Transcript Profiling in the Barley Mildew Pathogen *Blumeria graminis* by Serial Analysis of Gene Expression (SAGE)

Stephen W. Thomas,^{1,3} Mikkel A. Glaring,^{1,2} Søren W. Rasmussen,^{1,2} Julia T. Kinane,¹ and Richard P. Oliver³

¹Department of Physiology, Carlsberg Laboratory, Gamle Carlsberg Vej 10, Copenhagen Valby, DK-2500, Denmark;

²Department of Yeast Genetics, Carlsberg Laboratory, Gamle Carlsberg Vej 10, Copenhagen Valby, DK-2500, Denmark;

³Australian Centre for Necrotrophic Fungal Pathogens, Division of Science and Engineering, Murdoch University, WA 6150, Australia

Submitted 29 October 2001. Accepted 13 April 2002.

The fungal pathogen *Blumeria graminis* f. sp. *hordei* develops on the barley leaf via distinct, morphologically well-defined stages. After landing on a host plant, the conidia rapidly germinate to form a primary germ tube. Subsequently, an appressorial germ tube emerges from the conidium and differentiates an appressorium from which penetration of the host cell wall is attempted. We have used serial analysis of gene expression to provide a measurement of messenger RNA contents in ungerminated conidia, during conidial germination, and during appressorium formation. The resulting data provide a resource for the characterization of changes in transcript accumulation during early development of *B. graminis*.

Filamentous fungi impact numerous aspects of human activities. Some species provide enzymes for industrial use and food preparation. Others cause devastating diseases of agricultural crops, animals, and humans. Some produce antibiotics, or serve as model organisms for scientific research. Therefore, it is surprising that large-scale characterization of mRNA levels in filamentous fungi has not been performed. The present analysis depicts the characterization of mRNA abundance of individual genes during early development of the obligate, filamentous fungal plant pathogen *Blumeria* (syn. *Erysiphe*) *graminis* (DC.) Speer f. sp. *hordei*, the causal agent of barley powdery mildew.

Much of the current knowledge regarding events surrounding conidial germination and appressorium differentiation comes from studies of facultative plant pathogens. Analysis of gene deletion mutants has elucidated the roles of several genes and environmental factors in early development (Dean 1997; Hamer and Talbot 1998; Howard and Valent 1996; Mendgen et al. 1996; Xu 2000). Large-scale transcription profiling during conidial germination and appressorium formation are, however, entirely lacking.

Barley powdery mildew is caused by *B. graminis*, an obligate pathogen requiring the host for completion of its life cycle. Upon contact with a solid surface, the conidia produce a primary germ tube, whose role appears to be to sense the suitability of the surface for further fungal development (Kinane et al. 2000). On inductive surfaces, an appressorial germ tube

emerges that subsequently differentiates into the functional appressorium from which penetration of the underlying host cell is attempted. The characterization of gene expression and function in obligate pathogens is difficult due not only to an inability to recover nonpathogenic mutants, but also because the isolation of suitably defined material is problematic. Fortunately, some cellulose membranes support the efficient formation of *B. graminis* appressoria (Kinane et al. 2000; Kobayashi et al. 1991), allowing the preparation of suitable experimental material in the absence of contaminating plant tissue. Appressoria formed in vitro are known to be functional through the observation of penetration pores in cellulose membranes directly below the appressorial structures (Kobayashi et al. 1991). We have measured transcript abundance during *B. graminis* germination and appressorium development on inductive cellulose membranes using the serial analysis of gene expression (SAGE) procedure (Velculescu et al. 1995). Here, we analyze approximately 60,000 tags isolated from ungerminated conidia, germinating conidia, and appressoria of *B. graminis*. This work represents the first large-scale quantification of individual transcripts during development of a pathogenic fungus.

RESULTS

Analysis of gene number and expression during development.

Sequencing of approximately 20,000 tags from each of three SAGE libraries, representing ungerminated conidia, germinating conidia with a primary germ tube, and conidia with developing appressoria, identified 18,691 different 14-base tags. Ambiguous tags that were present only once were removed from the analysis as described (Lash et al. 2000). Seven tags that may have been derived from mispriming of residual ribosomal RNA were also removed from the analysis. This process identified 6,336 different tags that could be assumed to represent unique transcripts.

Statistical analyses of the differences in the number of specific tags in the three populations were performed and tags whose frequency changed significantly at the 95% confidence level between the three developmental stages were enumerated (Table 1). The determination of statistically robust (confidence interval 95%) alteration in specific tag frequencies, given that a total of approximately 20,000 tags were sequenced in each of three populations, requires that the representative tag must be identified a minimum of six times in at least one of the popula-

Corresponding author: R. P. Oliver, Telephone: +61 (8) 9360 7404; Fax: +61 (8) 9360 6303; E-mail: roliver@central.murdoch.edu.au.

tions studied. A total of 916 tags met the above criteria. In all, 463, 563, and 524 tags were present in the ungerminated conidia, germinated conidia, and appressorial populations, respectively, in six or more copies, indicating that many transcripts were abundant in more than one of the developmental stages under investigation. Totals of 106 and 94 tags displayed significantly increased frequency during germination and appressorial development, respectively, (Table 1.). A similar number of tags displayed a significant reduction in frequency during germination and appressorium development (82 and 105, respectively). It is important to note that the statistical analysis of the data allows inferences to be made only about the significance of observed differences in tag number from one population to another. Hence, the statistical analysis of a tag with a count of five tags in ungerminated conidia, one tag in germinated conidia, and eight tags in appressoria allows the conclusion of a significant increase in the transition from germination to appressorial development. It does not allow an inference to be made about the relative frequency of occurrence in ungerminated conidia, only that it is not significantly different from the level in germinated conidia or appressoria.

Mapping of tags to genes.

Comparison of the SAGE tags with the available expressed sequence tag (EST) sequences for *B. graminis* (Thomas et al. 2001) has allowed the assignment of 1,274 tags (20%) to sequenced EST clones. Of the 1,669 unique *B. graminis* ESTs, 80% of the representative tags have been detected in the SAGE analysis. Clearly, genes with abundant transcripts are more likely to be represented in both SAGE profiles and EST databases.

The 20 most frequently sequenced tags from each of the developmental stages studied and their corresponding EST clone are presented in Table 2. Many of the genes identified have high levels of mRNA present in all three stages studied, suggestive of an ongoing requirement for these gene products during development.

Statistical analysis of differential gene expression.

