

Metallochaperone-like genes in *Arabidopsis thaliana*

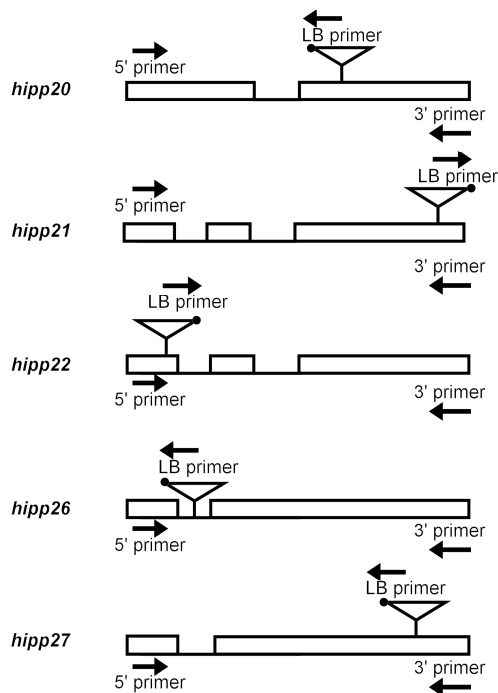
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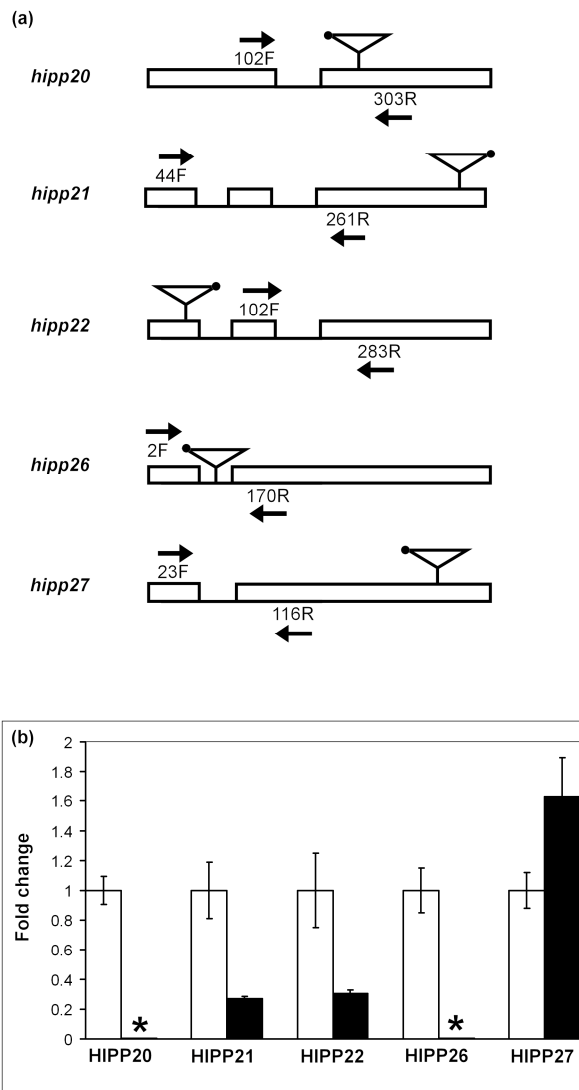
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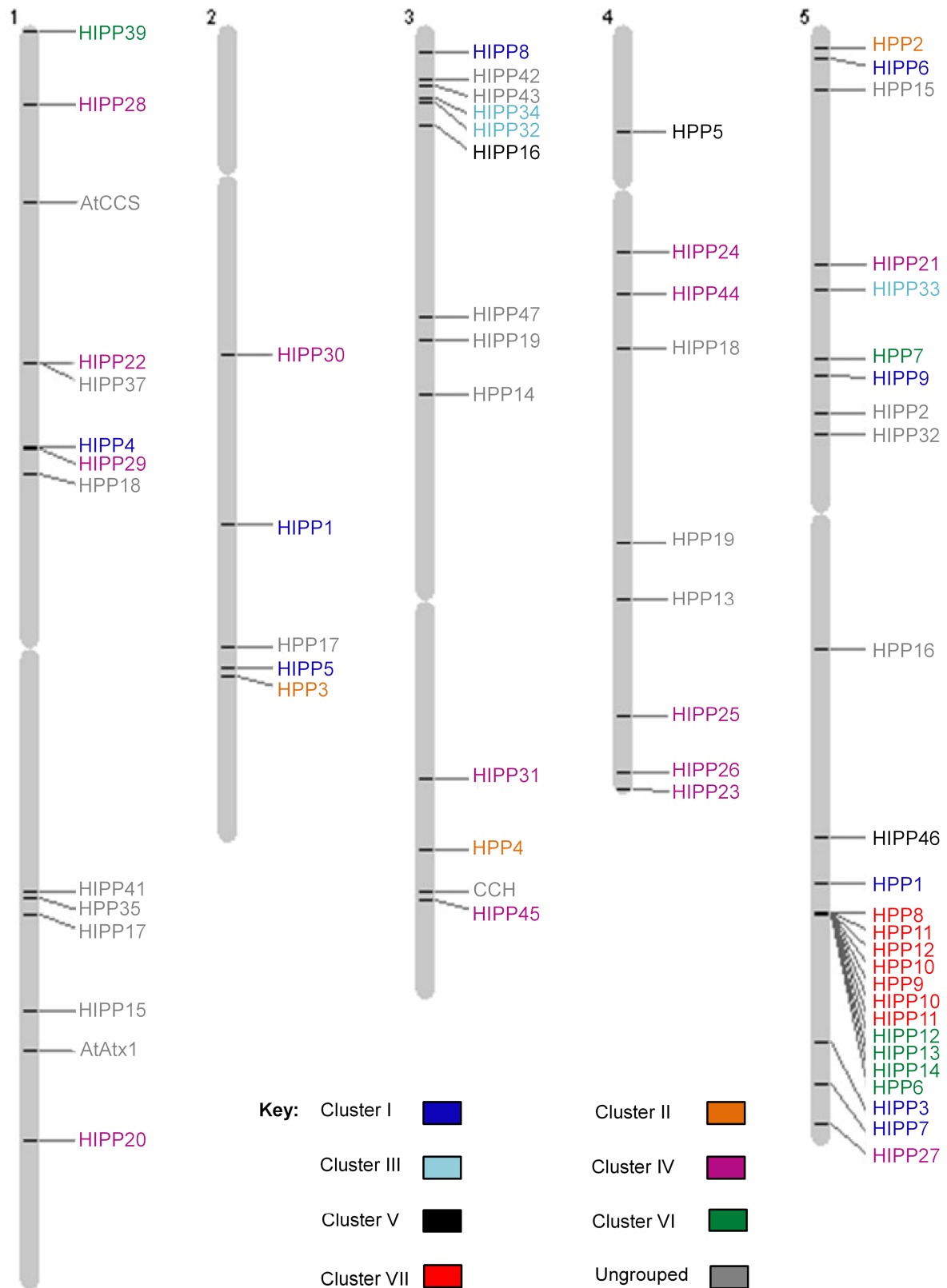


