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Analysis of the C-terminal domain of SccA, a putative cell wall stress receptor in *Aspergillus nidulans*

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This Honors paper by Jacqueline Ward has been read and approved for Honors in Biochemistry and Molecular Biology.

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CONTENTS

ABSTRACT

Analysis of the C-terminal domain of SccA, a putative cell wall stress receptor in *Aspergillus nidulans*

by

Jacqueline M. Ward

Aspergillus nidulans is a filamentous fungus that has been used as a model organism for many eukaryotic processes. Its research implications are far-reaching because fungi have both negative and positive effects on many aspects in medicine, industry, and the environment. Studying the cell wall specifically is important as this structure is not present in animal cells and could potentially serve as a target for medical treatment in many threatening fungal infections. This research aims to elucidate the function of the novel gene designated *SccA* which affects cell wall integrity in *A. nidulans*. Plasmid-based, overexpression of *SccA* can suppress the *calC2* mutation in protein kinase C (PkcA), which results in hypersensitivity to the chitin-binding agent Calcofluor White (CFW). It has previously been shown that hypersensitivity to CFW is indicative of cell wall integrity defects in both yeast and filamentous fungi. SccA is predicted to have a single transmembrane domain, an extracellular domain rich in serine and threonine amino acids, and a short cytoplasmic C-terminus of 59 amino acids. No sequence homologues exist in yeast, but structurally there is great similarity between SccA and other stress receptors. The C-terminal domains of yeast stress receptors like Wsc1 and Mid2 have been shown to play important roles in signal transduction upstream from Pkc. In an analysis of C-terminal truncation mutants, we have shown for the first time in *A. nidulans* that the C-terminal domain of SccA is also essential in proper cell wall integrity function.

Introduction

Implications of fungal research

The fungi are a broadly diverse range of organisms, which have both positive and negative impacts on many fields. The filamentous fungi are characterized by their ability to grow by extending long, branching cells called hyphae. Hyphae are divided by cross walls called septa which allow the flow of organelles between cells. Filamentous fungi play a role in industry, medicine, and the environment. For example, a species of *Aspergillus* is the largest biological producer of citric and gluconic acid, materials used as flavorings and preservatives in numerous products (Magnuson, 2004). Fungi are also used in other industrial processes such as cheese-ripening, soy sauce production, and certain fermentation processes (Saunders, 1989). Medically, fungal infections cause problems for many people. Healthy individuals are not as susceptible to severe mycoses, but immunocompromised patients are at very high risk for developing life-threatening fungal infections. Aspergillosis infection, caused by *Aspergillus fumigatus,* is now the leading cause of death among leukemia patients (Nierman, 2005). As a pathogen, filamentous fungi can also infect many crops and have far-reaching consequences on food supply and economics. Fungal infections have also been implicated in contributing to loss of coral in the Caribbean and Indo-Pacific reefs (Francini-Filho, 2008).

Aspergillus nidulans serves as a tractable model organism partially due its well-characterized sexual reproduction, which has made it a useful genetic model. The study of *A. nidulans* has elucidated many eukaryotic processes such as the cell cycle and microtubule formation (Morris, 1975; 1978). Our lab focuses on elucidation of the genes and proteins involved in cell wall metabolism. Because animal cells lack cell walls, the

fungal cell wall is an important target for antifungal drugs. Though this field of study is very significant, much is still left to be understood. The exact mechanism of how the cell wall is assembled and how this process is regulated has yet to be fully elucidated in filamentous fungi. This basic knowledge is necessary for the development of chemotherapeutic agents that target fungal pathogens.

Cell wall

The cell wall is a complex and unique organelle that defines the cell's shape, serves as its connection to the outside world through the extracellular matrix, and mediates osmotic and structural stress (Lesage, 2006; Svoboda, 2004). In filamentous fungi, the cell wall is constantly being remodeled as hyphae continue to extend throughout the cell's lifetime (Adams, 2004). In general, the cell wall consists of a layer of fibrous polysaccharides, including the polymer chitin, followed by a layer of glycosylated proteins, though the composition differs amongst species (Ruiz-Herrera, 1992). A generalized illustration of the cell wall is shown in Figure 1. Though cell wall chemistry and mechanical elasticity has been investigated in *A. nidulans* (Bull, 1970; Zhao, 2005), many of the genes and subsequent proteins that play a role in the synthesis and repair of the cell wall have yet to be studied.

One method of identifying novel components of cell wall metabolism is discovering the genetic basis of mutant strains associated with hypersensitivity to wall-compromising agents. One such wall-compromising agent is Calcofluor White (CFW), which has previously been used to identify genes involved in cell wall synthesis and regulation in both yeast and filamentous fungi (Lussier, 1997; Hill, 2006). CFW binds to

chitin in fungal hyphal walls (Maeda, 1967), disrupting these polymers which causes a reduced growth rate through hyphal tip lysis (Roncero, 1985). In addition, CFW has been shown to disrupt mannoprotein incorporation in *Candida albicans*, another filamentous fungus (Murgui, 1985). Normally, wild-type *Aspergillus nidulans* strains do not show sensitivity to CFW, and can grow on media treated with this chemical. A mutant strain that is hypersensitive to CFW suggests that the mutation is in a gene that codes for a protein necessary in cell wall metabolism.

Teepe et al (2006) identified a strain harboring a point mutation in the *A. nidulans* orthologue of protein kinase C (PkcA). This *calC2* mutation confers hypersensitivity to CFW. When media was treated with 10 μg/mL CFW, a wild type strain was able to sustain the cell wall stress caused by CFW but the strain with the *calC2* mutation showed no visible growth after two days. This hypersensitivity is demonstrated in Figure 2. Through complementation testing and gene mapping, this mutation was identified as a point mutation at position 2537 in exon 5 of PkcA. This base pair change replaces a glycine with a positively-charged arginine in a highly conserved region (C1B domain) of PkcA, and thereby confers sensitivity to CFW. A representation of the PkcA gene and the *calC2* mutation is shown in Figure 3.

Our lab has identified a novel protein, SccA, that suppresses the *calC2* mutation in PkcA. Plasmid-borne copies of *SccA* were transformed into the strain containing the *calC2* mutation and the addition of *SccA* allowed the mutant strain to grow on CFW. Protein analysis programs (such as ExPASy), predict that SccA is a 271-amino acid transmembrane protein with a very serine- and threonine-rich extracellular domain (Figure 4). It is also predicted that a cleavable N-terminal signaling peptide, consisting of

the first 11 residues, targets the protein to the endoplasmic reticulum. In unpublished data from our lab, an SccA::GFP chimera has also been shown to localize to the plasma membrane of hyphae and is very clearly present at septa, probably due to the double layer of membrane at these structures (Figure 5).

Cell Wall Integrity (CWI) pathway in Saccharomyces cerevisiae

BLAST search revealed that the translated open reading frame (ORF) of AN4897 (SccA) showed no homology to any proteins in the database. Interestingly though, the C-terminal domain of SccA is highly conserved amongst the filamentous fungi (Figure 6). There is a 34% identity with other filamentous fungi throughout the entire protein, but within the C-terminal domain there is a 69% identity. Though there is no exact sequence homology, the predicted structure of SccA is very much like certain stress receptors in the *Saccharomyces cerevisiae* Cell Wall Integrity (CWI) pathway (Figure 7). These cell wall stress receptors serve as the connection between the cell wall and the cytoplasm. So far, five stress receptors (Wsc1-3, Mid2, Mtl1) have been identified in *S. cerevisiae* and all of them share structural similarity. Like SccA, they possess serine- and threonine-rich extracellular regions which are hypothesized to be highly glycosylated and act as rigid probes extending into the cell wall (Rajavel, 1999). They also each have a single transmembrane domain, with a short, cytoplasmic C-terminus (Verna, 1997; Ketela, 1999; Rajavel, 1999). The three Wsc receptors ("wall stress component") are characterized by a cysteine-motif at the amino-terminus (Verna, 1997). Mid2 and Mtl1—the two other yeast cell wall stress receptors—lack this motif (Ketela, 1999; Rajavel, 1999), as does SccA.

These stress receptors work upstream from the protein kinase C orthologue (Pkc1) in *S. cerevisiae.* As shown in Figure 7, Pkc1 initiates a mitogen-activated protein (MAP) kinase cascade that has many important downstream cellular effects on transcription of cell wall- and morphogenesis-related genes including regulating responses in mating, hyphal growth, high osmolarity conditions, sporulation, and cell wall stress (Levin, 2005). In yeast, it is the cell wall stress receptors that trigger the signal transducation pathway to activate Pkc1 by interacting with the guanine exchange factor, Rom1/2, that activates the GTPase, Rho1. Because overexpression of SccA is able to suppress the *calC2* mutation in PkcA and thereby allow growth in the presence of CFW, this indicates that a direct or indirect connection exists between these two proteins. This link, along with the structural similarity between SccA and known yeast cell wall stress receptors, suggests the existence of an *A. nidulans* pathway similar to the CWI pathway in yeast.

Further conservation of the CWI pathway was shown by Fujioka (2007) who reported the identification of 3 Wsc homologues in *A. nidulans*. Kriangkripipat and Momany (2009) have shown that the Wsc homologue, AN5660/WscA, is mannosylated in a fashion similar to the Wsc proteins in *S. cerevisiae*. Although there are Wsc homologues in *A. nidulans*, the SccA protein is not one of them and does not show any specific sequence homology to the other known yeast stress receptors. The way *A. nidulans* responds to cell wall stress is not expected to be identical to yeast. Budding yeast and filamentous fungi have very different morphology and modes of growth, which could be reflected by differences in how their cell wall is made and modified. Potentially, SccA is similar in function to the other receptors, Mid2 or Mtl1, or it could be a completely novel *A. nidulans* stress receptor.

Importance of the cytoplasmic, C-terminal region

If SccA is a cell wall stress receptor, its cytoplasmic region will likely be very important in signaling. In *S. cerevisiae*, Vay (2004) showed that the complete C-terminal region of the yeast stress receptor, Wsc1, is essential for complementation of a *wsc1*Δ mutant. Strategic truncation mutations also revealed three separate regions of the C-terminus that both positively and negatively interact with Rom2, the downstream effector of Wsc1. Straede and Heinisch (2007) showed through Wsc1 and Mid2 chimera studies that the cytoplasmic tails of those sensors were the essential domains for determining the cell's response to cell wall stress. The importance of the C-terminal domain is not surprising considering this is the sole region of the protein that is cytoplasmic. In yeast, the cell wall stress receptors are hypothesized to act as mechanosensors in which their extracellular domain monitors the external environment, and the internal domain is responsible for the cytoplasmic signaling in the CWI pathway (Levin, 2005). When these studies deleted or altered the receptors' C-termini, the signaling was abolished and the cell could not appropriately respond to cell wall stress.

The goal of this project was to elucidate the functional significance of the cytoplasmic domain of SccA. It is likely that if SccA serves a function similar to a yeast cell wall stress receptor, then its C-terminus will also be essential. To determine the regions of the C-terminal region imperative for function, six different truncation mutants were constructed, in addition to a construct with the full-length C-terminus, in order to determine each truncation mutant's respective suppression of the *calC2* mutation in the presence of CFW. Each of these constructs was also tagged with Green Fluorescence

Protein (GFP) in order to determine general expression of the construct and to monitor cellular localization of SccA.