A total of 394 different tags displayed a significant change in the frequency of occurrence during the transition from the ungerminated conidium into an appressorial infection structure. The individual tags that have linked ESTs are listed in Table 3. This analysis identifies 183 genes displaying a flux in mRNA quantity between at least two of the stages analyzed. Blast analysis (Altschul et al. 1990, 1997; Thomas et al. 2001) identified putative homologues of 126 of these genes in other organisms; 57 genes have no previously characterized homologue.

Comparison to known expression profiles.

Prior to this study, there had been few attempts to measure the expression of individual genes during development of *B. graminis*. The quantification of transcripts of two chitin synthase genes, *BgChs1* and *BgChs2*, has been determined by semiquantitative reverse transcription polymerase chain reaction (RT-PCR), which indicated that *BgChs1* mRNA accumulated during germination and decreased slightly during appres-

sorium formation (Zhang et al. 2000). *BgChs2* had a similar profile but at a much lower transcript abundance. The occurrence of the tag for *BgChs1* in the SAGE profiles, albeit in low numbers (ungerminated, three tags; germinated, nine; appressoria, four), and the absence of a tag for *BgChs2* are entirely consistent with the published transcript analysis (Zhang et al. 2000). RT-PCR is evidently a more sensitive technique than SAGE (on 20,000 tags) for the detection of mRNA of individual genes.

Northern analysis data is available for glyceraldehyde 3-phosphate dehydrogenase (*Gpd*), *gEgh7*, and *gEgh16* (Justesen et al. 1996). *gEgh7* originally was reported to be a single copy gene, although we have since identified one other homologue, *gEgh7.2* (Thomas et al. 2001). *gEgh16* represents a small gene family comprising *gEgh16*, *gEgh16.2*, and *gEgh16.3*. Furthermore, the original study utilized spores germinated on glass pates, a noninductive surface. Insofar as the data are comparable, the trends in the SAGE data are consistent with the earlier Northern data. These results highlight the facility of SAGE to separate transcript profiles of individual members of gene families. Transcripts of *gEgh16* homologues recently have been shown to be abundant in appressoria of *Magnaporthe grisea* (Kamakura et al. 1999), suggesting that *gEgh16* has a role in appressorium maturation.

DISCUSSION

Array-based hybridization (Schena et al. 1995) and SAGE (Velculescu et al. 1995) represent the main techniques for high-throughput transcriptional profiling. Both techniques correlate well in terms of determining absolute levels of specific mRNAs as well as the identification of differential mRNA accumulation in different materials (Ishii et al. 2000). We chose the SAGE technique because it can measure the abundance of all transcripts with precision and because SAGE data is generated in a reusable, digital format from uncharacterized genes and is thus particularly suited to nonmodel systems and to collaborative studies. The data is viewed as a resource, allowing current and future researchers to rapidly assess particular transcript abundance during early development of newly identified genes arising from both EST and genome sequencing data. This is particularly important in light of continued sequencing of ESTs during development of the mildew colony and the ongoing plans for genome sequencing of *B. graminis*. (Eckert et al. 2001; M. Glaring, *unpublished data*). Thus far, we have analyzed the data against an EST database currently comprising 1,669 unigene sequences (Thomas et al. 2001). This preliminary analysis identifies some of the genes having changes in mRNA content during development and therefore gives an insight into some of the processes that may be important during infection structure formation. While 20% of tags are identified in the current EST analysis, ongoing sequencing efforts will undoubtedly allow more identifications to be made. In addition, the genes corresponding to unknown tags with interesting patterns of transcript accumulation may be rapidly isolated using one of the current PCR methods of tag-to-gene cloning (Polyak et al. 1997; Yu et al. 1999).

Identification of genes from SAGE tags.

Of the 1,669 EST sequences that are available, 1,274 were identified as SAGE tags. Identification of the most sequenced tags from each developmental stage (Table 2) showed that the majority displayed high transcript abundance in all three stages examined. The most sequenced tag was representative of a putative metallothionein, a protein associated with oxidative and heavy metal stress (Andrews 2000). Metallothionein homologues have been identified as specifically expressed

Table 1. Numbers of tags displaying significant changes in transcript abundance during germination and development of the appressorium^a

Transcriptional regulation	Ungerminated → germinated conidia	Germinated conidia → appressoria
Up	106 (57)	94 (46)
Down	82 (34)	105 (51)

^a Numbers in parentheses are tags with corresponding genes in the *Blumeria* expressed sequence tag database (Thomas et al. 2001).

during appressorium formation in *Colletotrichum gloeosporioides* (Hwang and Kolattukudy 1995). The presence of a heat-shock protein and a cytochrome p450 gene support the view that stress responses are fully activated during development. Other genes with considerable transcript levels, as judged by SAGE, are involved in protein synthesis, modifica-

tion, and degradation that may indicate that early germling development involves rapid protein synthesis and degradation (McCafferty and Talbot 1998).

Of the 1,274 genes with linked ESTs, 183 had significant changes in transcript abundance during development. These genes have been divided among 10 classes representing different

Table 2. Identification of the twenty genes with the highest transcript levels in each of the developmental stages studied^a