Supp Figure 1: Schematic representation of intron/exon boundaries and the location of T-DNAs in *hipp20*, *hipp21*, *hipp22*, *hipp26* and *hipp27*.

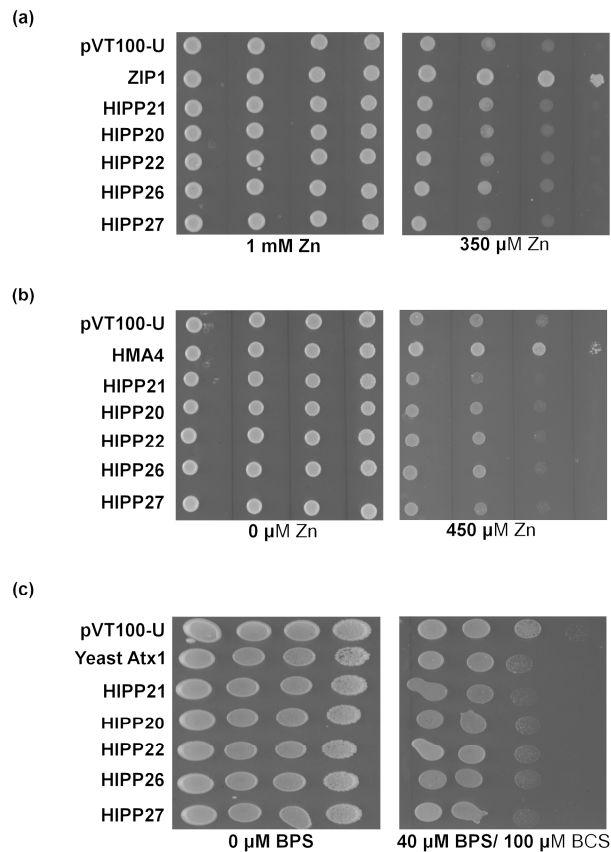
A schematic representation of the predicted transcribed regions of *hipp20*, *hipp21*, *hipp22*, *hipp26*, *hipp27*, showing approximate locations of exons (white boxes) and introns (black lines). ▽ Indicate the T-DNA insertion and • indicates the Left Border of the T-DNA. Arrows show the location and orientation of primers used for genotyping in each T-DNA mutant allele along with corresponding WT.



Supp Figure 2: Relative amount of transcript of mutant alleles in the *hipp20*, *hipp21*, *hipp22*, *hipp26* and *hipp27* mutants measured by RT-PCR. (a) A schematic outline of primer sets used in quantitative RT-PCR. Arrows denote the direction and position of PCR primer. **(b)** Relative amount of transcript of each *hipp* mutant allele (black bars) measured relative to wild-type (white bars) and normalised to *ACT2* expression. The graph represents the mean relative expression \pm SE of three independent biological samples of each mutant each measured in quadruplicate.



Supp Figure 3: The relative locations of the *Arabidopsis* HIPP and HPP genes were mapped on to the five *Arabidopsis* chromosomes using the Chromosome Map Tool (<http://www.Arabidopsis.org/jsp/ChromosomeMap/tool.jsp>) at TAIR. Members of each cluster are shown by different colour as shown in the Key.



Supp Figure 4: Heterologous expression of *HIPP20*, *HIPP21*, *HIPP22*, *HIPP26* and *HIPP27* cDNAs in *zrc1cot1* and *zrt1zrt2* and Δ *atx1*. (a) *zrt1zrt2* (Zn-dependent) expressing HIPPs, ZIP1 (positive control) and vector-only (pVT100-U) after 4 d growth at 30°C on LZM-U media with different concentrations of Zn. (b) *zrc1cot1* (Zn-sensitive) expressing HIPPs, AtHMA4 (positive control) and vector-only (pVT100-U) after 4 d growth at 30°C on SD-U media with and without Zn. (c) Yeast Δ *atx1* cells transformed with HIPPs, Yeast Atx1 (positive control) and vector-only (pVT100-U) were assayed for growth on SD/-U containing 30-100 μ M BPS and 100 μ M BCS (BPS/BCS) at 30°C.

Cells were spotted as one-tenth serial dilutions starting at $A_{600 \text{ nm}}$ 1.6.

