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Characterizing the role of NADP-malic enzyme in stomatal conductance

Abstract

A reliable supply of water is required to maintain virtually all forms of life. Plants, which lack the ability to migrate away from harsh environments, have evolved numerous mechanisms to cope with natural fluctuations of water availability. One type of modification is known as *stomata*, taken from the Greek word for “mouth”. Stomata resemble tiny mouths on the underside of leaves, opening and closing to regulate cellular water loss. Research has shown that over expression of NADP Malic Enzyme in transgenic plants causes the stomata to remain more closed. This decreases the amount of organismal water loss via transpiration, ultimately resulting in plants that consume less water.

Little is known about the link between NADP-ME and stomatal conductance. In order further characterize this relationship, we have compared enzyme expression between the guard cells and various other tissues of *Arabidopsis thaliana*. Using gene-specific primers for each of the six isoforms of *Arabidopsis* malic enzyme, RT-PCR was performed on RNA extracted from isolated guard cell protoplasts. The expression pattern of malic enzyme was then analyzed using gel electrophoresis. It was discovered that At5g11670 was the most highly expressed isoform in *Arabidopsis* guard cells, with significant expression of At5g25860 and At2g13566. Expression of all six isoforms was observed in root, stem, and leaf tissues.

We have developed a strategy to more accurately determine the mechanism by which NADP ME over-expression induces a decrease in stomatal conductance. Both the catalytic function and ion-binding activity of NADP-ME have the potential to affect guard cell activity of these transgenic plants, but it is not known which actually plays the significant role. To explore this, we have started work to create a deactivated form of maize NADP Malic Enzyme that retains ion-binding capabilities without catalyzing the malate to pyruvate reaction. The incapacitated enzyme will be introduced into an expression vector and expressed first in *E. coli*., and then in *Arabidopsis thaliana*. Stomatal conductance will be measured, answering questions regarding the role of the enzyme in guard cell activity. We hope that these findings may be used in engineering plant water use, further contributing to the success of world-wide agriculture.

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First Advisor

Dr. Marianne Laporte

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Dr. Jim Vandenbosch

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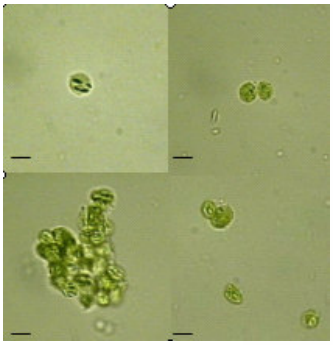

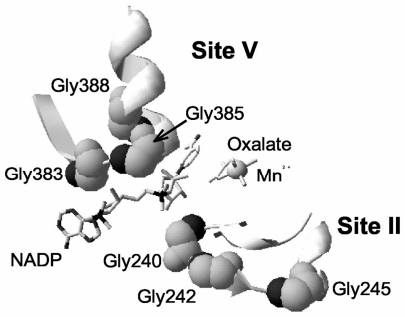

Work performed July 2003 through March 2004

Submitted April 2004

EASTERN MICHIGAN
UNIVERSITY

Characterizing The Role of NADP-Malic Enzyme in Stomatal Conductance

Honors Senior Thesis by
Jason Coliadis

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Honors Program Director, Joe Rankin _____

In partial fulfillment of the requirements of Eastern Michigan University for graduation with honors in the field of Biology

EASTERN MICHIGAN
UNIVERSITY

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Section 1

Malic Enzyme, Stomata, and Drought Resistant Plants

Earth's environment provides countless challenges for all species: an oxidative atmosphere, a relentless shower of heat and UV radiation, vast fluctuations in temperature and moisture, and so on. Organisms are constantly forced to respond appropriately in order to ensure the survival of their populations. With sufficient time and pressure, evolution can optimize these responses in the development of specific structures that enable a species to overcome these obstacles.

One such modification is the stomata: an elegantly simple organelle found on the undersurface of photosynthetic leaves. Stomata consist of two guard cells that swell and relax, regulating the size of a central opening. This process is known as conductance, and it provides a unique opportunity for the plant: it balances the necessary intake of carbon dioxide with the interest of water retention. During periods of excessive heat, the stomata favors a closed conformation, decreasing the pore size to hinder transpiration and conserve water. In order to capitalize on this ability, many organisms only open their stomata at night, when the cooler temperatures allow the acquisition of carbon dioxide without risking dehydration.

Fig 1. An open stoma

NADP-Malic Enzyme (NADP-ME) is a respiratory enzyme. It is found in both plants and animals, often with multiple isoforms per genome. NADP-ME functions to oxidize the four-carbon sugar malate to form the three-carbon sugar pyruvate, generating carbon dioxide. It also binds bivalent cations in this process, generally Mg^{2+} and Mn^{2+}.

$$NADP^+ + \text{malate} \xrightarrow{\text{Malic Enzyme} + Mg^{2+}} NADPH + \text{pyruvate} + CO_2$$

Eq 1. NADP-ME catalyzes the oxidation of malate.

