Title: Cold shock as a screen for genes involved in cold acclimatization in *Neurospora crassa*.

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Keywords: Neurospora, cold shock, cold adaptation, morphology, branching

ABSTRACT

When subjected to rapid drops of temperature (cold shock), Neurospora responds with a dramatic, but temporary shift in its branching pattern. While the cold shock response has been described morphologically, it has yet to be examined genetically. This project aims to begin the genetic characterization of the cold shock response and the associated acclimatization to cold environments. We report here the results of a screen of mutants from the Neurospora knockout library for alterations in their morphological response to cold shock and thus, their ability to acclimatize to the cold. Three groups of knockouts were selected to be subject to this screen: genes previously suspected to be involved in hyphal development as well as knockouts resulting in morphological changes; transcription factors; and genes homologous to E. coli genes known to alter their expression in response to cold shock. Several strains were identified with altered responses. The genes impacted in these mutants are listed and discussed. A significant percentage (81%) of the knockouts of genes homologous to those previously identified in E. coli showed altered cold shock responses in Neurospora – suggesting that the response in these two organisms is largely shared in common.

The environmental conditions that life must contend with can vary widely. Organisms have evolved a wide range of mechanisms for contending with these changing conditions. For the filamentous fungus Neurospora, growth continues through nearly the entire range of temperatures (above freezing) that is observed in this environment. Although the rate of tip extension varies linearly with temperature (Watters et al 2000), the branch density (the statistical distribution of distances between branch sites along a linear growing hypha) remains constant across this range (Watters *et al*. 2000) allowing the fungus to continue to infiltrate its environment at the same density. Temperatures progressing through this range would be expected to have dramatic impacts on enzyme activity generally (and thus overall metabolism), but also directly on features critical to growth such as membrane fluidity, DNA/RNA stability and the rates of transcription and translation.

Response to cold shock

When the apparent independence of branching and temperature is tested by rapid temperature shifts, a 3-phase response is observed (Figure 1, Watters *et al.* 2000, Watters 2013). The initial response to cold shock is the growth of a single longer than normal unbranched segment. This was termed the "Lag" phase of the response. This phase is followed by a series of closely spaced apical branch points, termed the "Apical" phase. Apical branch formation has been previously associated with the disruption and attempted reorganization of the normal tip-growth apparatus (Reynaga-Peña *et al*. 1995, Riquelme & Bartnicki-Garcia 2004), a mechanism distinct from that thought to be involved in lateral branching. Finally, with continued incubation at the lower temperature, the colony returns to lateral branching, termed the "Recovery" phase. Growth in this phase of the response resembles that which would be seen had the colony been grown at 4°C continuously. The same density is observed for growth subjected to constant incubation at 4°C (or any other fixed temperature) as well (Watters et al 2000). Thus, the cold shock response appears to be a temporary disturbance to a homeostatic system which maintains branch density at a constant, evolutionarily favored, value.

Thus, Neurospora appears to incorporate a system which has the effect of maintaining homeostasis for critical cellular characteristics. At least one impact of this system is that branch density is maintained at widely different temperatures. The morphological effects of cold shock are the indirect consequence of this system's staged process of adjusting cellular conditions in order to compensate for the new growth temperature.

Cold shock response of bacteria

Homeostasis in the face of temperature changes and more specifically the response to cold shock has been extensively studied in bacterial systems for over 20 years. The effect of cold shock is manifest in multiple cellular systems including: membrane rigidity (Shivaji & Prakash 2010), stability of secondary structures in DNA/RNA (Phadtare 2004), efficiency of protein folding (Phadtare 2004) and ribosome function (Gualerzi *et al*. 2011). While much remains to be described in these systems, cold shock appears to result in a multi-stage response (Phadtare 2004). First, a lag period in which growth and translation of proteins generally cease. This is followed by an adjustment phase in which specific cold-shock proteins which compensate for the changes brought on by the cold are preferentially translated (Giuliodori *et al*. 2004). In the final stage, growth continues otherwise normally, but at a reduced rate. DNA microarray transcription profiling of the cold shock response in E. coli by Phadtare and Inouye (2004) has shown that

several hundred genes respond to cold shock, either being transiently induced/repressed or showing prolonged induction/repression. Analogous responses to cold shock and/or cold acclimation have been observed in diverse organisms including plants (Guy 1999) and animals (Canclini & Esteves 2007). Attempts to uncover cold shock proteins in fungi (Fang & Leger 2010) have met with mixed success.

Connections between cold shock in bacteria and Neurospora

It is tempting to draw parallels between what is known about cold shock in bacterial systems and the observed response of Neurospora to similar cold shocks. Many of the systems affected during bacterial cold shock would be expected to impact fungal tip growth and branching (most obviously, membrane fluidity). Beyond that however, the nature and timing of the two responses are similar. Both responses can be adjusted by changing the intensity of the cold shock with more mild shocks (lower temperature differences) producing more mild responses and more severe shocks (larger temperature differences) producing more severe responses. Furthermore, the dynamics of the responses parallel each other. In each, there is a multistage response. There is an initial response which is transient in nature, followed by a more long-term response which largely represents a return to normal growth.

Guiding Hypotheses

The hypothesis of this project was that the observed cold shock response of Neurospora is a consequence of a cellular response homologous to that induced by cold shock in bacteria. Under this hypothesis, the observed, transient morphological changes are an aspect of the primary response which is that of the fungal cell adjusting itself to growth in the cold via a manner which is shared in common with simpler organisms. This hypothesis was tested by screening Neurospora knockout strains impacting genes homologous to those identified in E. coli which alter their expression patterns in response to cold shock. Of 68 knockouts screened, 55 (81%) showed changes in the morphological response to cold shock, suggesting a strong connection between the responses of E. coli and Neurospora to cold shock.

Additionally, Neurospora strains with knockouts of known transcription factors, genes impacting morphology, or genes homologous to those in yeast known to affect polar growth were similarly screened. In this screen, of 357 knockouts examined, 69 (19%) showed changes in the response to cold shock. Together, these screens provide the first molecular underpinning to the cold shock response in Neurospora,

MATERIALS AND METHODS

The Neurospora targeted deletion collection

As part of the Neurospora Genome Project, a collection of strains containing disruptions in presumptive genes has been constructed (Colot *et al*. 2006). Strains representing deletions of most of the genes of the Neurospora genome are available from the Fungal Genetics Stock Center (McCluskey 2003). As each deletion strain has been altered in a single, previously identified, presumptive gene – going from phenotype to sequence is greatly simplified.

The accession numbers listed in Table 1 represent the locus number of the gene subject to inactivation in the knockout strain under test. Every annotated gene in *Neurospora crassa* has been assigned a locus number of the form NCU####. The functions reported (Tables 1 – 3) are

those associated with the genes as annotated on the FungiDB database as of July 2017: fungidb.org/fungidb/. The reported functions are based solely on the annotations currently associated with those strains and have not been independently confirmed by the authors of this study. The genes identified in this study have been annotated on FungiDB to reflect these results.

Selection of knockout strains to screen

A screen of the entire library was determined to be impractical. We instead screened an abbreviated subsection of the library chosen to be more likely to yield positive responses.

First, knockouts of genes homologous to those which show altered transcription in E. coli subjected to cold shock. The protein sequences of E. coli genes identified by (Phadtare and Inouye 2004) were retrieved from the E. coli database (ecocyc.org/). These amino acid sequences were then fed into a BLAST search on the NIH NCBI site (blast.ncbi.nlm.nih.gov/Blast.cgi) with the output limited to Neurospora sequences in order to identify their nearest Neurospora homologs. These homologs were then searched on FungiDB to determine which had knockout strains available. From this final list, 68 were selected randomly for screening in this study. This set was selected to determine the degree of relationship between the cold shock response in E. coli and Neurospora.

Second, two sets of knockouts from the library (hyphal growth and morphological) were included in this screen. One plate (identified as "plate 29 – morphologicals" by the FGSC) contained 71 strains with knockouts known to cause morphological changes. The second plate (identified as "Hyphal Growth Set" by the FGSC) contained 78 strains with knockouts in genes homologous to genes in yeast known to affect polar growth.

Lastly, knockouts of known transcription factors in Neurospora were selected for cold shock screening. This collection is available as a set from the Fungal Genetics Stock Center (McCluskey 2003). It was selected for this screen to determine which transcription factors play a role in signaling to the cell that cold adaptation genes must be activated.

Media

Media and culturing procedures were those described in Davis & deSerres (1970). Growth described as being on "minimal" was on Vogel's minimal medium (Davis & deSerres 1970).

Screen

The selected knockout strains were subjected to a screen looking for altered responses to cold shock. Wild-type Neurospora progresses through a three-stage response following a shift into the cold. As detailed above, these are the Lag, Apical and Recovery phases. As the cold shock response is known to be stronger following more dramatic temperature shifts (Watters et al. 2000) we initially grew strains at 33°C and shifted to 4°C. Strains were inoculated onto Vogel's Minimal Medium and incubated overnight at 33°C. The next morning plates were moved to 4°C. After an overnight incubation at 4°C, the strain's response to cold shock was photographed and evaluated. Variations in the cold shock response from that of wild-type Neurospora were judged qualitatively and based primarily on the morphology of the "apical" phase of branching.

Photomicroscopy

Growing cultures were examined and photographed using a Motic 10MP digital camera attached to a Wolfe Beta Elite trinocular microscope. Photographs were taken of well separated, leading hyphae.

RESULTS AND DISCUSSION

During the initial study of the cold shock response in Neurospora (Watters et al 2000) it was observed that several classical morphological mutants (most notably "granular" and "delicate" produced altered responses to cold shock, demonstrating that mutants could be obtained which influenced this process. We chose to screen mutants from the Neurospora knockout library for their cold shock response in order to provide a genetic grounding to this process which has, thus far, been lacking. We chose to use the mutants of the knockout library instead of the products of a random mutagenesis as the knockouts allow an immediate identification of gene function in most cases.

Knockout strains displaying an altered morphological response to cold shock were classified according to the specific variation they displayed. Examples are shown in Figure 2. The "burst" phenotype was defined as displaying a large number of growing tips which stop growing, swell and then structurally fail leaving a pool of cytoplasm at the tip. The "fail" phenotype was defined as failing to display the apical branch phase characteristic of cold shock. In the "fail" response, growth proceeds normally with lateral branching following cold shock. The "thin" phenotype was defined by a very rapid decrease in hyphal diameter following cold shock. It was common to observe "thin" in combination with other altered cold shock responses. The "dense" phenotype was defined by displaying apical branching with visibly shorter distances between branch points following cold shock relative to the response in wild-type. The "weak" phenotype was defined as the opposite – an apical branch phase with visibly longer distances between branch points relative to wild-type following cold shock. Finally, the "cot-like" phenotype was characterized by a lack of apical branching, but a shift to tightly spaced lateral branches which morphologically resembled the growth of the traditional *cot* mutants at the restrictive temperature.