Materials and Methods

Strains, media, and culture methods

The R78 strain which has been previously described in Hill et al (2006) was used in this study. Strains are listed in Table 1. Complete medium (CM) consisted of 1% glucose, 0.2% peptone, 0.1% yeast extract, 0.1% casamino acids, 5% nitrate salts, 1% trace elements, 0.1% vitamin mix, 1.2 mM L-arginine, 10 mM uracil, 5 mM uridine, and 50 µg/mL ampicillin. Vitamin mix and nitrate salts were previously described in Kafer (1977). Trace elements were previously described in Hill and Kafer (2001). Minimal medium (MM) consisted of 1% glucose, 5% nitrate salts, 1% trace elements, 0.001% thiamine hydrochloride, 25 ppb biotin, and and 50 µg/mL ampicillin. Solid media contained 1.5% agar. CM with CFW was made by adding a stock solution of 1% in 25 mM KOH, filter-sterilized and stored frozen, to melted media at 55°C. CM + CFW was made in the following dilutions: 5 µg/mL, 7 µg/mL, and 10 µg/mL.

SccA and $(GA)_5$-GFP expression plasmids

To construct each of the C-terminal truncation mutants of *SccA*, the ORF AN4897.1 (*SccA*) along with 870 base pairs of upstream sequence (Appendix I), was PCR-amplified through the final amino acid desired in the truncation using the R78-XF2 plasmid as template. For each truncation mutant, 30 base pairs that correspond to 10 successive amino acid residues were deleted until the barrier of the predicted hydrophobic, transmembrane region at residue 214 (Figure 8). The stop codon at the end of each truncation was not included, so that GFP could be appropriately ligated to each construct at the 3' end. PCR was performed using the high fidelity DNA polymerase

PfuTurbo® and primer sets FL, T1, T2, T3, T4, T5, and T6. Primer sequences are outlined in Table 2. All of the resultant PCR products were column purified with the QIAquick PCR Purification Kit and digested with *Kpn1* and *Not1* (QIAGEN). The digested products were separated on a 0.65% agarose gel, the appropriate size pieces were confirmed and excised, then purified with the QIAquick Gel Extraction Kit (QIAGEN). The fragments were then ligated into the pRG3 plasmid (Osherov, 2000; Fungal Genetics Stock Center) via the *Kpn1* and *Not1* restriction sites. Sequencing is currently under way to verify each truncation mutant.

The *$(GA)_5$-GFP* sequence was amplified from plasmid pFNO3 (Yang, 2004; Fungal Genetics Stock Center) with the GFP primer set and prepared as above and digested with *Not1* and *Sph1*. *$(GA)_5$-GFP* is an alternate form of Green Fluorescent Protein that contains five extra sets of the Gly-Ala repeat in frame, at the N-terminus of the GFP protein. This provides a "hinge" region between the SccA target gene and GFP, which has been shown to improve function of the chimera (Yang, 2004). The fragments were ligated into each of the pRG3 truncation constructs at the *Not1* and *Sph1* restriction sites. A diagram of the final plasmid design is shown in Figure 9. Two controls were constructed: a full length SccA containing a stop codon and a full C-terminal truncation [T6 (1-214)] containing a stop codon. These two constructs served as controls to assess the effects of the addition of GFP. Strain R78 that contains the *calC2* mutation in PkcA was transformed with all constructs. In addition, the strain R429, which contains the pRG3 empty vector without GFP was used as a control.

Microscopic Methods

Six transformants from each SccA-GFP construct were observed with a fluorescence microscope to determine expression and correct protein localization. Transformants were streaked on MM and the newest growing hyphae were observed with an Olympus BX51 epifluorescence microscope in both fluorescence and transmitted-light mode. Qualitative colony-wide observations were made with a 20x objective. Qualitative cellular morphology observations were made with a 100x objective. These observations were then used to group together transformants which showed similar, moderate levels of GFP glow, which corresponded to levels of SccA expression. Transformants with similar expression were then tested for CFW sensitivity. Photos were taken with a SPOT RT-SE digital camera.

Screening for CFW sensitivity

Relative sensitivity to CFW was tested in two ways: the toothpick method and spore drop method. The toothpick method consisted of gently poking a colony and then poking at a gridded spot on either a control CM or CM + CFW agar plate to transfer an indefinite number of spores. The spore drop method consisted of applying 5-µL drops of spores consisting of 10,000 spores per drop to a gridded CM agar control plate and to the varying concentrations of CM + CFW agar plates (5 µg/mL, 7 µg/mL, and 10 µg/mL). Three transformants of each construct (FL, T1, T2, T3, T5, and T6) were tested in duplicate on the four sets of plates. Cultures were incubated for 4 days at 30°C. CFW sensitivity was determined by relative growth in comparison to the CM plate without CFW and to the empty vector control.

Results

GFP localization and expression

Figure 10 shows an example of the GFP localization of both full-length (FL) SccA compared to construct T6 SccA (1-214) that contained the most extreme truncation, a full deletion of the cytoplasmic domain. Both full length SccA and and T6 SccA (1-214) localize to the plasma membrane, as is indicated by the glow at the outer edges of the hyphae and the septa. All of the truncation mutants between FL and T6 were also tested with varying results. Of the 6 transformants tested for each truncation mutant, there were varying levels of GFP expression and localization. Some transformants demonstrated a very bright glow throughout a majority of hyphae, other transformants that were treated and transformed the exact same way demonstrated no glow whatsoever. This is due to the plasmid-based system as it is impossible to regulate the number of plasmids taken up by each transformant. Due to these constraints, three transformants with each construct were grouped together that demonstrated similar localization patterns and moderate levels of expression. However no transformants containing the T4 construct (SccA-GFP [1-241]) showed any level of GFP expression, thus this construct was removed from comparison. The other groups of similar transformants were then tested for their CFW sensitivity.

CFW sensitivity

Figure 11 shows full length SccA with and without GFP in comparison to the full truncation [T6 (1-214)] with and without GFP in an R78 strain background (which possesses the *calC2* mutation in PkcA). These tests were performed on 5 µg/mL CFW,

which is half the concentration used to first identify SccA's suppression of the *calC2* mutation, yet at this lower concentration the truncated version of SccA does not suppress the mutation. Figure 11A shows spore drop tests for two transformants and Figure 11B shows toothpick tests for over 30 transformants for each construct. The addition of GFP to both the constructs decreased strain growth on CFW. When the R78 strain was transformed with the full C-terminal truncation construct, there was an evident decrease in ability to grow on CFW.

Figure 12 shows each truncation mutants' ability to suppress the *calC2* mutation in comparison to full length SccA-GFP and empty vector on 5 µg/mL CFW. Each construct was transformed into the *calC2* mutant strain R78. Each of the three transformants shown exhibited equivalent expression patterns based on GFP signal. All transformants grew comparably on control CM without CFW. The transformants with the full length SccA-GFP construct were able to grow relatively well on 5 µg/mL CFW compared to empty vector, as previously exhibited in Figure 11. Transformants with the T1 SccA (1-261)-GFP, T2 SccA (1-251)-GFP, T3 SccA (1-241)-GFP, and T5 SccA (1-221)-GFP constructs all showed variable ability to grow on CFW. None of the transformants with the T6 SccA (1-214)-GFP construct showed any growth, indicating they are extremely sensitive to CFW. The strain containing the empty vector showed a limited amount of growth. Toothpick tests were also performed to test a larger number of transformants for the various truncation contrsucts; results were much more inconclusive than the toothpick tests shown in Figure 11B.

Discussion

Despite the variability in GFP expression for both the full length and the truncation mutant constructs, it is clear that both full length SccA and the C-terminal truncation mutants localize to the plasma membrane. There was variability in GFP expression and cellular morphology in each transformant but it was not characteristic of a specific truncation. Instead, this variability is probably due to the plasmid-based nature of the experiment. It is impossible to ensure that every spore from each of the transformants retains the plasmid. Regardless, it appears that the C-terminus has no significant effect on localization of SccA to the plasma membrane. This was to be expected since there is predicted to be a cleavable N-terminal signaling domain.

In a comparison of the full length and mutant SccA construct with and without GFP, it seems that the addition of GFP affects SccA's suppression of the *calC2* mutation. Because the addition of GFP affects the function of SccA, it was necessary to test the constructs without GFP. However it is impossible to normalize the expression levels of the constructs that do not contain GFP because GFP expression is the method we used to group transformants together with similar expression. With this in mind, this data is difficult to draw conclusions from when only a few transformants are tested. However when we tested many transformants across the board through toothpick testing, it seems that the C-terminal full truncation construct (T6SccA [1-214]) grows similarly to empty vector (as shown in Figure 11B). This means that the transformants with the complete C-terminus deletion [T6 SccA (1-214)] were not able to suppress the PkcA *calC2* mutation.

An interesting and somewhat unexpected finding is that the full C-terminal truncation construct with GFP is completely dead in comparison to a low level of growth

with the empty vector (as shown in Figure 12). That is to say that plasmid-borne copies of SccA-GFP without a C-terminus cause more CFW sensitivity than endogenous wild type copies alone. This explains why there is a certain amount of limited growth when the CFW-sensitive R78 strain is transformed with an empty vector. The PkcA *calC2* mutation in the R78 strain confers CFW sensitivity and since there are some endogenous copies of SccA, a low level of growth occurs on plates treated with low concentrations of CFW. When full length copies of SccA-GFP are transformed into the R78 strain on plasmids, the extra copies of the protein are able to suppress the *calC2* mutation and confer a resistance to the CFW sensitivity that is normally inherent within the R78 strain. However when the full C-terminal deletion [T6 SccA (1-214)-GFP] construct is present on a plasmid, this somehow causes the R78 strain to lose the low level of *calC2* suppression and be completely dead on plates treated with CFW. There are several theories as to why this would happen.

One theory involves normal SccA versus truncated SccA competition. Perhaps the C-terminally truncated SccA is localizing correctly and integrating into the plasma membrane where it competes away the upstream effectors of the normal, endogenous copies of SccA. The broken copies of SccA-GFP could be binding upstream activating molecules so that the normal, functioning copies of SccA are no longer activated, thereby arresting its endogenous ability to interact with further downstream proteins and eliminating its low level of suppression of the *calC2* mutation. In effect, the broken copy would be competing away the molecules with which the normal copy of SccA interacts. Another possibility includes competition with downstream effectors. In yeast, the downstream effector of the cell wall stress receptors is Rom2 (Vay, 2004).

However deletion of the C-terminus in the yeast stress receptors eliminates interaction with Rom2. Thus the idea that the C-terminal deletion of SccA is competing away a Rom2-like protein seems doubtful as the cytoplasmic region is likely the region that interacts with that downstream effector.

