

Structure of *Bacillus amyloliquefaciens* subtilisin Bpn' from NCBI protein structure site

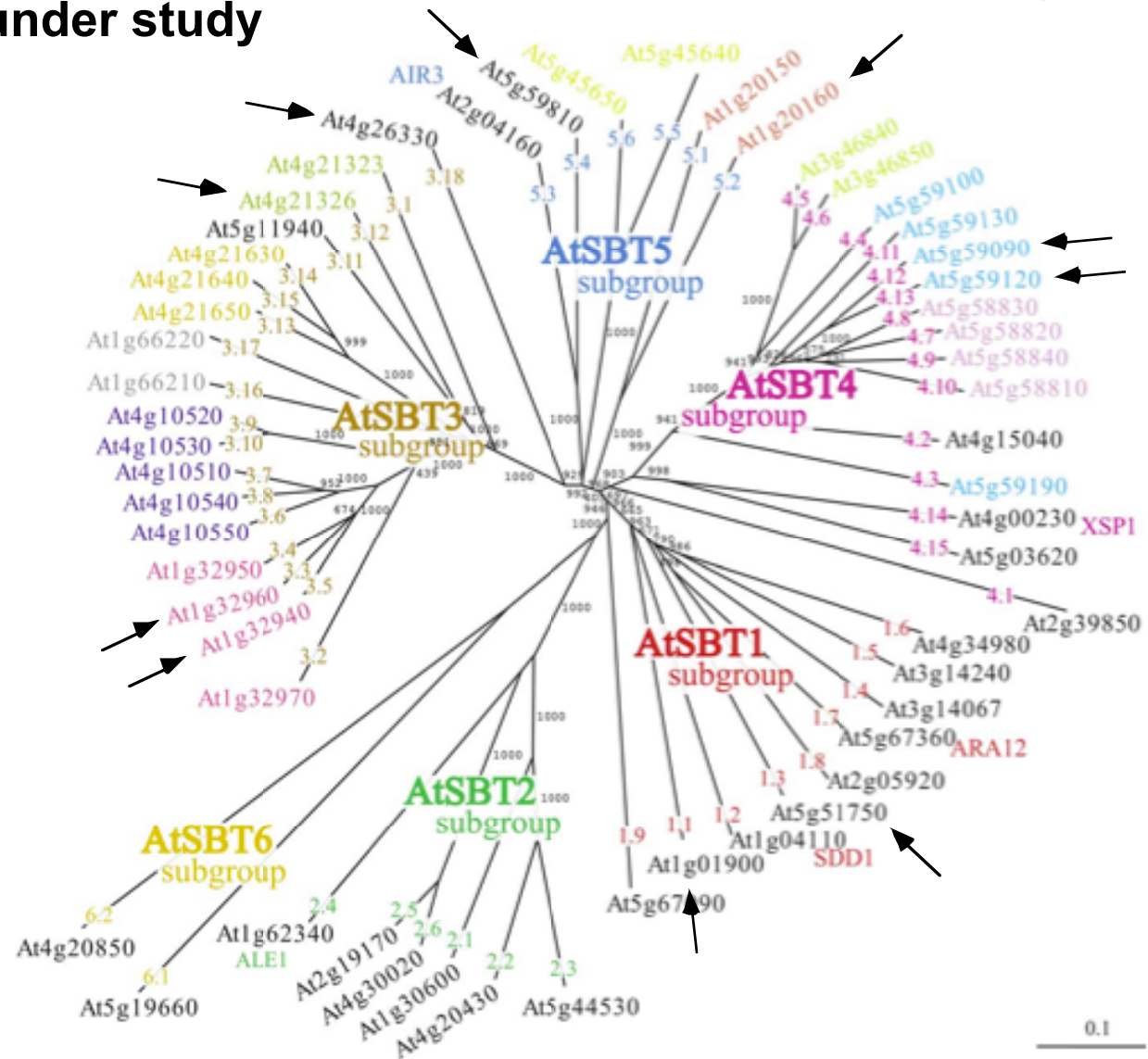
Howell Laboratory – U.S. National Science Foundation 2010 Project