We can imagine two distinct groups of genes to be identified by these screens. The first would be genes directly involved in cold adaptation which are responsible for altering the cell to accommodate the altered environment. This first group of functions, we could very well expect to be generalized to a wide variety of organisms. The second would be genes coding for proteins which are individually temperature sensitive and compensate for temperature changes by either altering regulation to compensate for the change in activity or shifting activity to a paralog which functions better at the new temperature. This second group we might expect to be more species specific.

Screen of E. coli cold shock gene homolog knockouts

A total of 68 Neurospora strains with knockouts of genes homologous to E. coli genes which alter transcription in response to cold shock (Phadtare and Inouye 2004) were screened. A total of 55 (81%) showed altered morphology to cold shock (Table 1). The knockouts displaying altered response to cold shock represent a variety of cellular functions. There does not to be any

correspondence between the response of a gene in Ecoli (up regulated, down regulated, transient or sustained), or its function (membrane metabolism, ROS control etc) (Phadtare and Inouye 2004) and the morphology displayed during cold shock.

The screen of cold shock orthologs provides a test of the hypothesis that the cold shock response in both E. coli and Neurospora share a great deal of their cold shock response in common. The very high percentage of overlap between genes playing a role in these two widely separated organisms argues that the two responses are functionally related to a large degree. The large fraction of overlap also argues that the majority of the genes identified in these two organisms are involved generally in cold adaptation. The results also suggest that screening for deviations from the normal cold shock response morphology is an effective tool for detecting genes important in cold adaptation.

No correlation was observed between the transcription change observed in E. coli (Phadtare and Inouye 2004) and the observed cold shock phenotype observed in the knockout strain of its Neurospora ortholog. Similarly, no correlation was observed between the cold shock phenotype observed and the annotated function of the genes affected in these strains.

Screen of Morphological/Hyphal plates

A total of 149 selected mutant strains from the Neurospora knockout library were previously segregated into two collections. The "Morphological" collection resulted in known morphological variations in the knockout strains. The "Hyphal" collection consisted of knockouts of genes previously suspected to play a role in hyphal growth. These two collections were screened for alterations to their response to cold shock. In total, 35 (23%) strains were identified (Table 2) that displayed variant cold shock responses. The altered responses fell into several phenotypic categories. The genes impacted in each knockout strain are identified in Table 2.

As with the E. coli orthologs, the genes identified from this collection which displayed altered response to cold shock appear to be involved in a number of different cellular functions. In this case they include lipid/membrane metabolism, protein degradation/turnover, gene regulation, protein regulation, and reactive oxygen control.

The morphological/hyphal knockouts were previously screened for temperature-dependent branch density (Watters et al. 2011). Comparing the strains identified above with alterations to their cold shock response to those previously determined to show temperature-dependent branching we find only a modest overlap with the following strains showing altered phenotypes in both: NCU02333, NCU00830, NCU04242, NCU02114, NCU04264, and NCU03076. Examining the overlap statistically via Chi-square (calculations not shown) yields a p value greater than 0.9, strongly suggesting that the overlap is random. This suggests that these two screens (cold shock vs temperature sensitive branching during steady-state growth) are independent. This leads us to conclude that the cold shock response and temperature-dependent branching are independent aspects of cold adaptation, highlighting the different genes involved in short-term adaptation to the cold as opposed to those required for sustained growth in cold environments. Additional screens of the knockout library for strains displaying growth rate

dependent branching, and comparing them to those with an altered cold shock response will allow us to further examine the apparent independence of these two morphological screens.

Screen of transcription factor knockouts

A total of 208 Neurospora strains with knockouts in genes which function as transcription factors were screened for their response to cold shock. In all, 34 (16%) showed altered morphology to cold shock (Table 3).

The transcription factor screen identifies a number of genes which may function in a broad way to regulate multiple genes to the purpose of cold adaptation.

As with the knockouts of orthologs of E. coli cold shock responding genes, the mutant strains identified in the additional screens show no observed correlations between the phenotypes observed and the annotated functions of the genes with a variety of functions being associated with the observed cold shock variations.

In conclusion, the gene functions highlighted by these screens (Table 1-3) are diverse. It is unclear how the diverse gene network, partially exposed here, coordinates for the function of temperature acclimatization. The results presented here demonstrate a strong relationship between the cold shock responses of E. coli and *Neurospora crassa*. The phenotype under examination here (morphological response to cold shock) appears to be influenced by a diverse network of genes. Similar diversity of function has been observed in other examinations of morphogenesis in Neurospora (Seiler & Plamann 2003). Further work on cold acclimatization, including a broad survey of the full Neurospora knockout library should help clarify these connections.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Indiana Space Grant Consortium (INSGC). We would also like to thank Kevin McCluskey and everyone at the Fungal Genetics Stock Center for supplying the knockout strains and their diligent work in support of the fungal genetics community over the years.

Table 1: Results of screening 68 knockouts selected as orthologs of genes known to alter expression in response to cold shock in E coli:

NCU#	Phenotype	Gene ID	Gene symbol
NCU01213	burst	superoxide dismutase-2	sod-2
NCU01782	burst	Ras guanyl-nucleotide exchange factor RasGEF	
NCU05410	dense	arginine-5	arg-5
NCU04899	dense	tricarboxylic acid-15	tca-15
NCU09120	fail	lysine-specific histone demethylase Aof2	
NCU00528	fail	hyphal anastomosis-4	ham-4
NCU03415	fail	aldehyde dehydrogenase	CBS-3
NCU09975	fail	multidrug resistance protein 3	
NCU05927	fail	GTP-binding protein GUF1	GTP-7
NCU07832	fail	pre-mRNA processing splicing factor 8	msp-39
NCU00056	fail	condensing enzyme with mitochondrial function	cem-1
NCU10008	fail	tricarboxylic acid-14	tca-14
NCU01004	fail	phosphatidylserine decarboxylase proenzyme	CHOL-15
NCU11289	fail	aldo-keto reductase	
NCU05770	thin	catalase-2	cat-2
NCU04140	thin	FK506 resistant-2	fkr-2
NCU02556	thin	histone acetyl transferase-2	hat-2
NCU02630	thin	heat shock protein 78	hsp78
NCU02055	thin	uridine nucleosidase Urh1	NUS-1
NCU05151	thin	phosphoketolase	PHK-1
NCU06005	thin	glycerol kinase	GLK-1
NCU05606	thin	glucosidase 2 subunit beta	GHX-4
NCU09767	thin	membrane transporter	
NCU04791	thin	menadione-induced gene-10	mig-10
NCU06342	thin	phospholipase D	PLA-5
NCU07156	thin	histidine-6	his-6
NCU09930	thin	folic acid synthesis protein	fol-9
NCU08968	thin	dimethyladenosine transferase	

NCU#	Phenotype	Gene ID	Gene symbol
NCU01744	thin	enhancer-2 of am	en(am)-2
NCU01772	thin	DNA-directed RNA polymerase III polypeptide	rpo-10
NCU01528	thin	glyceraldehyde-3-phosphate dehydrogenase-1	gpd-1
NCU08216	thin	cystathionine beta-synthase	MET-11
NCU06659	thin&fail	GTP-binding protein	GTP-3
NCU10760	thin&fail	jumonji domain-containing protein 5	
NCU07732	thin&fail	arginine-2	arg-2
NCU08693	thin&fail	heat shock protein 70-5	hsp70-5
NCU08858	thin&fail	MFS alpha-glucoside transporter	SUT-1
NCU00793	thin&fail	trehalose phosphate synthase	GT20-2
NCU00771	thin&fail	UBX domain-containing protein 7	
NCU04117	thin&fail	ATP-dependent permease MDL2	ABC-7
NCU08336	thin&fail	tricarboxylic acid-12	tca-12
NCU04583	weak	acetyltransferase	
NCU06017	weak	thiosulfate sulfurtransferase	TST-1
NCU01589	weak	heat shock protein 60	hsp60
NCU08439	weak	leptomycin B resistance protein pmd1	ABC-2
NCU01625	weak	DNA repair helicase RAD3	DNR-10
NCU04303	weak	asparagine synthetase 2	asn-1
NCU04339	weak	ribokinase	RIK-8
NCU00919	weak	ATP-dependent RNA helicase rok-1	drh-16
NCU00565	weak	lipoic acid synthetase	LIA-1
NCU07027	weak	glycogen phosphorylase	GYP-1
NCU10053	weak	thymidylate synthase	pyr-8
NCU00567	weak	arginine-6	arg-6
NCU06523	weak	glycosylhydrolase family 13-4	gh13-4
NCU08933	weak	cellular nucleic acid-binding protein	

Table 2: Results of screening 148 knockouts on the "morphological" and "hyphal growth" plates:

NCU #	phenotype	Gene ID	symbol
NCU02133.2	burst	superoxide dismutase-1	sod-1
NCU03623.2	burst	ubiquitin-conjugating enzyme E	
NCU03938.2	burst	alternative oxidase-5	aod-5
NCU04242.2	burst/dense	period-6	prd-6
NCU07728.2	burst/thin	siderophore regulation	sre
NCU02333.2	thin	arginase-1	aga-1
NCU02542.2	thin	embden-meyerhof pathway-1	emp-1
NCU03013.2	thin	anchored cell wall protein-10	acw-10
NCU04264.2	thin	extracellular developmental signal biosynthesis protein FluG	
NCU05591.2	thin	ABC transporter CDR4	
NCU07075.2	thin	calcium exchanger	cax
NCU00830.2	thin	ctr copper transporter	tcu-1
NCU01613.2	thin	protoperithecia-2	pp-2
NCU03076.2	thin	delta-1-pyrroline-5-carboxylate dehydrogenase	
NCU04669.2	thin	hypothetical protein homologous to Bactericidal permeability-increasing protein	
NCU03184.2	thin	C2H2 conidiation transcription factor FlbC	
NCU02260.2	thin	regulatory particle, ATPase-like-3	rpt-3
NCU05295.2	thin	proteasome catalytic alpha-5	pca-5
NCU09366.2	thin	proteasome catalytic beta-6	pcb-6
NCU02636.2	failure	peroxin 4	pex4
NCU09830.2	failure	menadione-induced gene-12	mig-12
NCU00467.2	failure	COP9 signalosome-5	csn-5
NCU03314.2	failure	mob2-like-a	mob-2a
NCU03901.2	cot-like	peroxin 14	pex14
NCU09842.2	failure	mitogen activated protein kinase-1	mak-1
NCU03277.2	failure	peroxin 10	pex10
NCU06205.2	failure	regulator of conidiation-1	rco-1
NCU06145.2	failure	RING-6	RING-6
NCU07947.2	failure	glycolipid transfer protein HET-C2	
NCU01408.2	weak	COP9 signalosome-3	csn-3
NCU03894.2	weak	serine/threonine protein kinase-4	stk-4
NCU07617.2	dense	aconidiate-3	acon-3
NCU02114.2	dense	G1/S-specific cyclin Cln1	