Another theory is that SccA exists as a dimer. In this situation, the endogenous SccA dimers would be present at a low level, conferring a low level of *calC2* suppression. More copies would increase the shear amount of dimers or rate of dimerization, thereby increasing *calC2* suppression. However when the C-terminal fully-truncated copy of SccA-GFP is present, perhaps it dimerizes to the endogenous copies of SccA, inducing a change in their natural function, and losing their natural, albeit low, ability to suppress the *calC2* mutation. However because a survey of the literature has yielded no evidence that other cell-wall stress receptors exist in dimerized forms, and there is no indication that SccA contains a coiled-coil domain which often serves as a dimerization "tag," the dimerization theory is simply speculation.

Regardless of the mechanism, it is clear that SccA-GFP with a fully-truncated C-terminus has a rather dramatic negative effect on the suppression of the *calC2* mutation in PkcA. This deletion of the C-terminus eliminates the cell's ability to respond to cell wall stress. Unfortunately, it is difficult to draw any further conclusions from the intermediate truncation mutants. Each of the observed colonies within each set of mutants demonstrated variable CFW sensitivity. Even when the exact same transformant was tested in duplicate, the results were not always identical. This variability is evident in the differences between each transformant of each truncation mutant in Figure 12. More tests need to be performed to confirm these observations.

The plasmid-based system is inherently variable as it is difficult to regulate how many plasmids are taken up in a transformation. The GFP expression observations served to somewhat normalize the expression levels of each SccA construct, but these are not fool-proof. It is also exceedingly difficult to perform western blot analysis to monitor protein levels because of the membrane-based nature of SccA. Perhaps these limits are the cause of the variation in the ability to suppress the *calC2* mutation for each of the truncation mutants. Another limit is the small number of transformants tested. As only 3 transformants were tested via spore-drop test for each construct, it is possible that the small sample size is to blame for variability in *calC2* suppression. However, toothpick tests were also performed which also yielded extremely inconclusive results.

It is possible that these results are not necessarily inconclusive but rather indicative that there is not one certain section of the cytoplasmic region that is essential, but rather the entire cytoplasmic region as a whole. As the change in phenotype is so dramatic when the entire region is deleted, it seems odd that the other truncation mutants would not yield a certain spectrum of phenotypes. Perhaps each of the deleted regions contributes in some way to the whole functioning unit of the C-terminal cytoplasmic region.

Further research will focus on solidifying the results for each of the truncation mutants. More CFW spore-drop tests are necessary to draw specific conclusions. Future research will act in coordination with current work to determine if SccA is essential and to create a construct that could induce or repress its endogenous expression. Though the plasmid-based system was useful to initially identify SccA's ability to suppress the *calC2* mutation in PkcA, endogenous techniques should be explored. With this type of control

over endogenous expression, it will be possible to determine if plasmid-borne copies of SccA are sufficient to suppress the *calC2* mutation or if the endogenous copies work in coordination with the extraneous copies. This type of work could also be performed with SccA lacking a cytoplasmic region to determine if extraneous copies of this mutant maintain their extreme CFW sensitivity without the endogenous copies. Dimerization could be verified or disproven with split-fluorescent protein studies or two-hybrid analysis.

Figures

Figure 1: Generalized structure of the fungal cell wall. The black figures at top represent glycosylated proteins. The long gray figures below represent fibrous polysaccharides. The linear black figures below that represent chitin. The layered structure at the bottom represents the plasma membrane. Figure adapted from Grun, 2003.

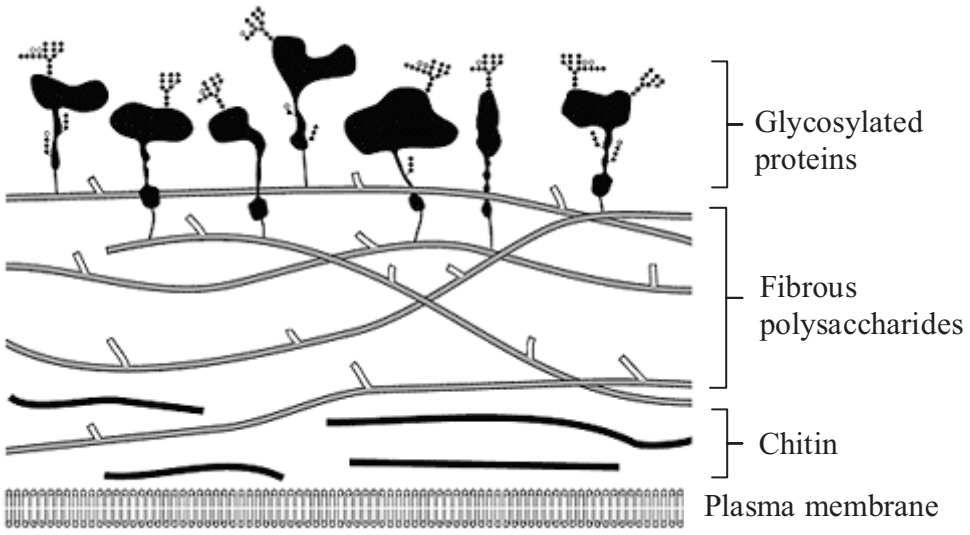


Figure 2: The *calC2* mutation confers sensitivity to CFW. Wild type strain on top row compared to strain with c*alC2* mutation (strain R78) on bottom row. Colonies were grown for 2 days at 30°C. Figure adapted from Hill, 2006.

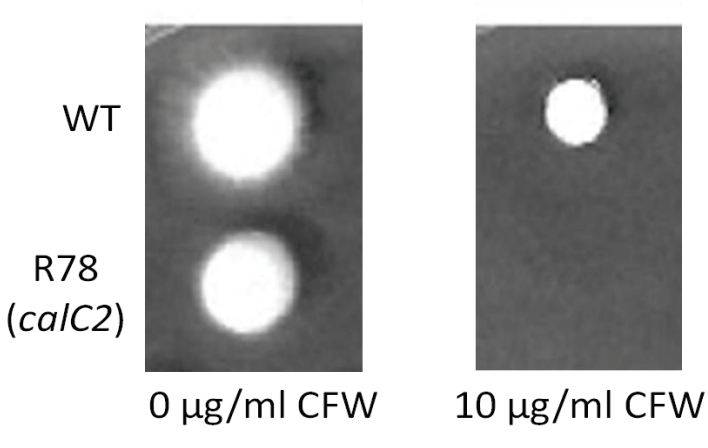


Figure 3: Domain structure of the PkcA gene and sequence comparison of C1B domain amongst multiple fungal and animal species. Gray shading in the sequence indicates conserved residues. The asterisk indicates the glycine residue that is replaced by arginine in the *calC2* mutation. Figure from Teepe, 2006.

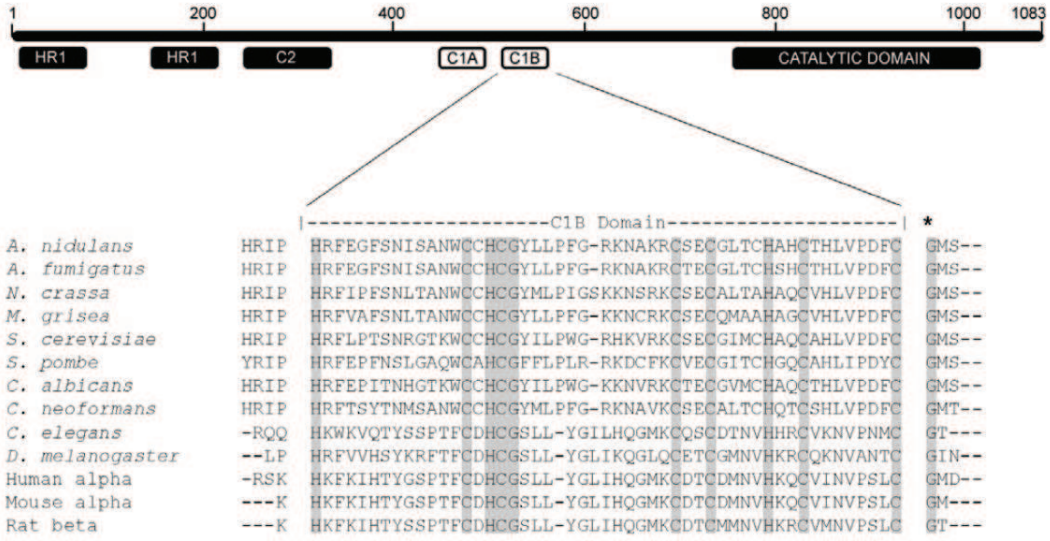


Figure 4: A) Primary structure of SccA. Underlined region represents cleavable N-terminal peptide. Italicized region indicates extracellular serine/threonine rich region. Bold sequence indicates transmembrane domain. Sequence underlined with dots indicates C-terminal, cytoplasmic domain. B) Predicted structure of SccA. Specific domains indicated by pattern key below.

A)

MRVTALPFFLY*SLSLVAVVAGQSTQVDSIS*
DVTDALPTPTQTSATDTDSDTETTTSESSST
TTTEPTTTSSSTSSSDDETSGTTTSSSSTSTSD
PTTTTTSDTDTTTTSSSTDPDPTTTSTSSSDD
DSSTTRTPTVTTITTTQTIDGTPVPTTYTSTNS
EASESESPGLSGSSGDSDSGLDSNQKK
IIIGVVVGVGGAILIGAIGVVAW
RIRARRRAAADDDATDLMSGTAVGSGV
REKAPSPSGGTPFRSTLDQYHNPGPVNA

B)

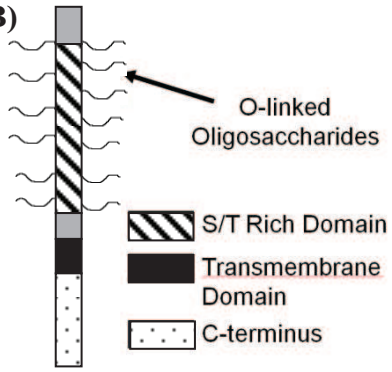


Figure 5: An SccA-GFP chimera localizes to the cell surface. The septum is indicated by the arrow. Image obtained from unpublished data by Terry Hill.

