Yeast-2-Hybrid Protein Analysis of *Arabidopsis Thaliana*Trichomes

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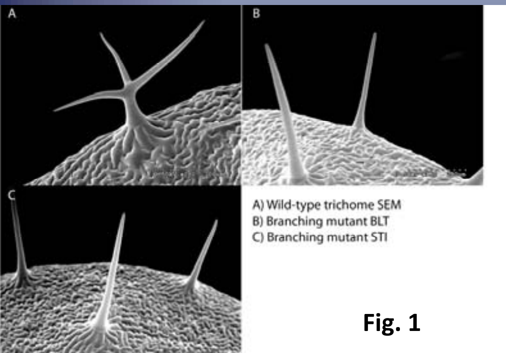
Introduction

Arabidopsis thaliana is a small flowering plant that's small genome makes it ideal for use as a model organism for genetic research. *A. thaliana's*genome has been completely sequenced, but many gene functions remain a mystery.

A feature of *A. thaliana* is the distinct branching structure of the trichomes on the epidermis of leaves. (Fig.1A) These trichomes, or hairs, are small unicellular structures whose function is not clear.

To better understand the function of trichomes, it is helpful to better understand their development, and the genes and proteins involved. Mutations in trichomes are utilized to identify genes required for their development. In this experiment, branching mutants will be analyzed.



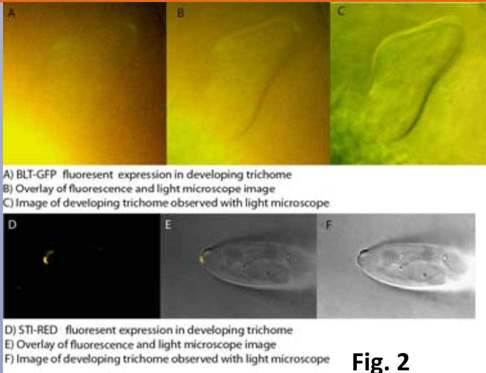
A) Wild-type trichome SEM
B) Branching mutant BLT
C) Branching mutant STI

Fig. 1

Hypothesis

Two mutants, BLT and Stichel (STI), both result in an altered trichome structure. In these mutants, trichomes fail to branch during development, so appear as single stalks as opposed to the wild-type tri-branched structure. (Fig. 1) Because these mutations share the same phenotype, the next step would be to analyze if the two genes have a related function. Creation of transgenic genes with fluorescent markers allow visualization of the where the encoded gene products are localized. Both BLT and STI are localized at the growing tip of developing trichomes. (Fig. 2)

It is therefore hypothesized that BLT and STI proteins interact during the development of trichomes. BLT and STI both contain a region of DNA that should form a coiled coil, (Fig.3) and could interact via that association. It will be investigated whether BLT and STI interact with each other, or if perhaps, they interact with themselves. Yeast-2-hybrid analysis of the proteins will be used to assay whether or not an interaction will occur.



A) BLT-GFP fluoresent expression in developing trichome
B) Overlay of fluorescence and light microscope image
C) Image of developing trichome observed with light microscope

D) STI-RED fluoresent expression in developing trichome
E) Overlay of fluorescence and light microscope image
F) Image of developing trichome observed with light microscope