The Howell Laboratory has received funding from the NSF 2010 program to study the function of members of the subtilase (subtilisin-like serine protease) gene family in *Arabidopsis* through a collaboration with European partners in The *Arabidopsis* Subtilase Consortium (TASC). The aim of the project in the Howell lab is to understand the role of ten subtilase genes during shoot regeneration in tissue culture. The Howell lab's interest in subtilase genes was sparked by the finding that the expression of three subtilase genes (At1g01900, At4g26330 and At5g59120) is highly associated with a major QTL determining shoot regeneration efficiency (Lall et al. 2004 *Genetics* 167:1883-1892). Another seven subtilase genes are differentially regulated during shoot or root regeneration in tissue culture.

Subtilase genes under study in the Howell Lab

Subtilase genes under study in the Howell lab in a project supported by the US National Science Foundation 2010 program. The Howell lab is focused on 10 subtilase genes as indicated by arrows in the subtilase gene family tree.

protein	locus
AtSBT1.1	At1g01900
AtSBT1.3	At5g51750
AtSBT3.3	At1g32960
AtSBT3.5	At1g32940
AtSBT3.12	At4g21326
AtSBT3.18	At4g26330
AtSBT4.12	At5g59090
AtSBT4.13	At5g59120
AtSBT5.2	At1g20160
AtSBT5.4	At5g59810