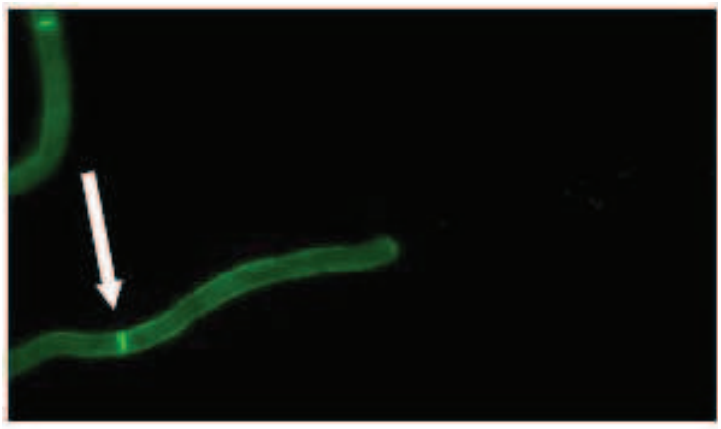


Figure 6: Conservation of C-terminal primary sequence of SccA amongst filamentous fungi. There is a 69% identity amongst the species in this region. Species names are indicated on the left. Asterisks (*) below each residue indicate complete conservation. Two dots (:) indicate a high level of conservation. One dot (.) indicates a moderate level of conservation. Numbers on the left and right side of each sequence indicate residue number at the beginning and end of the cytoplasmic domain, respectively.

```
A. oryzae      220 HARKRNAHDNDEATDLMSGTAVGSGLREKAPSPGAGGTPFKSTLDQYHNPGPVNAASNF 279
A. flavus      220 HARKRNAHDNDEATDLMSGTAVGSGLREKAPSPGAGGTPFKSTLDQYHNPGPVNAASNF 279
A. niger       215 HARK-AAQDNDEAADLMSGTAVGAGAREKAPSPGAGGTPFKTTLDQYHNPGPVNAASNF 273
A. terreus     193 HVRKRNALDQDDSADLMSGTAVGSGMREKAPSP-AQGTPFRSTLDQYHNPGPVNAASNF 251
N. fischeri    206 RARRRAAADNDEAADLMSGTAVGSGVREKAPSPGAGGTPFRSTLDQYHNPGPVNAASNF 265
A. fumigatus   208 RARRRAAADNDEAADLMSGTAVGSGAREKAPSPGAGSTPFRSTLDQYHNPGPVNAASNF 267
A. clavatus    226 HARR-KATDHDDAADLMSGTAVGSGVREKAPSPGAGGTPFRSTLDQYHNPGPVNAASNF 285
A. nidulans    214 RARRRAAAD-DDATDLMSGTAVGSGVREKAPSP-SGGTPFRSTLDQYHNPGPVNAASNF 271
                   :.*:  * * *:::*********:* ******* : .***::**************** 
```

Figure 7: Proposed Cell Wall Integrity (CWI) Pathway from *Saccharomyces cerevisiae*. The five cell wall stress receptors are Wsc1, Wsc2, Wsc3, Mid2, and Mtl1. Stress receptors are shown in the box, Pkc1 is encircled. Figure adapted from Levin (2005) and Fujioka (2007).

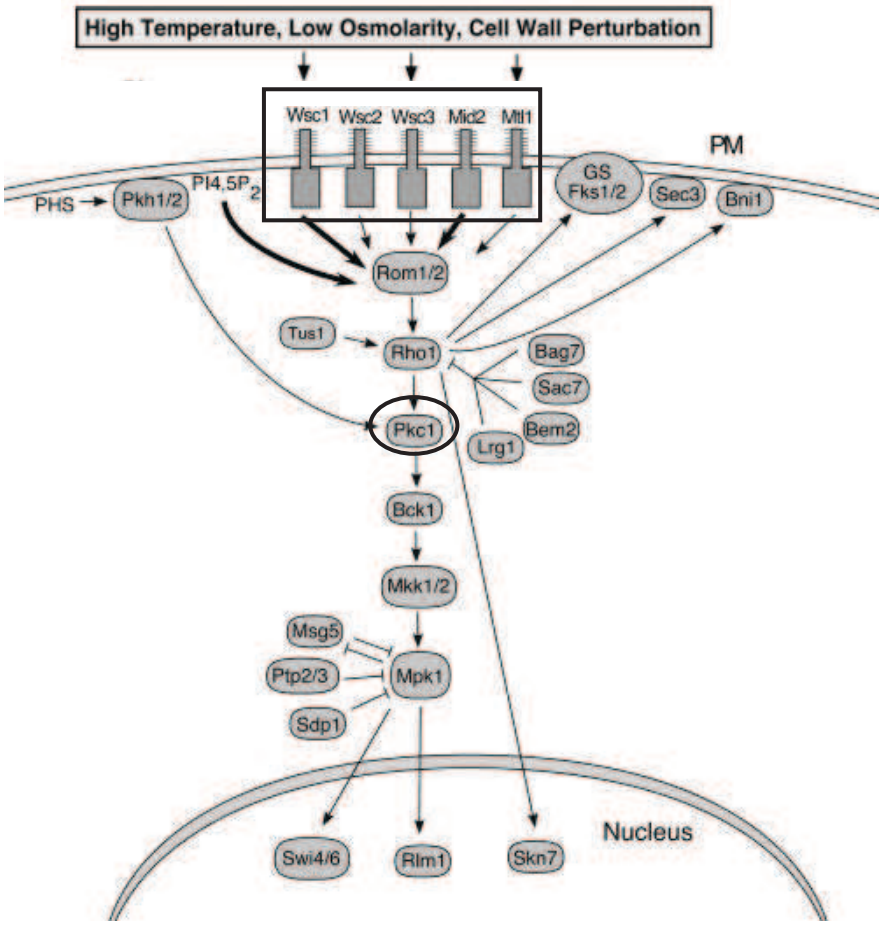


Figure 8: C-terminal truncation strategy for SccA. Labels on the left indicate each truncation (FL is "full length") and the respective residues present in each truncation. The gray rectangles represent the extracellular domain. The dashed rectangles represents the serine/threonine-rich region. The black rectangles represents the transmembrane domain. The dotted rectangles represent the cytoplasmic domain. "N" and "C" represent the N-terminus and C-termini, respectively.

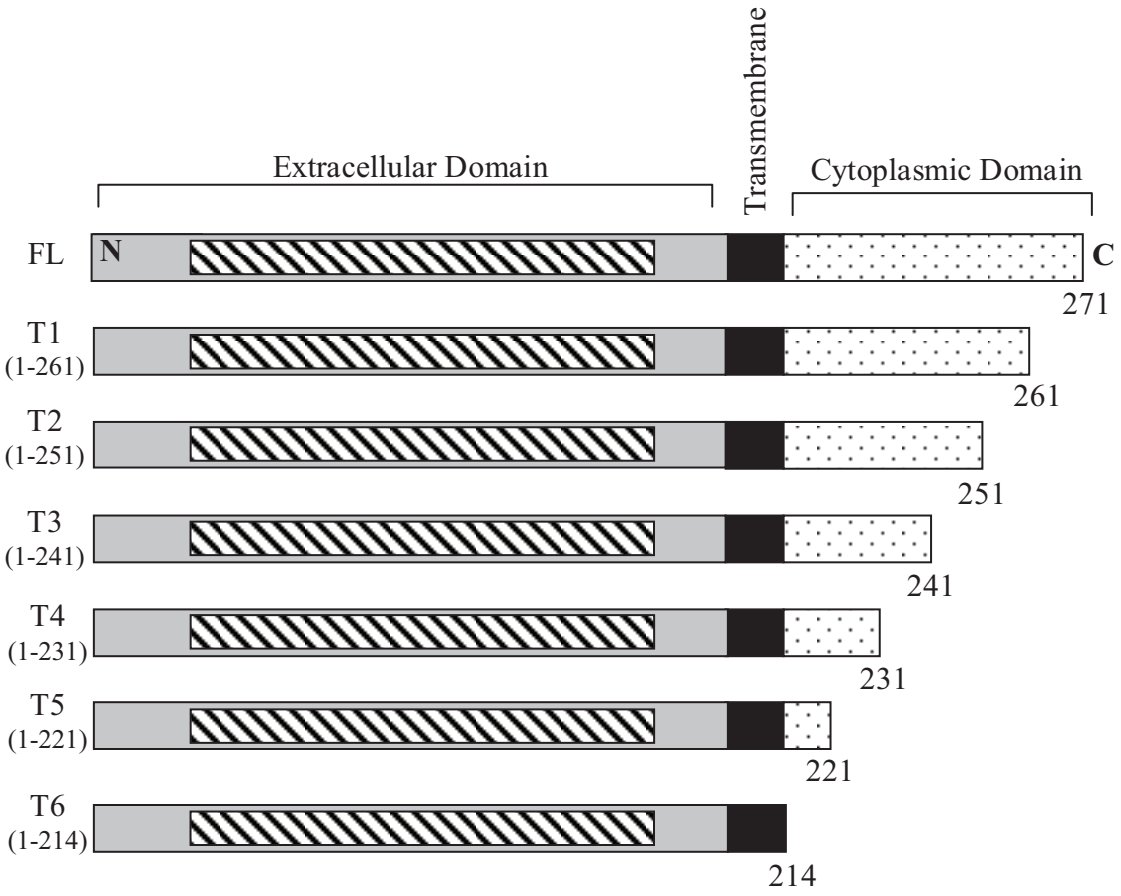


Figure 9: Diagram of SccA-GFP expression plasmid. The pRG3 plasmid was used as a vector. The boxes connected to cross lines indicate respective restriction sites. "Pyr4" indicates a pyrimidine selectable marker. "AmpR" indicates a selectable marker for ampicillin resistance. The gray box indicates the *SccA* gene or mutant. The black box indicates the *$(GA)_5$-GFP* gene.

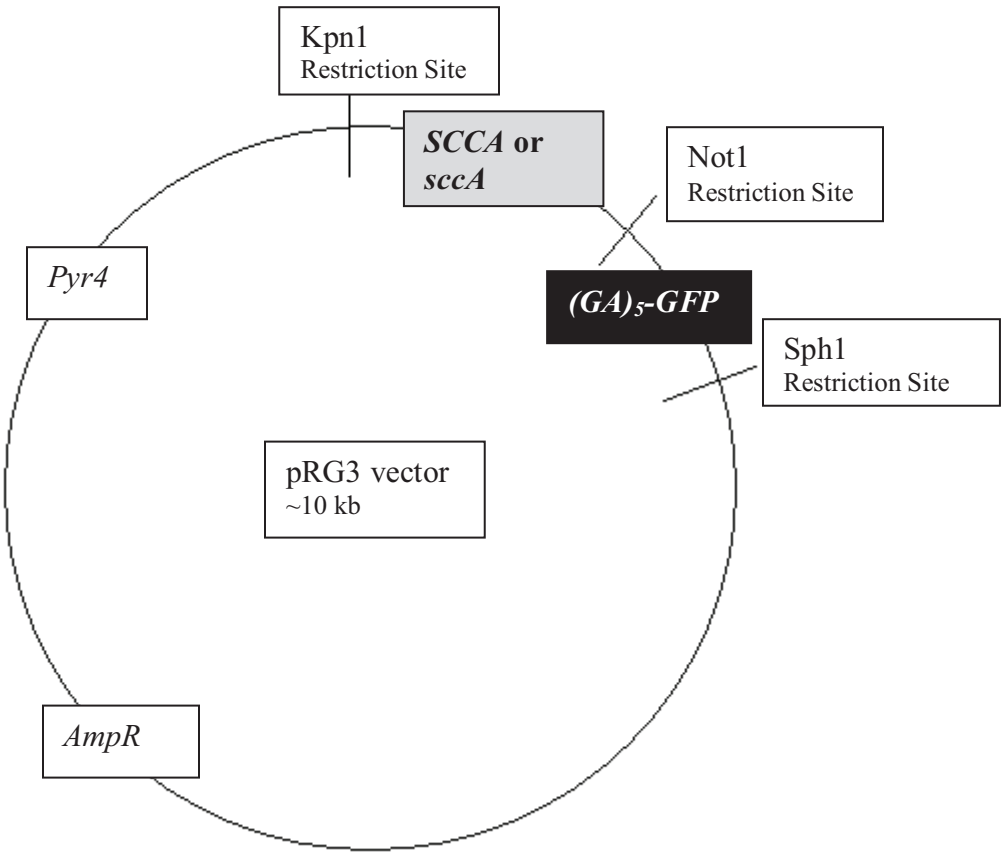
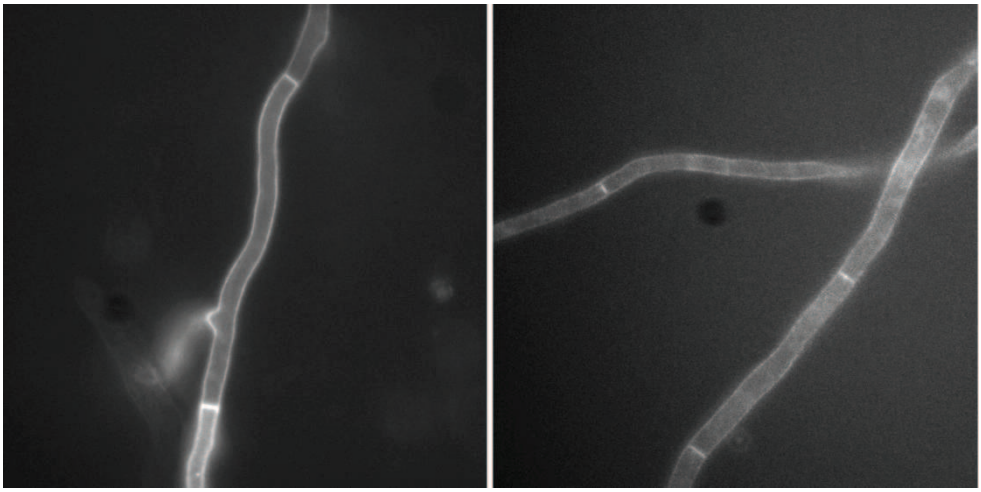