Table 3: Results of screening 208 knockouts of Neurospora transcription factors:

NCU#	Phenotype	Gene ID	Gene symbol
NCU05051	thin	COL-23	col-23
NCU01629	thin	hypothetical protein	
NCU04561	thin	melanization defective-1	mld-1
NCU05909	weak	hypothetical protein	
NCU00499	weak	all development altered-1	ada-1
NCU07945	weak	fungal specific transcription factor	tah-4
NCU08658	weak	zinc finger transcription factor-50	znf-50
NCU02017	fail	CBF/NF-Y family transcription factor	ada-2
NCU00097	fail	BEAK-1	bek-1
NCU07561	fail	hypothetical protein	
NCU00499	fail	all development altered-1	ada-1
NCU02173	fail	zinc finger transcription factor-52	znf-52
NCU02356	fail	white collar 1	wc-1
NCU08294	fail	nitrogen assimilation transcription factor nit-4	nit-4
NCU08294	fail	nitrogen assimilation transcription factor nit-4	nit-4
NCU06990	fail	hypothetical protein	
NCU07788	fail	fungal specific transcription factor	col-26
NCU06028	fail	quinic acid utilization activator	qa-1F
NCU07945	fail	fungal specific transcription factor	tah-4
NCU06068	fail	fungal specific transcription factor	col-25
NCU02214	fail	TAH-2	tah-2
NCU03070	burst	hypothetical protein	
NCU03962	dense	hypothetical protein	
NCU03356	dense	hypothetical protein	
NCU03905	dense	hypothetical protein	
NCU01154	dense	submerged protoperithecia-1	sub-1
NCU00144	dense	hypothetical protein	
NCU03417	dense	hypothetical protein	
NCU06990	dense	hypothetical protein	
NCU03120	dense	hypothetical protein	
NCU08000	thin & fail	cutinase transcription factor 1 alpha	far1
NCU08651	thin & fail	zinc binuclear cluster-type protein	col-27
NCU05536	thin & fail	hypothetical protein	
NCU07705	thin & fail	C6 finger domain-containing protein	clr-1

Figure 1: Cold shock response in wild-type Neurospora:

Figure 2: Alternate cold shock morphologies displayed:
Bursting tips, failure to cold shock, shift to thin hypha, extra dense apical branching, cot-like, weaker than normal apical branching

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ABSTRACT

When subjected to rapid drops of temperature (cold shock), Neurospora responds with a temporary shift in its morphology. This report is the first to examine this response genetically. We report here the results of a screen of selected mutants from the Neurospora knockout library for alterations in their morphological response to cold shock and thus, their ability to acclimatize to the cold. Three groups of knockouts were selected to be subject to this screen: genes previously suspected to be involved in hyphal development as well as knockouts resulting in morphological changes; transcription factors; and genes homologous to *E. coli* genes known to alter their expression in response to cold shock. A total of 115 strains were identified with altered responses. We report here the cold shock morphologies and GO categorizations of strains subjected to this screen. Of strains with knockouts in genes associated with hyphal growth or morphology, 30 of 129 tested (23%) showed an altered response to cold shock. Of strains with knockouts in transcription factor genes, 30 of 147 (20%) showed an altered response to cold shock. Of strains with knockouts in genes homologous to *E. coli* genes which display altered levels of transcription in response to cold shock, a total of 55 of 68 tested (81%) showed an altered cold shock response. This suggests that the response to cold shock in these two organisms is largely shared in common.

Introduction

The environmental conditions that life must contend with can vary widely. Organisms have evolved a wide range of mechanisms for contending with these changing conditions. For the filamentous fungus Neurospora, growth continues through nearly the entire range of temperatures (above freezing) that is observed in this environment. Although the rate of tip extension varies linearly with temperature (Watters et al 2000), the branch density (the statistical distribution of distances between branch sites along a linear growing hypha) remains constant across this range (Watters *et al*. 2000) allowing the fungus to continue to infiltrate its environment at the same density. Temperatures progressing through this range would be expected to have dramatic impacts on enzyme activity generally (and thus overall metabolism), but also directly on features critical to growth such as membrane fluidity, DNA/RNA stability and the rates of transcription and translation.

In both Neurospora and *E. coli*, there is a multistage response to cold shock. There is an initial response which is transient in nature, followed by a more long-term response which largely represents a return to normal growth. Neurospora grows via extension at a hyphal tip with periodic branching which is typically lateral (Figure 1A). However, when Neurospora is subjected to cold shock, a multi-phase morphological response is observed (Figure 1B, Watters *et al.* 2000, Watters 2013). The morphological effects of cold shock are the indirect consequence of this system's staged process of adjusting cellular conditions in order to compensate for the new growth temperature.

Homeostasis in the face of temperature changes and more specifically the response to cold shock has been extensively studied in bacterial systems for over 20 years. DNA microarray transcription profiling of the cold shock response in *E. coli* by Phadtare and Inouye (2004) identified a collection of genes which alter their level of transcription in response to cold shock.

The hypothesis of this project was that the observed cold shock response of Neurospora is a consequence of a cellular response homologous to that induced by cold shock in bacteria. Under this hypothesis, the observed, transient morphological changes are a consequence of the fungal cell adjusting itself to growth in the cold via a manner which is shared in common with simpler organisms. This hypothesis was tested by screening Neurospora knockout strains impacting genes homologous to those identified in *E. coli* which alter their expression patterns in response to cold shock. In addition, a broader collection of selected knockout strains were screened to identify additional genes which play a role in the cold shock response and thus cold acclimatization. Together, the results of this screen provides the first molecular underpinning to the cold shock response in Neurospora,

MATERIALS AND METHODS

The Neurospora targeted deletion collection

As part of the Neurospora Genome Project, a collection of strains containing disruptions in presumptive genes was constructed (Colot *et al*. 2006). Strains representing deletions of most of the genes of the Neurospora genome are available from the Fungal Genetics Stock Center (McCluskey 2003). As each deletion strain has been altered in a single, previously identified, presumptive gene – going from phenotype to sequence is greatly simplified.

The accession numbers listed in Tables 1&2 represent the locus number of the gene subject to inactivation in the knockout strain under test. Every annotated gene in *Neurospora crassa* has been assigned a locus number of the form NCU#####. The gene identities reported in the tables are those associated with the genes as annotated on the FungiDB database as of July 2017: fungidb.org/fungidb/. The gene identities reported are based solely on the annotations currently associated with those strains and have not been independently confirmed by the authors of this study. Gene Ontologies reported are those determined by pantherdb.org (Mi *et al*. 2016) as of December 2017.

Knockout sets selected to be subjected to screen.

A screen of the entire library was determined to be impractical. We instead screened an abbreviated subsection of the library chosen to be more likely to yield positive responses. These fall into three basic sets.

The first set are knockouts of genes homologous to those which show altered transcription in *E. coli* when subjected to cold shock (Phadtare and Inouye 2004). The protein sequences of *E. coli* genes identified by were retrieved from the *E. coli* database (ecocyc.org/). These amino acid sequences were then fed into a BLAST search on the NIH NCBI site (blast.ncbi.nlm.nih.gov/Blast.cgi) with the output limited to Neurospora sequences in order to identify their nearest Neurospora homologs. These homologs were then searched on FungiDB to determine which had knockout strains available. From this final list, 68 were selected for screening in this study. This set was selected to determine the degree of relationship between the cold shock response in *E. coli* and Neurospora.

Second, two previously organized sets of knockouts generally associated with hyphal growth and morphology and available from the FGSC were included in this screen. One set (identified as “plate 29 – morphologicals” by the FGSC) contained strains with knockouts known to cause morphological changes. The second set (identified as “Hyphal Growth Set” by the FGSC) contained strains with knockouts in genes homologous to genes in yeast known to affect polar growth. A total of 129 strains from these two sets were screened.

The last set consists of knockouts of known transcription factors in Neurospora. This collection is available as a set from the Fungal Genetics Stock Center (McCluskey 2003). It was selected for this screen to determine which transcription

factors play a role in signaling to the cell that cold adaptation genes must be activated. A total of 147 strains from this set were screened.

Media

Media and culturing procedures were those described in Davis & deSerres (1970). Growth described as being on "minimal" was in plates containing Vogel's minimal medium (Davis & deSerres 1970) with 2% agar.

Screen

The selected knockout strains were subjected to a screen looking for altered responses to cold shock. Wild-type Neurospora progresses through a three-stage response following a shift into the cold. To induce the cold shock response, we initially grew strains at 33°C and shifted to 4°C. We selected 33°C as our "normal" temperature as the cold shock response has previously been demonstrated to be dependent on the degree of the temperature shift the hypha are subjected to (Watters et al. 2000). The larger temperature shift used here would be expected to result in tighter branching during the apical phase. We decided this was desirable as it would make any variations from the normal cold shock response more visible and easier to identify in the screen. Strains were inoculated by dropping a suspension of conidia onto Vogel's Minimal Medium and incubated overnight at 33°C. The next morning plates were moved to 4°C. After an overnight incubation at 4°C, the strain's response to cold shock was photographed and evaluated. Variations in the cold shock response from that of wild-type Neurospora were judged qualitatively. Knockouts were subjected to cold shock and photographed a minimum of three independent runs on separate days to assure consistency of the response within a strain.

Photomicroscopy

Growing cultures were examined and photographed using a Motic 10MP digital camera attached to a Wolfe Beta Elite trinocular microscope. Photographs were taken of well separated, leading hyphae. All photomicrographs were taken using 40x magnification.

Phenotypes scored

The morphology of strains following cold shock was scored visually by comparing collections of photographs of cold shock in a given strain to the response seen with a wild type strain (*Neurospora crassa* Oak Ridge). Those with altered responses were then further categorized visually into the groups reported in Table 1 "CS phenotype."

