Plant DPPs III:

NUDIX/M49 proteins from *Physcomitrella patens* and *Arabidopsis thaliana*

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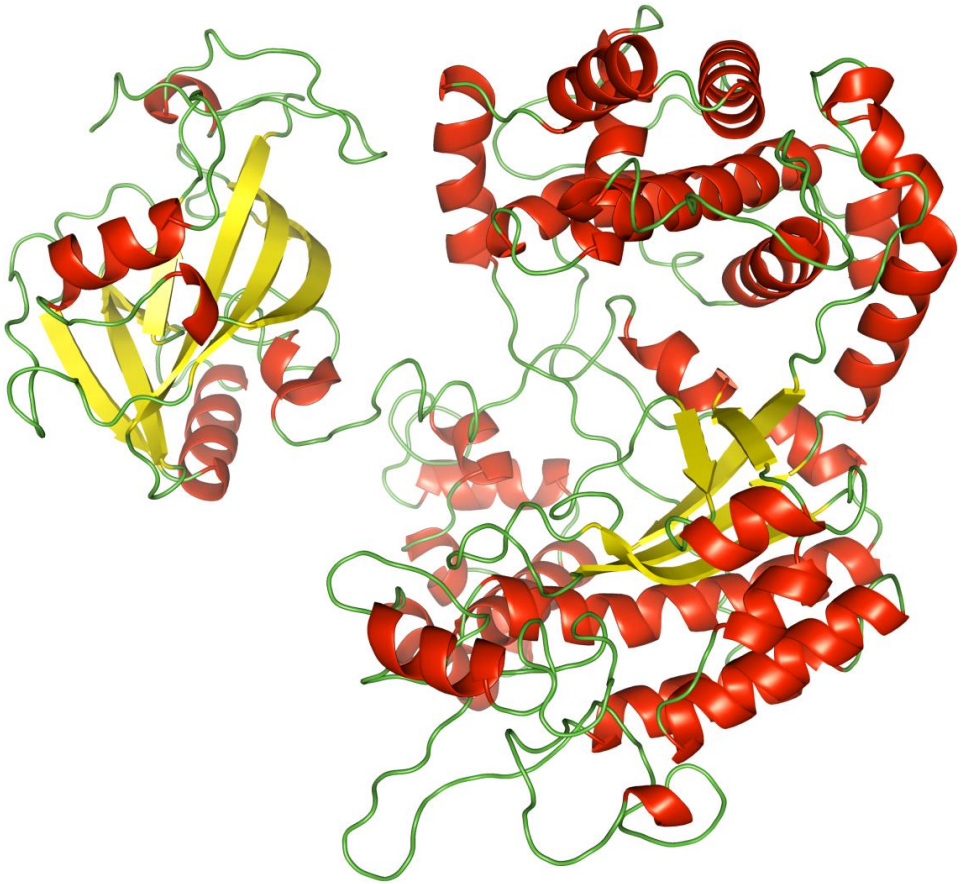
DPP III Minisymposium

Zagreb, March 21st 2016

One protein, two domains, three predicted activities



NUDIX – a fold;
a phosphatase superfamily
with nucleoside diphosphates
linked to X as substrates

M49* – DPP III with atypical
active site motif HEXXH

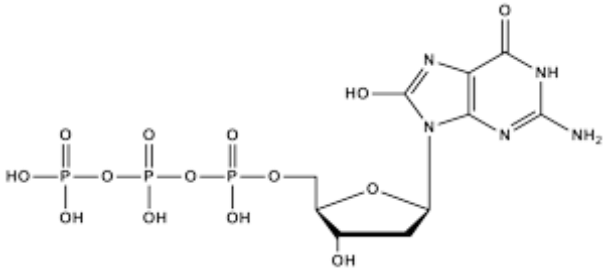
NUDIX/DPP III → ND

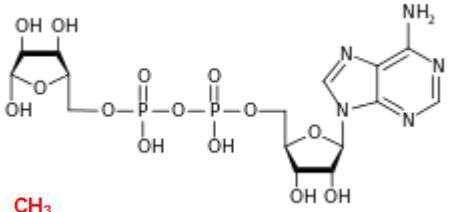
Predicted activities

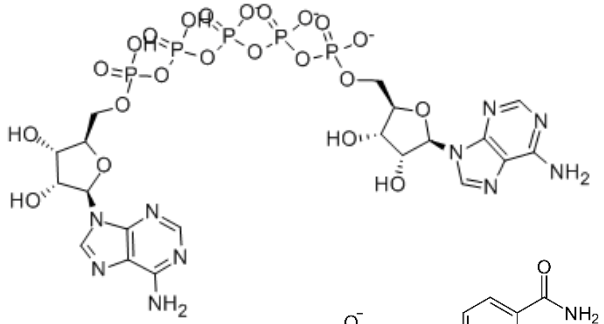
domain	activity	conserved residues	substrate	assay
M49	peptidase – DPP III	E592	Arg_2-2NA	yes
NUDIX	phosphatase	E92, E96	?	yes
NUDIX	isomerase – IDI	?	IPP	yes

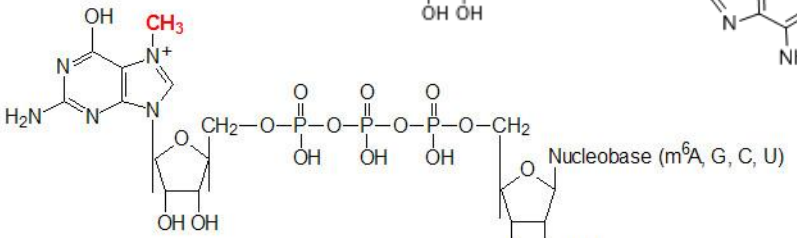
Gunawardana (2009) Comp Func Genomics
Ogawa (2005) JBC