In April of 2002, Laporte et. al published a paper in the Journal of Experimental Botany entitled, "Engineering for drought avoidance: expression of maize NADP-malic enzyme in tobacco results in altered stomatal function". These researchers created transgenic tobacco plants that over expressed *Zea mays* NADP-ME in their stomata, and observed the rate of growth, stomatal conductance and water consumption of these organisms. They found that overexpressing maize NADP-ME in guard cells resulted in a smaller stomatal aperture. As a result, the transgenic organisms lost less water via transpiration through the more closed stomata, and showed a remarkable level of water efficiency. They gained significantly more fresh mass per unit of water consumed than the wild type, while depleting less of the amount of available moisture in the soil. In addition, the transgenic tobacco plants grew at statistically similar rates as unmodified tobacco plants, indicating that the improvement in water use did not come at the expense of organismal development (Fig. 2).

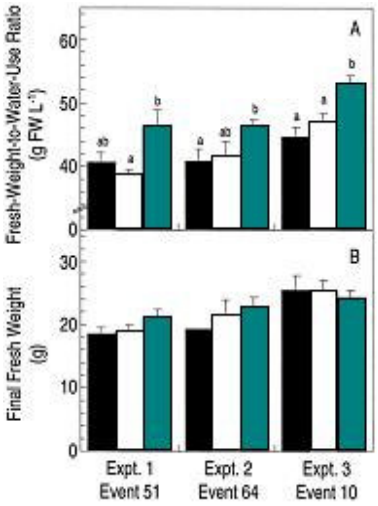


Fig.2. Experimental plants (**cyan**) showed improved water usage and normal development. (from Laporte et. al, 2002)

This study establishes a link between NADP-ME and stomatal conductance, indicating that malic enzyme overexpression induces stomatal closure. These findings also imply that this connection may be modified through genetic engineering and used to improve plant water use. Such man-made adaptations would allow organisms unable to survive in hot, arid regions to have an increased level of tolerance for such conditions.

This is an ideal modification for agriculture in areas where water is scarce. Unfortunately, before these findings can be further developed, we must learn more about the connection between malic enzyme and stomatal activity. Little is known about the activity of stomatal malic enzyme under normal conditions. Furthermore, we do not know what effect that overexpressing NADP-ME has on guard cell physiology, nor how such effects influence stomatal conductance. In fact, precious little is known about this system, and it is to this that I turn my attention.

Section 2

The Expression of NADP-ME in Arabidopsis thaliana

Background

In order to begin characterizing the relationship of NADP-ME and stomatal conductance, we began examining the expression of malic enzyme in wild-type *Arabidopsis thaliana*. In addition to a short gestation time and minute stature, there is a vast wealth of knowledge available on this organism, making an ideal subject to study. Our primary interest was the location and expression of NADP-malic enzyme in this plant, particular in its relevance to guard cells. Is NADP-ME expressed in this organism? If so, where is it most highly expressed, and which isoforms are present in guard cells? To answer these questions, we designed an experiment that would give us a clear picture of the NADP-ME expression pattern in *Arabidopsis thaliana*.

Fig 3. *Arabidopsis thaliana*

The procedure of this experiment hinged greatly on the modern face of molecular genetics: the field of bioinformatics. Modern studies on genome mapping and protein

characterization are generating vast quantities of DNA and protein data. Sequencing the genome of a single species yields millions of pieces of nucleotide data, leaving the scientific community desperately in need of a way to standardize and organize it all. Enter bioinformatics: a hybrid between the fields biology and computer science. Computers have simplified this organizational nightmare, and the globalization of the internet makes access to these wells of information extremely convenient for researchers.

"The *Arabidopsis* Information Resource" (TAIR, http://www.arabidopsis.org) is one such bastion of genetic information and is completely dedicated to *Arabidopsis thaliana*, our organism of interest. Using this bioinformatics tool, we were able to identify six forms of malate oxidoreductase enzymes in the *Arabidopsis* genome. These genes are classified by locus number, a universal system that identifies all of the sequenced genes for a particular organism with standardized letter/number combinations. Based on sequence homology and motif analysis, we believe that these six genes are isoforms of NADP-Malic enzyme. The six forms of NADP-ME in *Arabidopsis* are listed in fig. 4, along with predicted cellular location. Using the sequence data from the TAIR resource, we were able to develop a procedure to determine if specific mRNA transcripts of the various NADP-ME isoforms were present in Arabidopsis tissues samples. The presence of mRNA transcripts for a particular protein indicates that its corresponding gene is being expressed.

Locus #	Predicted Localization
At1g79750	Chloroplast
At2g13560	Cytosol
At2g19900	Cytosol
At4g00570	Mitochondrion
At5g11670	Cytosol
At5g25880	Cytosol

Fig. 4. Table of *Arabidopsis* NADP-ME Loci

Procedure

Our methods of determining NADP-ME expression in Arabidopsis thaliana are outlined in figure 5. Our strategy was to isolate total RNA from the tissues being examined, use RT-PCR and PCR to generate partial cDNAs for each gene, and run the amplification products on an electrophoresis gel. More detailed information regarding our procedures is in the following pages, intended to serve as a reference for future research.