RESULTS AND DISCUSSION

When Neurospora is subjected to a rapid temperature downshift, a 3-phase response is observed (Figure 1, Watters *et al.* 2000, Watters 2013). The initial response to cold shock is the growth of a single longer than normal unbranched segment. This was termed the "Lag" phase of the response. This phase is followed by a series of closely spaced apical

branch points, termed the "Apical" phase. Apical branch formation has been previously associated with the disruption and attempted reorganization of the normal tip-growth apparatus (Reynaga-Peña *et al*. 1995, Riquelme & Bartnicki-Garcia 2004), a mechanism distinct from that thought to be involved in lateral branching. Finally, with continued incubation at the lower temperature, the colony returns to lateral branching, termed the "Recovery" phase. Growth in this phase of the response resembles that which would be seen had the colony been grown at 4°C (or any other fixed temperature) continuously (Watters et al 2000). Thus, the cold shock response appears to be a temporary disturbance to a homeostatic system which maintains branch density at a constant, evolutionarily favored, value. The morphological effects of cold shock are the indirect consequence of this system's staged process of adjusting cellular conditions in order to compensate for the new growth temperature.

Cold shock response of *E. coli*

Homeostasis in the face of temperature changes and more specifically the response to cold shock has been extensively studied in bacterial systems for over 20 years. The effect of cold shock is manifest in multiple cellular systems including: membrane rigidity (Shivaji & Prakash 2010), stability of secondary structures in DNA/RNA (Phadtare 2004), efficiency of protein folding (Phadtare 2004) and ribosome function (Gualerzi *et al*. 2011). While much remains to be described in these systems, cold shock appears to result in a multi-stage response (Phadtare 2004). First, a lag period in which growth and translation of proteins generally cease. This is followed by an adjustment phase in which specific cold-shock proteins which compensate for the changes brought on by the cold are preferentially translated (Giuliodori *et al*. 2004). In the final stage, growth continues otherwise normally, but at a reduced rate. DNA microarray transcription profiling of the cold shock response in *E. coli* by Phadtare and Inouye (2004) has shown that several hundred genes respond to cold shock, either being transiently induced/repressed or showing prolonged induction/repression. Analogous responses to cold shock and/or cold acclimation have been observed in diverse organisms including plants (Guy 1999) and animals (Canclini & Esteves 2007). Attempts to uncover cold shock proteins in fungi (Fang & Leger 2010) have met with mixed success.

Connections between cold shock in bacteria and Neurospora

It is tempting to draw parallels between what is known about cold shock in bacterial systems and the observed response of Neurospora to similar cold shocks. Many of the systems affected during bacterial cold shock would be expected to impact fungal tip growth and branching (e.g. membrane fluidity). In addition, the nature and timing of the two responses are similar. Both can be adjusted by changing the intensity of the cold shock with more mild shocks (lower temperature differences) producing more mild responses and more severe shocks (larger temperature differences) producing more severe responses. Furthermore, the dynamics of the responses parallel each other. In each, there is a multistage response. There is an initial response which is transient in nature, followed by a more long-term response which largely represents a return to normal growth.

During the initial study of the cold shock response in Neurospora (Watters et al 2000), it was observed that two classical morphological mutants (most notably "granular" and "delicate") produced altered responses to cold shock (not reported), demonstrating that mutants could be obtained which influenced this process. We chose to screen mutants from the Neurospora knockout library for their cold shock response in order to provide a genetic grounding to this process which has, thus far, been lacking. We chose to use the mutants of the knockout library instead of the products of a random mutagenesis as the knockouts allow an immediate identification of gene function in most cases.

Knockout strains displaying an altered morphological response to cold shock were classified according to the specific variation they displayed. Examples are shown in Figure 1. The "burst" phenotype was defined as displaying a large number of growing tips which stop growing, swell and then structurally fail leaving a pool of cytoplasm at the tip. The "fail" phenotype was defined as failing to display the apical branch phase characteristic of cold shock. In the "fail" response, growth proceeds normally with lateral branching following cold shock. The "thin" phenotype was defined by a very rapid decrease in hyphal diameter following cold shock. It was common to observe "thin" in combination with other altered cold shock responses. The "dense" phenotype was defined by displaying apical branching with visibly shorter distances between branch points following cold shock relative to the response in wild-type. The "weak" phenotype was defined as the opposite – an apical branch phase with visibly longer distances between branch points relative to wild-type following cold shock. Finally, the "cot-like" phenotype was characterized by a lack of apical branching, but a shift to tightly spaced lateral branches which morphologically resembled the growth of the traditional *cot* mutants at the restrictive temperature.

Screen of *E. coli* cold shock gene homolog knockout set

A total of 68 Neurospora strains with knockouts of genes homologous to *E. coli* genes which alter transcription in response to cold shock (Phadtare and Inouye 2004) were screened. A total of 55 (81%) showed altered morphology to cold shock (Knockouts presenting alterations to the cold shock response are reported together in Table 1, sorted by phenotype). The knockouts displaying altered response to cold shock represent a variety of cellular functions. Phadtare and Inouye report genes which respond to cold shock by altering their transcription levels. Comparisons (Chi^2 not shown) between these transcription changes in *E. coli* and the cold shock phenotype displayed by these genes orthologs in Neurospora do not suggest there are any clear associations between transcription changes and cold shock morphology.

The screen of cold shock orthologs provides a test of the hypothesis that the cold shock response in both *E. coli* and Neurospora share a great deal of their cold shock response in common. The very high percentage of overlap between genes playing a role in these two widely separated organisms argues that the two responses are functionally related.

Screen of Morphological/Hyphal plate knockout sets

A total of 129 selected mutant strains from the Neurospora knockout library were previously segregated into two collections. The "Morphological" collection resulted in known morphological variations in the knockout strains. The "Hyphal" collection consisted of knockouts of genes previously suspected to play a role in hyphal growth. These two collections were screened for alterations to their response to cold shock. In total, 30 (23%) strains were identified (Table 2) that displayed variant cold shock responses. The altered responses fell into several phenotypic categories (Table 1).

The morphological/hyphal knockouts were previously screened for temperature-dependent branch density (Watters et al. 2011). Comparing the strains identified above with alterations to their cold shock response to those previously determined to show temperature-dependent branching we find only a modest overlap with the following strains showing altered phenotypes in both: NCU02333, NCU00830, NCU04242, NCU02114, NCU04264, and NCU03076. Examining the overlap statistically via Chi-square (calculations not shown) yields a p value greater than 0.9, strongly suggesting that the overlap is random. This suggests that these two screens (cold shock vs temperature sensitive branching during steady-state growth) are independent. This leads us to conclude that the cold shock response and temperature-dependent branching are independent aspects of cold adaptation, highlighting the different genes involved in short-term adaptation to the cold as opposed to those required for sustained growth in cold environments. Additional screens of the knockout library for strains displaying growth rate dependent branching, and comparing them to those with an altered cold shock response will allow us to further examine the apparent independence of these two morphological screens.

Screen of transcription factor knockout set

A total of 147 Neurospora strains with knockouts in genes which function as transcription factors were screened for their response to cold shock. In all, 30 (20%) showed altered morphology to cold shock (Table 1).

As with the knockouts of orthologs of *E. coli* cold shock responding genes, the mutant strains identified in the additional screens show no observed correlations between the phenotypes observed and the annotated functions of the genes with a variety of functions being associated with the observed cold shock variations.

Frequency of knockouts yielding alterations in the cold shock was dependent on the source of the knockout

As detailed above, mutants screened represented three different sets of knockouts: *E. coli* cold-shock responding orthologs, Neurospora morphological/hyphal growth mutants, and Neurospora transcription factors. These three groups displayed altered cold shock responses at different rates with the majority (81%) of the *E. coli* orthologs showing altered responses and much lower frequencies (23% and 20% respectfully) of the morph/hyphal and transcription factor knockouts showing altered responses (Table 1). Additionally, the phenotypes of the altered cold shock response showed a non-random distribution with regard to the knockout set the mutant was associated with using Chi^2. Comparing knockout set vs cold shock phenotype among those with alterations yields a Chi^2 of 32.2 and an associated p value < 1%.

Much of the significance is coming from an over-representation of "dense" cold shock responses among otherwise unidentified (i.e. "hypothetical protein") transcription factors.

Cold shock phenotype was not correlated to GO categorization of the knockouts

The cold shock phenotype of knockouts was compared to their gene ontology categorizations via Chi^2 analysis. Comparing cold shock phenotype to either its Molecular Function or Biological Process categorization failed to produce significant differences (p values of ~0.75 and ~0.5 respectfully). This fails to support the suggestion that knockouts with specific GO categories are associated with specific altered phenotypes in the cold shock response.

The data was also examined to determine if there was a non-random association between knockouts which show any alteration to their cold shock response (regardless of the specific phenotype) and those that show the wild type response vs their GO categorization. For both "Molecular Function" and "Biological Process" GO categories, no significant association was seen (via Chi^2, p=0.4 and 0.5 respectfully), similarly failing to support the suggestion that knockouts with specific GO categorizations are tied to the cold shock response.

Cold shock phenotype was weakly associated with growth rate among transcription factor knockouts

Linear growth rates for the transcription factor knockouts reported by Carrillo et al (2017) were compared via T-test for knockouts showing altered cold shock responses vs those showing no alteration to the response. One possible association between growth rate and altered cold shock phenotype was found for the knockouts displaying a dense phenotype which showed statistically faster growth rates than those with no alterations to cold shock (T-test, p=0.019). This is consistent with previous observations between growth rate and cold shock (Watters et al 2000), however the opposite association (slow growth rates among mutants displaying weak cold shock responses or failure to respond) is not observed, as would be expected if growth rate was a key factor among the knockouts. Taken together, there appears to be, at best, a weak association between growth rate and alterations to the cold shock phenotype among the transcription factor knockout mutants. This stands in contrast to the observation in wild type Neurospora (Watters et al 2000) that the morphology of the cold shock response was directly dependent on growth rate changes. This suggests that the altered morphologies observed among the knockout mutants are due to changes in gene activity associated with the knockouts and not simply the consequence of changes in growth rates in these mutants.

In conclusion, the gene functions highlighted by these screens (Table 1) are diverse. It is unclear how the diverse gene network, partially exposed here, coordinates for the function of temperature acclimatization. The results presented here demonstrate a strong relationship between the cold shock responses of *E. coli* and *Neurospora crassa*. The phenotype under examination here (morphological response to cold shock) appears to be influenced by a diverse network of genes. Similar diversity of function has been observed in other examinations of morphogenesis in Neurospora (Seiler & Plamann 2003). Further work on cold acclimatization should help clarify these connections.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Indiana Space Grant Consortium (INSGC). We would also like to thank Kevin McCluskey and everyone at the Fungal Genetics Stock Center for supplying the knockout strains and their diligent work in support of the fungal genetics community over the years.