Supp Table 1: The *Arabidopsis* HIPP and HPP Proteins

Chromosomal locus	Number of introns	Protein details						
		Number of CXXC motifs	Number of HMA domains	Putative ^a prenylation site	Length (a. a)	$\beta\alpha\beta\alpha\beta$ ^b Fold	Predicted ^c Localization	
I								
At5g24580	HIPP9	3	2	2	Yes	318	1) Yes 2) ?	?
At1g29000	HIPP4	3	2	2	Yes	287	1) ? 2) ?	Cyt
At5g50740	HPP1	4	2	2	No	290	1) Yes 2) Yes	?
At5g63530	HIPP7	4	2	2	Yes	355	1) Yes 2) Yes	?
At3g02960	HIPP8	4	2	2	Yes	246	1) Yes 2) ?	?
At2g28090	HIPP1	3	2	2	Yes	245	1) ? 2) Yes	Cyt
At5g60800	HIPP3	4	2	2	Yes	283	1) Yes 2) Yes	?
At2g36950	HIPP5	2	2	2	Yes	386	1) Yes 2) ?	?
At5g03380	HIPP6	4	2	2	Yes	392	1) Yes 2) Yes	Cyt
II								
At5g02600	HPP2	2	1	1	No	319	Yes	Plast
At2g37390	HPP3	2	1	1	No	259	Yes	Mito
At3g53530	HPP4	2	1	1	No	248	Yes	?
III								
At3g05220	HIPP34	2	1	1	Yes	577	Yes	Mito
At5g19090	HIPP33	2	1	1	Yes	587	Yes	?
At3g06130	HIPP32	2	1	1	Yes	473	Yes	?
IV								
At4g38580	HIPP26	1	1	1	Yes	153	Yes	Cyt
At4g35060	HIPP25	1	1	1	Yes	153	Yes	?

a: Presence or absence of isoprenylation sequence at the C-terminus of the HIPP/HPPs. The predictions were conducted through PrePS-Prenylation Prediction Suite (<http://mendel.imp.ac.at/sat/PrePS/index.html>) (Maurer-Stroh and Eisenhaber, 2005).

b: $\beta\alpha\beta\alpha\beta$ -fold structure was determined by using Conserved Domain Database (CDD) (<http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml>) (Marchler-Bauer et al., 2008). Description abbreviations: Yes, proteins predicted to contain the $\beta\alpha\beta\alpha\beta$ -fold and ? programme fails to predict. Where the protein has two HMA domains (1) indicates first domain and (2) indicates second.

c: Seven different localisation prediction programmes were used (iPSORT, MitoPred, LOctree, PeroxiP, Predator, SubLoc and TargetP). Proteins with three or more programmes predicting the same localisation are marked: Cyt, Cytoplasm; Plast, Plastid; Mito, Mitochondrion. ? three or more prediction programmes fail to reach the consensus.

Table 4.1 Continued

Chromosomal locus		Number of introns	Protein details					Predicted Localization
			Number of CXXC motifs	Number of HMA domains	Putative prenylation site	Length (a. a)	$\beta\alpha\beta\alpha\beta$ Fold	
At5g66110	HIPP27	1	1	1	Yes	145	Yes	?
At1g71050	HIPP20	1	1	1	Yes	152	Yes	Cyt
At4g08570	HIPP24	1	1	1	Yes	150	Yes	?
At4g39700	HIPP23	1	1	1	Yes	158	Yes	?
At1g06330	HIPP28	1	1	1	Yes	158	Yes	?
At1g29100	HIPP29	1	1	1	Yes	141	Yes	?
At5g17450	HIPP21	2	1	1	Yes	149	Yes	?
At1g22990	HIPP22	2	1	1	Yes	152	Yes	Cyt
At3g48970	HIPP31	2	1	1	Yes	140	?	?
At3g56891	HIPP45	2	1	1	Yes	166	Yes	?
At2g18196	HIPP30	2	1	1	Yes	142	Yes	Cyt
At4g10465	HIPP44	2	1	1	Yes	183	Yes	?
V								
At5g48290	HIPP46	2	0	1	Yes	181	?	?
At3g07600	HIPP16	2	0	1	Yes	157	?	?
At4g05030	HPP5	3	1	1	No	110	?	Mito
VI								
At1g01490	HIPP39	2	0	1	Yes	177	Yes	?
At5g23760	HPP7	2	0	1	No	103	?	?
At5g52770	HPP6	1	0	1	No	111	Yes	?
At5g52750	HIPP13	2	0	1	Yes	139	?	?
At5g52760	HIPP14	2	0	1	Yes	126	?	?
At5g52740	HIPP12	2	0	1	Yes	118	Yes	?
VII								
At5g52720	HIPP10	2	0	1	Yes	111	Yes	?
At5g52730	HIPP11	1	0	1	Yes	185	Yes	?
At5g52670	HPP8	2	0	1	No	103	Yes	Cyt