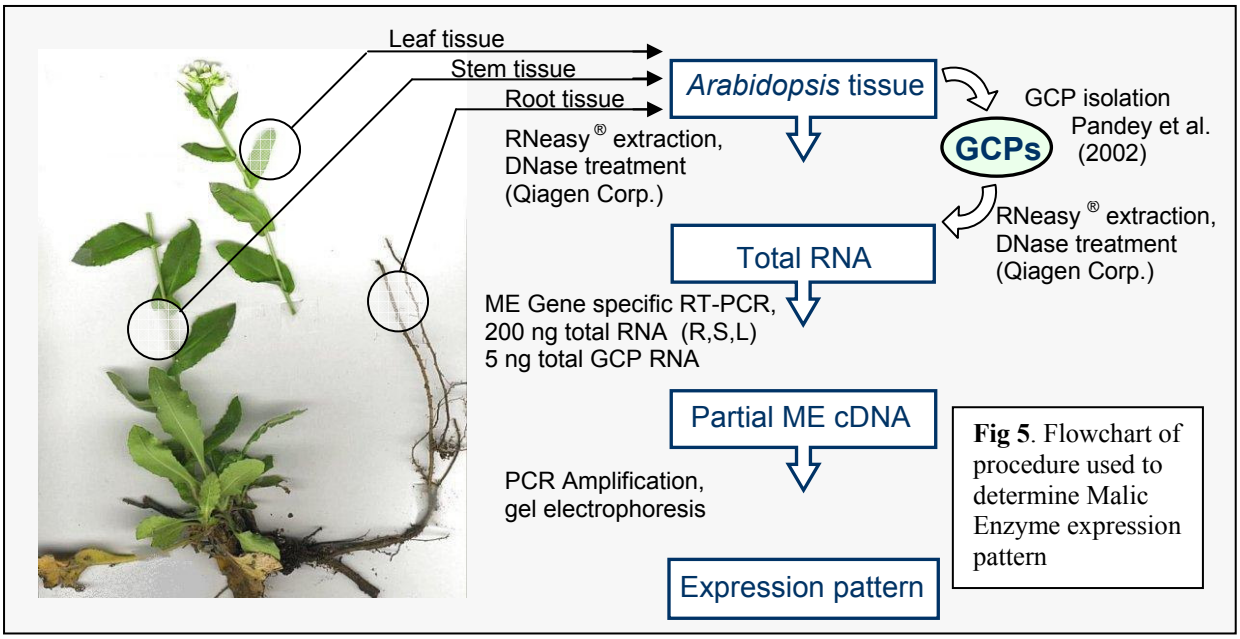


Fig 5. Flowchart of procedure used to determine Malic Enzyme expression pattern

In order to determine the NADP-ME expression pattern in Arabidopsis, we extracted the total RNA from the individual tissues we intended to study. This was accomplished by isolating leaf, root, and stem tissue samples from fresh *Arabidopsis* plants. These tissues were separately subjected to RNeasy® extraction (Qiagen corp.), providing us with a sample of the total RNA present in each of the tissues at the time of the extraction.

In order to detect NADP-ME expression in *Arabidopsis* stomata, we performed an overnight guard cell protoplast (GCP) isolation procedure in accordance with Pandey et. al. (2002) The major veins were excised from one hundred young Arabidopsis leaves, which were subsequently blended for 30 seconds in a Waring blender. The resulting leaf peels were poured through a 200-µm mesh, and large chunks of mesophyl cells were removed from the preparation with forceps. The peels were then rinsed with basic solution containing 5mM Mes-Tris (pH 5.5), 0.5 mM Ascorbic acid, 0.5 mM $CaCl_2$, 10 µM KH_2PO_4, 0.55 M sorbitol.

The peels were then placed in an enzymatic solution consisting of 50 mL basic solution, 0.65% Onozuka RS Cellulase, 0.35% Macerozyme R10, 0.25% (w:v) BSA, and 0.001% Kanamycin. The solution and peels were then shaken at 40 excursions/min. for 12-13 hours. Following this digestion, the guard cell protoplasts were filtered through a 20-µm mesh, and the remaining peels were washed with approximately 150 mL of basic solution to release any clinging GCPs. The filtrate was centrifuged at 200g for 5 min, and the pellet was resuspended in 25 mL of fresh basic solution. An identical centrifugation was performed, and the supernatant was removed to a final volume of 2-3 mL, containing suspended guarded cell protoplasts. These protoplasts were then used as guard cell tissue, and RNA was isolated from the GCPs in the same manner as the root, stem, and leaf tissue.

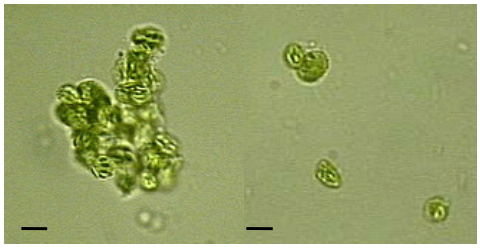


Fig. 6. Our isolated guard cell protoplasts

In subsequent steps, we used RT-PCR to amplify nucleic acid portions of NADP-ME template, but it is essential that we can differentiate between genomic DNA and

messenger RNA amplification products. Primers designed to bind to the mRNA will also bind to the complementary DNA strand, but amplifying genomic DNA will only reassert that these tissues contain the genes for NADP-ME isoforms, not that they are actively being transcribed.To control for this, our RNA extractions were then subjected to a DNase treatment, which digested any remaining genomic DNA from our samples. In addition, our primers were designed to flank genomic introns, which would generate a size difference between genomic and mRNA amplification, detectable via gel electrophoresis.

