately 14 kDa) that are distantly related to the expansin family (Kende *et al.* 2004; Ludidi *et al.* 2002). Expansins have major roles in regulating cell extension (Cosgrove *et al.* 2002). AtPNP-A and AtPNP–B show homology with the N-terminus of expansins but are considerably shorter as they lack the wall binding domain (Ludidi *et al.* 2002). A related transcript from citrus CjBAp12 has tested negative for expansin activity suggesting a role other than wall loosening (Ceccardi *et al.* 1998).

Unlike expansins, PNPs appear to be involved in regulating solute and water homeostasis (Gehring and Irving 2003). This is supported by observations that both native irPNPs and recombinant AtPNP-A (at nanomolar concentrations) stimulate protoplast swelling and stomatal opening (Maryani *et al.* 2001; Morse *et al.* 2004; Rafudeen *et al.* 2003; Wang *et al.* 2007) in addition to rapid changes in ion fluxes in roots (Ludidi *et al.* 2004; Pharmawati *et al.* 1999). Recombinant AtPNP-A directly increases stomatal conductance and transpiration rates and this is correlated with increased efficiency of light use during photosynthetic CO_2 fixation enhancing photosynthetic rates (Gottig *et al.* 2008). Native irPNPs levels are increased in NaCl stressed whole plants and Arabidopsis suspension culture cells exposed to high salt or osmoticum (Rafudeen *et al.* 2003). Furthermore, analysis of Arabidopsis microarray data through Genevestigator (Zimmermann *et al.* 2004) also indicates that AtPNP-A transcripts are up-regulated in response to many abiotic and biotic stresses such as osmotic, salt, mineral deficiencies, ozone and plant pathogens. Co-expression and promoter content analyses indicate

that AtPNP-A may function alongside other pathogenesis-related proteins as a component of plant defense responses (Meier *et al.* 2008). Further, recombinant AtPNP-A modulates the effect of ABA on stomatal aperture (Wang *et al.* 2007). Since both compounds are up-regulated in times of environmental stress (e.g. drought), it is possible that one of the physiological roles of PNP-A is to act as an antagonist to ABA. This would result in promote limited gas exchange as PNP promotes stomatal opening. Collectively, these findings lead to the hypothesis that PNP-like molecules form part of the stress response and possibly function as extracellular signalling molecules that directly affect water and solute transport.

The N terminus of PNP molecules contains a domain that is conserved between plant species and animal NPs. Protoplast expansion assays have been used to show that this domain is critical for the action of PNP (Morse *et al.* 2004; Wang *et al.* 2007). Although the PNP receptor has not been identified, both native irPNPs and PNP-A induce rapid and transient increases in intracellular cyclic GMP levels (Pharmawati *et al.* 1998; Pharmawati *et al.* 2001; Wang *et al.* 2007) indicating the receptor is in some way associated with guanylate cyclase catalytic activity. Interestingly, the receptors for ANP in animals are membrane bound receptor guanylate cyclases (Potter *et al.* 2006).

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Phytosulfokines (PSKs) and other sulphated peptides

Phytosulfokine-α (PSK-α) is a pentapeptide that is sulphated on its two tyrosine residues ($Y(SO_3H)IY(SO_3H)TQ$). PSK-α was first discovered as a cell proliferation agent essential for low density cell culture from cultures of asparagus (Matsubayashi and Sakagami 1996) and later

from rice and carrot suspension cultures (Kobayashi *et al.* 1999; Matsubayashi *et al.* 1997). The sulphation of the tyrosine residues is required to obtain nanomolar activity (Matsubayashi and Sakagami 1996). The identification of the growth promoting activities of this peptide has commercial implications as some taxol producing cultures respond to PSK-α (Kim *et al.* 2006).

Several precursor proteins for PSK (proPSK) have been identified in different species and each is ~80–90 kDa long with an N terminal secretory signal sequence and a PSK sequence near the C terminus (Lorbiecke and Sauter 2002; Yang *et al.* 1999; Yang *et al.* 2001). ProPSK is probably sulphated as the protein is processed through the Golgi network by enzymes such as tyrosylprotein sulphotransferase (Hanai *et al.* 2000) before being secreted. Dibasic residues occur upstream from the PSK sequence in proPSK and these are cleaved by specific subtilsin serine proteases in the apoplast (Srivastava *et al.* 2008) before further processing to the active pentapeptide. Thus several enzymes and ProPSK need to meet in the apoplast and presumably will need to be secreted from adjacent or the same cells for this to occur. The PSK receptor is a conserved leucine rich repeat receptor-like kinase (LRR-RLK) protein and is involved in regulating root elongation (Matsubayashi *et al.* 2006; Matsubayashi *et al.* 2002). In plants, PSK is released from the various proPSK proteins and acts in a paracrine or autocrine fashion on nearby cells expressing the receptor which is most clearly shown in roots where PSK enhances root elongation by controlling cell size (Kutschmar *et al.* 2009). PSKs also have a role in attenuating expression of stress response genes during differentiation of tracheary elements (Motose *et al.* 2009).

The family of sulphated signalling peptides is likely to expand following the recent discovery of an 18 amino acid tyrosine sulphated glycopeptide called PSY1 for PLANT PEPTIDE CONTAINING SULPHATED TYROSINE (Amano *et al.* 2007). PSY1 is upregulated by wounding and promotes cellular expansion and proliferation and binds to a LRR-RLK protein AtPSYR1 that is closely related to AtPSKR1 (Amano *et al.* 2007).