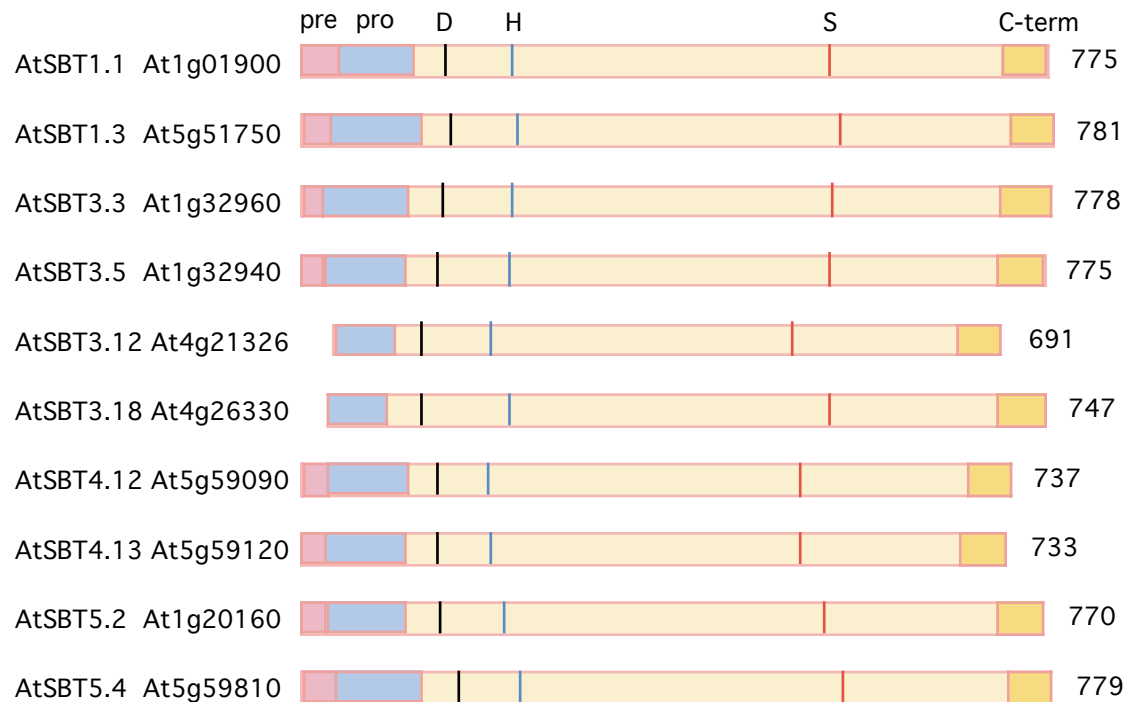


0.1

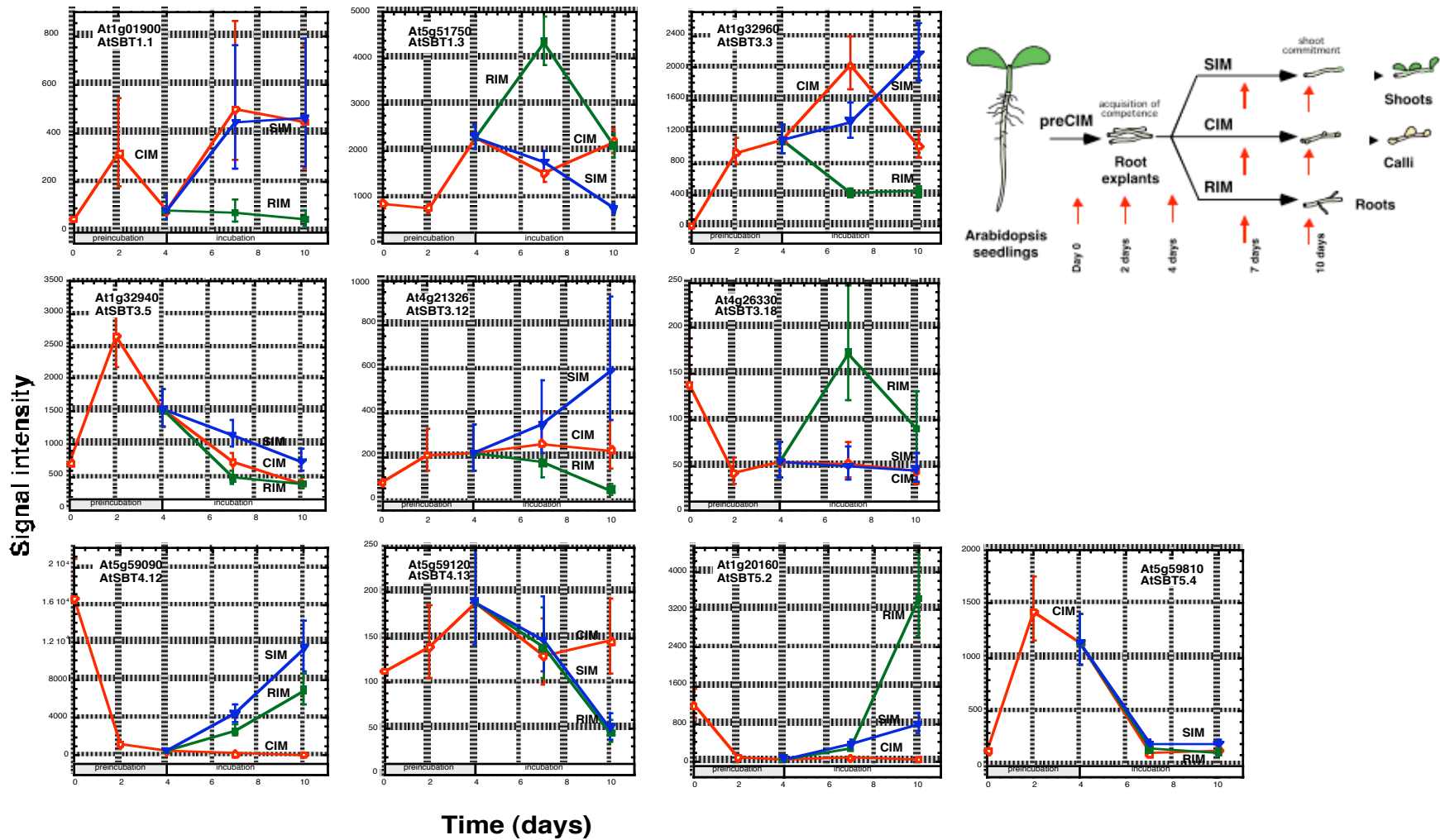
from Rautengarten et al., 2005

Predicted structure of subtilase proteins under study in the Howell Lab

Most subtilases have a prepro structure and critical Asp, His and Ser residues form a catalytic triad at the active site of the enzymes. Of the 10 subtilases under study in the Howell lab, 8 have predicted presequences.

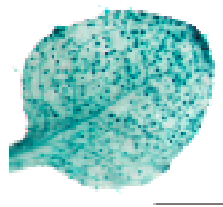


Expression profiles of subtilase genes during the regeneration of shoots, callus and roots in tissue culture

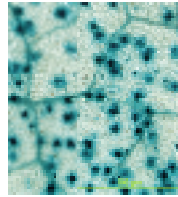


Expression patterns of subtilase promoter:GUS constructs in Arabidopsis

AtSBT5.4 At5g59810



Leaf



Leaf (detail)



Root

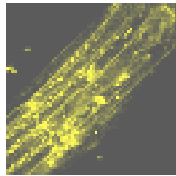


Flowers

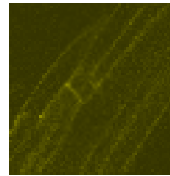
SBT name	AGI #	Transcript expression pattern Rautengarten et al.	Transcript expression pattern AtGen	Promoter:GUS expression pattern
AtSBT1.1	At1g01900	strong everywhere, esp flowers, siliques	embryo	Cotyl & leaf guard cells, cotyl, leaf & root veins, shoot apex, flower sepals, upper style
AtSBT1.3	At5g51750	strong everywhere	flower, inflorescence	Cotyl & leaf guards cells, root zone of cell division, flower sepals, upper style
AtSBT3.3	At1g32960	strong, low in siliques, seeds	pathogen, harpin	Cotyl, leaf & root veins, leaf guard cells, flower sepals