Table 1: Of 334 knockouts screened 115 were observed to alter the phenotype of the cold shock response. For each knockout strain tested ("ID"/NCU#####) we report the Cold Shock phenotype, the annotated gene function and gene abbreviation, the set of mutants the knockout came from (*E. coli* cold shock mutant ortholog, the Morphological or Hyphal growth plates from the FGSC, or the Transcription Factor plates from the FGSC) and the Gene Ontology categorizations for both Molecular Function and Biological Process.

ID	CS Phenotype	Gene Function	Gene	Knockout set	GO: Molecular Function	GO: Biological Process
NCU03938	burst	alternative oxidase-5	aod-5	Morph/Hyph		
NCU03070	burst	hypothetical protein		Transc Factors	Binding	Biological Regulation
NCU01782	burst	Ras guanyl-nucleotide exchange factor RasGEF		*E. coli* CS Orth		Ccellular Process
NCU02133	burst	superoxide dismutase-1	sod-1	Morph/Hyph	antioxidant/binding/catalytic Activity	Cellular Process/ Response to Stimulus
NCU01213	burst	superoxide dismutase-2	sod-2	*E. coli* CS Orth	Binding	Developmental Process
NCU03623	burst	ubiquitin-conjugating enzyme E		Morph/Hyph	Binding	Cellular Process/ Metabolic Process
NCU04242	burst/dense	period-6	prd-6	Morph/Hyph	Binding/Catalytic Activity	Biological Regulation/ Cellular Process/ Metabolic Process
NCU07728	burst/thin	siderophore regulation	sre	Morph/Hyph		
NCU03901	cot-like	peroxin 14	pex14	Morph/Hyph	Binding	Cellular Component Organization or Biogenisis/ Cellular Process/ Localization/ Metabolic Process
NCU07617	dense	aconidiate-3	acon-3	Morph/Hyph		Biological Regulation/ Developmental Process/ Reproduction
NCU05410	dense	arginine-5	arg-5	*E. coli* CS Orth	Binding	Cellular Process/ Metabolic Process
NCU02114	dense	G1/S-specific cyclin Cln1		Morph/Hyph	Binding	Cellular Process
NCU00144	dense	hypothetical protein		Transc Factors		
NCU03120	dense	hypothetical protein		Transc Factors		
NCU03356	dense	hypothetical protein		Transc Factors		
NCU03417	dense	hypothetical protein		Transc Factors		

NCU03905	dense	hypothetical protein		Transc Factors		
NCU03962	dense	hypothetical protein		Transc Factors	Binding	Cellular Process/ Metabolic Process
NCU06990	dense	hypothetical protein		Transc Factors		
NCU01154	dense	submerged protoperithecia-1	sub-1	Transc Factors		
NCU04899	dense	tricarboxylic acid-15	tca-15	*E. coli* CS Orth	Catalytic Activity	Metabolic Process
NCU03415	fail	aldehyde dehydrogenase	CBS-3	*E. coli* CS Orth		
NCU11289	fail	aldo-keto reductase		*E. coli* CS Orth	Catalytic Activity/transporter Activity	
NCU00097	fail	BEAK-1	bek-1	Transc Factors		
NCU02017	fail	CBF/NF-Y family transcription factor	ada-2	Transc Factors		
NCU00056	fail	condensing enzyme with mitochondrial function	cem-1	*E. coli* CS Orth		
NCU00467	fail	COP9 signalosome-5	csn-5	Morph/Hyph	Binding	Metabolic Process
NCU06068	fail	fungal specific transcription factor	col-25	Transc Factors		
NCU07788	fail	fungal specific transcription factor	col-26	Transc Factors		
NCU07945	fail	fungal specific transcription factor	tah-4	Transc Factors		
NCU07947	fail	glycolipid transfer protein HET-C2		Morph/Hyph		Localization/ Metabolic Process
NCU05927	fail	GTP-binding protein GUF1	GTP-7	*E. coli* CS Orth		
NCU00528	fail	hyphal anastomosis-4	ham-4	*E. coli* CS Orth		
NCU07561	fail	hypothetical protein		Transc Factors		
NCU09120	fail	lysine-specific histone demethylase Aof2		*E. coli* CS Orth	Binding	Cellular Process/ Metabolic Process
NCU09830	fail	menadione-induced gene-12	mig-12	Morph/Hyph	Catalytic Activity	Cellular Process/ Metabolic Process
NCU09842	fail	mitogen activated protein kinase-1	mak-1	Morph/Hyph	Catalytic Activity/signal transducer activity	Biological Regulation/ Cellular Process/ Response to Stimulus/ Metabolic Process

NCU03314	fail	mob2-like-a	mob-2a	Morph/Hyph	Binding/Catalytic Activity	Cellular Process
NCU09975	fail	multidrug resistance protein 3		*E. coli* CS Orth		
NCU08294	fail	nitrogen assimilation transcription factor nit-4	nit-4	Transc Factors		
NCU03277	fail	peroxin 10	pex10	Morph/Hyph		Cellular Component Organization or Biogenisis/ Cellular Process/ Localization
NCU02636	fail	peroxin 4	pex4	Morph/Hyph		
NCU01004	fail	phosphatidylserine decarboxylase proenzyme	CHOL-15	*E. coli* CS Orth	Catalytic Activity	Cellular Process/ Metabolic Process
NCU07832	fail	pre-mRNA processing splicing factor 8	msp-39	*E. coli* CS Orth	Catalytic Activity	Cellular Component Organization or Biogenisis/ Cellular Process/ Metabolic Process
NCU06028	fail	quinic acid utilization activator	qa-1F	Transc Factors		
NCU06205	fail	regulator of conidiation-1	rco-1	Morph/Hyph		
NCU06145	fail	RING-6	RING-6	Morph/Hyph		
NCU02214	fail	TAH-2	tah-2	Transc Factors		
NCU10008	fail	tricarboxylic acid-14	tca-14	*E. coli* CS Orth		Cellular Process/ Metabolic Process
NCU02356	fail	white collar 1	wc-1	Transc Factors	Binding	
NCU02173	fail	zinc finger transcription factor-52	znf-52	Transc Factors		
NCU05591	thin	ABC transporter CDR4		Morph/Hyph	Catalytic Activity/transporter Activity	Cellular Process/ Metabolic Process
NCU03013	thin	anchored cell wall protein-10	acw-10	Morph/Hyph	antioxidant/binding/catalytic Activity	Cellular Process/ Response to Stimulus
NCU02333	thin	arginase-1	aga-1	Morph/Hyph	Binding/Catalytic Activity	Cellular Process/ Metabolic Process
NCU03184	thin	C2H2 conidiation transcription factor FlbC		Morph/Hyph		

NCU07075	thin	calcium exchanger	cax	Morph/Hyph	transporter activity	Biological Regulation/ Cellular Process
NCU05770	thin	catalase-2	cat-2	*E. coli* CS Orth	antioxidant/binding	Cellular Process/ Response to Stimulus/ Metabolic Process
NCU05051	thin	COL-23	col-23	Transc Factors		
NCU00830	thin	ctr copper transporter	tcu-1	Morph/Hyph		
NCU08216	thin	cystathionine beta-synthase	MET-11	*E. coli* CS Orth	Binding	Cellular Process/ Metabolic Process
NCU03076	thin	delta-1-pyrroline-5-carboxylate dehydrogenase		Morph/Hyph		
NCU08968	thin	dimethyladenosine transferase		*E. coli* CS Orth		Cellular Component Organization or Biogenisis/ Cellular Process/ Metabolic Process
NCU01772	thin	DNA-directed RNA polymerase III polypeptide	rpo-10	*E. coli* CS Orth		Cellular Process/ Metabolic Process
NCU02542	thin	embden-meyerhof pathway-1	emp-1	Morph/Hyph	Catalytic Activity	Biological Regulation/ Cellular Process/ Metabolic Process
NCU01744	thin	enhancer-2 of am	en(am)-2	*E. coli* CS Orth	Catalytic Activity	Cellular Process/ Metabolic Process
NCU04264	thin	extracellular developmental signal biosynthesis protein FluG		Morph/Hyph	Binding	Cellular Process/ Metabolic Process
NCU04140	thin	FK506 resistant-2	fkr-2	*E. coli* CS Orth	Binding/Catalytic Activity	Cellular Process/ Metabolic Process
NCU09930	thin	folic acid synthesis protein	fol-9	*E. coli* CS Orth	transporter activity	Cellular Process/ Metabolic Process
NCU05606	thin	glucosidase 2 subunit beta	GHX-4	*E. coli* CS Orth	Binding/Catalytic Activity	Biological Regulation/ Metabolic Process
NCU01528	thin	glyceraldehyde-3-phosphate dehydrogenase-1	gpd-1	*E. coli* CS Orth	Catalytic Activity	Metabolic Process
NCU06005	thin	glycerol kinase	GLK-1	*E. coli* CS Orth		
NCU02630	thin	heat shock protein 78	hsp78	*E. coli* CS Orth		
NCU07156	thin	histidine-6	his-6	*E. coli* CS Orth		

NCU02556	thin	histone acetyl transferase-2	hat-2	*E. coli* CS Orth		Cellular Component Organization or Biogenisis/ Cellular Process/ Metabolic Process
NCU01629	thin	hypothetical protein		Transc Factors		
NCU04669	thin	hypothetical protein homologous to Bactericidal permeability-increasing protein		Morph/Hyph		
NCU04561	thin	melanization defective-1	mld-1	Transc Factors		
NCU09767	thin	membrane transporter		*E. coli* CS Orth		
NCU04791	thin	menadione-induced gene-10	mig-10	*E. coli* CS Orth		
NCU05151	thin	phosphoketolase	PHK-1	*E. coli* CS Orth		
NCU06342	thin	phospholipase D	PLA-5	*E. coli* CS Orth		Cellular Process/ Localization/ Locomotion
NCU05295	thin	proteasome catalytic alpha-5	pca-5	Morph/Hyph		Cellular Process/ Metabolic Process
NCU09366	thin	proteasome catalytic beta-6	pcb-6	Morph/Hyph	Catalytic Activity	Cellular Process/ Metabolic Process
NCU01613	thin	protoperithecia-2	pp-2	Morph/Hyph	Catalytic Activity	Biological Regulation/ Cellular Component Organization or Biogenisis/ Cellular Process/ Metabolic Process
NCU02260	thin	regulatory particle, ATPase-like-3	rpt-3	Morph/Hyph		
NCU02055	thin	uridine nucleosidase Urh1	NUS-1	*E. coli* CS Orth		
NCU07705	thin/fail	C6 finger domain-containing protein	clr-1	Transc Factors		
NCU08000	thin/fail	cutinase transcription factor 1 alpha	far1	Transc Factors		
NCU05536	thin/fail	hypothetical protein		Transc Factors	Binding/Catalytic Activity	Cellular Component Organization or Biogenisis/ Cellular Process/ Response to Stimulus/ Metabolic Process