Figure 10: Localization for GFP-tagged full-length SccA and T6 SccA (1-214). Both the full length and the extreme truncation mutant appear to localize to the plasma membrane. Photos were taken with a SPOT RT-SE digital camera and an Olympus BX51 epifluorescence microscope with a 100x objective.



Full Length SccA(1-271)-GFP T6 SccA (1-214)-GFP

Figure 11: The R78 strain (with the *calC2* mutation in PkcA) transformed with full length SccA with and without GFP compared to transformants with full C-terminal truncation [SccA (1-214)] with and without GFP. The transformants with SccA-GFP (1-214) are not able to suppress the *calC2* mutation to grow on CFW. *A)* Two transformants were tested in spore drop tests at 10,000 spores per colony *B)* Many transformants were tested in toothpick tests.

A)

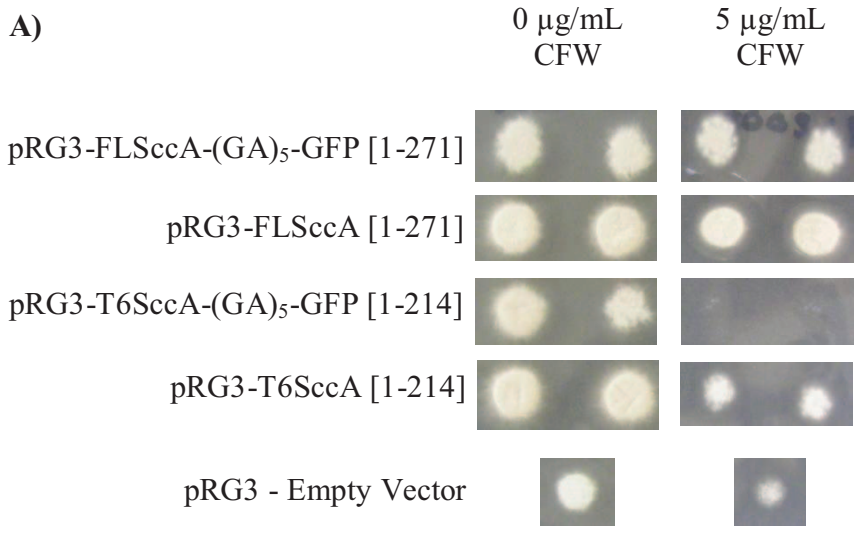


Figure 11 (cont'd)

B)

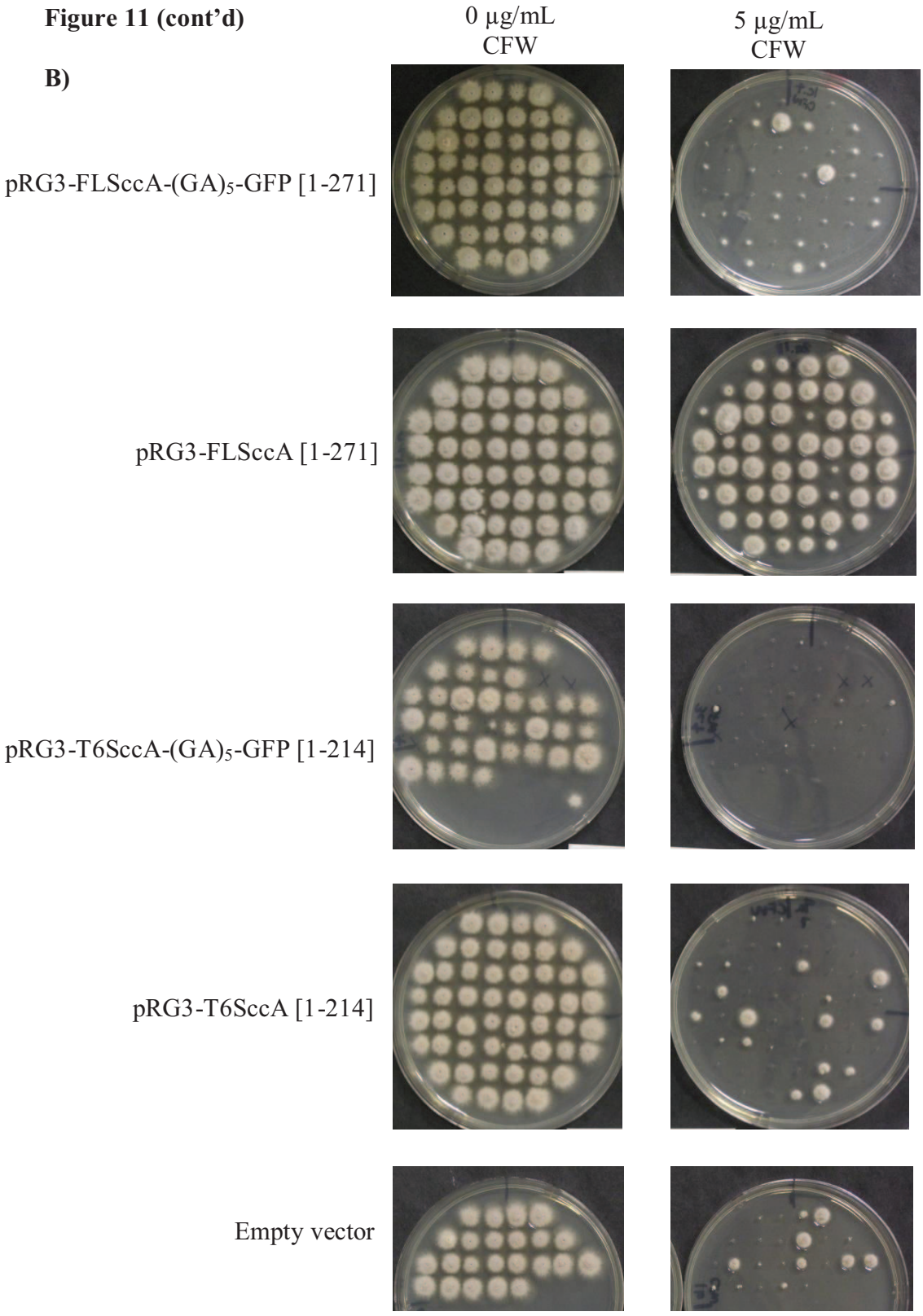
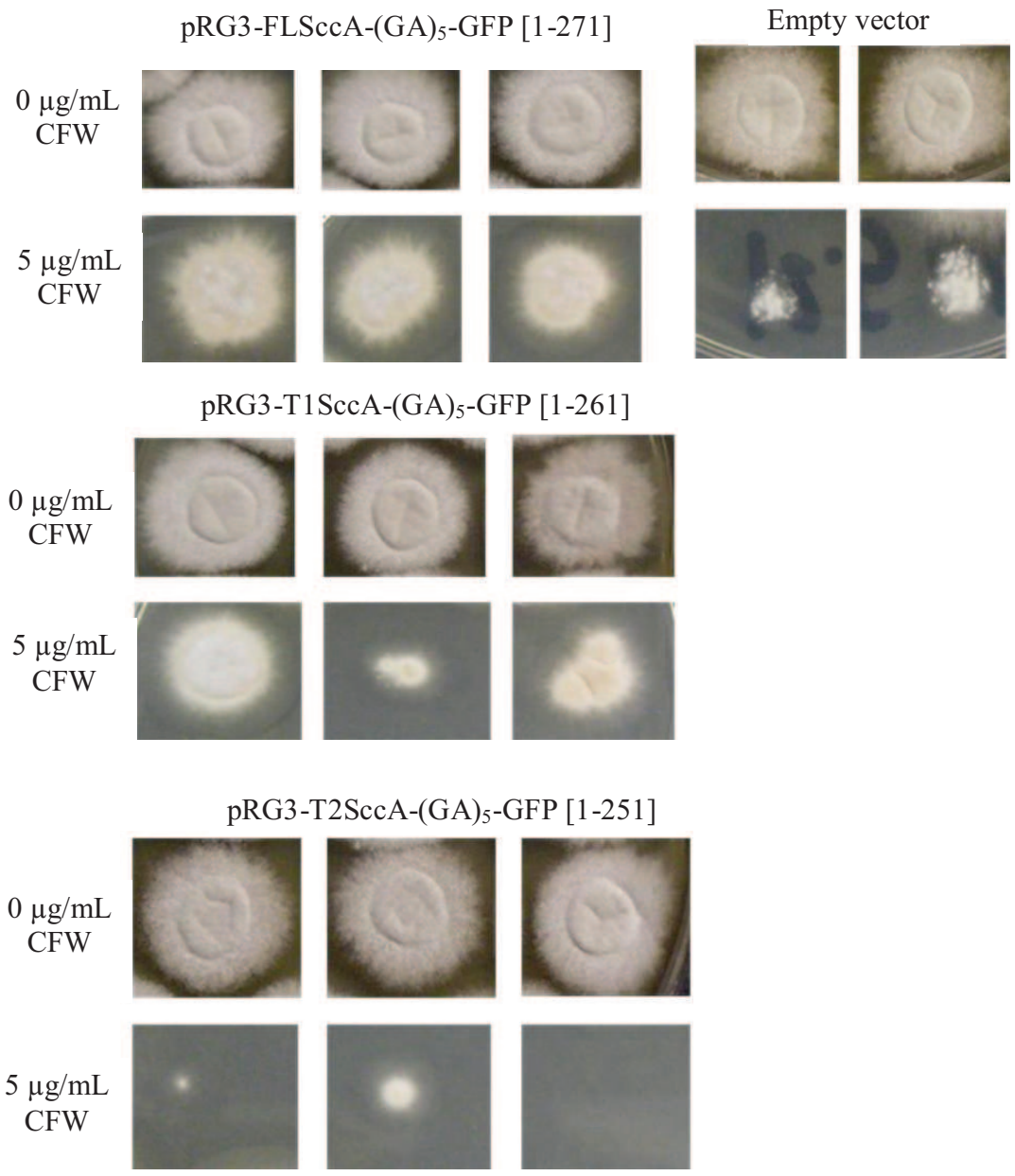
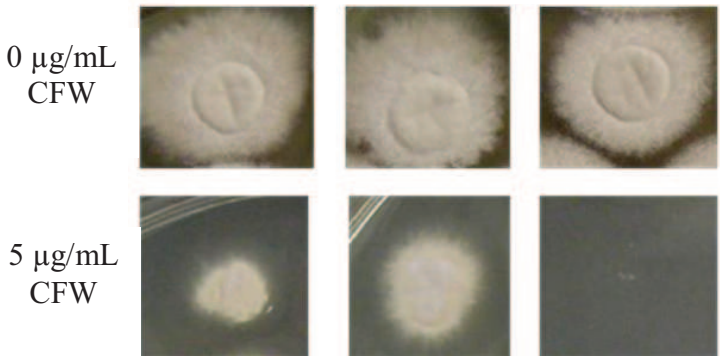