Table 4.1 Continued

Chromosomal locus		Number of introns	Protein details					Predicted Localization
			Number of CXXC motifs	Number of HMA domains	Putative prenylation site	Length (a. a)	βββββ Fold	
At5g52710	HPP9	5	0	2	No	451	1) ? 2) ?	?
At5g52700	HPP10	3	0	2	No	370	1) ? 2) ?	?
At5g52680	HPP11	3	0	2	No	238	1) ? 2) Yes	?
At5g52690	HPP12	4	0	2	No	177	1) Yes 2) Yes	Cyt
Ungrouped								
At3g24450	HPP14	2	0	1	No	140	Yes	?
At1g12520	AtCCS	5	1	1	No	254	Yes	Plast
At2g35730	HPP17	2	1	1	No	115	Yes	?
At3g04900	HIPP42	0	0	1	Yes	208	Yes	?
At4g23882	HPP19	3	1	1	No	284	Yes	?
At1g30473	HPP18	3	1	1	No	239	?	?
At5g26690	HIPP2	2	1	1	Yes	114	Yes	?
At1g56210	HIPP35	2	1	1	Yes	364	Yes	?
At3g56240	AtCCH	2	1	1	No	121	Yes	Cyt
At1g66240	AtAtx1	2	1	1	No	106	Yes	Cyt
At4g27590	HPP13	1	0	1	No	156	Yes	Cyt
At5g27690	HIPP36	2	1	1	Yes	352	Yes	?
At5g05365	HPP15	2	1	1	No	77	Yes	?
At5g37860	HPP16	2	1	1	No	262	Yes	?
At1g63950	HIPP15	1	0	1	Yes	113	Yes	?
At1g23000	HIPP37	2	1	1	Yes	358	Yes	?
At1g55780	HIPP41	1	0	1	Yes	133	?	?
At3g20180	HIPP47	1	0	1	Yes	118	?	?
At4g13380	HIPP18	2	0	1	Yes	195	?	?
At1g57780	HIPP17	4	0	2	Yes	264	1) ? 2) ?	?
At3g21490	HIPP19	2	1	1	Yes	136	Yes	?
At3g05920	HIPP43	2	1	1	Yes	126	?	?

Supp Table 2: hipp mutant alleles identified in this study

Allele ^a	Ecotype ^b	Position ^c	Gene T-DNA boundary sequence ^d
<i>hipp20</i>	Col	exon 2 (391)	AGT GGA TAC GTG GAA CCA ttgacgcttagacaac S G Y V E P
<i>hipp21</i>	Col	exon 3 (1251)	gttgtctaagcgtcaa AC ATC CGT AAT CCC ACT CAA I R N P T Q
<i>hipp22</i>	Col	exon 1 (53)	gaaaaaagaacatcac A ATA CGA AAA CGG AAG AAA CGT I R K R K K R
<i>hipp26</i>	Ws	intron 1 (208)	<u>GTAGAAACTAGCTTCGAT</u> tgtactgaatcggattttca
<i>hipp27</i>	Ler	exon 2 (356)	CTG TGG CCT TAC GTA CCT TA catagggatgaaaacggt L T P T V P

^a: *hipp20* (SALK_048115), *hipp21*(SALK_131715), *hipp22* (CS112068) and *hipp27* (CS180249) were obtained from SALK and *hipp26* (FLAG_631G11) was obtained from INRA.

^b: Ws, Wassilewskija; Col, Columbia; Ler, *Landsberg erecta*.

^c: numbers indicate the base pair immediately adjacent to the left-border of the T-DNA insertion relative to the A of the predicted initiation codon of the respective gene.

^d: the vertical bar identifies the boundary between gene sequence and insertion sequence; gene sequences are in upper case, intron sequences are underlined and coding sequences are translated below; insertion sequences are in lower case.