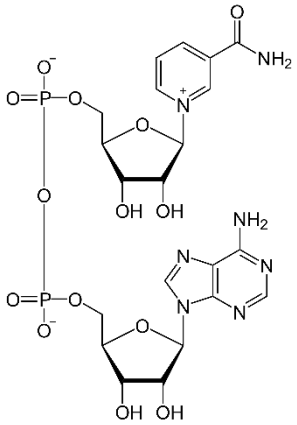
Nudix phosphatase substrates





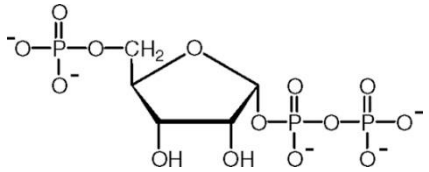


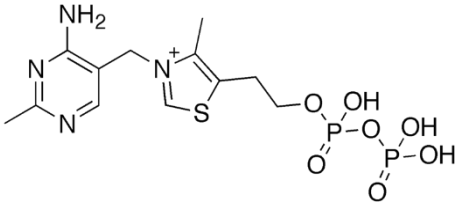


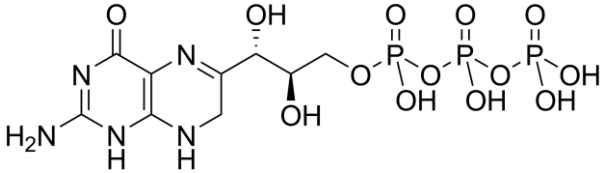


substrate ambiguity: nucleotide and non-nucleotide substrates

function: housecleaning and metabolic regulation

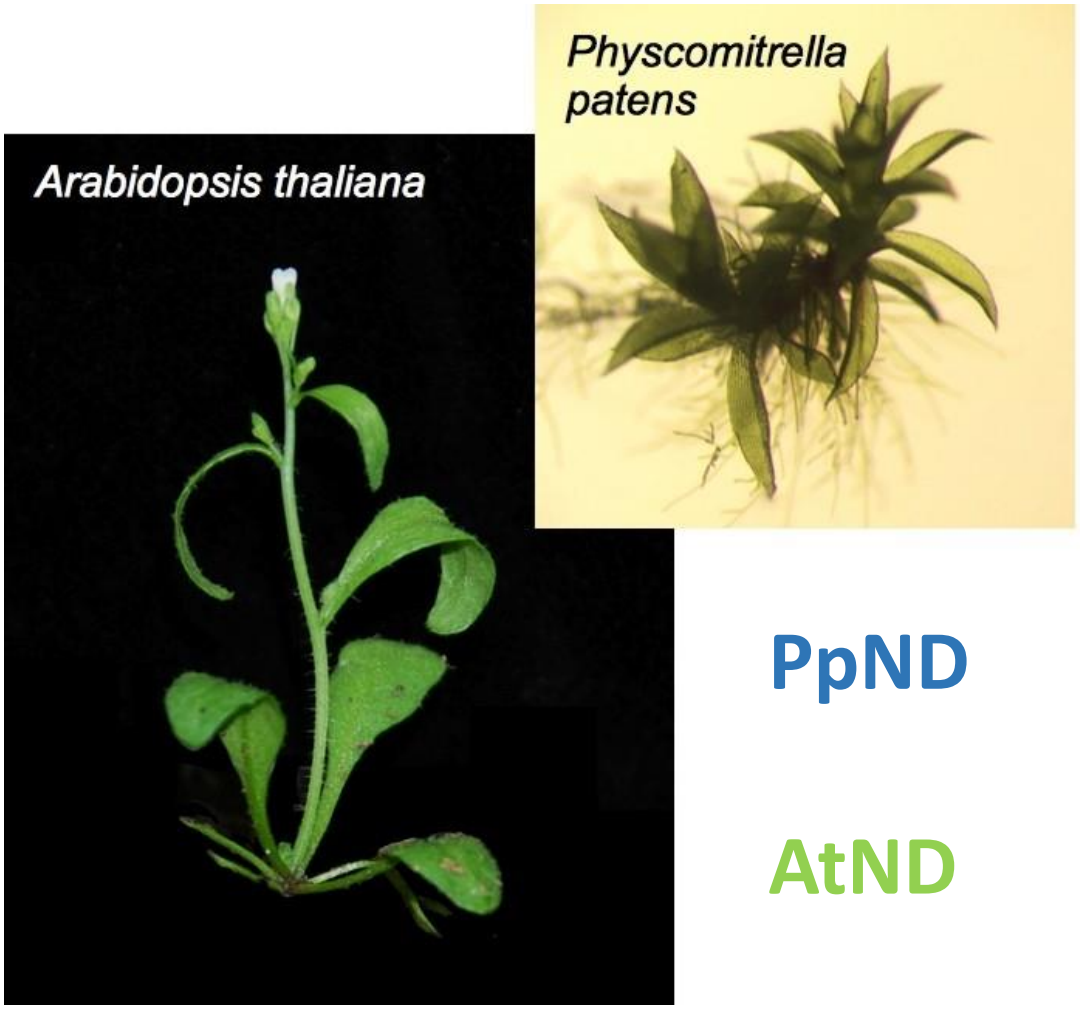






McLennan (2006) Cell Mol Life Sci

Model organisms

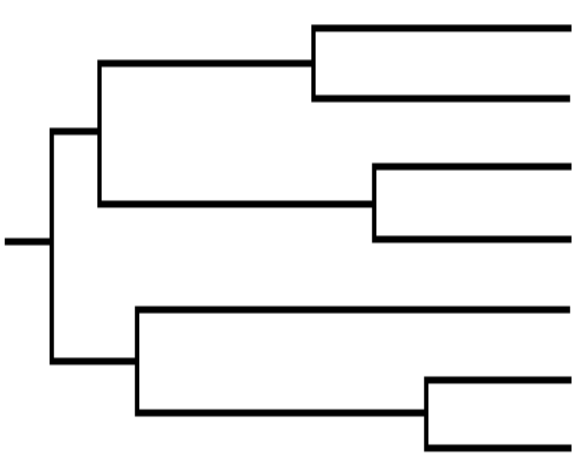


PpND

AtND

NUDIX/DPP III conserved in streptophytes!

Peptidase activity

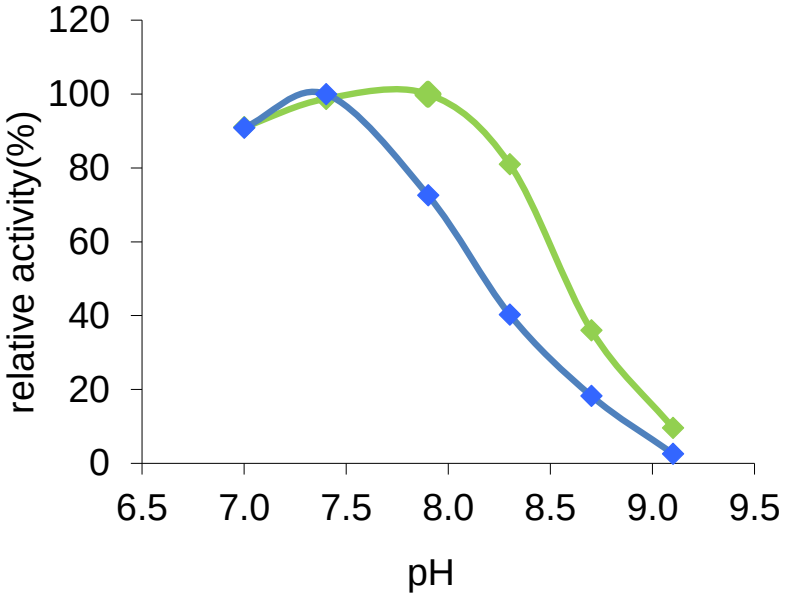


enzyme from	Specific activity with Arg_2-2NA (U/mg)	
human	1000	HEXXGH
yeast	20	
Porphyromonas	15	
Bacteroides	60	
Caldithrix	4	HEXXH
Physcomitrella	2	
Arabidopsis	0.2	