NCU08651	thin/fail	zinc binuclear cluster-type protein	col-27	Transc Factors		
NCU07732	thin/fail	arginine-2	arg-2	*E. coli* CS Orth	Binding/Catalytic Activity	Cellular Process/ Metabolic Process
NCU04117	thin/fail	ATP-dependent permease MDL2	ABC-7	*E. coli* CS Orth		
NCU06659	thin/fail	GTP-binding protein	GTP-3	*E. coli* CS Orth		Cellular Process/ Metabolic Process
NCU08693	thin/fail	heat shock protein 70-5	hsp70-5	*E. coli* CS Orth	Binding	Cellular Process/ Response to Stimulus/ Metabolic Process
NCU10760	thin/fail	jumonji domain-containing protein 5		*E. coli* CS Orth		
NCU08858	thin/fail	MFS alpha-glucoside transporter	SUT-1	*E. coli* CS Orth	transporter activity	Cellular Process/ Localization
NCU00793	thin/fail	trehalose phosphate synthase	GT20-2	*E. coli* CS Orth	Catalytic Activity	Cellular Process/ Response to Stimulus/Metabolic Process
NCU08336	thin/fail	tricarboxylic acid-12	tca-12	*E. coli* CS Orth	Catalytic Activity	Cellular Process/ Localization/ Metabolic Process
NCU00771	thin/fail	UBX domain-containing protein 7		*E. coli* CS Orth		
NCU04583	weak	acetyltransferase		*E. coli* CS Orth	Catalytic Activity	
NCU00499	weak	all development altered-1	ada-1	Transc Factors		
NCU00567	weak	arginine-6	arg-6	*E. coli* CS Orth	Binding	Cellular Process/ Metabolic Process
NCU04303	weak	asparagine synthetase 2	asn-1	*E. coli* CS Orth	Binding/Catalytic Activity	Cellular Process/ Metabolic Process
NCU00919	weak	ATP-dependent RNA helicase rok-1	drh-16	*E. coli* CS Orth		
NCU08933	weak	cellular nucleic acid-binding protein		*E. coli* CS Orth		
NCU01408	weak	COP9 signalosome-3	csn-3	Morph/Hyph		Cellular Process/ Metabolic Process
NCU01625	weak	DNA repair helicase RAD3	DNR-10	*E. coli* CS Orth	Binding/Catalytic Activity	Cellular Process/ Metabolic Process

NCU07027	weak	glycogen phosphorylase	GYP-1	*E. coli* CS Orth	Binding/Catalytic Activity	Cellular Process/ Metabolic Process
NCU06523	weak	glycosylhydrolase family 13-4	gh13-4	*E. coli* CS Orth	Catalytic Activity	Metabolic Process
NCU01589	weak	heat shock protein 60	hsp60	*E. coli* CS Orth	Binding	Cellular Component Organization or Biogenisis/ Cellular Process/ Metabolic Process
NCU05909	weak	hypothetical protein		Transc Factors		
NCU08439	weak	leptomycin B resistance protein pmd1	ABC-2	*E. coli* CS Orth	transporter activity	Cellular Process/ Metabolic Process
NCU00565	weak	lipoic acid synthetase	LIA-1	*E. coli* CS Orth	Catalytic Activity	Cellular Process/ Metabolic Process
NCU04339	weak	ribokinase	RIK-8	*E. coli* CS Orth	Catalytic Activity	Cellular Process/ Metabolic Process
NCU03894	weak	serine/threonine protein kinase-4	stk-4	Morph/Hyph	Binding/signal transducer activity	Biological Regulation/ Cellular Process/ Developmental Process/ Response to Stimulus
NCU06017	weak	thiosulfate sulfurtransferase	TST-1	*E. coli* CS Orth		Localization/ Response to Stimulus/ Metabolic Process
NCU10053	weak	thymidylate synthase	pyr-8	*E. coli* CS Orth		
NCU08658	weak	zinc finger transcription factor-50	znf-50	Transc Factors		

Table 2: Of knockouts screened, 229 presented no change to the cold shock morphology. Columns are the same as for Table 1.

ID	Gene Function	Gene	Knockout set	GO: Molecular Function	GO: Biological Process
NCU00017	hypothetical protein		Transc Factors		
NCU00019	Fork head protein homolog 1	FKH1	Transc Factors		
NCU00038	zinc finger transcription factor-32	znf-32	Transc Factors		
NCU00081	DNA topoisomerase 3-beta	dnt-3	*E. coli* CS Orth		
NCU00090	pH-response transcription factor pacC/RIM101	pacc-1	Transc Factors		
NCU00105	ribosome biogenesis-58	rbg-58	Morph/Hyph		Cellular Component Organization or Biogenisis
NCU00135	Phosphatidyl synthase, phosphatidyl synthase, variant 1	gpl-1	Morph/Hyph		Cellular Process/Metabolic Process
NCU00157	COP9 signalosome-1	csn-1	Morph/Hyph		
NCU00204	hypothetical protein		Morph/Hyph		
NCU00217	hypothetical protein		Transc Factors		
NCU00233	glycosyl hydrolase family 16-15	gh16-15	Transc Factors		
NCU00285	hypothetical protein		Transc Factors		
NCU00289	tall aerial hyphae-1	tah-1	Transc Factors		
NCU00329	vegetative asexual development-1	vad-1	Transc Factors		
NCU00355	catalase-3	cat-3	Morph/Hyph	Antioxidant Activity/ Binding/Catalytic Activity	Response to Stimulus/ Cellular Process/ Metabolic Process
NCU00396	pre-mRNA-splicing factor rse-1	msp-5	Morph/Hyph	Binding	Cellular Process/Metabolic Process
NCU00406	velvet	vel	Morph/Hyph	Binding/Signal Transducer Activity/Catalytic Activity	Biological Regulation/Developmental Process/ Response to Stimulus/Cellular Process

NCU00554	Aspartate-semialdehyde dehydrogenase	hom-1	Morph/Hyph		
NCU00609	initiation-specific alpha-1,6-mannosyltransferase	och-1	Morph/Hyph	Catalytic Activity	Cellular Process/ Metabolic Process
NCU00631	chromatin remodelling factor 9-1	crf9-1	Transc Factors		
NCU00634	Ribosomal protein L14	crp-47	Morph/Hyph	Structural Molecule Activity	Cellular Component Organization or Biogenisis
NCU00694	hypothetical protein		Transc Factors		
NCU00749	conidiation at high carbon dioxide-1	chc-1	Transc Factors		
NCU00768	mRNA binding post-transcriptional regulator		Morph/Hyph		
NCU00808	zinc finger transcription factor-48	znf-48	Transc Factors		
NCU00810	Beta-galactosidase	gh2-3	Morph/Hyph	Catalytic Activity	Cellular Process/ Metabolic Process
NCU00824	histone deacetylase-3	hda-3	Morph/Hyph	Binding/Catalytic Activity	Biological Regulation/Cellular Compoonent Organization or Biogenisis/ Cellular Process
NCU00902	zinc finger white collar protein WC2	wc-2	Transc Factors		
NCU00923	topogenesis of outer membrane beta barrel protein 37	tob37	Morph/Hyph		
NCU00945	fungal specific transcription factor	col-20	Transc Factors		
NCU00959	succinate dehydrogenase iron-sulfur protein	tca-10	*E. coli* CS Orth		Cellular Process/ Metabolic Process
NCU01020	hypothetical protein		Morph/Hyph		
NCU01033	hypothetical protein related to regulatory protein wetA		Morph/Hyph	Binding	
NCU01037	hypothetical protein		Morph/Hyph		
NCU01097	hypothetical protein		Transc Factors		
NCU01122	hypothetical protein		Transc Factors		
NCU01181	acyl-CoA dehydrogenase family member 11	acd-3	Morph/Hyph		
NCU01197	cell wall biogenesis protein phosphatase Ssd1	gul-1	Morph/Hyph		

NCU01213	superoxide dismutase-2	sod-2	Morph/Hyph	Antioxidant Activity/Binding/Catalytic Activity	Developmental Process
NCU01225	ubiquitin conjugating enzyme - 13	uce-13	Morph/Hyph		
NCU01312	myb-like DNA-binding protein myb-1	rca-1	Morph/Hyph		
NCU01368	proteasome component C11	pcb-4	Morph/Hyph	Catalytic Activity	Cellular Process/ Metabolic Process
NCU01478	fungal specific transcription factor domain-containing protein		Transc Factors		
NCU01642	hypothetical protein homologous to Neurofibromin		Morph/Hyph		
NCU01833	Two-component histidine kinase CHK-1	nik-2	Morph/Hyph		
NCU01994	transcription factor-1	tcf-1	Transc Factors		
NCU02057	autoinducer 2 sensor kinase/phosphatase luxQ		Morph/Hyph		
NCU02094	vegetative asexual development-2	vad-2	Transc Factors	Binding	Cellular Process/Metabolic Process
NCU02111	myosin-5	myo-5	Morph/Hyph	Binding/ Structural Molecule Activity/Catalytic Activity	Cellular Component Organization or Biogenisis/Localization Process/ Cellular Process
NCU02142	hypothetical protein		Transc Factors		
NCU02160	small GTPase RAC	rac-1	Morph/Hyph	Binding/Signal Transducer Activity/Catalytic Activity	Biological Regulation/Cellular Compoonent Organization or Biogenisis/ Developmental Process/ Response to Stimulus/ Cellular Process/ Metabolic Process
NCU02226	methylthioribose-1-phosphate isomerase	met-23	Morph/Hyph	Catalytic Activity	Cellular Process/ Metabolic Process

