

Use of yeast-based surrogate gene expression system to study plant genes that protect against apoptosis

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Abstract: A screen of a tomato cDNA library for genes that protect yeast from ced3-induced death resulted in the isolation of 24 tomato genes. The protective mechanism is not known. Ced3, like all caspases, is first synthesized as an inactive pro-enzyme that is then processed to an active protease. One possibility for the protective mechanism is that the tomato genes interfere with ced3 processing. The status of ced3 processing was investigated in yeast co-expressing different genes.

Introduction: Apoptosis, a form of programmed cell death (PCD), is needed for the proper development of an organism. When triggered inappropriately, PCD often leads to disease. Most forms of PCD induce the activity of a family of proteases called caspases. Caspases are first made in cells as inactive proteins that are then activated by proteolytic cleavage to result in smaller active proteases. Scientists have discovered that p35, an insect virus gene, inhibits the activity of all known caspases. When p35 is expressed in plants, it blocks PCD and results in decreased susceptibility to many pathogens. Public misperception of p35 is that if eaten p35 is "cancer causing" because it can promote cancer of mammalian cells when expressed inside those cells by preventing death. Therefore p35 is not yet suitable for the use in crops. It is known that organisms can inhibit their own caspases with endogenous gene products. Our lab has screened for tomato genes which may inhibit caspases by using a yeast expression system.



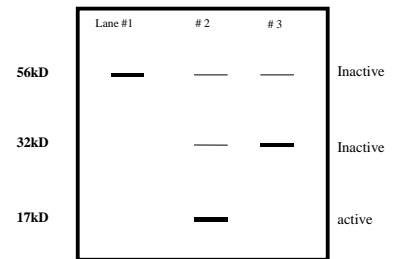
Apoptosis. The process of a dismantling cell by programmed cell death.

Materials and previous results:

- 1) A His-tagged caspase for expression in yeast. The six histidine (His6) epitope was fused to the ced3 protein by PCR. The His6 epitope is needed for western detection of ced3 processing in yeast.
- 2) Expression of ced3 in yeast results in no growth. Ced3 induced growth arrest can be relieved by co-expression of p35.
- 3) Our lab has screened 500,000 different tomato "test genes" for genes that also protect yeast from ced3-induced growth arrest. We are further characterizing 24 of these genes.

During my internship, I participated in the quest for a plant gene that blocks the processing of caspases (ced3) just like p35.

Possible results of ced3 processing



Lane #1 no processing;

#2 complete processing;

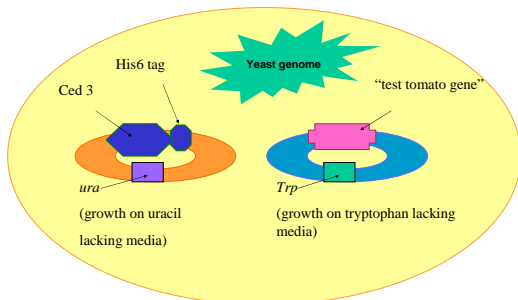
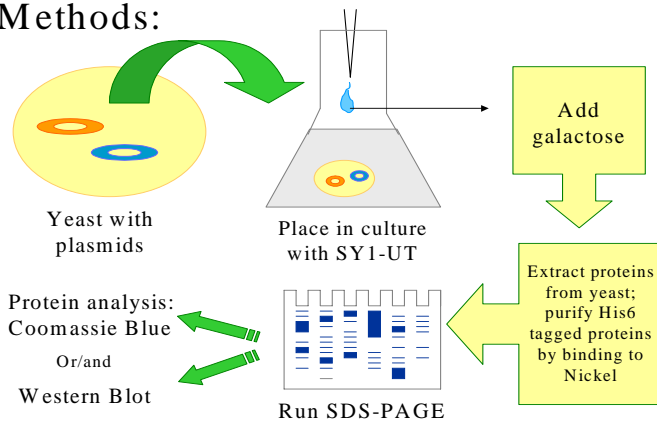
#3 partial processing

Objective: To investigate the mechanism by which tomato genes protect yeast from caspase activity.

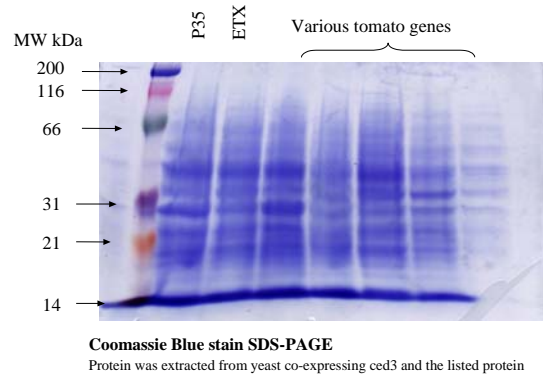
Specific techniques used:

- Induce ced3 expression in yeast.
- Purify protein from yeast.
- Analyze ced3 protein by western blotting.

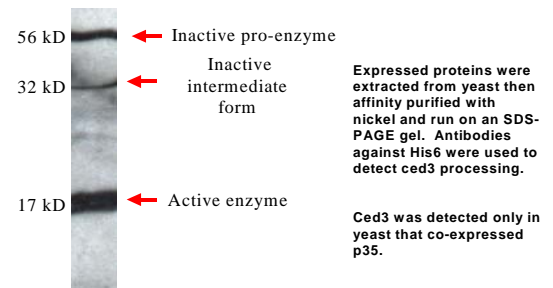
Methods:



Schematic of yeast containing two plasmids. Two plasmids are simultaneously maintained in yeast by growth on media lacking uracil and tryptophan. Expression of ced3 and the "test gene" are induced by addition of galactose to the media



Western analysis of ced3 protein



Conclusion:

1. Co-expression of p35 does not stop ced3 processing.
2. Growth on glycerol (SY1) probably does not keep ced3 off completely allowing mutations to block ced3 expression.
3. Activity assays for ced3 will be developed.