Replacing the HECCH motif in PpND with a hexapeptide

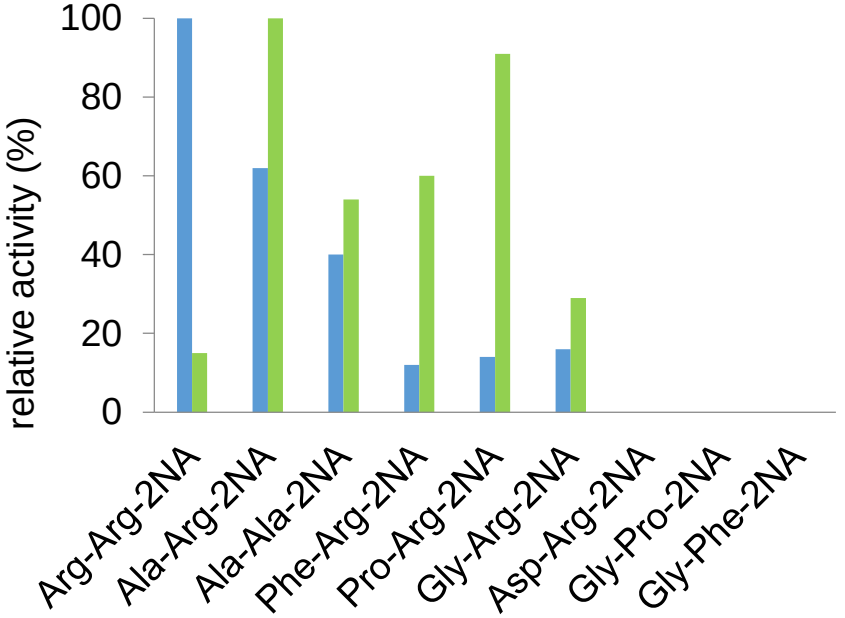
HECCGH	0.08
HECLGH	0.04

Differences between plant DPPs III

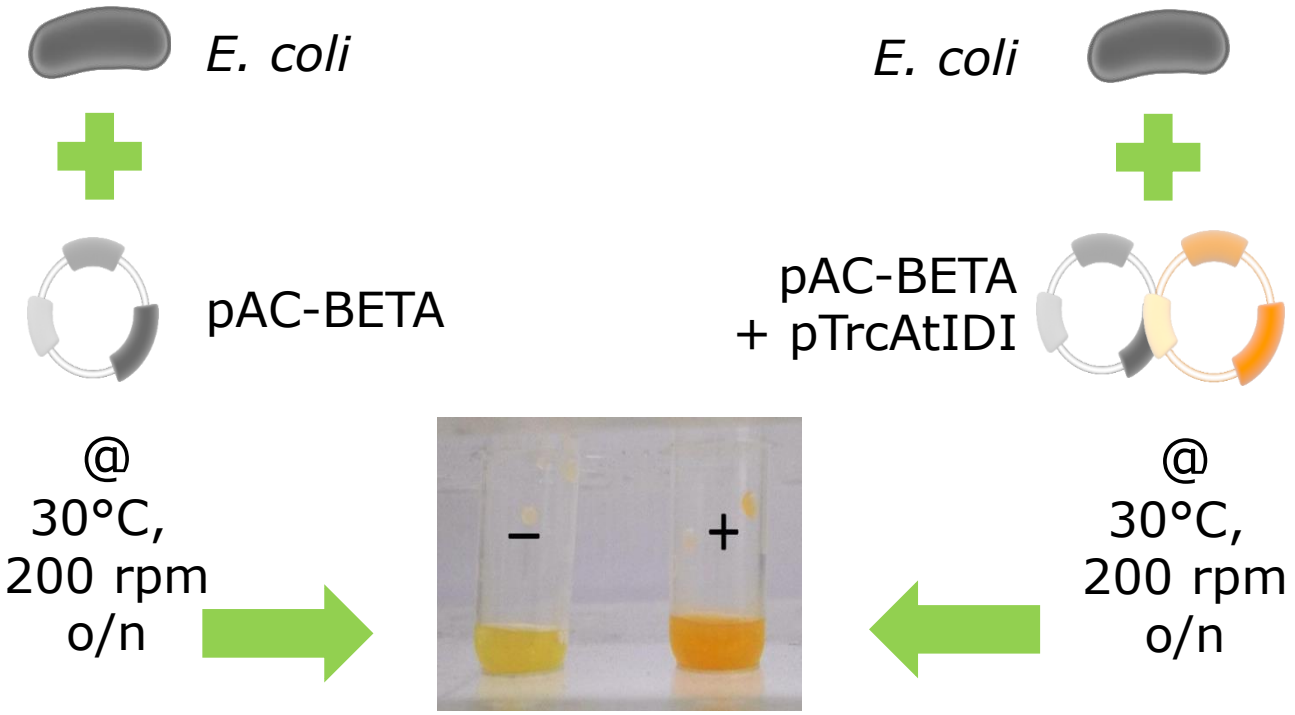


PpND

AtND



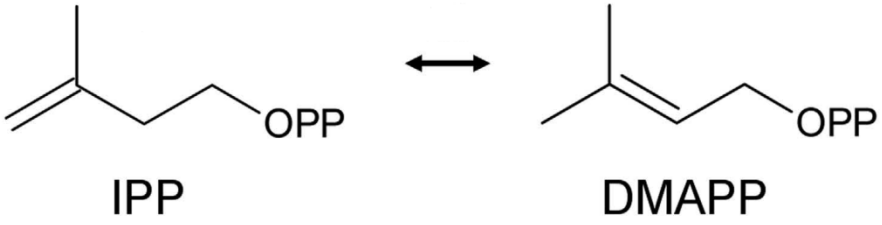
Color complementation assay for IDI activity