Following RNA isolation and DNA digestion, we performed RT-PCR on our RNA samples using primers specific to six NADP-ME isoforms we are investigating. Primer pairs and expected product sizes for RNA and genomic amplification are listed in figure 7. The partial cDNAs generated through our RT reactions were amplified using PCR, and the results were loaded into a 1.5% agarose gel and ran at 120 volts for apx. 45 minutes. We also amplified 16s rRNA and beta-tubulin, genes that tend to be transcribed in all tissues. This served as a control; as long as these reactions generated a product, we can be sure that our protocol was effective in amplifying gene transcripts.

Fig. 7 Primer pairs and product sizes

Locus	RNA Size	Genomic Size
16 s rRNA	268 bp	432 bp
At1g79750	295 bp	504 bp
At2g13560	292 bp	992 bp
At2g19900	213 bp	369 bp
At4g00570	238 bp	580 bp
At5g11670	295 bp	504 bp
At5g25880	242 bp	481 bp

RT-PCR Reaction Conditions

5 µL 2x mastermix (Invitrogen)
0.4 µL each primer
0.2 µL Reverse transcriptase
10 ng RNA
H_2O to 10 µL

RT-PCR Program

1. 48°C for 45 min.
2. 94°C for 2 min.
3. 94°C for 30 sec.
4. 60°C for 1 min
5. 68°C for 2 min, GoTo 3 39x
6. 68°C for 7 min.

Results: Analysis of our PCR products by gel electrophoresis **(Fig. 8)** shows that all six forms of NADP-ME are clearly expressed in the leaf, stem, and root tissue of *Arabidopsis thaliana*. All products were loaded by locus number, in the order of leaf, stem, and root samples. Bright bands indicate gene expression, and 100 bp ladder was used as a size reference.

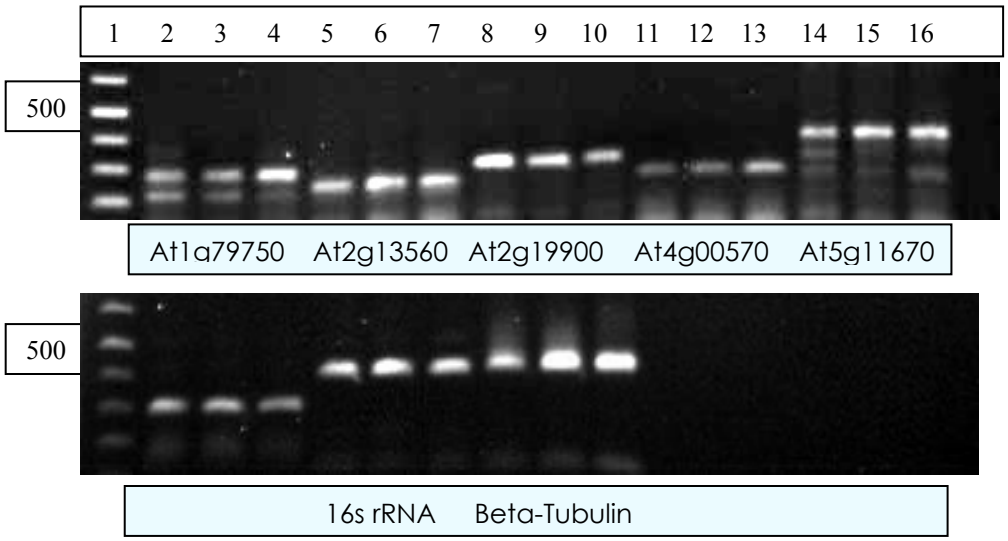


Fig.8 Root, stem, and leaf NADP-ME expression

We found that At5g11670 (**Fig. 9**, lane 5) was the only isoform highly expressed in guard cells. We also saw some expression of At1g79750, At2g13560 and At2g19900 (lanes 1, 2 and 3), although to a lesser extent. We found no detectable expression of At4g00570 or At5g25880 in our preparations. All major products correspond with the expected product size from mRNA amplification. The reactions were loaded in order of locus number for guard cell protoplasts and whole leaves. Five ng of total RNA was used for whole leaf and guard cell protoplast RT-PCR reactions.

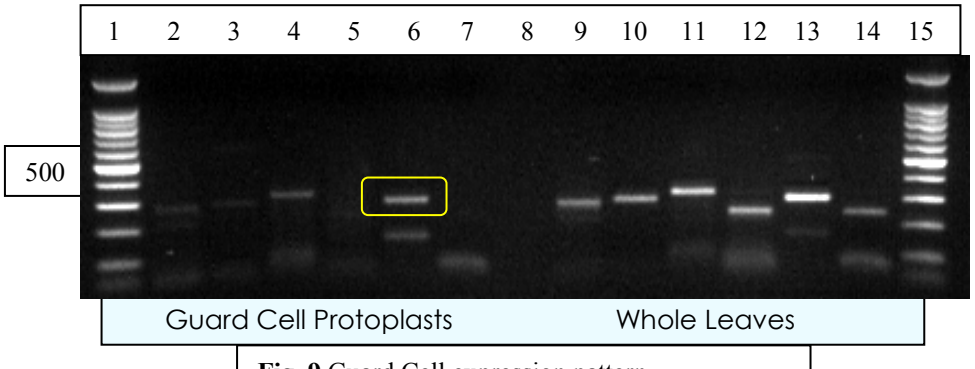


Fig. 9 Guard Cell expression pattern

Discussion

We conclude from this study that only one isoform of NADP-Malic Enzyme, At5g11670 is highly expressed in *Arabidopsis thaliana* guard cells. This distinctly contrasts the leaf, stem, and root tissues examined, which indicated that all six isoforms are expressed to a considerable extent. But what insight have we gained from this? By singling out which isoforms of NADP-ME are expressed in *Arabidopsis* stomata and other tissues, we have targeted one specific gene that may be modified to alter malic enzyme activity in guard cells, our organelle of interest. This may be the starting point for a host of other experiments. Disabling or overexpressing the most active isoform of NADP-ME in guard cells may further promote understanding of the correlation between malic enzyme expression and drought resistance. The At5g11670 promoter may be cloned, allowing us to test the effect of expressing various other proteins in tandem with guard cell malic enzyme.