NCU02250	ATP synthase subunit ATP9	oli	Morph/Hyph	Transporter Activity/Catalytic Activity	Cellular Process/Metabolic Process
NCU02265	period clock protein FRQ	frq	Morph/Hyph		
NCU02307	hypothetical protein		Transc Factors		
NCU02387	nuclear import and export protein Msn5		Morph/Hyph	Binding/ Transporter Activity	Biological Regulation/Localization Process/ Cellular Process
NCU02406	nuclear protein		Morph/Hyph	Binding	Cellular Component Organization or Biogenisis
NCU02498	Cullin-3	cul-3	Morph/Hyph	Binding	Cellular Process/Metabolic Process
NCU02576	zinc finger transcription factor-39	znf-39	Transc Factors		
NCU02604	U3 small nucleolar RNA-associated protein 10	rbg-7	Morph/Hyph	Binding	Biological Regulation/Cellular Compoonent Organization or Biogenisis/Cellular Process/ Metabolic Process
NCU02639	Argininosuccinate synthase	arg-1	*E. coli* CS Orth	Catalytic Activity	Cellular Process/ Metabolic Process
NCU02666	zinc finger transcription factor-58	znf-58	Transc Factors		
NCU02671	cutinase G-box binding protein	msn-1	Transc Factors		
NCU02699	zinc finger transcription factor-14	znf-14	Transc Factors		
NCU02712	acetate-10	ace-10	*E. coli* CS Orth		
NCU02713	conidial separation-1	csp-1	Transc Factors	Binding	Cellular Process/ Metabolic Process
NCU02724	transcription factor-21	tcf-21	Transc Factors		
NCU02752	zinc finger transcription factor-47	znf-47	Transc Factors		

NCU02768	transcription factor-20	tcf-20	Transc Factors		
NCU02794	Fso1	so	Morph/Hyph		
NCU02826	sodium/calcium exchanger protein	trm-16	Morph/Hyph	Transporter Activity	
NCU02896	all development altered-3	ada-3	Transc Factors		
NCU02934	hypothetical protein		Transc Factors		
NCU02948	non-anchored cell wall protein-4	ncw-4	*E. coli* CS Orth	Catalytic Activity	
NCU02957	hypothetical protein		Transc Factors		
NCU02994	hypothetical protein		Transc Factors		
NCU03033	transcription factor-26	tcf-26	Transc Factors	Binding	Biological Regulation/Response to Stimulus
NCU03043	C2H2 finger domain-containing protein FlbC	acon-4	Transc Factors		
NCU03073	DNA polymerase epsilon, subunit D	pole-4	Transc Factors		
NCU03077	hypothetical protein		Transc Factors		
NCU03096	bromodomain associated domain-containing protein		Morph/Hyph		
NCU03110	hypothetical protein		Transc Factors		
NCU03125	NIMA-interacting protein TinC		Morph/Hyph		
NCU03164	two-component system response regulator		Morph/Hyph		
NCU03184	zinc finger transcription factor-21	znf-21	Transc Factors		
NCU03206	zinc finger transcription factor-22	znf-22	Transc Factors		
NCU03244	WD repeat protein		Transc Factors		
NCU03281	transport of copper-2	tcu-2	Morph/Hyph		
NCU03320	all development altered-4	ada-4	Transc Factors		
NCU03479	endoribonuclease ysh-1	paa-5	Morph/Hyph	Binding/Catalytic Activity	Metabolic Process
NCU03489	colonial-21	col-21	Transc Factors		
NCU03576	conidiophore development protein hymA	hym-1	Morph/Hyph	Binding	
NCU03593	homeobox domain-containing protein	kal-1	Transc Factors		
NCU03643	fatty acid regulation-2	far-2	Transc Factors		

NCU03669	AdoMet-dependent rRNA methyltransferase spb1	rmt-3	*E. coli* CS Orth	Catalytic Activity	Cellular Component Organization or Biogenisis/ Cellular Process/ Metabolic Process
NCU03686	oxidase assembly protein 2	tah-3	Transc Factors		
NCU03699	zinc finger transcription factor-13	znf-13	Transc Factors		
NCU03702	rRNA 2'-O-methyltransferase fibrillarin	rbg-16	Morph/Hyph		
NCU03725	vegetative incompatibility blocked-1	vib-1	Morph/Hyph	Binding	Biological Regulation/Cellular Process/ Metabolic Process
NCU03931	all development altered-5	ada-5	Transc Factors		
NCU04001	female fertility-7	ff-7	Transc Factors	Catalytic Activity	
NCU04096	serine/threonine-protein kinase 3	prk-9	Morph/Hyph	Binding/Signal Transducer Activity/Catalytic Activity	Biological Regulation/Developmental Process/ Multicellular Organismal Process/ Response to Stimulus/ Cellular Process
NCU04142	heat shock protein 80	hsp80	*E. coli* CS Orth		Response to Stimulus/ Metabolic Process
NCU04179	C2H2 transcription factor	sah-1	Transc Factors		
NCU04211	hypothetical protein		Transc Factors		
NCU04302	ubiquitin-conjugating enzyme E	nup-22	Morph/Hyph		
NCU04359	hypothetical protein		Transc Factors		
NCU04390	fungal specific transcription factor	col-22	Transc Factors		
NCU04513	ubiquitin conjugating enzyme Ubc14	uce-14	Morph/Hyph	Catalytic Activity	Metabolic Process
NCU04533	DUF1881 domain-containing protein	app	Morph/Hyph		
NCU04619	hypothetical protein		Transc Factors		

NCU04628	hypothetical protein		Transc Factors		
NCU04731	Sterol regulatory element binding protein sah-2	sah-2	Transc Factors		
NCU04733	UvrD/REP helicase	mus-50	*E. coli* CS Orth		
NCU04834	sensor histidine kinase/response regulator	phy-1	Morph/Hyph		
NCU04851	hypothetical protein		Transc Factors		
NCU04866	all development altered-6	ada-6	Transc Factors		
NCU05046	calcium-transporting ATPase 3	ena-1	Morph/Hyph	Transporter Activity/Catalytic Activity	Cellular Process/ Metabolic Process
NCU05210	postreplication repair E3 ubiquitin-protein ligase rad-18	uvs-2	Transc Factors	Catalytic Activity	Response to Stimulus/ Cellular Process/ Metabolic Process
NCU05242	zinc finger transcription factor-25	znf-25	Transc Factors		
NCU05250	nuclear division-76	div-76	Transc Factors	Binding	Biological Regulation/Cellular Compoonent Organization or Biogenisis/Localization Process/ Response to Stimulus/Cellular Process/Metabolic Process
NCU05294	zinc finger transcription factor-40	znf-40	Transc Factors		
NCU05383	fungal specific transcription factor	col-24	Transc Factors		
NCU05411	pathway-specific nitrogen regulator		Transc Factors		
NCU05637	hypothetical protein		Transc Factors		
NCU05767	zinc finger transcription factor-10	znf-10	Transc Factors		
NCU05790	phytochrome-like histidine kinase 2	phy-2	Morph/Hyph		
NCU05854	hypothetical protein		Morph/Hyph		
NCU05858	fatty acid oxygenase	fam-2	Morph/Hyph	Catalytic Activity	

NCU05891	arid/bright domain-containing protein		Morph/Hyph	Binding	Biological Regulation/Cellular Process/ Metabolic Process
NCU05956	Beta-galactosidase	gh2-2	Morph/Hyph	Catalytic Activity	Cellular Process/ Metabolic Process
NCU05993	hypothetical protein		Transc Factors		
NCU05994	transcription factor-10	tcf-10	Transc Factors	Binding	Cellular Component Organization or Biogenisis/Localization Process/Response to Stimulus/Cellular Process/ Metabolic Process
NCU06049	DNA damage response protein RcaA	nbs1	Morph/Hyph	Binding/Catalytic Activity	Biological Regulation/Cellular Compoonent Organization or Biogenisis/Response to Stimulus/ Cellular Process/Metabolic Process
NCU06145	RING-6		Morph/Hyph		
NCU06173	hypothetical protein		Transc Factors		
NCU06175	Peroxisomal membrane protein	pex3	Morph/Hyph		
NCU06186	hypothetical protein		Transc Factors		
NCU06205	transcriptional repressor rco-1	rco-1	Transc Factors		
NCU06213	zinc finger transcription factor-9	znf-9	Transc Factors		
NCU06265	Hyphal anastamosis-13 protein	ham-13	Morph/Hyph		
NCU06407	zinc finger transcription factor 1	vad-3	Transc Factors		
NCU06411	vegetative asexual development-4	vad-4	Transc Factors	Binding/Catalytic Activity	Metabolic Process

NCU06419	map kinase kinase	mek-1	Morph/Hyph	Binding/Signal Transducer Activity/Catalytic Activity	Biological Regulation/Developmental Process/ Response to Stimulus/Cellular Process
NCU06429	alpha-actinin		Morph/Hyph		
NCU06440	proteasome component PRE6	pca-4	Morph/Hyph	Catalytic Activity	Cellular Process/ Metabolic Process
NCU06454	Rho-type GTPase	cdc42	Morph/Hyph	Binding/Signal Transducer Activity/Catalytic Activity	Biological Regulation/Cellular Compoonent Organization or Biogenisis/ Developmental Process/ Response to Stimulus/ Cellular Process/ Metabolic Process
NCU06503	zinc finger transcription factor-24	znf-24	Transc Factors		
NCU06531	hypothetical protein		Morph/Hyph		
NCU06605	DNA damage-binding protein 1	dim-8	Morph/Hyph	Binding	Response to Stimulus/ Cellular Process/ Metabolic Process
NCU06650	secretory phospholipase A2	spp-3	Morph/Hyph		
NCU06656	transcriptional activator protein acu-15	acu-15	Transc Factors		
NCU06695	cytochrome c oxidase polypeptide VI	cox-6	Morph/Hyph	Transporter Activity/Catalytic Activity	Cellular Process/ Metabolic Process
NCU06714	para-aminobenzoic acid synthetase	pab-1	Morph/Hyph		
NCU06744	hypothetical protein		Transc Factors		
NCU06764	20S proteasome subunit Y7	pca-2	Morph/Hyph	Catalytic Activity	Cellular Process/ Metabolic Process
NCU06799	fungal specific transcription factor	vad-5	Transc Factors		
NCU06845	short chain dehydrogenase/reductase		Morph/Hyph		