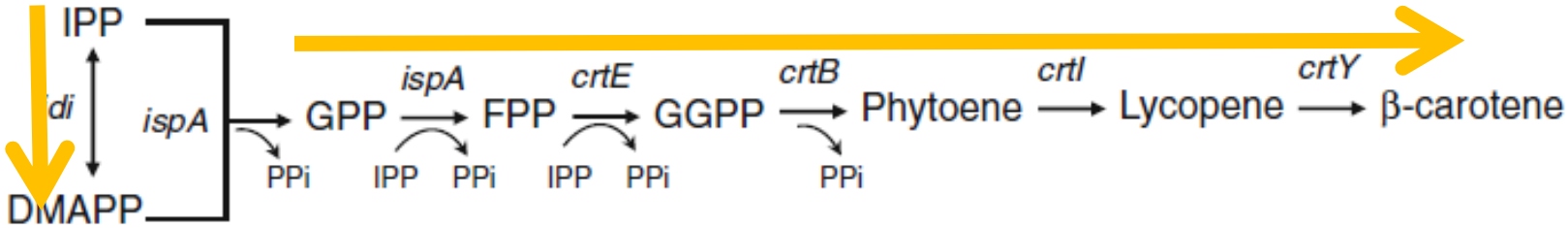


Sun (1996) JBC

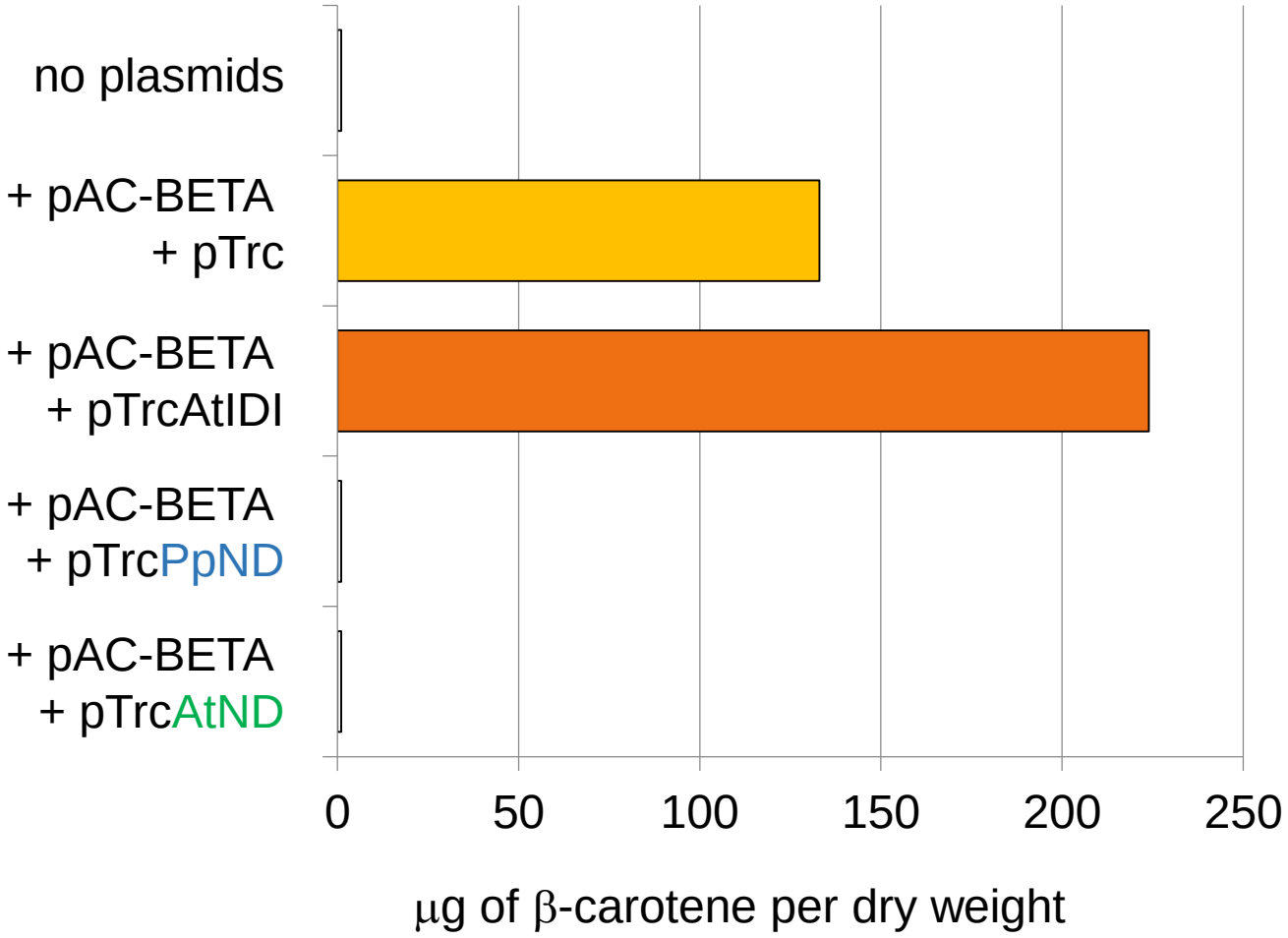
IDI increases β-carotene content

IDI: isopentenyl diphosphate isomerase

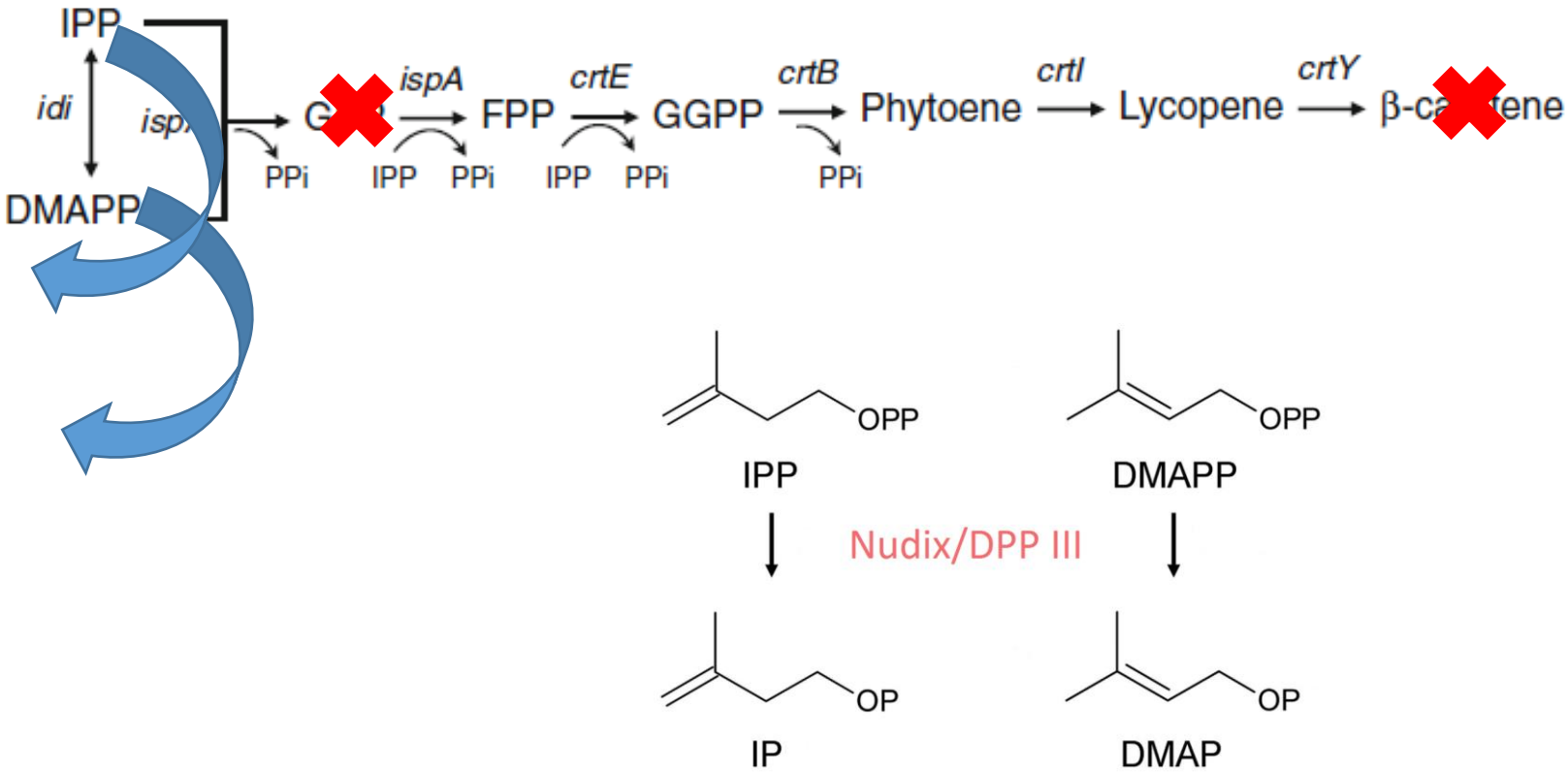




NDs are not isomerases!



IPP loss causes β-carotene loss?



Nudix hydrolase

NUDIX motif: **GXXXXXEXXXXXXXREUXEEXGU** (U = I, L or V)