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Rapid Alkalization Factor (RALF)

The first RALF peptide was isolated from tobacco leaf extracts as part of a screen to identify defense induced peptides that stimulate rapid alkalinisation of tobacco suspension cell culture medium. NaRALF is a small (5 kDa) 49 amino acid polypeptide derived from an 115 amino acid preproprotein (Pearce *et al.* 2001). Dibasic residues occur upstream from the RALF sequence in proRALF and these are cleaved by specific subtilsin serine proteases to release the active RALF peptide (Srivastava *et al.* 2009). RALF and RALF-like (RALFL) annotated peptide sequences are composed of an N-terminal signal and small divergent charged or polar mature peptide with a highly conserved cysteine rich motif (CX{4,14}CX{22,51}CX{6,12}CX{5,14}CX{5, 6}C) (Pearce *et al.* 2001; Silverstein *et al.* 2007). RALFL sequences have been found in many plant families including tobacco, tomato, Arabidopsis, alfalfa, poplar and rice (Germain *et al.* 2005; Haruta and Constabel 2003; Olsen *et al.* 2002; Pearce *et al.* 2001; Silverstein *et al.* 2007) suggesting a ubiquitious role in plants (Haruta *et al.* 2008; Pearce *et al.* 2001). There are over 39 annotated RALFL genes in Arabidopsis and these are clustered within the genome as they are in rice (Olsen *et al.* 2002; Silverstein *et al.* 2007).

A link between alkalinization and defense response has been suggested as the small glycoprotein of *Phytophthora megasperma,* pep-13, causes the rapid media pH alkalinization of parsley cells which is followed by the induction of phenylalanine ammonia lyase (PAL) and the formation of defensive phytoalexins (Nürnberger *et al.* 1994). Although NaRALF has been associated with activation of mitogen activated protein (MAP) kinase in tobacco leaves it did not induce tobacco *PAL* transcripts as systemin does suggesting it is not a defensive wound signal peptide (Haruta

and Constabel 2003; Pearce *et al.* 2001). Moreover, no difference in transcript levels of seven Arabidopsis *RALF* genes in both *mpk1* (constitutive systemic acquired resistance) or *ctr1* (constitutive ethylene response) mutants (Olsen *et al.* 2002) and neither mechanical wounding nor exposure to insect oral secretions in *Solanum chacoense* affected *RALFL* expression levels (Germain *et al.* 2005). However, RALFL transcripts have been found to be differentially expressed in most plant organs examined in Arabidopsis, poplar and *S. chacoense* and are over represented in the reproductive organs (ovaries and fruit) of *Medicago truncutula* and Arabidopsis suggesting RALFL peptides play a role in development (Germain *et al.* 2005; Haruta and Constabel 2003; Olsen *et al.* 2002; Silverstein *et al.* 2007). Specifically, RALFL proteins may play a role in root development as exogenous RALFL applied to Arabidopsis and tomato seedlings inhibited root growth (Pearce *et al.* 2001) and silencing of RALF in tobacco disrupted root hair development (Wu *et al.* 2007). In tobacco the silenced RALF root phenotype was partially restored when mutant plants were grown on low-pH buffered medium (pH 5.7) and the root phenotype was reproduced when wild-type plants were grown on high-pH buffered medium. It was found that RALF was required to regulate root hair extracellular pH and the transition from root hair initiation to tip growth and plant growth in basic soils (Wu *et al.* 2007). In tomato suspension cells two 25 and 120kDa cell surface proteins were found to bind specifically to LeRALF and a similar 120kDa binding protein was also found in tobacco and alfalfa cell membranes suggesting the interactions of these proteins play a role in an intracellular signaling pathway (Scheer *et al.* 2005). Interestingly, nanomolar amounts of AtRALF1 caused cytoplasmic Ca^{2+} levels in the surface cells of Arabidopsis seedling roots to increase within forty seconds (Haruta *et al.* 2008), where it may play a role in eliciting events linked to stress response or the modulation of growth. Recently, overexpression of AtRALF23 was shown to impair brassinosteroid effects on growth and brassinosteroids themselves down regulate AtRALF23 expression in wild type (Srivastava *et al.* 2009).

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TAPETUM DETERMINANT1 (TPD1)

TPD1 was first identified by mutant screens selecting for male sterility and found to play a role in anther development. The *tpd1* mutants exhibit a phenotype of anthers lacking a tapetum while producing excess microsporocytes (Yang *et al.* 2003) and has an identical phenotype to *ems1* (*EXCESS MICROSPOROCYTES1* also known as *EXTRA SPOROGENOUS CELLS* (*EXS*)) (Zhao *et al.* 2002). A complementary expression pattern of TPD1 and EMS1 in the microspores and tapetum was evident (Yang *et al.* 2003) suggesting that the gene products interact. This was further supported by overexpression studies where the effects of TPD1 were dependent upon the expression of EMS1 (Jia *et al.* 2008; Yang *et al.* 2003). *TPD1* encodes a small protein with a predicted secretory N terminal signal sequence and orthologues have been identified in other plants such as rice (Zhao *et al.* 2008). EMS1 encodes a leucine rich repeat receptor like kinase (LRR-RLK) (Zhao *et al.* 2002) and binds to TPD1 at a specific site within its extracellular leucine rich domain and this in turn activates EMS1 receptor auto-phosphorylation (Jia *et al.* 2008).

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