Figure 12: The R78 strain (with the *calC2* mutation in PkcA) was transformed with the full length and mutant constructs, as is indicated by the title above each set of pictures. Each of the three pictures for each truncation mutant is a separate transformant, all of which showed similar GFP expression and localization.

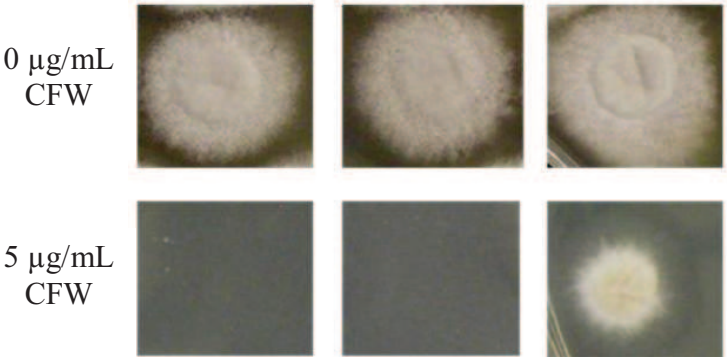


(Figure 12 cont'd)

pRG3-T3SccA-$(GA)_5$-GFP [1-241]



pRG3-T5SccA-$(GA)_5$-GFP [1-221]



pRG3-T6SccA-$(GA)_5$-GFP [1-214]

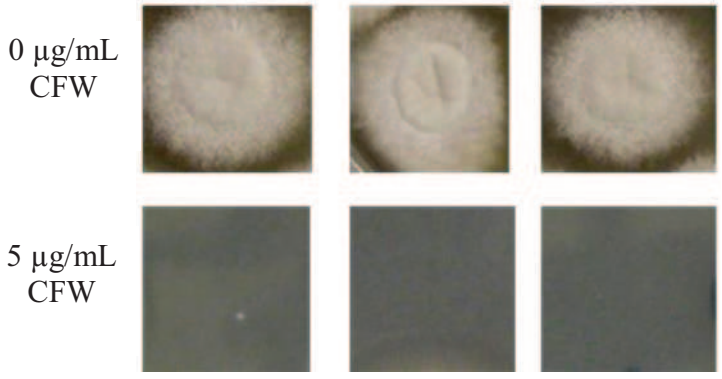


Table 1: Strain Table

Strains	Genotype
R78	*calC2; pyrG89; wA3; pyroA4*
R429	*pyrG89; wA3; pyroA*

Table 2: Primer Sequences

Set	Primer name	Oligonucleotide Sequence (5′-3′)[†]	Rest. site
	GFP primers		
GFP	$(GA)_5$-GFP 5′	ATAGCGGCCGCCGGAGCTGGTGCAGGCG	*Not*I
	$(GA)_5$-GFP 3′	ATAGCATGCTATTTGTATAGTTCATCCATGCCATG	*Sph*I
	Cloning primers		
FL	*SccA* (1-271) 5′	ATAGGTACCTGGGGTGCCTTGATTATTCC	*Kpn*I
	SccA (1-271) 3′	ATAGCGGCCGCAAAGTTTGACGCAGCATTGAC	*Not*I
T1	*SccA* (1-261) 5′	ATAGGTACCTGGGGTGCCTTGATTATTCC	*Kpn*I
	SccA (1-261) 3′	ATAGCGGCCGCGTTGTGGTACTGGTCGAGCG	*Not*I
T2	*SccA* (1-251) 5′	ATAGGTACCTGGGGTGCCTTGATTATTCC	*Kpn*I
	SccA (1-251) 3′	ATAGCGGCCGCGGGAGTGCCCCCAGAA	*Not*I
T3	*SccA* (1-241) 5′	ATAGGTACCTGGGGTGCCTTGATTATTCC	*Kpn*I
	SccA (1-241) 3′	ATAGCGGCCGCCTCACGGACACCAGAGCC	*Not*I
T4	*SccA* (1-231) 5′	ATAGGTACCTGGGGTGCCTTGATTATTCC	*Kpn*I
	SccA (1-231) 3′	ATAGCGGCCGCGCTCATAAGGTCGGTAGCGTC	*Not*I
T5	*SccA* (1-221) 5′	ATAGGTACCTGGGGTGCCTTGATTATTCCCTTT	*Kpn*I
	SccA (1-221) 3′	ATAGCGGCCGCAGCAGCCCGCCTCCG	*Not*I
T6	*SccA* (1-214) 5′	ATAGGTACCTGGGGTGCCTTGATTATTCCCTTT	*Kpn*I
	SccA (1-214) 3′	ATAGCGGCCGCCCTCCAAGCAACCACCCC	*Not*I

[†] The underlined sequence introduces the indicated restriction site. Bold sequence indicates a stop codon.

Table 3: Plasmid Table

Plasmid Name	Characteristics	Source
pFNO3	*(GA)$_5$-GFP; pyrG; Kan^R*	Yang, 2004
pRG3	*pyr4; Amp^R*	Osherov, 2000
R78-XF2	*SccA ; Amp^R*	Hill/Loprete
pRG3-FLSccA-(GA)$_5$-GFP	*SccA*(1-271) *-(GA)$_5$-GFP; pyr4; Amp^R*	This study
pRG3-T1SccA-(GA)$_5$-GFP	*sccA*(1-261) *-(GA)$_5$-GFP; pyr4; Amp^R*	This study
pRG3-T2SccA-(GA)$_5$-GFP	*sccA*(1-251) *-(GA)$_5$-GFP; pyr4; Amp^R*	This study
pRG3-T3SccA-(GA)$_5$-GFP	*sccA*(1-241) *-(GA)$_5$-GFP; pyr4; Amp^R*	This study
pRG3-T4SccA-(GA)$_5$-GFP	*sccA*(1-231) *-(GA)$_5$-GFP; pyr4; Amp^R*	This study
pRG3-T5SccA-(GA)$_5$-GFP	*sccA*(1-221) *-(GA)$_5$-GFP; pyr4; Amp^R*	This study
pRG3-T6SccA-(GA)$_5$-GFP	*sccA*(1-214) *-(GA)$_5$-GFP; pyr4; Amp^R*	This study

Appendix I: Gene Sequences

Gene/Mutant Name	Sequence[†]
(GA)$_5$-GFP	GGAGCTGGTGCAGGCGCTGGAGCCGGTGCCAGTAAAGGAGAAGA ACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGAT GTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGAT GCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGA AAACTACCTGTTCCATGGCCAACACTTGTCACTACTTTCACCTATG GTGTTCAATGCTTTTCAAGATACCCAGATCATATGAAGCGGCACG ACTTCTTCAAGAGCGCCATGCCTGAGGGATACGTGCAGGAGAGG ACCATCTTCTTCAAAGACGACGGGAACTACAAGACACGTGCTGA AGTCAAGTTTGAGGGAGACACCCTCGTCAACAGGATCGAGCTTA AGGGAATCGATTTCAAGGAGGACGGAAACATCCTCGGCCACAAG TTGGAATACAACTACAACTCCCACAACGTATACATCATGGCCGAC AAGCAAAAGAACGGCATCAAAGCCAACTTCAAGACCCGCCACAA CATCGAAGACGGCGGCGTGCAACTCGCTGATCATTATCAACAAA ATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATT ACCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAAGA GAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTA CACATGGCATGGATGAACTATACAAATA
SCCA [AN4897.1]	TGGGGTGCCTTGATTATTCCTTTCATAGGCCCGGCGCCGGAGGAG AGGCATCCATGGCCCCTGGAGTCATGAACCATGGCCCGCTGCTTA AAATAGTAGTACTCCAAATGTGAGGGATAATACTCAGATTCCAGT CTCGACTGGGAAAAACTCACTGGATCGACACGGTTGCAATGAAG GATGCAATCATGCAGAGGATAGTCTCGCACCTCTGACGACTGACG AGAAGGAGTCGCTTTCCTTGTTCATCGCTCTGACAGCTGATAAAC TGTCGTTCTTGAAACACTGCCCCCAGCGTGTCTCACGCGACGAGC CTTGAATGGCTAGAAAGCCCCTTAGTGCCTGCGCTAAGAATCGAC CGCAGACCCATGATCAGGCTTGAAAGAATCCAAAAGCAAAATGG ACTTGGCGCCTTATGCTGTGCGCATTACAGAGTAGTATTCCCTGA CCGAGATTCCGCACTAGTCCACTTGAGCCTTAGGGCTTTTTGCCGT CTCATCCAGGCTGGGCCAGCTGTGTTGTCGGGGGGCGTGACTCCC AAGTCCTTTTATCCTTTCCCTCCCATTCTCATCCCACCTCCTTACA ATCTTTTCCTCAATACCTTCATCGCTGTTATTATCGCCTTGACGGA TTCCATCGAGGTGATAGTCGCTCGTTAAAGACTTCCTTTGCTCCCT TGCCTACCTCGCGCTTAGGCCCCGAATCAGACCCTCGTTAAATCT CTCCGCTTCTTCGCCACCCGCATTTCTCCTAGAGTGATCGTCAGCC TGGGCTTATACGACGCTCGAGTTCTTCGATCGTTGTCATCTCGATC TATCCATCGCAACATCGAATAGCCCATC**ATG**CGGGTCACTGCTCT CCCGTTCTTTCTCTACTCGCTCTCTCTCGTGGCTGTGGTAGCGGGT CAGAGCACACAGGTCACAGATTCCATCTCGGATGTCACCGACGCT CTTCCGACACCCACCCAGACCTCTGCTACAGACACCGATTCTGAC ACCGAAACCACTACCTCTGAATCTTCCAGTACGACGACGACAGAG CCAACAACAACGTCGAGTTCAACAAGCTCGTCCGATGATGAAAC CAGCTCTGGCACTACTACATCATCCTCGTCTACCTCAACGTCGGA TCCCACAACCACAACGACGAGCGACACTGATACCACGACCACTTC ATCAAGCACTGATCCGGATCCCACAACCACTAGCACGAGCTCTTC AGATGATGACAGCTCGACGACACGCACTCCGACCGTGACCACCA TCACAACAACCCAGACTATTGATGGAACGCCAGTTCCTACCACAT ACACTAGCACAAACAGTGAAGCCAGTGAATCTACGGAATCCCCC