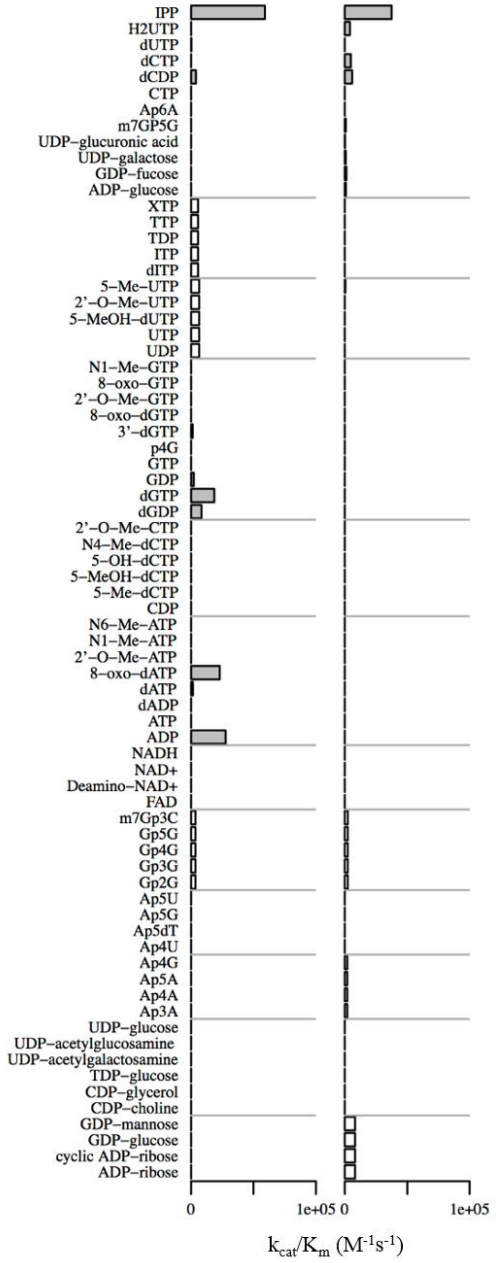
NDs: **GHISAGDXSLXXAXRELXEELGU**

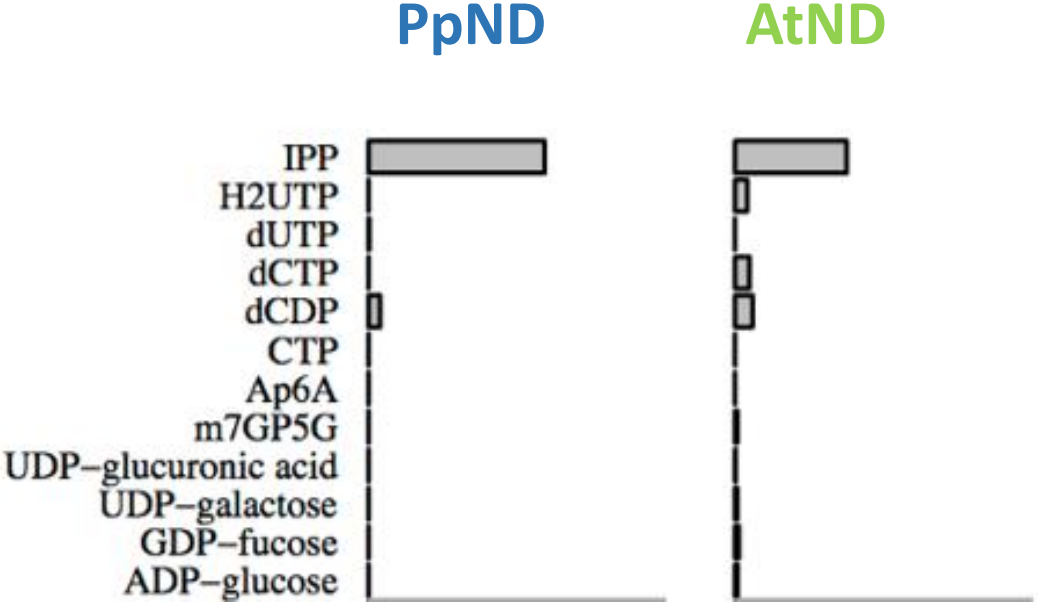
metal binding and catalytic site

substrate specificity determined by the N-terminal extension or by residues in variable loop regions

PpND and AtND sent to prof. Brenner's lab in Berkeley

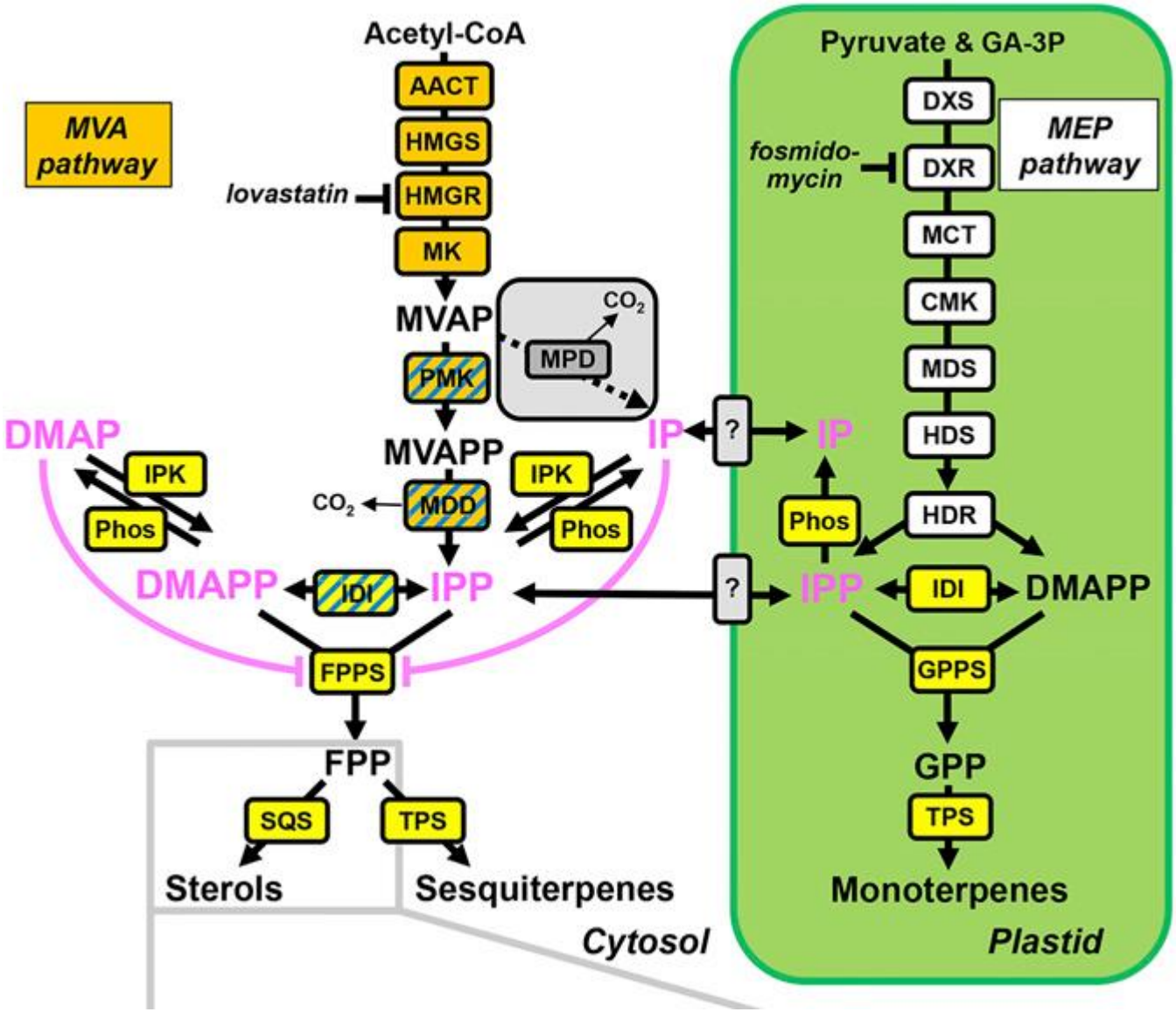
McLennan(2006) Cell Mol Life Sci





	$k_{cat}/K_M(M^{-1}\ s^{-1})$	
IPP	$1.9 \cdot 10^4$	$1.8 \cdot 10^4$
8-oxo-dATP	$1.1 \cdot 10^4$	
ADP	280	
dGDP	170	
dGTP	185	

A role for IPP phosphatase?



!