Tag	GenBank accession no.	Putative homologue	Ug	Ge	Ap
Ungerminated conidia					
AAGGGAGGAT	AW791711	Metallothionein	286	209	501
CCGCAGGGCT	AW790864	Subtilisin-like serine protease	159	135	212
GCTAACGCAG	AW791080	Peptidyl-prolyl <i>cis-trans</i> isomerase	158	143	217
TCACCCCACT	AW791647	Novel EST (D00719)	156	88	129
AATTAGCTAA	AW791518	Glucose-repressible gene	113	70	46
TCAATTCTCT	AW791559	Elongation factor 1- α	111	117	115
TGTCATAGCT	AW790559	Retrotransposon	95	144	86
TCGAAGGGTG	AW788687	GEL1 protein	70	49	75
ATACCAAAAT	AW791955	30-kDa heat shock protein	63	10	3
GCTGCAACAG	...	Unknown	61	0	0
GAGGTCGGGC	AW791580	Male sterility 2-like protein	58	86	112
GCACTATTGA	AW788796	<i>Mycosphaerella graminicola</i> EST AW181057	56	91	141
TTAGTCTGTT	AW792295	Novel EST (D01117)	55	16	14
CTTCGATGGT	AW791136	Mannosyl-oligosaccharide α -1,2-mannosidase	55	25	86
CGAGCGGTGA	AW791967	Ubiquitin conjugating enzyme E2-16 kDa	53	77	67
ATGGGTGGAC	AW792426	Related to chitinase 3	52	45	90
GGGTTGCAAA	AW792221	<i>Aspergillus nidulans</i> EST AI211830	51	15	16
TCCCTATTAA	...	Unknown	49	12	6
GAGCGCGTGA	AW790920	Isotrichodermin C15 hydroxylase	49	13	36
CGTGGGGATA	AW791719	Cytochrome p450-like TBP	49	63	70
Germinated conidia					
AAGGGAGGAT	AW791711	Metallothionein	286	209	501
TGTCATAGCT	AW790559	Retrotransposon	95	144	86
GCTAACGCAG	AW791080	Peptidyl-prolyl <i>cis-trans</i> isomerase	158	143	217
CCGCAGGGCT	AW790864	Subtilisin-like serine protease	159	135	212
TTTCAATCTG	AW790713	Lipase 2 precursor	32	134	135
AAAAAAAAAA	...	Unidentifiable	41	131	21
TCAATTCTCT	AW791559	Elongation factor 1- α	111	117	115
GCACTATTGA	AW788796	<i>Mycosphaerella graminicola</i> EST AW181057	56	91	141
TCACCCCACT	AW791647	Novel EST (D00719)	156	88	129
GAGGTCGGGC	AW791580	Male sterility 2-like protein	58	86	112
CAGAGAGGCA	AW789769	Novel EST (C01328)	44	85	41
GTGAAATCCG	AW790071	gEgh7.2	27	80	84
CGAGCGGTGA	AW791967	Ubiquitin conjugating enzyme E2-16 kDa	53	77	67
GGTTTCGGGC	AW788286	60s ribosomal protein p2	46	74	52
ACACCTAGAT	AW789656	Enolase	39	73	13
AATTAGCTAA	AW791518	Glucose-repressible gene	113	70	46
TCTACATAG	AW787954	Retrotransposon	23	67	32
CGTGGGGATA	AW791719	Cytochrome p450-like TBP	49	63	70
ATATAGGCGG	AW791010	Novel EST (D00348)	10	50	31
TCGAAGGGTG	AW788687	GEL1 protein	70	49	75
Appressoria					
AAGGGAGGAT	AW791711	Metallothionein	286	209	501
GCTAACGCAG	AW791080	Peptidyl-prolyl <i>cis-trans</i> isomerase	158	143	217
CCGCAGGGCT	AW790864	Subtilisin-like serine protease	159	135	212
CGCGCAATGA	AW788949	<i>Neurospora crassa</i> EST AI397742	16	27	149
TGTGTGCGCG	AW791142	Myb-like transcription factor	14	27	142
GCACTATTGA	AW788796	<i>Mycosphaerella graminicola</i> EST AW181057	56	91	141
TTTCAATCTG	AW790713	Lipase 2 precursor	32	134	135
TCACCCCACT	AW791647	Novel EST (D00719)	156	88	129
TCAATTCTCT	AW791559	Elongation factor 1- α	111	117	115
GAGGTCGGGC	AW791580	Male sterility 2-like protein	58	86	112
GGCCCATAGG	AW789547	Novel EST (C01191)	34	34	91
ATGGGTGGAC	AW792426	Related to chitinase 3	52	45	90
CTCGGCCAG	AW791162	<i>Hex1</i>	12	44	89
TGTCATAGCT	AW790559	Retrotransposon	95	144	86
CTTCGATGGT	AW791136	Mannosyl-oligosaccharide α -1,2-mannosidase	55	25	86
GTGAAATCCG	AW790071	gEgh7.2	27	80	84
TCGAAGGGTG	AW788687	GEL1 protein	70	49	75
TGACCACGTT	AW791307	Novel EST (D00580)	33	48	72
CGTGGGGATA	AW791719	Cytochrome p450-like TBP	49	63	70
CGAGCGGTGA	AW791967	Ubiquitin conjugating enzyme E2-16kD	53	77	67

^a Homology is based on expect values of BlastX, BlastN, or tBlastX matches $P(N) \leq 1e^{-5}$. EST = expressed sequence tag. Number of tags for ungerminated conidia (Ug), germinated conidia (Ge), and appressoria (Ap) are given.

Table 3. *Blumeria graminis* genes displaying significant changes in transcript abundance during germination and appressorium differentiation^a

Tag sequence ^b	GenBank accession no.	Homology ^c	Putative function ^d	Ug	Ge	Ap	TP
Increase, both^e							
Clones with homologues only in pathogenic fungi							
GCACTATTGA	AW788796	<i>Mycosphaerella graminicola</i> EST AW181057	...	56	91	141	1
Clones with homologues only in fungi							
CTCGGCCAG	AW791162	20-kDa subunit V-ATPase (Hex1)	Cell structure	12	44	89	2
Clones with homologues in fungi and other organisms							
TGTGTGCGCG	AW791142	Myb-like transcription factor	Transcription	14	27	142	3
Increase, Ge^f							
Clones with homologues only in pathogenic fungi							
ACACCAACAA	AW790842	Infected barley EST BE215687	...	17	34	23	1
AGTGCACCTA	AW791209	Retrotransposon	Transposon	13	31	23	2
CAAGCGAATA*	AW787876	<i>Magnaporthe grisea</i> EST AI068602	...	24	45	45	3
GTGAAATCCG	AW790071	gEgh7.2 protein	...	27	80	84	4
Clones with homologues only in fungi							
AAGATGAGTT*	AW788544	<i>Aspergillus nidulans</i> EST AA785380	...	1	9	6	5
GACAGAAATC*	AW788989	Flavoprotein	Electron trans.	4	14	9	6
GATAGTCTTA	AW791824	Ste12 homologue	Transcription	0	7	5	7
TAAATAGATT	AW791476	Rasp f 7	...	2	16	7	8
TTTCAATCTG	AW790713	Lipase 2 precursor	Fatty acid met.	32	134	135	9
Clones with homologues in fungi and other organisms							
AAAAGCGAAA	AW792122	S-adenosylmethionine synthetase	Amino acid met.	1	12	12	10
AAGTTGGTTG	AW788464	60S ribosomal protein Rp23	Protein syn.	6	20	22	11
AATTATGGGC	AW791241	<i>Triticum aestivum</i> EST BE402096	...	7	19	12	12
ACCCAGAAG*	AW788491	NADH-ubiquinone oxidoreductase	Electron trans.	0	6	2	13
ACTTGAGCCT	AW792262	F1n21.11; similar to glyoxal oxidase	...	19	38	47	14
ATGCTTCTCA	AW789444	40S ribosomal protein S12	Protein syn.	3	12	6	15
ATTGCAGCTG*	AW792127	Pyruvate dehydrogenase E1 component	Glycolysis	2	13	9	16
CGAGCGGTGA	AW791967	Ubiquitin conjugating enzyme	Proteolysis	53	77	67	17
GAGGTCGGGC	AW791580	Male sterility 2-like protein	Cell structure	58	86	112	18
GCATCTTTCT	AW790787	Saccharopine dehydrogenase	Amino acid met.	6	17	9	19
GTCAGTTTTA*	AW788571	Glycogen debranching enzyme	Metabolism	3	12	10	20
TAAAGCCTGC	AW789051	Ribosomal protein L27	Protein syn.	0	6	5	21
TGCTGTCGAA*	AW788777	Plasma membrane H ⁺ -ATPase	Transport	2	11	4	22
TGTCAGCGGA	AW792301	186K protein (CgMMV)	...	0	6	14	23
TTAAATTGGC	AW788299	ADP, ATP carrier protein	Transport	4	21	14	24
TTGGTCTTGG	AW791393	40S ribosomal protein S4	Protein syn.	4	15	13	25
Clones with no known homologues							
AAGCGAATTA	AW789659	Novel EST (C01261)	...	0	6	2	26
AATCCTTCTT	AW790162	Novel EST (C01556)	...	7	23	18	27
ACACTCTTTT	AW792729	Novel EST (D01357)	...	22	41	35	28
CGGAGCTTCC	AW791331	Novel EST (D00478)	...	13	29	45	29
CTCGGATTTG	AW791259	Novel EST (D00555)	...	5	18	10	30
GAAGTGTAGT	AW790551	Novel EST (D00034)	...	8	22	14	31
GGGCGTTATT*	AW789271	Novel EST (C01025)	...	0	8	2	32
TGGTTCGTCT	AW792527	Novel EST (D01245)	...	0	6	1	33
TTACCTGGCG*	AW790603	Novel EST (D00063)	...	9	26	14	34
Increase, Ug to Ap^g							
Clones with homologues only in pathogenic fungi							
ACCCAGCTTT*	AW788054	Retrotransposon	Transposon	0	2	6	1
CAAAATGCAG*	AW792327	gEgh16.2 protein	...	9	12	24	2
GGCCAACTTG*	AW788630	Retrotransposon	Transposon	2	5	12	3