	GGCTTGAGCGGGTCTTCTGGCGATTCCGACTCTGGCCTGGACTCC AACCAGAAGAAAATAATCATCGGTGTCGTCGTTGGTGTTGGTGGT GCCATCCTGATTGGTGCTATTGGGGTGGTTGCTTGGAGGATACGT GCCCGGAGGCGGGCTGCTGCGGATGACGACGCTACCGACCTTAT GAGCGGCACTGCCGTCGGCTCTGGTGTCCGTGAGAAAGCCCCAA GCCCTTCTGGGGGCACTCCCTTCAGGTCTACGCTCGACCAGTACC ACAACCCTGGCCCCGTCAATGCTGCGTCAAACTTT
Truncation 1 *sccA* (1-261)	TGGGGTGCCTTGATTATTCCTTTCATAGGCCCGGCGCCGGAGGAG AGGCATCCATGGCCCCTGGAGTCATGAACCATGGCCCGCTGCTTA AAATAGTAGTACTCCAAATGTGAGGGATAATACTCAGATTCCAGT CTCGACTGGGAAAAACTCACTGGATCGACACGGTTGCAATGAAG GATGCAATCATGCAGAGGATAGTCTCGCACCTCTGACGACTGACG AGAAGGAGTCGCTTTCCTTGTTCATCGCTCTGACAGCTGATAAAC TGTCGTTCTTGAAACACTGCCCCCAGCGTGTCTCACGCGACGAGC CTTGAATGGCTAGAAAGCCCCTTAGTGCCTGCGCTAAGAATCGAC CGCAGACCCATGATCAGGCTTGAAAGAATCCAAAAGCAAAATGG ACTTGGCGCCTTATGCTGTGCGCATTACAGAGTAGTATTCCCTGA CCGAGATTCCGCACTAGTCCACTTGAGCCTTAGGGCTTTTTGCCGT CTCATCCAGGCTGGGCCAGCTGTGTTGTCGGGGGGCGTGACTCCC AAGTCCTTTTATCCTTTCCCTCCCATTCTCATCCCACCTCCTTACA ATCTTTTCCTCAATACCTTCATCGCTGTTATTATCGCCTTGACGGA TTCCATCGAGGTGATAGTCGCTCGTTAAAGACTTCCTTTGCTCCCT TGCCTACCTCGCGCTTAGGCCCCGAATCAGACCCTCGTTAAATCT CTCCGCTTCTTCGCCACCCGCATTTCTCCTAGAGTGATCGTCAGCC TGGGCTTATACGACGCTCGAGTTCTTCGATCGTTGTCATCTCGATC TATCCATCGCAACATCGAATAGCCCATC**ATG**CGGGTCACTGCTCT CCCGTTCTTTCTCTACTCGCTCTCTCTCGTGGCTGTGGTAGCGGGT CAGAGCACACAGGTCACAGATTCCATCTCGGATGTCACCGACGCT CTTCCGACACCCACCCAGACCTCTGCTACAGACACCGATTCTGAC ACCGAAACCACTACCTCTGAATCTTCCAGTACGACGACGACAGAG CCAACAACAACGTCGAGTTCAACAAGCTCGTCCGATGATGAAAC CAGCTCTGGCACTACTACATCATCCTCGTCTACCTCAACGTCGGA TCCCACAACCACAACGACGAGCGACACTGATACCACGACCACTTC ATCAAGCACTGATCCGGATCCCACAACCACTAGCACGAGCTCTTC AGATGATGACAGCTCGACGACACGCACTCCGACCGTGACCACCA TCACAACAACCCAGACTATTGATGGAACGCCAGTTCCTACCACAT ACACTAGCACAAACAGTGAAGCCAGTGAATCTACGGAATCCCCC GGCTTGAGCGGGTCTTCTGGCGATTCCGACTCTGGCCTGGACTCC AACCAGAAGAAAATAATCATCGGTGTCGTCGTTGGTGTTGGTGGT GCCATCCTGATTGGTGCTATTGGGGTGGTTGCTTGGAGGATACGT GCCCGGAGGCGGGCTGCTGCGGATGACGACGCTACCGACCTTAT GAGCGGCACTGCCGTCGGCTCTGGTGTCCGTGAGAAAGCCCCAA GCCCTTCTGGGGGCACTCCCTTCAGGTCTACGCTCGACCAGTACC ACAAC
Truncation 2 *sccA* (1-251)	TGGGGTGCCTTGATTATTCCTTTCATAGGCCCGGCGCCGGAGGAG AGGCATCCATGGCCCCTGGAGTCATGAACCATGGCCCGCTGCTTA AAATAGTAGTACTCCAAATGTGAGGGATAATACTCAGATTCCAGT CTCGACTGGGAAAAACTCACTGGATCGACACGGTTGCAATGAAG GATGCAATCATGCAGAGGATAGTCTCGCACCTCTGACGACTGACG AGAAGGAGTCGCTTTCCTTGTTCATCGCTCTGACAGCTGATAAAC TGTCGTTCTTGAAACACTGCCCCCAGCGTGTCTCACGCGACGAGC

	CTTGAATGGCTAGAAAGCCCCTTAGTGCCTGCGCTAAGAATCGAC CGCAGACCCATGATCAGGCTTGAAAGAATCCAAAAGCAAAATGG ACTTGGCGCCTTATGCTGTGCGCATTACAGAGTAGTATTCCCTGA CCGAGATTCCGCACTAGTCCACTTGAGCCTTAGGGCTTTTTGCCGT CTCATCCAGGCTGGGCCAGCTGTGTTGTCGGGGGGCGTGACTCCC AAGTCCTTTTATCCTTTCCCTCCCATTCTCATCCCACCTCCTTACA ATCTTTTCCTCAATACCTTCATCGCTGTTATTATCGCCTTGACGGA TTCCATCGAGGTGATAGTCGCTCGTTAAAGACTTCCTTTGCTCCCT TGCCTACCTCGCGCTTAGGCCCCGAATCAGACCCTCGTTAAATCT CTCCGCTTCTTCGCCACCCGCATTTCTCCTAGAGTGATCGTCAGCC TGGGCTTATACGACGCTCGAGTTCTTCGATCGTTGTCATCTCGATC TATCCATCGCAACATCGAATAGCCCATC**ATG**CGGGTCACTGCTCT CCCGTTCTTTCTCTACTCGCTCTCTCTCGTGGCTGTGGTAGCGGGT CAGAGCACACAGGTCACAGATTCCATCTCGGATGTCACCGACGCT CTTCCGACACCCACCCAGACCTCTGCTACAGACACCGATTCTGAC ACCGAAACCACTACCTCTGAATCTTCCAGTACGACGACGACAGAG CCAACAACAACGTCGAGTTCAACAAGCTCGTCCGATGATGAAAC CAGCTCTGGCACTACTACATCATCCTCGTCTACCTCAACGTCGGA TCCCACAACCACAACGACGAGCGACACTGATACCACGACCACTTC ATCAAGCACTGATCCGGATCCCACAACCACTAGCACGAGCTCTTC AGATGATGACAGCTCGACGACACGCACTCCGACCGTGACCACCA TCACAACAACCCAGACTATTGATGGAACGCCAGTTCCTACCACAT ACACTAGCACAAACAGTGAAGCCAGTGAATCTACGGAATCCCCC GGCTTGAGCGGGTCTTCTGGCGATTCCGACTCTGGCCTGGACTCC AACCAGAAGAAAATAATCATCGGTGTCGTCGTTGGTGTTGGTGGT GCCATCCTGATTGGTGCTATTGGGGTGGTTGCTTGGAGGATACGT GCCCGGAGGCGGGCTGCTGCGGATGACGACGCTACCGACCTTAT GAGCGGCACTGCCGTCGGCTCTGGTGTCCGTGAGAAAGCCCCAA GCCCTTCTGGGGGCACTCCC
Truncation 3 *sccA* (1-241)	TGGGGTGCCTTGATTATTCCTTTCATAGGCCCGGCGCCGGAGGAG AGGCATCCATGGCCCCTGGAGTCATGAACCATGGCCCGCTGCTTA AAATAGTAGTACTCCAAATGTGAGGGATAATACTCAGATTCCAGT CTCGACTGGGAAAAACTCACTGGATCGACACGGTTGCAATGAAG GATGCAATCATGCAGAGGATAGTCTCGCACCTCTGACGACTGACG AGAAGGAGTCGCTTTCCTTGTTCATCGCTCTGACAGCTGATAAAC TGTCGTTCTTGAAACACTGCCCCCAGCGTGTCTCACGCGACGAGC CTTGAATGGCTAGAAAGCCCCTTAGTGCCTGCGCTAAGAATCGAC CGCAGACCCATGATCAGGCTTGAAAGAATCCAAAAGCAAAATGG ACTTGGCGCCTTATGCTGTGCGCATTACAGAGTAGTATTCCCTGA CCGAGATTCCGCACTAGTCCACTTGAGCCTTAGGGCTTTTTGCCGT CTCATCCAGGCTGGGCCAGCTGTGTTGTCGGGGGGCGTGACTCCC AAGTCCTTTTATCCTTTCCCTCCCATTCTCATCCCACCTCCTTACA ATCTTTTCCTCAATACCTTCATCGCTGTTATTATCGCCTTGACGGA TTCCATCGAGGTGATAGTCGCTCGTTAAAGACTTCCTTTGCTCCCT TGCCTACCTCGCGCTTAGGCCCCGAATCAGACCCTCGTTAAATCT CTCCGCTTCTTCGCCACCCGCATTTCTCCTAGAGTGATCGTCAGCC TGGGCTTATACGACGCTCGAGTTCTTCGATCGTTGTCATCTCGATC TATCCATCGCAACATCGAATAGCCCATC**ATG**CGGGTCACTGCTCT CCCGTTCTTTCTCTACTCGCTCTCTCTCGTGGCTGTGGTAGCGGGT CAGAGCACACAGGTCACAGATTCCATCTCGGATGTCACCGACGCT CTTCCGACACCCACCCAGACCTCTGCTACAGACACCGATTCTGAC ACCGAAACCACTACCTCTGAATCTTCCAGTACGACGACGACAGAG CCAACAACAACGTCGAGTTCAACAAGCTCGTCCGATGATGAAAC CAGCTCTGGCACTACTACATCATCCTCGTCTACCTCAACGTCGGA