- cytosolic enzyme
- activity on IPP
- conserved in all plants

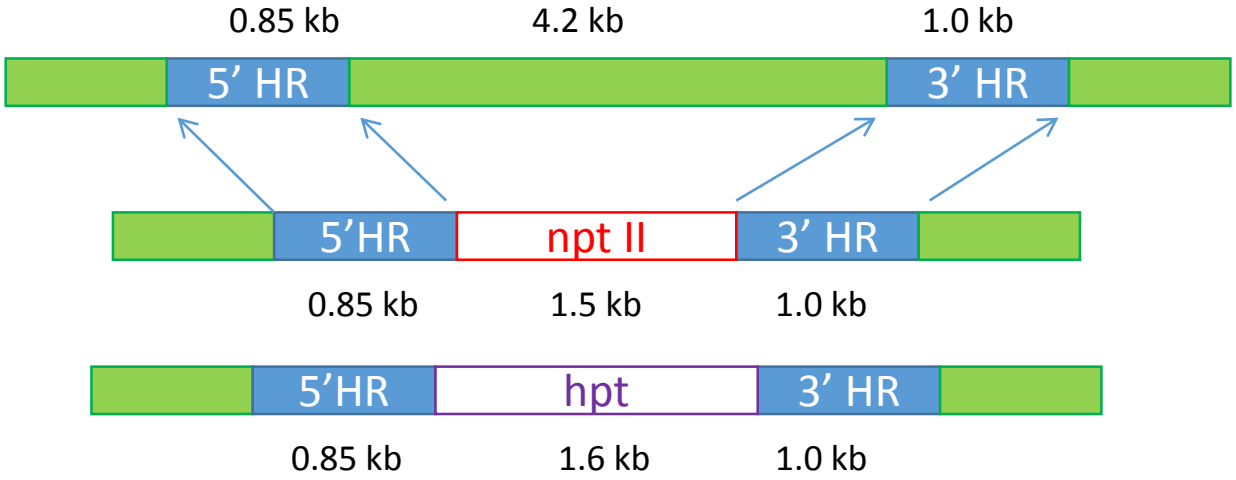
?

- terpenoid synthesis regulation
- transport of IP/DMAP
- regulation of ND

Henry (2015) PNAS

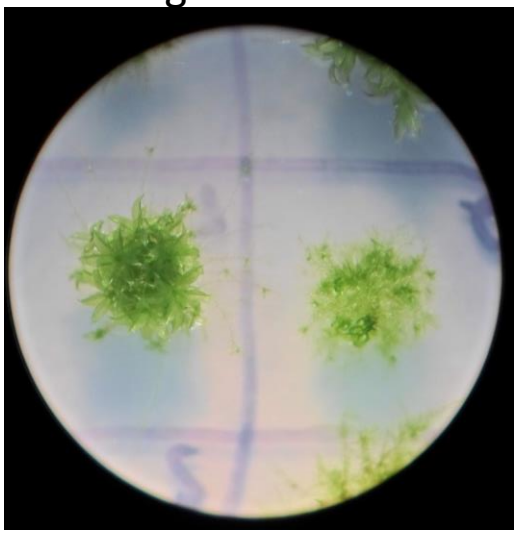
Knockout produced in *Physcomitrella patens*

nptII and hpt constructs used to transform protoplasts

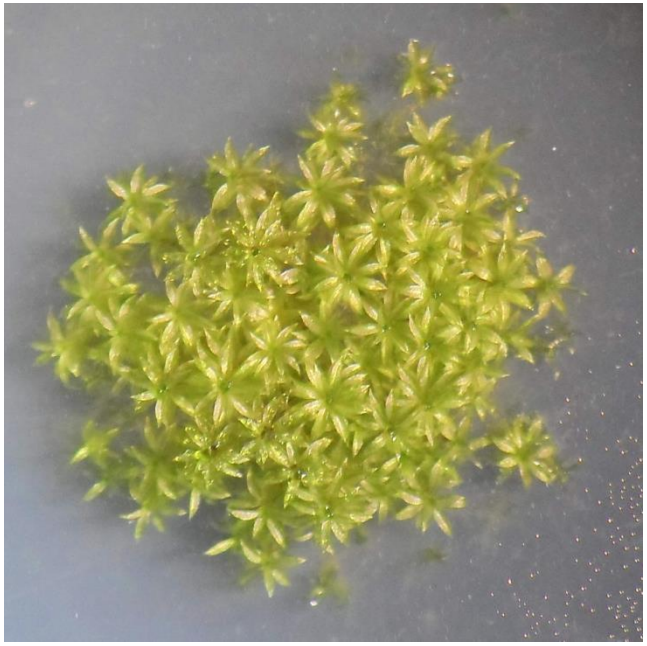


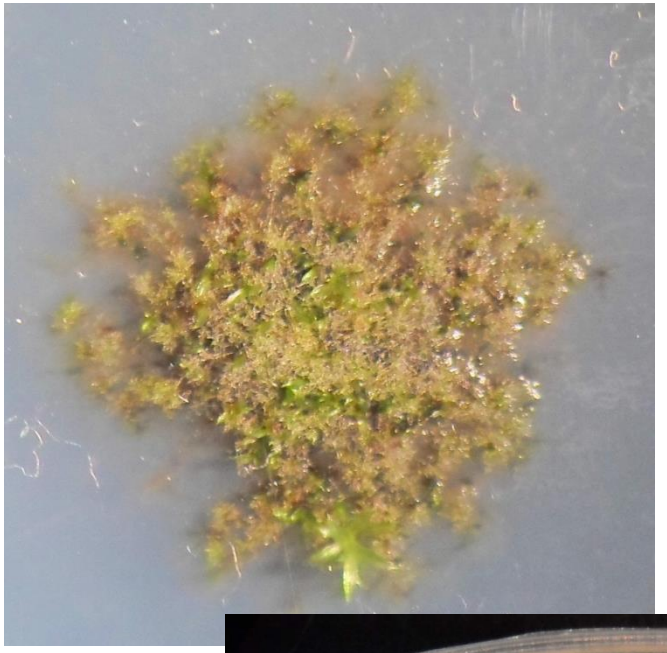
antibiotic resistance cassettes inserted into genome by homologous recombination
removed START codon and exons 1-12 (of total 20)

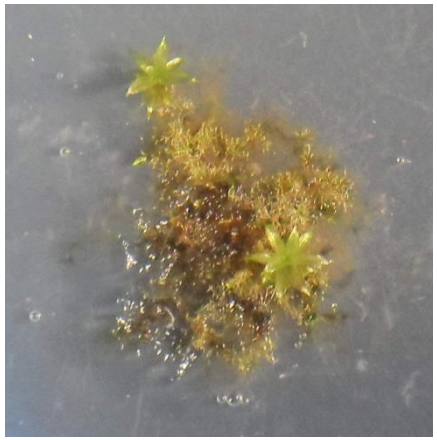
RT-PCR results still to come, but 8 lines
show proper integration of HRs,
and we need 3 independent lines!