(continued on next page)

^a Number of tags for ungerminated conidia (Ug), germinated conidia (Ge), and appressoria (Ap) are given. The transcript profile (TP) divides changes in transcriptional abundance into one of 10 profiles as described.

^b Tags matching clones lacking a polyA tail are labeled with an asterisk.

^c Homology is based on expect values of BlastX, BlastN, and tBlastX matches of less than e^{-5} ; EST = expressed sequence tag. Differences in transcript levels are significant at the 95% ($P \leq 0.05$) confidence interval.

^d Electron trans. = electron transport, met. = metabolism, syn. = synthesis, degrad. = degradation, mod. = modifications, FA = fatty acid, chrom. = chromosome.

^e Genes displaying a significant increase in transcript abundance both during germination and during appressorium formation.

^f Genes displaying a significant increase in transcript abundance during germination only.

^g Genes displaying a significant increase in transcript abundance between ungerminated conidia and appressoria but not attributable to germination or appressorium formation only.

^h Genes displaying a significant increase in transcript abundance during appressorium formation only.

ⁱ Genes displaying a significant increase in transcript abundance during germination followed by a significant decrease during appressorium formation.

^j Genes displaying a significant decrease in transcript abundance during germination followed by a significant increase during appressorium formation.

^k Genes displaying a significant decrease in transcript abundance during appressorium formation only.

^l Genes displaying a significant decrease in transcript abundance between ungerminated conidia and appressoria but not attributable to germination or appressorium formation only.

^m Genes displaying a significant decrease in transcript abundance during germination only.

ⁿ Genes displaying a significant decrease in transcript abundance both during germination and during appressorium formation.

Table 3. (continued from preceding page)