	TCCCACAACCACAACGACGAGCGACACTGATACCACGACCACTTC ATCAAGCACTGATCCGGATCCCACAACCACTAGCACGAGCTCTTC AGATGATGACAGCTCGACGACACGCACTCCGACCGTGACCACCA TCACAACAACCCAGACTATTGATGGAACGCCAGTTCCTACCACAT ACACTAGCACAAACAGTGAAGCCAGTGAATCTACGGAATCCCCC GGCTTGAGCGGGTCTTCTGGCGATTCCGACTCTGGCCTGGACTCC AACCAGAAGAAAATAATCATCGGTGTCGTCGTTGGTGTTGGTGGT GCCATCCTGATTGGTGCTATTGGGGTGGTTGCTTGGAGGATACGT GCCCGGAGGCGGGCTGCTGCGGATGACGACGCTACCGACCTTAT GAGCGGCACTGCCGTCGGCTCTGGTGTCCGTGAG
Truncation 4 *sccA* (1-231)	TGGGGTGCCTTGATTATTCCTTTCATAGGCCCGGCGCCGGAGGAG AGGCATCCATGGCCCCTGGAGTCATGAACCATGGCCCGCTGCTTA AAATAGTAGTACTCCAAATGTGAGGGATAATACTCAGATTCCAGT CTCGACTGGGAAAAACTCACTGGATCGACACGGTTGCAATGAAG GATGCAATCATGCAGAGGATAGTCTCGCACCTCTGACGACTGACG AGAAGGAGTCGCTTTCCTTGTTCATCGCTCTGACAGCTGATAAAC TGTCGTTCTTGAAACACTGCCCCCAGCGTGTCTCACGCGACGAGC CTTGAATGGCTAGAAAGCCCCTTAGTGCCTGCGCTAAGAATCGAC CGCAGACCCATGATCAGGCTTGAAAGAATCCAAAAGCAAAATGG ACTTGGCGCCTTATGCTGTGCGCATTACAGAGTAGTATTCCCTGA CCGAGATTCCGCACTAGTCCACTTGAGCCTTAGGGCTTTTTGCCGT CTCATCCAGGCTGGGCCAGCTGTGTTGTCGGGGGGCGTGACTCCC AAGTCCTTTTATCCTTTCCCTCCCATTCTCATCCCACCTCCTTACA ATCTTTTCCTCAATACCTTCATCGCTGTTATTATCGCCTTGACGGA TTCCATCGAGGTGATAGTCGCTCGTTAAAGACTTCCTTTGCTCCCT TGCCTACCTCGCGCTTAGGCCCCGAATCAGACCCTCGTTAAATCT CTCCGCTTCTTCGCCACCCGCATTTCTCCTAGAGTGATCGTCAGCC TGGGCTTATACGACGCTCGAGTTCTTCGATCGTTGTCATCTCGATC TATCCATCGCAACATCGAATAGCCCATC**ATG**CGGGTCACTGCTCT CCCGTTCTTTCTCTACTCGCTCTCTCTCGTGGCTGTGGTAGCGGGT CAGAGCACACAGGTCACAGATTCCATCTCGGATGTCACCGACGCT CTTCCGACACCCACCCAGACCTCTGCTACAGACACCGATTCTGAC ACCGAAACCACTACCTCTGAATCTTCCAGTACGACGACGACAGAG CCAACAACAACGTCGAGTTCAACAAGCTCGTCCGATGATGAAAC CAGCTCTGGCACTACTACATCATCCTCGTCTACCTCAACGTCGGA TCCCACAACCACAACGACGAGCGACACTGATACCACGACCACTTC ATCAAGCACTGATCCGGATCCCACAACCACTAGCACGAGCTCTTC AGATGATGACAGCTCGACGACACGCACTCCGACCGTGACCACCA TCACAACAACCCAGACTATTGATGGAACGCCAGTTCCTACCACAT ACACTAGCACAAACAGTGAAGCCAGTGAATCTACGGAATCCCCC GGCTTGAGCGGGTCTTCTGGCGATTCCGACTCTGGCCTGGACTCC AACCAGAAGAAAATAATCATCGGTGTCGTCGTTGGTGTTGGTGGT GCCATCCTGATTGGTGCTATTGGGGTGGTTGCTTGGAGGATACGT GCCCGGAGGCGGGCTGCTGCGGATGACGACGCTACCGACCTTAT GAGC
Truncation 5 *sccA* (1-221)	TGGGGTGCCTTGATTATTCCTTTCATAGGCCCGGCGCCGGAGGAG AGGCATCCATGGCCCCTGGAGTCATGAACCATGGCCCGCTGCTTA AAATAGTAGTACTCCAAATGTGAGGGATAATACTCAGATTCCAGT CTCGACTGGGAAAAACTCACTGGATCGACACGGTTGCAATGAAG GATGCAATCATGCAGAGGATAGTCTCGCACCTCTGACGACTGACG AGAAGGAGTCGCTTTCCTTGTTCATCGCTCTGACAGCTGATAAAC

	TGTCGTTCTTGAAACACTGCCCCCAGCGTGTCTCACGCGACGAGC CTTGAATGGCTAGAAAGCCCCTTAGTGCCTGCGCTAAGAATCGAC CGCAGACCCATGATCAGGCTTGAAAGAATCCAAAAGCAAAATGG ACTTGGCGCCTTATGCTGTGCGCATTACAGAGTAGTATTCCCTGA CCGAGATTCCGCACTAGTCCACTTGAGCCTTAGGGCTTTTTGCCGT CTCATCCAGGCTGGGCCAGCTGTGTTGTCGGGGGGCGTGACTCCC AAGTCCTTTTATCCTTTCCCTCCCATTCTCATCCCACCTCCTTACA ATCTTTTCCTCAATACCTTCATCGCTGTTATTATCGCCTTGACGGA TTCCATCGAGGTGATAGTCGCTCGTTAAAGACTTCCTTTGCTCCCT TGCCTACCTCGCGCTTAGGCCCCGAATCAGACCCTCGTTAAATCT CTCCGCTTCTTCGCCACCCGCATTTCTCCTAGAGTGATCGTCAGCC TGGGCTTATACGACGCTCGAGTTCTTCGATCGTTGTCATCTCGATC TATCCATCGCAACATCGAATAGCCCATC**ATG**CGGGTCACTGCTCT CCCGTTCTTTCTCTACTCGCTCTCTCTCGTGGCTGTGGTAGCGGGT CAGAGCACACAGGTCACAGATTCCATCTCGGATGTCACCGACGCT CTTCCGACACCCACCCAGACCTCTGCTACAGACACCGATTCTGAC ACCGAAACCACTACCTCTGAATCTTCCAGTACGACGACGACAGAG CCAACAACAACGTCGAGTTCAACAAGCTCGTCCGATGATGAAAC CAGCTCTGGCACTACTACATCATCCTCGTCTACCTCAACGTCGGA TCCCACAACCACAACGACGAGCGACACTGATACCACGACCACTTC ATCAAGCACTGATCCGGATCCCACAACCACTAGCACGAGCTCTTC AGATGATGACAGCTCGACGACACGCACTCCGACCGTGACCACCA TCACAACAACCCAGACTATTGATGGAACGCCAGTTCCTACCACAT ACACTAGCACAAACAGTGAAGCCAGTGAATCTACGGAATCCCCC GGCTTGAGCGGGTCTTCTGGCGATTCCGACTCTGGCCTGGACTCC AACCAGAAGAAAATAATCATCGGTGTCGTCGTTGGTGTTGGTGGT GCCATCCTGATTGGTGCTATTGGGGTGGTTGCTTGGAGGATACGT GCCCGGAGGCGGGCTGC
Truncation 6 *sccA* (1-14)	TGGGGTGCCTTGATTATTCCTTTCATAGGCCCGGCGCCGGAGGAG AGGCATCCATGGCCCCTGGAGTCATGAACCATGGCCCGCTGCTTA AAATAGTAGTACTCCAAATGTGAGGGATAATACTCAGATTCCAGT CTCGACTGGGAAAAACTCACTGGATCGACACGGTTGCAATGAAG GATGCAATCATGCAGAGGATAGTCTCGCACCTCTGACGACTGACG AGAAGGAGTCGCTTTCCTTGTTCATCGCTCTGACAGCTGATAAAC TGTCGTTCTTGAAACACTGCCCCCAGCGTGTCTCACGCGACGAGC CTTGAATGGCTAGAAAGCCCCTTAGTGCCTGCGCTAAGAATCGAC CGCAGACCCATGATCAGGCTTGAAAGAATCCAAAAGCAAAATGG ACTTGGCGCCTTATGCTGTGCGCATTACAGAGTAGTATTCCCTGA CCGAGATTCCGCACTAGTCCACTTGAGCCTTAGGGCTTTTTGCCGT CTCATCCAGGCTGGGCCAGCTGTGTTGTCGGGGGGCGTGACTCCC AAGTCCTTTTATCCTTTCCCTCCCATTCTCATCCCACCTCCTTACA ATCTTTTCCTCAATACCTTCATCGCTGTTATTATCGCCTTGACGGA TTCCATCGAGGTGATAGTCGCTCGTTAAAGACTTCCTTTGCTCCCT TGCCTACCTCGCGCTTAGGCCCCGAATCAGACCCTCGTTAAATCT CTCCGCTTCTTCGCCACCCGCATTTCTCCTAGAGTGATCGTCAGCC TGGGCTTATACGACGCTCGAGTTCTTCGATCGTTGTCATCTCGATC TATCCATCGCAACATCGAATAGCCCATC**ATG**CGGGTCACTGCTCT CCCGTTCTTTCTCTACTCGCTCTCTCTCGTGGCTGTGGTAGCGGGT CAGAGCACACAGGTCACAGATTCCATCTCGGATGTCACCGACGCT CTTCCGACACCCACCCAGACCTCTGCTACAGACACCGATTCTGAC ACCGA AACCACTACCTCTGAATCTTCCAGTACGACGACGACAGA GCCAACAACAACGTCGAGTTCAACAAGCTCGTCCGATGATGAAA CCAGCTCTGGCACTACTACATCATCCTCGTCTACCTCAACGTCGG ATCCCACAACCACAACGACGAGCGACACTGATACCACGACCACT

	TCATCAAGCACTGATCCGGATCCCACAACCACTAGCACGAGCTCT TCAGATGATGACAGCTCGACGACACGCACTCCGACCGTGACCACC ATCACAACAACCCAGACTATTGATGGAACGCCAGTTCCTACCACA TACACTAGCACAAACAGTGAAGCCAGTGAATCTACGGAATCCCC CGGCTTGAGCGGGTCTTCTGGCGATTCCGACTCTGGCCTGGACTC CAACCAGAAGAAAATAATCATCGGTGTCGTCGTTGGTGTTGGTGG TGCCATCCTGATTGGTGCTATTGGGGTGGTTGCTTGGAGG

† The underlined sequences indicate primer complements. Bold sequences indicate start codons.

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