Laporte et. al. (2002) clearly showed that overexpressing NADP-ME has an effect on stomatal conductance. However, does malic enzyme influence guard cell activity under normal conditions, or does overexpression induce artifact-type effects that do not play a role in wild-type organisms? I believe that the unusual specificity of At5g11670 may help answer to answer this question. Guard cells have a unique expression pattern: one particular isoform is highly expressed, while three isoforms that are expressed in *all other tissues* are completely suppressed. One interpretation of this is that that evolution has played a role: the specific expression of At5g11670 in guard cells hints that it may play a role in guard cell activity, or that expressing only half of the malic enzyme isoforms is important in stomatal function. Further experimentation is necessary to

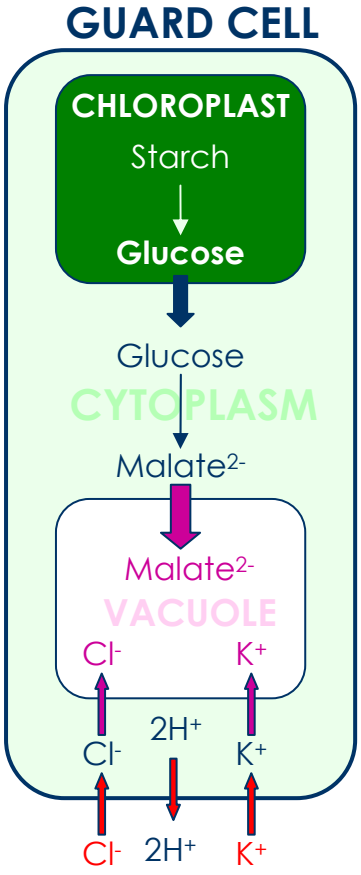
address this question. The remainder of my research has been addressing a different problem: *by what mechanism does over-expression of NADP-ME affect stomatal conductance?*

Section 3

Site-Directed Mutagenesis of NADP-Malic Enzyme

The expression of maize NADP Malic Enzyme in the guard cells of tobacco was shown to have an effect on stomatal conductance, resulting in a more water-efficient organism (Laporte et. al. 2002) These developments suggest a strategy for genetic engineering of drought-resistant plants, and imply a role of Malic Enzyme in stomatal function. It is to this role that I turn my attention: the precise mechanism by which the over-expression of this enzyme alters stomatal function remains unknown.

Fig. 10 Guard cell physiology



Both *malate* metabolism and Mg^{2+} binding are key components in malic enzyme activity (see page 2). Unfortunately, both of these components have the potential to affect stomatal conductance, making things a bit complicated. During the opening of the stomata, potassium cations are imported into the cytoplasm of the guard cell. To balance the positive charge influx, $malate^{2-}$ is synthesized from glucose and the cell takes up Cl- ions, and all are stored in the vacuole. (Fig. 10)[1] The turgor pressure of the guard cell increases and swells with incoming water, promoting a more open stomata.

[1] From "Detecting the Expression of NADP+-Malic Enzyme in *Arabidopsis thaliana* Guard Cells" Mũniz and Laporte, 2003

We believe that during stomatal closure, the converse occurs: malate is decomposed or otherwise removed and ion pathways are reversed, decreasing turgor pressure and relaxing the guard cells. Since both malate and ion flow are critical to this process, which one truly plays the greater role in decreasing stomatal activity when NADP-ME is overexpressed?

Hypothesis 1

Overexpression of maize NADP-ME gene in the guard cells of tobacco increases its catalytic activity, breaking down the cellular malate stores into pyruvate, which is further metabolized. This occurs to a greater extent in transgenic plants; abnormal amounts of malate are degraded. This decreases intracellular tension, allowing the guard cells to "relax" and promoting more closed stomata. Less water is lost through the increasingly closed stomata, which promotes a decreased rate of organismal water consumption and a more efficient organism.

Hypothesis 2

The ion-binding properties of NADP-ME play a greater role in this mechanism than the catalytic functions. Overexpressing NADP-ME would also increase the ion binding capabilities of the system, reducing the amount of free Mg^{2+}. Magnesium ions have been identified as contributing to the inhibition of potassium ion channels in the vacuole (Pei et. al. 1999): less magnesium means less inhibition, and results in overactive, "leaky" ion channels. As K^{+} escapes through these channels, the turgor pressure of the guard cell drops, the guard cells relax, and the stomata close.

These two hypotheses indicate two different directions in which further research on this system will be directed. If the catalytic function of NADP-ME is critical to the

success of Laporte et al.'s (2002) transgenics, then perhaps other enzymes or plant modifications can be introduced to support malate deconstruction in the guard cell. However, we cannot confidently make attempts to optimize these modified organisms without being sure that catalysis itself is necessary to induce water conservation.