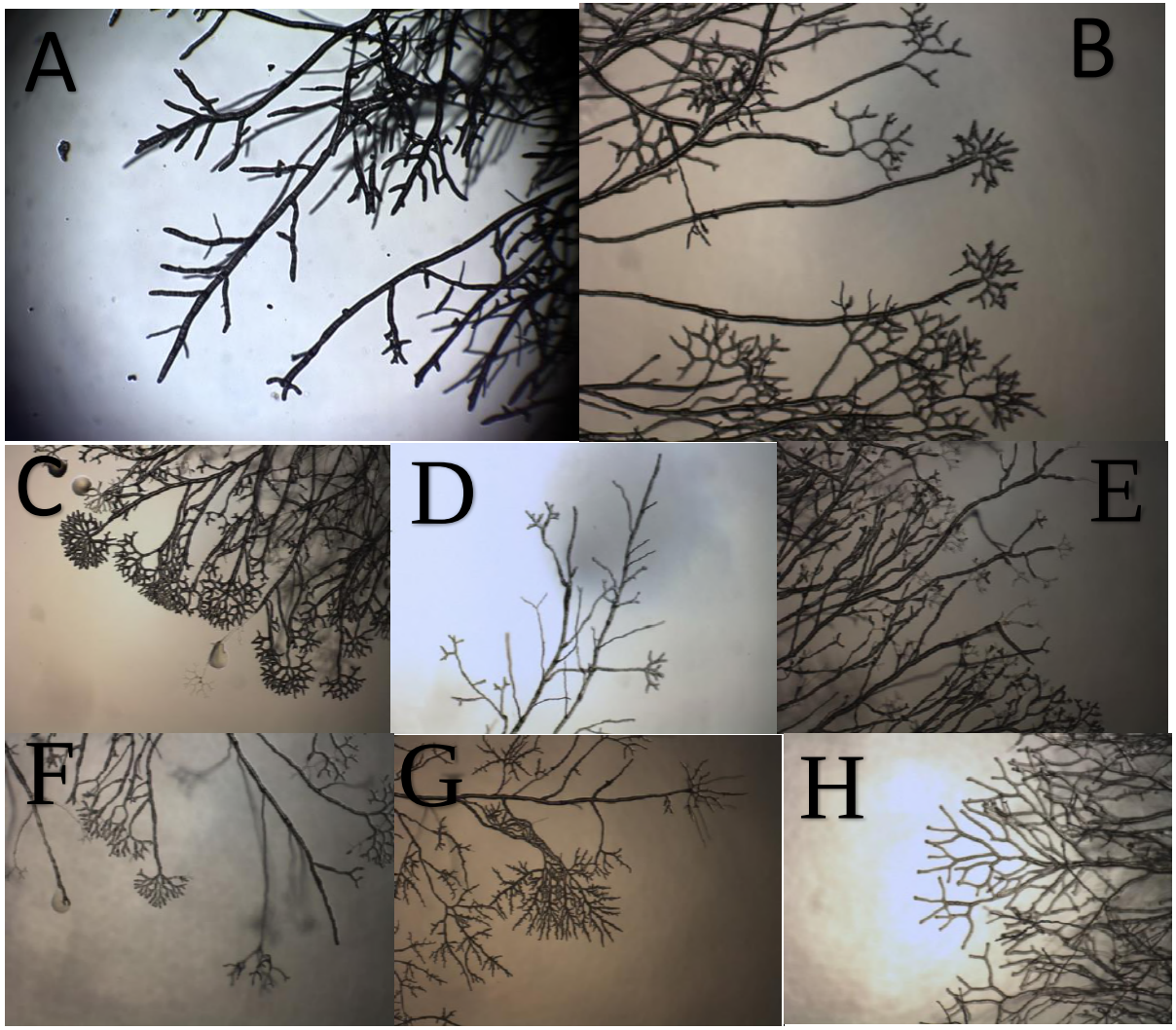
NCU06910	Cell wall integrity and stress response component 1	wsc-1	Morph/Hyph		
NCU06919	hypothetical protein		Transc Factors		
NCU06971	transcriptional activator xlnR	xlr-1	Transc Factors		
NCU07007	submerged protoperithecia-2	sub-2	Transc Factors		
NCU07039	GATA type zinc finger protein Asd4	asd-4	Transc Factors		
NCU07139	BEAK-2	bek-2	Transc Factors		
NCU07221	two-component system protein A	hcp-1	Morph/Hyph		
NCU07237	hypothetical protein		*E. coli* CS Orth		
NCU07281	glucose-6-phosphate isomerase	gpi-1	*E. coli* CS Orth	Catalytic Activity	Metabolic Process
NCU07374	hypothetical protein		Transc Factors		
NCU07378	serine threonine protein kinase	stk-12	Morph/Hyph	Catalytic Activity	Biological Regulation/Cellular Compoonent Organization or Biogenisis/Response to Stimulus/ Cellular Process/Metabolic Process
NCU07379	transcription factor-5	tcf-5	Transc Factors		
NCU07392	transcriptional regulatory protein pro-1	adv-1	Transc Factors		
NCU07420	eIF4A	eif4A	Morph/Hyph		
NCU07535	SAH-3	sah-3	Transc Factors	Catalytic Activity	Cellular Process/ Metabolic Process
NCU07589	acetyltransferase		Morph/Hyph		
NCU07591	Integral membrane protein		Morph/Hyph		
NCU07605	hypothetical protein		Morph/Hyph		
NCU07621	zinc-regulated transporter 1	tzn-1	Morph/Hyph	Transporter Activity	Cellular Process
NCU07728	siderophore regulation protein	sre	Transc Factors		
NCU07900	hypothetical protein		Transc Factors		
NCU07952	zinc finger transcription factor-37	znf-37	Transc Factors		

NCU08049	hypothetical protein		Transc Factors		
NCU08050	hypothetical protein		Morph/Hyph		
NCU08055	zip-like-1	zip-1	Transc Factors	Binding	Response to Stimulus/ Cellular Process/ Metabolic Process
NCU08063	kinetochore protein-18	kpr-18	Transc Factors	Catalytic Activity	Cellular Process/ Metabolic Process
NCU08093	hypothetical protein		Morph/Hyph	Transporter Activity/Catalytic Activity	Cellular Component Organization or Biogenisis/ Cellular Process/ Metabolic Process
NCU08147	Na or K P-type ATPase	ph7	Morph/Hyph	Transporter Activity/Catalytic Activity	Cellular Process/ Metabolic Process
NCU08148	H+/nucleoside cotransporter		*E. coli* CS Orth	Transporter Activity	Localization Process/ Cellular Process
NCU08225	high affinity nickel transporter nic1	trm-34	Morph/Hyph		
NCU08289	DNA methylation modulator-2	dmm-2	Transc Factors	Binding	
NCU08290	Ku70/Ku80 family protein	mus-51	*E. coli* CS Orth	Binding	Biological Regulation/Cellular Compoonent Organization or Biogenisis/Response to Stimulus/ Cellular Process/Metabolic Process
NCU08443	hypothetical protein		Transc Factors		
NCU08516	aldose 1-epimerase	aep-1	*E. coli* CS Orth	Catalytic Activity	Metabolic Process
NCU08634	hypothetical protein		Transc Factors		
NCU08652	hypothetical protein		Transc Factors		
NCU08726	fluffy	fl	Transc Factors		

NCU08741	Hyphal anastamosis protein 3	ham-3	Morph/Hyph		
NCU08744	hypothetical protein		Transc Factors		
NCU08791	catalase-1	cat-1	Morph/Hyph	Antioxidant Activity/Binding/Catalytic Activity	Response to Stimulus/ Cellular Process/ Metabolic Process
NCU08848	hypothetical protein		Transc Factors		
NCU08875	Cullin binding protein CanA		Morph/Hyph		Cellular Component Organization or Biogenisis/ Metabolic Process
NCU08891	hypothetical protein		Transc Factors		Cellular Process
NCU08899	hypothetical protein		Transc Factors		
NCU08901	hypothetical protein		Transc Factors		
NCU08927	dihydroceramide delta(4)-desaturase	dcd	Morph/Hyph	Catalytic Activity	Cellular Process/ Metabolic Process
NCU08992	hypothetical protein		Morph/Hyph	Binding	Cellular Component Organization or Biogenisis/ Cellular Process/ Metabolic Process
NCU09033	zinc finger transcription factor-46		Transc Factors		
NCU09068	nitrogen catabolic enzyme regulatory protein	nit-2	Transc Factors		
NCU09071	AGC/NDR protein kinase	dbf2	Morph/Hyph	Catalytic Activity	Biological Regulation/Cellular Compoonent Organization or Biogenisis/Response to Stimulus/ Cellular Process/Metabolic Process

NCU09123	Ca/CaM-dependent kinase-1	camk-1	Morph/Hyph		
NCU09201	hypothetical protein		Morph/Hyph		
NCU09205	nitrate assimilation regulatory protein nirA	vad-6	Transc Factors		
NCU09248	transcription factor-27	tcf-27	Transc Factors		
NCU09252	hypothetical protein		Transc Factors		
NCU09315	phosphorus acquisition-controlling protein	nuc-1	Transc Factors		
NCU09333	Zinc finger transcription factor ace-1	ace-1	Transc Factors		
NCU09364	Hsp30-like protein	hsp30	Morph/Hyph		Response to Stimulus/ Metabolic Process
NCU09423	secreted protein related to phopholipase A2		Morph/Hyph		
NCU09450	26S proteasome regulatory subunit Rpn2	rpn-2	Morph/Hyph	Catalytic Activity	Cellular Process/Metabolic Process
NCU09494	hypothetical protein		Morph/Hyph		
NCU09529	hypothetical protein		Transc Factors	Binding	Cellular Process/Metabolic Process
NCU09549	zinc finger transcription factor-51	znf-51	Transc Factors		
NCU09655	hypothetical protein		Morph/Hyph		
NCU09739	all development altered-7	fld	Transc Factors		
NCU09804	zinc finger transcription factor-43	znf-43	Transc Factors		
NCU09829	hypothetical protein		Transc Factors		
NCU09842	mitogen-activated protein kinase MKC1	mak-1	Morph/Hyph	Signal Transducer Activity/ Catalytic Activity	Biological Regulation/ Response to Stimulus/ Cellular Process/ Metabolic Process
NCU09866	thyroid hormone receptor interactor 12		Morph/Hyph		
NCU09882	metacaspase-1A	mcp-1	Morph/Hyph		
NCU10006	hypothetical protein		Transc Factors		

Figure 1: Conventional growth vs cold shock in wild-type and mutant Neurospora:
A) Wild-type (Oak Ridge) Neurospora growth at 33ºC, B) cold shock response in wild-type Neurospora, While many of the knockout strains tested displayed a morphological response to cold shock indistinguishable from that of wild-type, alternative morphologies were observed. These were classified into categories, examples of which are shown here. Examples of the alternate cold shock phenotypes displayed with the identity of the mutant shown as the example are shown: C) Burst: tips of growing hypha burst commonly (NCU02133, superoxide dismutase-1), D) Fail: a failure to display any morphological response to cold shock (NCU02636, peroxin-4), E) Thin: hyphal diameter narrows on cold shock (NCU03013, anchored cell wall protein-10), F) Dense: apical branching tighter than that normally displayed during cold shock (NCU07617, aconidiate-3), G) Cot-like: phenotype resembles that seen at the restrictive temperature of a temperature-sensitive colonial (cot) mutant strain. (NCU03901, peroxin-14), H) Weak: apical branching during cold shock which is less dense than normally observed (NCU01408, COP9 signalosome-3). Combinations of the above were sometimes observed as noted in Table 1.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