Tag sequence ^b	GenBank accession	Homology ^c	Putative function ^d	Ug	Ge	Ap	TP
GTAAGATGCA	AW789153	gEgh16 protein	...	0	4	11	4
TTACACCAA*	AW788481	Acid proteinase Eapc	Protein degrad.	0	3	6	5
Clones with homologues only in fungi							
GGCGAAGGGT	AW792470	Hypothetical protein (<i>S. pombe</i>)	...	2	5	10	6
Clones with homologues in fungi and other organisms							
AGTCAACGAA	AW789369	<i>Sorghum bicolor</i> EST BE362624	...	3	6	13	7
CGGTCCTGGA*	AW790176	Calnexin	Protein mod.	0	4	6	8
GAGGTCCCCG*	AW791952	Pol polyprotein	Transposon	2	7	12	9
GGCCGGATGG	AW790637	Serine/threonine protein kinase	Signaling	5	7	15	10
GGGCGGTGTC	AW790400	ORF g (<i>Drosophila</i> sp.)	...	2	6	10	11
Clones with no known homologues							
AAATGCATCG	AW789908	Novel EST (C01409)	...	0	2	9	12
Increase, Ug to Ap^g							
AGATAAGGGG*	AW792557	Novel EST (D01262)	...	1	3	10	13
AGCTGGCGCC	AW791652	Novel EST (D00722)	...	1	2	8	14
CATCGCGGAC	AW790517	Novel EST (D00015)	...	4	7	15	15
CGACAGGGAT	AW789916	Novel EST (C01414)	...	0	1	6	16
GCTGCACGCT	AW792218	Novel EST (D01075)	...	13	19	27	17
GTGAATGCGT	AW787981	Novel EST (C00012)	...	3	6	12	18
TTGGAGCAGG*	AW788004	Novel EST (C00031)	...	12	19	29	19
TTGGCTTCAG	AW791104	Novel EST (D00401)	...	3	8	12	20
Increase, Ap only^h							
Clones with homologues only in pathogenic fungi							
AGTGCTCTTC*	AW788879	Retrotransposon	Transposon	17	18	40	1
GACCGCTTGG*	AW791069	<i>Metarrhizium anisopliae</i> EST AJ273279	...	14	19	43	2
GGAGTTTGAA*	AW789327	gEgh16.3 protein	...	1	0	8	3
Clones with homologues only in fungi							
CGCGCAATGA*	AW788949	<i>Neurospora crassa</i> EST AI397742	...	16	27	149	4
GCTATCCGTG	AW791931	<i>Aspergillus nidulans</i> EST AA785675	...	0	0	7	5
TCCAAGGGTG	AW790942	GEL 1	Cell structure	70	49	75	6
Clones with homologues in fungi and other organisms							
ATGGGTGGAC	AW792426	Related to chitinase 3	Cell structure	52	45	90	7
CAGGTCAAGT	AW791038	Succinyl-CoA:3-ketoacid-CoA transferase	Lipid/FA met.	13	6	25	8
CCCATTTAAC*	AW791418	Synaptic glycoprotein SC2 spliced variant	...	1	0	9	9
CCGCAGGGCT	AW790864	Subtilisin-like serine protease	Protein mod.	159	135	212	10
CGGACCAAAG*	AW788593	Histone H3	Chrom. structure	5	6	21	11
GCCGCGGTAT	AW790645	3-ketoacyl-CoA thiolase	Fatty acid met.	5	7	17	12
GCGCGTCAAT*	AW789921	Triosephosphate isomerase	Glycolysis	24	25	47	13
GCTAACGCAG	AW791080	Peptidyl-prolyl <i>cis-trans</i> isomerase	Protein mod.	158	143	217	14
GGCCTGGCTC*	AW788163	Serine proteinase Stubble	Protein mod.	16	12	25	15
TGTGGGACGA	AW788278	Chitinase	Cell structure	1	3	12	16
Clones with no known homologues							
AAGTCGATCG	AW788723	Novel EST (C00658)	...	5	9	37	17
ACAGGACAAA	AW792757	Novel EST (D01374)	...	18	27	49	18
ATCAAGTGAC	AW790594	Novel EST (D00057)	...	6	14	30	19
ATCTTGCCG	AW788016	Novel EST (C00041)	...	1	0	6	20
CACAATTTCC	AW792144	Novel EST (D01030)	...	0	0	6	21
CCCTCTGGGT*	AW790222	Novel EST (C01591)	...	2	0	7	22
CTCGACGGAG	AW789531	Novel EST (C01180)	...	9	16	46	23
GAAGCTGGGA	AW790697	Novel EST (D00133)	...	11	12	37	24
GCGGCTTGCT	AW789993	Novel EST (C01455)	...	2	7	20	25
GCTGGACAGG	AW791982	Novel EST (D00936)	...	30	30	53	26
GGATACACTG	AW792383	Novel EST (D01163)	...	5	9	27	27
GGCCCATAGG	AW789547	Novel EST (C01191)	...	34	34	91	28
GGGATGGGTA	AW792418	Novel EST (D01183)	...	0	0	9	29
GTCCGCTTTC*	AW791182	Novel EST (D00447)	...	6	7	18	30
TATCGGCGCT	AW791253	Novel EST (D00552)	...	31	19	38	31
TGACCACGTT	AW791307	Novel EST (D00580)	...	33	48	72	32
TTACTCCACT	AW792226	Novel EST (D01079)	...	16	26	50	33
Increase, decreaseⁱ							
Clones with homologues only in pathogenic fungi							
TATGAAATTA	AW790659	<i>Mycosphaerella graminicola</i> EST AW179988	...	23	40	1	1
TCCTACATAG*	AW787954	Retrotransposon	Transposon	23	67	32	2
TGTCATAGCT	AW790559	Retrotransposon	Transposon	95	144	86	3
Clones with homologues only in fungi							
TAAAGTTGAT	AW791231	Sterigmatocystin biosynthesis p450 monooxygenase	Secondary met.	6	18	7	4
Clones with homologues in fungi and other organisms							
ACAAAAA	AW788980	60S ribosomal protein Y16	Protein syn.	7	21	6	5
ACACCTAGAT	AW789656	Enolase	Glycolysis	39	73	13	6
AGTGTTACAC*	AW787959	Bip protein	Protein mod.	5	34	15	7

(continued on next page)

patterns of mRNA flux (Table 3). Approximately 60% of these genes have functionally characterized homologues from other organisms, giving an insight into their putative roles in mildew development. As more EST and genome sequences becomes available, it is envisaged that many of the ESTs of unknown function will have identifiable homologues in pathogenic or nonpathogenic filamentous fungi.

It is evident that genes encoding proteins with associated functions tend to display similar fluxes in mRNA content. The genes for several ribosomal proteins fall into classes B, E, and G. Overall, this may imply an increased demand for protein synthesis during germination and a subsequent decline during further development. In *Aspergillus nidulans*, cloning of genes with increased accumulation of mRNA during conidial germination compared with mycelial growth resulted in the isolation

of 10 cDNAs, all of which encoded ribosomal proteins (d'Enfert 1997).

The supply of glycerol and conversion of stored energy during conidial germination and host penetration has been the subject of recent study (Thines et al. 2000). Several catabolic pathways are represented in the SAGE analysis. Increased presence of transcript for a glycogen debranching enzyme (B20, Table 3) during germination is suggestive of the breakdown of glycogen. Three glycolytic enzymes, enolase (E6, Table 3), fructose biphosphate aldolase (E11, Table 3), and pyruvate kinase (E15, Table 3), display increasing mRNA levels during germination that subsequently fall during appressorium formation. In addition, lower quantities of mRNA for glyceraldehyde 3-phosphate dehydrogenase are detected during appressorium formation (G19, Table 3), whereas one of the com-

Table 3. (continued from preceding page)