AtSBT3.5	At1g32940	medium strong everywhere	pathogen	General leaf, flower sepals, silique abscission zone
AtSBT3.12	At4g21326	strong everywhere	pollen, photoperiod	Leaf veins, flower sepals
AtSBT3.18	At4g26330	weak root	pollen?	General leaf (light)
AtSBT4.12	At5g59090	medium strong everywhere	sulfate, root	Leaf guard cells, leaf petioles, root veins, flower sepals
AtSBT4.13	At5g59120	flower, root	flower, embryo	Leaf guard cells, flower sepals, upper style
AtSBT5.2	At1g20160	strong everywhere	ARR22ox, root	Cotyl, leaf & root veins, upper style
AtSBT5.4	At5g59810	flower, root	flower	Cotyl & leaf guard cells, root veins, flower sepals, stigma

Subcellular localization of subtilase-YFP constructs in Arabidopsis cells

AtSBT5.4
At5g59810



Untransformed
control



Roots

SBT name	AGI #	pSORT localization prediction	Subcell localization (35Sp:GENE-YFP)
AtSBT1.1	At1g01900	sec, type Ib memb	apoplast
AtSBT1.3	At5g51750	extracell	not yet defined
AtSBT3.3	At1g32960	extracell	not yet defined
AtSBT3.5	At1g32940	plas memb, type Ia	apoplast
AtSBT3.12	At4g21326	peroxisome? no sig seq	not yet defined
AtSBT3.18	At4g26330	peroxisome? no sig seq	not yet defined
AtSBT4.12	At5g59090	extracell	not yet defined
AtSBT4.13	At5g59120	extracell	apoplast, cytoplasmic aggregates
AtSBT5.2	At1g20160	extracell	not yet defined
AtSBT5.4	At5g59810	mito? type II memb	mito