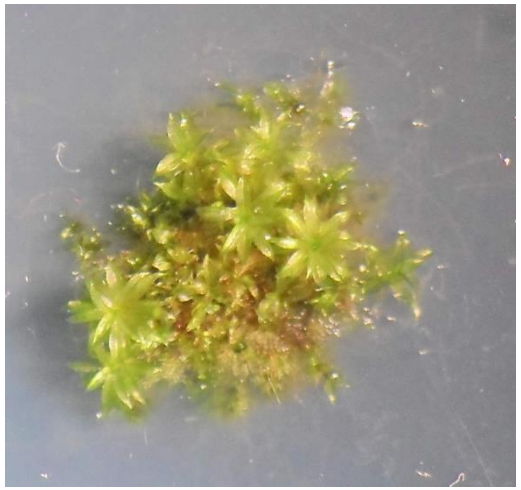


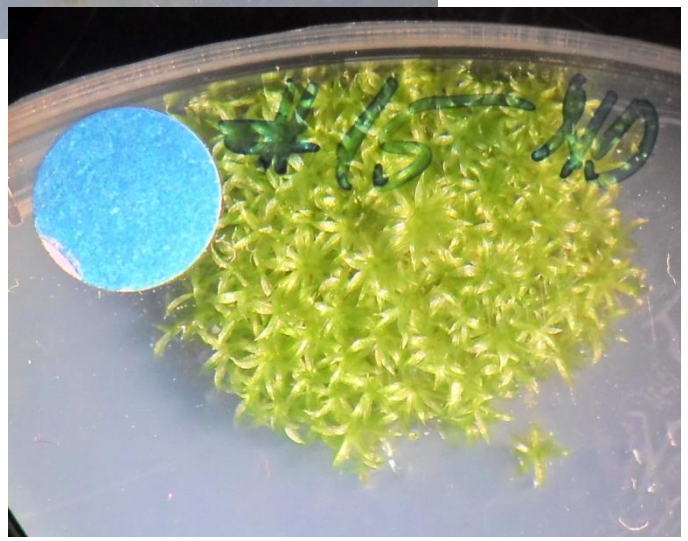
Possible phenotype difference

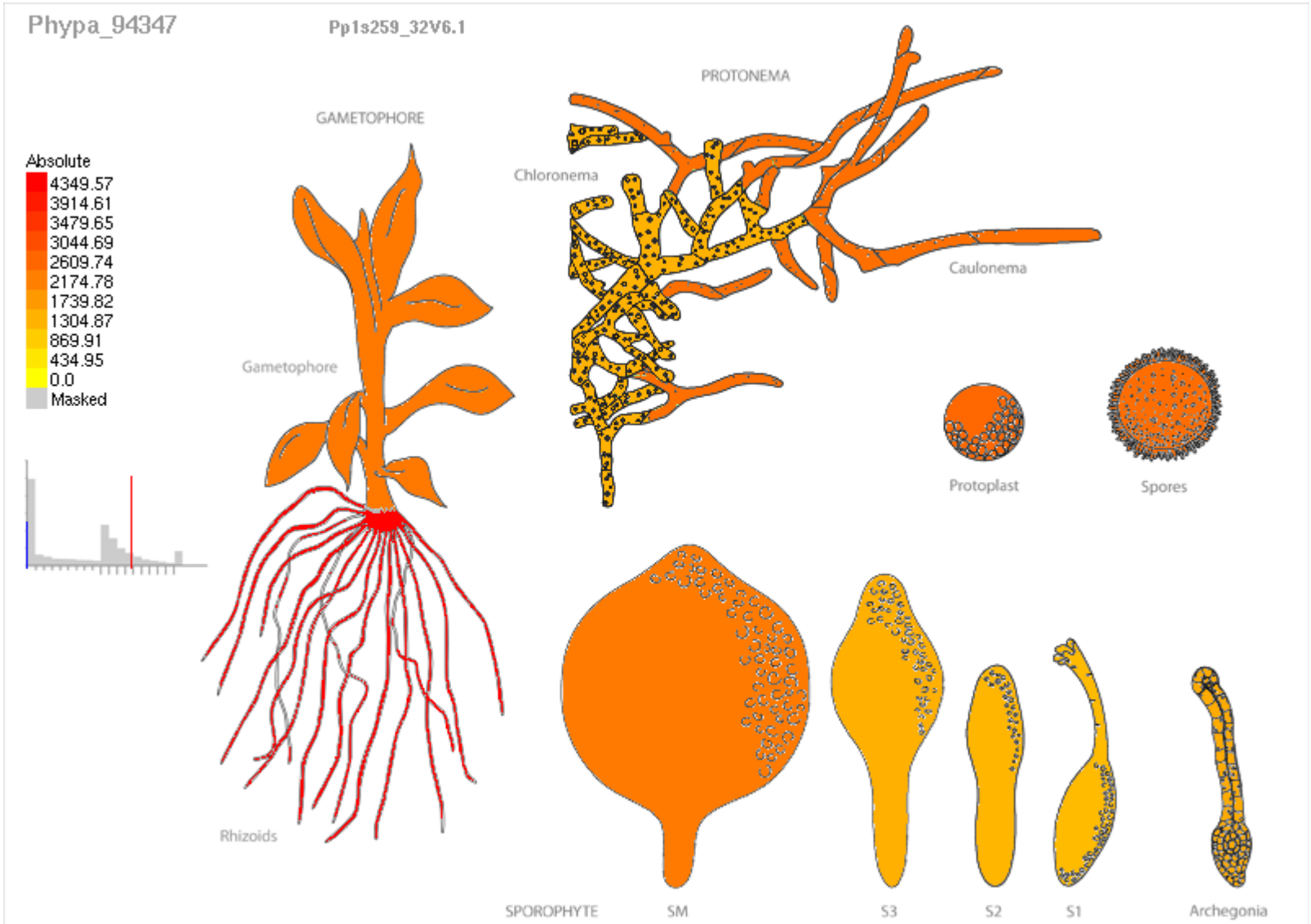












Physcomitrella eFP Browser at bar.utoronto.ca Ortiz-Ramírez *et al.*, Molecular Plant, 2015 / Winter *et al.*, 2007

The different tissue types were isolated from wildtype Physcomitrella patens (Gransden) grown in controlled conditions at 25 °C with 16h light and 50% humidity. Induction of gametangia and sporophyte development was conducted in short day conditions at 17 °C and 50% humidity. Tissues were sampled in triplicate and processed for hybridization on NimbleGen v1.6 P. patens 135k arrays (32741 probe sets). Expression data were normalized by RMA. Drawings by Marcela H. Coronado.

Future experiments:

describing and explaining the phenotype of the knockout

gene and protein expression under stress conditions

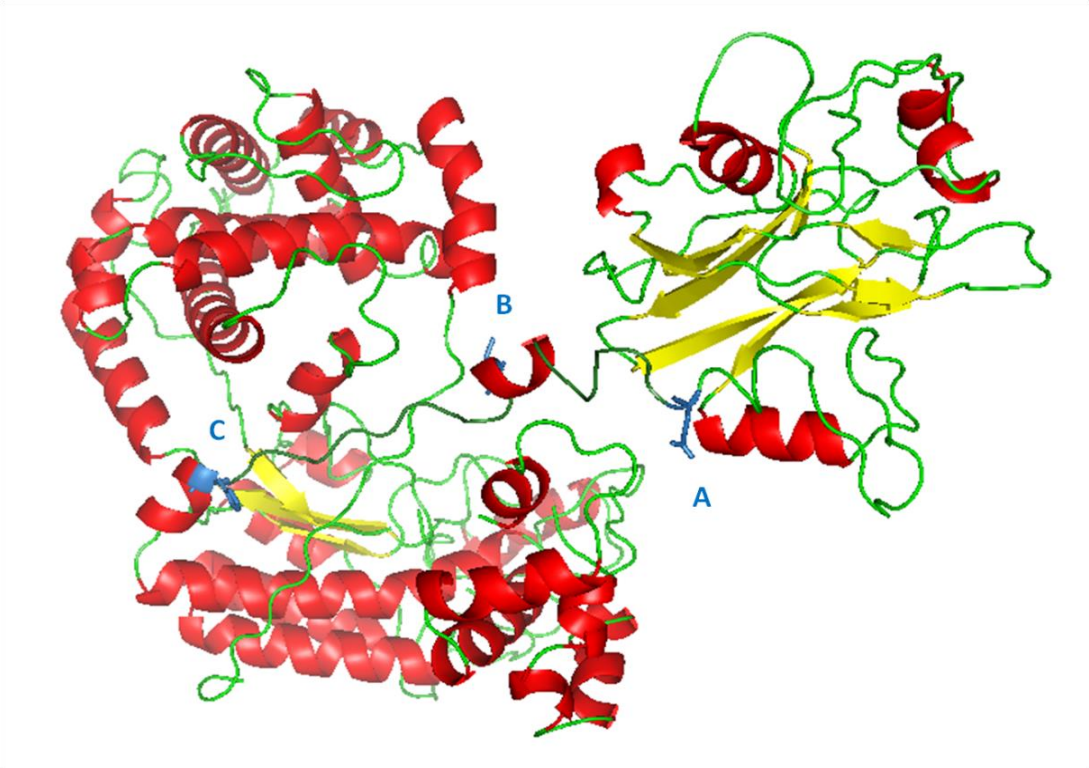
knockout metabolomics (plant hormones, terpenoids, peptidome?)