Tag sequence ^b	GenBank accession	Homology ^c	Putative function ^d	Ug	Ge	Ap	TP
ATGGAAGTAA	AW789651	Ribosomal protein L14	Protein syn.	14	28	11	8
CAATGGAATT	AW790608	40s ribosomal protein S17	Protein syn.	9	24	9	9
GCGAACATTG	AW788822	Peroxisomal hydratase-dehydrogenase-epimerase	Fatty acid met.	12	24	10	10
GGAAATAGAG	AW790281	Fructose-bisphosphate aldolase	Glycolysis	13	32	12	11
GGTTTCGGGC	AW788286	60S ribosomal protein P2	Protein syn.	46	74	52	12
GTGTAACATA*	AW787924	ATP synthase γ chain	Electron trans.	3	13	2	13
TAGTAAATTT	AW789452	Mannosyl-oligosaccharide α -1,2-mannosidase	Protein mod.	10	23	3	14
TCATTTTCA	AW791226	Pyruvate kinase	Glycolysis	11	34	3	15
TTCTGGTACA*	AW789493	Protein disulphide isomerase	Protein mod.	9	23	5	16
Clones with no known homologues							
AAATAAAAAAC*	AW789299	Novel EST (C01043)	...	11	23	7	17
AACTTGAATT	AW790156	Novel EST (C01553)	...	1	8	0	18
ATATAGGCGG	AW791030	Novel EST (D00348)	...	10	50	31	19
CAGAGAGGCA*	AW789769	Novel EST (C01328)	...	44	85	41	20
Decrease, increase^j							
Clones with homologues only in fungi							
AAATACCCTG	AW791784	Cellulose growth specific protein	Host cell degrad.	32	16	38	1
AAGGGAGGAT	AW791711	Metallothionein	...	286	209	501	2
ACCAAACCTCA	AW790393	Ribosomal protein S28	Protein syn.	8	1	13	3
CCAACGTTTG	AW790498	Thioredoxin	Redox reactions	41	21	38	4
CTTCGATGGT*	AW791136	Mannosyl-oligosaccharide α -1,2-mannosidase	Protein mod.	55	25	86	5
Decrease, increase^j							
GAGCGCGTGA	AW790920	Isotrichodermin C-15 hydroxylase	Other met.	49	13	36	6
GTTCGAAGTTG*	AW790846	Peroxisomal membrane protein pmp20p	Detoxification	21	6	25	7
TATCGCGGTC*	AW789111	Phosphatidylserine synthase	Cell structure	8	1	8	8
Clones with no known homologues							
TCACCCCACT	AW791647	Novel EST (D00719)	...	156	88	129	9
TCCACTGGAT	AW791134	Novel EST (D00419)	...	46	28	53	10
Decrease, Ap only^k							
Clones with homologues only in fungi							
AAGAGAATTT	AW788489	Hypothetical protein (<i>S. pombe</i>)	...	2	6	0	1
CGAAAAAATA	AW791335	<i>Neurospora crassa</i> EST AA901986	...	9	12	2	2
GAAAAAATA	AW789324	Aut1	Transport	6	13	3	3
GATAAAAAGT*	AW792656	<i>Neurospora crassa</i> EST AW712490	...	30	30	10	4
GATCCTAGCT	AW790464	Hypothetical protein (<i>S. pombe</i>)	...	3	9	1	5
GTTCAATTTT*	AW789202	NADH-ubiquinone oxidoreductase	Electron trans.	1	6	0	6
TAAAACCTCT	AW792722	Tropomyosin	Cytoskeleton	5	13	3	7
TACTACATCT	AW790651	QDE2-gene silencing	Transcription	3	7	0	8
Clones with homologues in fungi and other organisms							
AAGGAAAGCG	AW790531	14-3-3 protein	Transcription	10	16	5	9
AATAGATGAA*	AW790370	Cyclophilin	Protein mod.	5	8	1	10
AATTTCAAAT	AW788912	Nucleoside diphosphate kinase	Metabolism	13	23	6	11
ACGCATTAAT	AW792002	Profilin A	Cytoskeleton	7	16	6	12
AGGTATAGTT	AW791459	Pyruvate carboxylase	Gluconeogenesis	18	29	9	13
ATATCGAACT	AW787872	60S ribosomal protein L22	Protein syn.	9	19	4	14
CAAAAAAATA	AW791903	GTP-binding nuclear protein GSP1/CNR1	Protein mod.	25	31	10	15
CACAGGCGTT	AW790930	GABA-specific permease	Transport	2	9	1	16
GACTCAATCT	AW792466	Serine hydroxymethyltransferase/ ABC transporter	Amino acid met. /detoxification	11	20	6	17
GATTGAAAAT*	AW789551	Catalase/peroxidase	Detoxification	9	15	2	18
GTACGATAAT	AW788939	Glyceraldehyde 3-phosphate dehydrogenase	Glycolysis	30	39	23	19
GTCCCTTACA	AW792607	Carboxypeptidase Y precursor	Protein mod.	18	29	11	20
TAGCTCATTG	AW790216	Myosin 2	Cytoskeleton	2	8	1	21

(continued on next page)

ponents of pyruvate dehydrogenase (B16, Table 3) displays an increase in transcript level during germination only. Together, the observed changes are indicative of an active participation of glycolysis during germination that is subsequently down-regulated during appressorium formation. An exception, the gene for triosephosphate isomerase (D13, Table 3), exhibits higher transcript abundance during appressorium differentiation only. This may reflect shunting of glycolytic intermediates to membrane synthesis or possibly glycerol formation suggested to be associated with the increased turgor pressure observed in the appressoria of *M. grisea* (de Jong et al. 1997). Conversely, decreasing transcript levels for two genes encoding enzymes associated with gluconeogenesis, pyruvate carboxylase (G13, Table 3) and fructose-1,6-bisphosphatase (H5, Table 3), during appressorial development is apparent. Transcripts of a lipase precursor (B9, Table 3) accumulate during

germination and appressorium formation, whereas two β -oxidation enzymes, succinyl CoA:3-ketoacid transferase (D8, Table 3) and 3-ketoacyl-CoA thiolase (D12, Table 3), show increases in mRNA content during the appressorial development phase only. Interestingly, succinyl CoA:3-ketoacid transferase is associated with ketone body catabolism. This suggests a possible active utilization of lipids in preference to carbohydrates in the later stages of germling development.

The *Hex1* gene of *Neurospora crassa* recently has been identified as a core component of the Woronin body (Jedd and Chua 2000). Woronin bodies have been observed in many filamentous fungi, including *B. graminis* (McKee 1971), and are thought to act in plugging septal pores after damage to hyphae in order to prevent uncontrolled leakage of the cytoplasm (Jedd and Chua 2000). The increased presence of transcripts of the *B. graminis Hex1* homologue observed during germination

Table 3. (continued from preceding page)