I am currently working to differentiate between these two possible roles of NADP-ME, and more precisely determine what is occurring in transgenic plants expressing maize malic enzyme. I will create a deactivated form of maize NADP Malic Enzyme that maintains ion-binding capabilities without catalyzing the malate to pyruvate reaction. We will create transgenic plants that express the mutant protein in their stomata, allowing us to test the ion binding function of NADP-ME independent of its catalytic activity.

To create the mutated enzyme, will we be using a procedure from Detarsio et. al.'s (2003) work, entitled Maize C4 NADP-malic Enzyme: Expression in Escherichia Coli and Characterization of Site-directed Mutants at the Putative Nucleoside-binding Sites. These researchers used the process of site-directed mutagenesis to explore active site characteristics of Maize NADP-ME. They generated multiple variations of malic enzyme by introducing abnormal amino acids at various locations, expressed their abnormal proteins in *E. coli*, and measured the catalytic activity of the mutants. They found that two such mutations, titled the Gsite2V and Gsite5V mutations, completely knocked out the reaction of malate to pyruvate in their expression assays. Both mutations replaced a glycine residue with a valine, extending the R-group by three carbons. (Fig. 11.) Detarsio et. al. (2003) reasoned that introducing these mutations must have obstructed the active site of the enzyme, preventing malate from binding and inhibiting

catalysis. For our purposes, this mutation should be ideal: it prevents catalysis of malate, but does not cause any major structural changes that would interfere with the ion binding properties of NADP-ME.

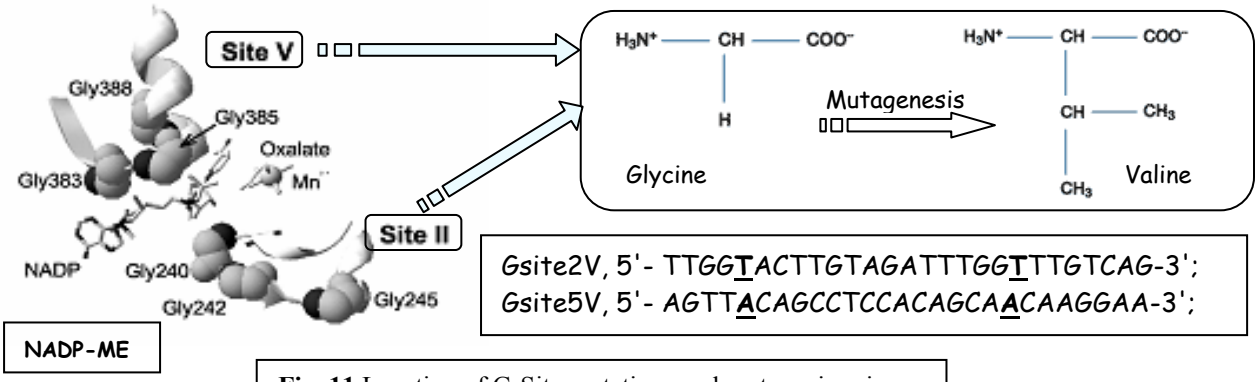


Fig. 11 Location of G-Site mutations and mutagenic primers

I have spent the last six months working to recreate this mutant. The first step in the process is clone the cDNA for the maize malic enzyme by isolating Maize RNA from fresh tissue. I am accomplishing this with an RNeasy extraction protocol from Qiagen corp. Following RNA isolation, I use a Beckman-Coultier Spectrophotometer to quantify the extractions: this machine uses light absorbance to determine the concentration and quality of the extracted nucleic acid solution. If both the concentration and purity are sufficient (conc > 25 ng/μL, Abs 260/280 > 2.0), a reverse transcriptase reaction is performed to generate a cDNA from malic enzyme transcripts present in the RNA extraction. This is known as first-strand synthesis: the initial cDNA will be amplified to make double stranded cDNA molecules.

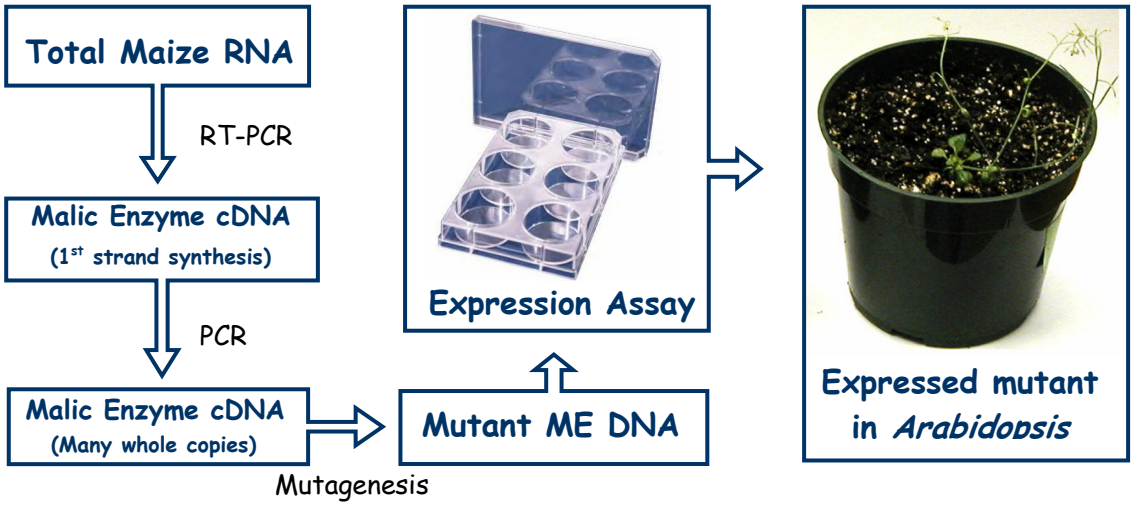
Following first-strand synthesis, an RNase H treatment (Invitrogen) is performed to digest any remaining RNA from our cDNA strands, preparing the template strand for PCR amplification. PCR is then performed using primers spanning the open reading