Separation of domains

„fragments” cloned with C-terminal His-tags

NUDIX fragments (23-26 kDa) not soluble

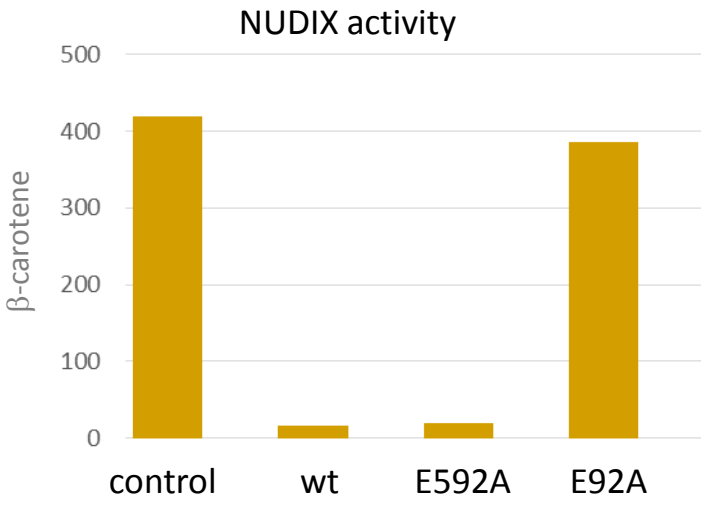
M49 fragments (63-65 kDa) soluble with lower activity than wild-type,but lost activity at -8 °C



fragment	specific activity
A	0.0
B	0.11
C	0.06

Preparation of inactive mutants

			phosphatase	peptidase
wild type	R**E**LQEE	H**E**XXH	YES	YES
E592A	R**E**LQEE	HACCH	YES	NO
E92A	RALQEE	H**E**XXH	NO	YES



	DPP III activity
wild type	4,0
E592A	0,0
E92A	3,0

Acknowledgments

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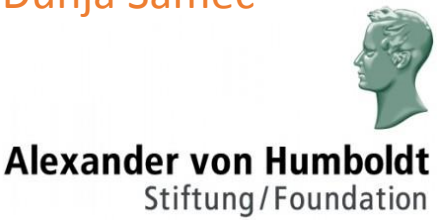

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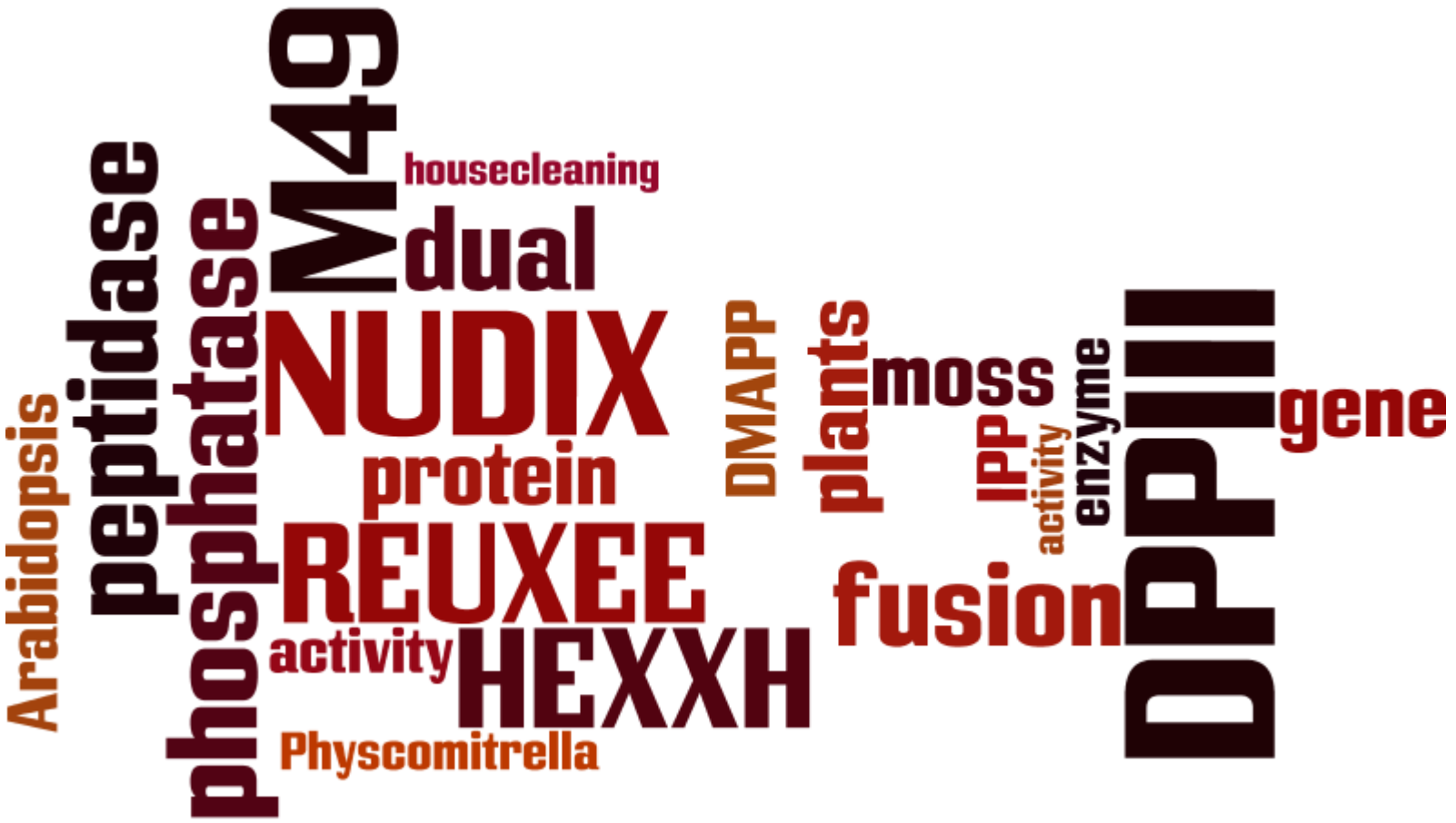
M49
housecleaning
peptidase
dual
phosphatase
NUDIX
protein
DMAPP
plants
moss
gene
DPPIII
IPP
activity
enzyme
Arabidopsis
REUXEE
fusion
activity
HEXXH
Physcomitrella