Tag sequence ^b	GenBank accession	Homology ^c	Putative function ^d	Ug	Ge	Ap	TP
TATTTTGAT	AW791728	Bleomycin hydrolase	Detoxification	1	7	0	22
TCGTAAAGGT	AW792640	Rho1	Signaling	18	17	6	23
TTGGCTCAAT	AW789848	40s ribosomal protein S5	Protein syn.	12	20	5	24
Clones with no known homologues							
ACACATTTT*	AW789718	Novel EST (C01294)	...	8	12	3	25
ATGATTTTCT	AW791805	Novel EST (D00816)	...	3	7	0	26
ATTTTCTCTC	AW791656	Novel EST (D00724)	...	3	7	0	27
GTAGAAAAAG	AW791721	Novel EST (D00765)	...	5	14	2	28
TAAAAAATA	AW791319	Novel EST (D00586)	...	10	10	2	29
TATTGGAAGT*	AW789289	Novel EST (C01036)	...	2	8	1	30
Decrease, Ug to Ap^l							
Clones with homologues only in pathogenic fungi							
ATATAATGTG	AW791976	Cap20 (<i>Colletotrichum gloeosporioides</i>)	...	17	9	6	1
Clones with homologues only in fungi							
CATTGAGGAA*	AW789717	<i>Aspergillus niger</i> EST BE759613	...	21	17	10	2
CCAAAAGCAT*	AW788721	NADH dehydrogenase	Electron trans.	7	5	0	3
Clones with homologues in fungi and other organisms							
AGGCAAACAG*	AW790435	Peptidyl-prolyl isomerase	Protein mod.	8	2	1	4
GTAAAGTTCA	AW792329	Fructose-1,6-bisphosphatase	Gluconeogenesis	15	11	3	5
GTTCTTGAGA	AW790515	MepB	Protein mod.	14	10	4	6
TATGCGCTCA	AW792070	Vacuolar ATP synthase subunit E	Transport	9	3	1	7
Clones with no known homologues							
ACAAAAAATC	AW791325	Novel EST (D00475)	...	10	5	2	8
GAAAGAAGAA	AW791461	Novel EST (D00604)	...	6	1	0	9
Decrease, Ge only^m							
Clones with homologues only in fungi							
ACAACCTGTA	AW791156	<i>Aspergillus nidulans</i> EST AI210287	...	26	8	7	1
CGCTGTAAAA	AW792085	<i>Neurospora crassa</i> EST AI319045	...	14	2	1	2
GAAGAATTAC	AW792399	<i>Neurospora crassa</i> EST AA898852	...	17	3	6	3
GGCAATTGCT	AW792032	<i>Neurospora crassa</i> EST AA898854	...	6	0	3	4
GGGTTGCAAA*	AW792221	<i>Aspergillus nidulans</i> EST AI211830	...	51	15	16	5
Clones with homologues in fungi and other organisms							
AATGAAGATG	AW791855	Spore wall maturation protein DIT1	Cell structure	11	1	1	6
ACCATTAGGA	AW791200	Heat shock protein Hsp1	Stress response	16	3	10	7
ATACCAAAAT	AW791955	30-kDa heat shock protein	Stress response	63	10	3	8
ATACCGAAAG*	AW790177	30-kDa heat shock protein	Stress response	20	5	2	9
CCCCAACAGA	AW792024	Type III alcohol dehydrogenase	Other met.	34	9	16	10
GACAGAATCA*	AW792532	Ice plant EST AW053761	...	37	4	6	11
GCTGCCGCGG*	AW791646	EFA27 for EF hand	Transcription	18	0	1	12
TCTACTGCGG	AW790429	α hemolysin	...	10	2	0	13
TGATAAAGCG	AW790588	D-xylose reductase II, III	Metabolism	29	12	18	14
TTGTACGCGA	AW792507	Unknown protein <i>Homo sapiens</i>	...	10	1	2	15
Clones with no known homologues							
AAGGGTGTA	AW792009	Novel EST (D00951)	...	36	10	17	16
ATAGACCGTT	AW792043	Novel EST (D00972)	...	19	7	9	17
ATATATCAAA	AW791629	Novel EST (D00709)	...	9	0	0	18
ATCGGCGTAT*	AW789601	Novel EST (C01224)	...	6	0	2	19
ATTAATGCGG	AW792338	Novel EST (D01140)	...	40	22	19	20
TCTGTGCAAA	AW791483	Novel EST (D00617)	...	9	1	4	21
TTAGTCTGTT	AW792295	Novel EST (D01117)	...	55	16	14	22
TTGATTACTC*	AW788433	Novel EST (C00443)	...	13	4	5	23
Decrease bothⁿ							
Clones with homologues only in fungi							
AATTAGCTAA	AW791518	Glucose-repressible gene	Signaling	113	70	46	1

and appressorium formation (A2, Table 3) is consistent with a putative role of Woronin bodies in blocking the septal pore between the conidium and the appressorium, allowing the accumulation of sufficient appressorial turgor pressure required for breaching of the host cell wall. The observation of a *Hex1* homologue in *M. grisea* (Jedd and Chua 2000), where host penetration results from massive turgor generation in the appressorium (Howard et al. 1991), supports this theory.

Transcripts of a Myb-like transcription factor are identified with an increasing frequency during both germination and appressorium formation (A3, Table 3). It would be of interest to determine if the transcription factor is under the control of the cAMP pathway, which has been implicated in the differentiation of appressoria in many pathogenic fungi, including *B. graminis* (Hall et al. 1999; Kinane et al. 2000).

Gene families are a feature of the results and are well suited to analysis by SAGE because the technique relies on the isolation of a tag from the three prime end of the messenger, a region where most members of gene families display the highest degree of divergence. The *gEgh16* gene family has already been mentioned. Six peptidyl-prolyl *cis-trans* isomerase and cyclophilin genes also have been identified. Three of these genes display significant changes in transcript abundance and, interestingly, the profiles are complementary; the increased accumulation of transcripts of one gene during appressorium formation (D14, Table 3) being shadowed by a decreased presence of the other two (G10 and H4, Table 3). In comparison, the various retroelement gene families predominantly display significant increases in mRNA quantities during germination and appressorium formation.

During early development, the fungal germling must degrade the spore wall and synthesize new cell wall constituents to allow hyphal growth and appressorium differentiation. Therefore, it is to be expected that transcripts of two chitinase homologues became significantly more prevalent as development continued (D7 and D16, Table 3). The relatively high transcript abundance of a homologue of GEL1 (D6, Table 3), required for elongation of 1,3- β -glucan in *Aspergillus fumigatus* (Mouyna et al. 2000), is suggestive of the importance of 1,3- β -glucan synthesis in cell wall formation during early fungal development.

Currently available at the NCBI dbEST database are 24,626 sequences from *N. crassa*, 12,993 sequences from *A. nidulans*, and 1,693 sequences from *Metarhizium anisopliae*; as well as 1,158 sequences from *Mycosphaerella graminicola*, 2,681 sequences from *M. grisea*, and 600 sequences from *Cladosporium fulvum*. In addition, numerous genes from human pathogenic fungi are available for comparison, as well as the complete yeast genome and SAGE expression profiles for 4,665 yeast genes during cell cycle progression in yeast (Velculescu et al. 1997). There exists the opportunity to extend both gene identification and the transcript profiling to related fungi with the aim of building a global relational database comprising functional and expression data for a range of pathogens. Such a database would allow the identification of potential targets of future studies of the genes involved in filamentous fungal growth and pathogenicity.