frame of maize NADP-ME, amplifying all 2000+ base pairs of the maize NADP Malic Enzyme cDNA (in theory… see *Section 4: Problem Solving*).

The generation of a complete NADP-ME cDNA will provide the raw material for site-directed mutagenesis: we are currently working on this isolation/amplification process. A small volume of the malic enzyme cDNA will then be reamplified using mutagenic primers. These primers contain sequence "errors": the mutations we intend to introduce in the DNA sequence. Following the mutagenesis, we will sequence the malic enzyme mutants to ensure that our modifications were successful.

Finally, we will introduce these mutations into an expression vector, and transform the mutant cDNA into E. coli. This allows us to "trick" the bacteria into expressing a foreign protein: in this case, E. coli will generate whole NADP-ME proteins based on the mutant DNA we have supplied. We can then test the catalytic and ion binding activity of the protein. If our enzyme maintains its ion binding abilities without registering any catalytic activity, we will splice the mutant DNA into Arabidopsis plants, and grow the transgenic organisms. We will then measure the stomatal conductance and water consumption of these organisms, allowing us to draw conclusions about the role of malate catalysis in stomatal conductance.

Fig. 12 NADP-ME Mutagenesis schematic



Section 4

Problem Solving

RT-PCR and PCR are generally considered to be routine procedures, and are often the stepping-stones to any number of procedures in molecular biology. Unfortunately, both methods require numerous reagents in optimal concentrations, and a complicated series of cycling temperatures and times, all of which vary depending on the size, composition, purity, and secondary structure of the RNA or DNA involved. Failure of any one portion of the protocol will result in an empty or smeared gel picture, which gives almost no indication of what went wrong. This is usually resolved with a few days of tinkering: repeating all procedures, adjusting $MgCl_2$ concentration, adding more or less template nucleic acid, etc.

Unfortunately, the isolation of a full-length cDNA Malic Enzyme cDNA has proven to be a greater challenge than anticipated: early attempts to visualize our whole ME products through gel electrophoresis were not reassuring. The procedures were repeated, but we did not seem to get any significant amplification using primers spanning the entire enzyme (19L – 2075 R). Routine procedures did not correct the problem, so we analyzed one potential area of difficulty: secondary structure of the mRNA transcripts.

In order to successfully amplify RNA to a cDNA during first strand synthesis, the enzyme reverse transcriptase binds to primers that have annealed to complementary locations on the RNA template. Starting from the primers, the enzyme begins synthesizing cDNA and continues linearly down the RNA strand, processing one nucleotide at a time. (visualize PacMan gobbling up a series of beads on a string). Unfortunately, the RNA template does not always lay perfectly within a "beads-on-

string" position; thermodynamics within the molecule can force the RNA to wrap around itself, adopting conformations that inhibit systematic amplification into cDNA.

We are currently trying a few different things to alleviate this secondary structure, and promote amplification. We have been experimenting with the addition of adding formamide to the RT-PCR reactions, to the PCR reactions, and to both: this chemical has been known to relieve secondary nucleic acid structure. Our results indicate that this strategy may be working; the addition of formamide seems to decrease the amount of incomplete transcription. Running a series of incomplete products on a gel produces a smearing effect: this is notably reduced in some reactions with formamide.

We are also attempting to bypass this secondary structure by generating the cDNA in multiple pieces, and then using a ligase enzyme to attach them all together. We are working with a series of primers spanning approximately. every hundred base pairs, and are mix-and-matching them to determine which bands we can reliably generate. So far, the 108L-238R, 216L-442R, and 316L-2075R primer pairs reliably generate bands that correspond with the expected product sizes and can be combined to form a full length cDNA; we have begun sequencing each product to ensure that accurate transcription has occurred. If the sequence data is deemed acceptable, we will begin ligating the strands together, building our malic enzyme gene out of obtainable nucleic acid sections.

The ligase process may be difficult, however, and I am still looking for a way to generate the entire cDNA with one reaction. Very recent data shows that modification of the RT-PCR temperature conditions may be the key to our success. The 316L-2075R RT-PCR reaction, when run under the 42ºC synthesis temperature listed in the protocol,

consistently failed to produce a usable product. However, we have discovered that raising this temperature to 50ºC drastically improves the quality of our reaction, and we are left with 1600 bases of usable NADP-Malic Enzyme cDNA. We have purchased a thermostable reverse transcriptase (Superscript III, Invitrogen Corp) in order to facilitate this process. I have not attempted to rerun the 19L-2075R under these conditions, but it is most definitely next on my agenda. If it works, we will be closer than ever before to developing and expressing a site-directed mutant of NADP-ME, and answering our questions regarding this potential mechanism for improving water use in transgenic organisms.