Fig. 2

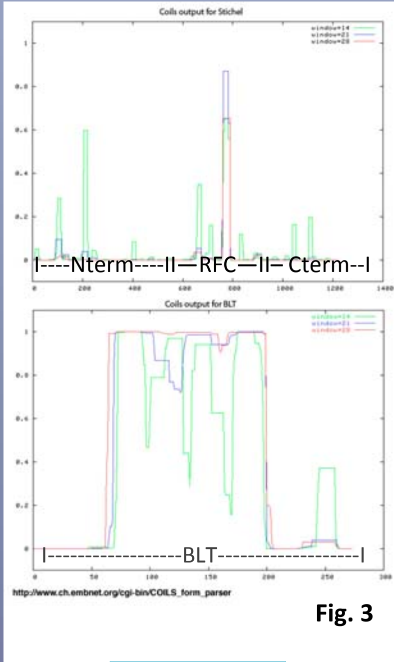


Fig. 3

Methods

To analyze protein-protein interactions, the genes involved must first be isolated and cloned into suitable vectors. STI was broken into 3 fragments with PCR primers (Fig. 3), the C-terminal end, the N-terminal end, and the target coiled coil region, called RFC. The BLT and the STI fragments were PCR amplified and separated by gel electrophoresis. The terminal ends of each fragments were digested with EcoRI and BamHI, were ligated into vectors pBridge and pGAD424 using a DNA ligase, and transformed into BioLineE.coli cells. Transformants carrying the desired product were identified by restriction enzyme mapping and DNA sequencing of isolated plasmids.

To analyze the interactions, the plasmids with inserts from E.coli clones were transformed into a yeast strain y-190. Empty vectors without inserts were used as a negative controls. The bait encoded product, containing a DNA binding domain, and the prey encoded product, containing the DNA activation domain, were co-transformed into y-190. The yeast transformants were tested for expression beta-galactosidase activity.

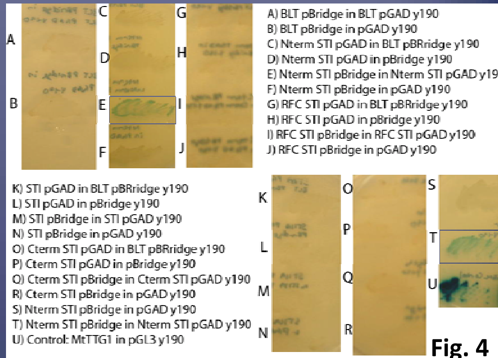
Methods Cont.

An assay for interaction was performed using the LacZ (B-galactosidase) gene as a reporter for protein-proteing interaction. This gene will be expressed if a bait–prey interaction occurs. An Xgal assay was used in which the yeast plates were flooded with an Xgal containing agarose solution to detect B-galactosidase activity. If the proteins interacted, the yeast would degradeXgal and turn a blue color. If they did not turn blue, they could not degradeXgal and, therefore, did not have interacting proteins.

Results

As shown in Figure 4, all of the proteins failed to interact except for one. The N-terminus of STI interacted with itself in yeast-2-hybrid. BLT failed to interact with STI's RFC region in yeast-2-hybrid, but because the N-terminus did interact with itself, it is possible that the proteins are simply too large to fold and interact properly in yeast.

An alternative approach to determine if the proteins do in fact interact with each other or with themselves, is to perform a co-immunopercipitaiton experiment. In this, one protein is tagged so that an antibody can recognize it, and is run through a column with an antibody that recognized the proteins associated antibody. The protein will elute last through the column, bringing associated proteins along with it. The complex can then be run out and separated on a gel, and submitted to mass-spectrometry to determine their size. This can help to identify whether a protein interacts with another, with itself, or with none at all, and is a reasonable next step to determining BLT's interactions.



A) BLT pBridge in BLT pGAD y190
B) BLT pBridge in pGAD y190
C) Nterm STI pGAD in BLT pBRidge y190
D) Nterm STI pGAD in pBridge y190
E) Nterm STI pBridge in Nterm STI pGAD y190
F) Nterm STI pBridge in pGAD y190
G) RFC STI pGAD in BLT pBRidge y190
H) RFC STI pGAD in pBridge y190
I) RFC STI pBridge in RFC STI pGAD y190
J) RFC STI pBridge in pGAD y190
K) STI pGAD in BLT pBRidge y190
L) STI pGAD in pBridge y190
M) STI pBridge in STI pGAD y190
N) STI pBridge in pGAD y190
O) Cterm STI pGAD in BLT pBRidge y190
P) Cterm STI pGAD in pBridge y190
Q) Cterm STI pBridge in Cterm STI pGAD y190
R) Cterm STI pBridge in pGAD y190
S) Nterm STI pBridge in pGAD y190
T) Nterm STI pBridge in Nterm STI pGAD y190
U) Control: MtTTG1 in pGL3 y190

Fig. 4

Acknowledgement

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