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Glossary of Terms

Anneal – the binding of primers to the template DNA strand

Arabidopsis thaliana - A C3 plant related to mustard, commonly used in molecular biology due to its short gestation time and small growth form

Bioinformatics – A field of study and commerce associated with the electronic storage, organization, and accessibility of genetic data.

cDNA – An exact copy of an extranuclear piece of RNA, contains only expressed sequences that are incorporated into the final protein

Electrophoresis - A method used to determine the size of a DNA fragment.

Exon – An area of DNA that is transcribed to mRNA that encodes for an amino acid (or stop codon) in the final protein.

Guard Cell - Plant cells that open and close to regulate the size of a central aperture, known as the stomate. Guard cells are analagous to the "lips" of the stoma al "mouth"

hnRNA – Heterogenous nuclear RNA: RNA transcripts from DNA prior to intron cleavage and final processing

Intron – An area of nucleotides that are present in genomic DNA, but excised out of hnRNA transcripts during mRNA processing.

Isoform – A protein that has the same function and similar or identical sequence to another protien, but is the product of a different gene

Malate - A four carbon sugar of the citric acid cycle, principle substrate of NADP-Malic Enzyme

mRNA – An RNA intermediate between DNA and protein; mRNA transcripts are synthesized from genomic DNA template, processed, and used as the final nucleic acid template that encodes for protein

PCR - Polymerase chain reaction, a method used to amplify known sequences of DNA, making hundreds of thousands of copies from a single piece

Primers – Short sections of RNA that bind (anneal) to DNA template during the intitiation of transcription

Pyruvate – A three carbon sugar of the citric acid cycle, principle product of the reaction of NADP-Malic Enzyme and malate

RT-PCR - Reverse transcriptase polymerase chain reaction, a method of amplifying RNA into cDNA copies. This is uaually followed by cDNA amplification via PCR.

Site-Directed Mutagenesis – The generation of mutant proteins by modifying the DNA that encodes for them at specific sites, and expressing the mutant DNA

Stomata – A mouth-like structure on the bottom surface of photosynthetic leaves. Mediates gas exchange and is an important site of organismal water loss.

TAIR – The ***A****rabidopsis* **I**nformation **R**esource, located at http://www.arabidopsis.org/. An invaluable service for anyone studying *Arabidopsis*, containing the complete Arabidopsis genome and a searchable gene directory.

NADP-Malic Enzyme (NADP-ME) – A respiratory enzyme that catalyzes the oxidation of malate, generating pyruvate and carbon dioxide, also binding bivalent cations in the process

Transpiration – The loss of water vapor, particular via evaporation through open stomata.

Turgor Pressure- Intracellular pressure resulting from the movement of water in accordance with solute concentration

Zea mays – Maize, or corn plants.

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AT1G79750	AT1G79750.1	malate oxidoreductase, putative, similar to malate oxidoreductase (NADP-dependent malic enzyme)	F19K16_27(orf) AT1G79750(orf) F19K16.27(orf)	yes	chloroplast, malic enzyme activity, oxidoreductase activity, acting on NADH or NADPH, NAD or NADP as acceptor, malate metabolism
AT2G13560	AT2G13560.1	malate oxidoreductase, putative, similar to NAD-dependent malic enzyme 62 kDa isoform, mitochondrial precursor	T10F5.10(orf) AT2G13560(orf) T10F5_10(orf)	yes	mitochondrion, mitochondrion, malic enzyme activity, malic enzyme activity, oxidoreductase activity, acting on NADH or NADPH, NAD or NADP as acceptor, malate metabolism, malate metabolism
AT2G19900	AT2G19900.1	malate oxidoreductase, putative, similar to NADP-dependent malic enzyme	F6F22.7(orf) F6F22_7(orf) AT2G19900(orf)	yes	cellular_component unknown, malic enzyme activity, malic enzyme activity, oxidoreductase activity, acting on NADH or NADPH, NAD or NADP as acceptor, transferase activity, malate metabolism, malate metabolism, metabolism
AT4G00570	AT4G00570.1	malate oxidoreductase, putative, similar to NAD-dependent malic enzyme 59 kDa isoform, mitochondrial precursor	F6N23_16(orf) F6N23.16(orf) AT4G00570(orf)	yes	mitochondrion, disulfide oxidoreductase activity, malic enzyme activity, malic enzyme activity, oxidoreductase activity, acting on NADH or NADPH, NAD or NADP as acceptor, electron transport, malate metabolism, malate metabolism
AT5G11670	AT5G11670.1	malate oxidoreductase, putative, similar to NADP-dependent malic enzyme	AT5G11670(orf) T22P22.60(orf) T22P22_60(orf)	yes	cellular_component unknown, malic enzyme activity, malic enzyme activity, oxidoreductase activity, acting on NADH or NADPH, NAD or NADP as acceptor, transferase activity, malate metabolism, malate metabolism, metabolism
AT5G25880	AT5G25880.1	malate oxidoreductase, putative, similar to NADP-dependent malic enzyme	T1N24_25(orf) AT5G25880(orf) T1N24.25(orf)	no	cellular_component unknown, malic enzyme activity, oxidoreductase activity, acting on NADH or NADPH, NAD or NADP as acceptor, malate metabolism, N-terminal protein myristoylation