MATERIALS AND METHODS

Fungal techniques.

All experiments were conducted on *Blumeria graminis* f. sp. *hordei* race C15. Maintenance of the fungus on the susceptible barley cv. Golden Promise has been described previously (Kinane et al. 2000). Isolation of the fungal material at different stages of development was achieved by inducing germination and appressorium formation on cellulose membranes as

previously described (Kinane et al. 2000). The mean percentage of conidia forming primary germ tubes or appressoria on artificial surfaces was calculated by microscopic evaluation. The SAGE profile for germinating conidia was constructed using RNA from conidia 4 h after application to cellulose membrane, at which point 82% had a primary germ tube. This is near the maximum degree of germination observed on either cellulose membranes or barley epidermis (Kinane et al. 2000) and, although slightly higher rates of germination can be established at later time points, 4 h was deemed appropriate because, at this stage, appressorial germ tube formation is negligible, so that the material is solely indicative of germination. The appressorial SAGE profile was constructed with RNA from conidia after 16 h of contact with cellulose membrane. At this point, 90% of the conidia had a primary germ tube, of which 77% also had swollen appressorial germ tubes and 66% had fully differentiated appressoria. Again, at this time, the percentage of appressorial germ tube emergence and differentiation was approaching the maximum observed on both cellulose membranes and barley epidermis (Kinane et al. 2000).

RNA techniques.

Total RNA was isolated for all developmental stages studied following the procedure of Chomczynski and Sacchi (1987) with the addition of 50 mM ascorbic acid to solution D to prevent polyphenol oxidation. PolyA⁺ RNA was purified on oligo(dT)-cellulose spin columns (Amersham Pharmacia Biotech) following the manufacturer's specifications. The purity and quality of both total RNA and mRNA preparations was confirmed by denaturing gel electrophoresis (data not shown).

Generation of SAGE profiles.

For each developmental stage, 2.5 to 5 μ g of polyA⁺ RNA was used to construct SAGE tag populations following the original protocol (Velculescu et al. 1995) and incorporating published improvements (Kenzelmann and Mühlemann 1999). Tag concatamers were cloned into pZero (Invitrogen) and the constructs introduced into *Escherichia coli* Top10 cells (Invitrogen). Recombinant plasmids were isolated from single colonies employing a Qiagen minikit and sequenced using BigDye terminator technology (Applied Biosystems, Foster City, CA, U.S.A.).

Handling of sequence data.

Raw sequence files were imported into the DNATools sequence handling program and ambiguous base calls were corrected manually when possible. Following sequence editing, ditags (Velculescu et al. 1995) were extracted using both the automatic extraction facilities within DNATools and a second software package, eSAGE (Margulies and Innis 2000). Both approaches yield a display of individual tags and their frequency of occurrence, and discrepancies between the outputs were resolved manually. Duplicate ditags arising from PCR bias as well as tags originating from the linker sequences automatically were removed from the analyses by the software employed. Tags sequenced only once across the three populations also were removed from the analysis according to Lash and associates (2000) to prevent overestimation of the number of different tags introduced by properties inherent to the SAGE procedure.

Statistical analysis of SAGE data.

Comparison of SAGE profiles between the three populations studied was accomplished by graphical display using the SAGE utilities available in the DNATools package as well as the statistical package within eSAGE (Margulies and Innis 2000). Statistical analysis using DNATools was performed by comparing the number of tags in two stages using the Z test as previously described (Altman 1991; Kal et al. 1999). The

eSAGE software employs the test statistic developed by Audic and Claverie (1997). To avoid erroneous analysis of differences in observed numbers of tags between populations when the number of observations was low (i.e., less than six tags in one population), the statistical significance of observed differences was calculated using the more conservative Fisher Exact test running on the SPSS statistical package (ver. 10 for Windows; SPSS, Chicago).

Statistical analysis of observed differences in tag number were made between ungerminated and germinating conidia, between germinating conidia and appressoria, and, when appropriate, between ungerminated conidia and appressoria. Applying these tests of statistical significance allowed the grouping of genes into several different classes, as follows. (i) A significant increase in transcript abundance was observed during germination (between ungerminated and germinated conidia) as well as during appressorium formation (between germinated conidia and appressoria). (ii) A significant increase in transcript abundance was observed during germination only. (iii) A significant increase in transcript abundance was not detectable between ungerminated and germinated conidia or between germinated conidia and appressoria, but was apparent between ungerminated conidia and appressoria. (iv) A significant increase in transcript abundance was detectable only during appressoria formation. (v) A significant increase in transcript abundance was apparent during germination, followed by a significant decrease during appressorium formation. (vi) A significant decrease in transcript abundance was apparent during germination and was followed by a significant increase in transcript abundance during appressorium formation. (vii) A significant decrease in transcript abundance was observed during appressorium formation only. (viii) A significant decrease in transcript abundance was not detectable between ungerminated and germinated conidia or between germinated conidia and appressoria, but was apparent between ungerminated conidia and appressoria. (ix) A significant decrease in transcript abundance was observed during germination only. (x) A significant decrease in transcript abundance was observed during germination as well as during appressorium formation.

Mapping of SAGE tags.

Prior to mapping to EST clones, tags were analyzed against the published ribosomal RNA sequences of *B. graminis* f. sp. *hordei* (Mori et al. 2000; Paulin and Harrington 2000; Saenz and Taylor 1999). Tags present in the ribosomal RNA sequences were regarded as having arisen due to mispriming of residual ribosomal RNA and were discarded from the analysis.

Identification of genes represented by SAGE tags was accomplished using DNATools following principles devised by Velculescu and associates (1995). Briefly, the entire complement of EST sequences from *B. graminis* initially was examined for the presence of a polyA tail. For those sequences containing a polyA tail, the closest *Nla*III site was identified and the tag sequences extracted. The extracted tags were labeled "A" to indicate that they arose from a polyA-containing EST. For clones where both the 5' and 3' sequence lacked a polyA tail, the most 3' *Nla*III sites in both sequences were identified and the tag immediately adjacent to the 3' end of the *Nla*III recognition site on both DNA strands was used for identification. Such clones, lacking a polyA tail, could yield a maximum of four tags, of which only one would represent the gene in question; therefore, the tags were labeled "W" or "C" to indicate from which strand the tag arose. Tags originating from the 5' and 3' sequences of such clones were used for mapping only in cases where the correct orientation of the gene could be verified by the alignment of the EST sequence to a homologous sequence (BlastX P(N) $\leq 1e^{-5}$) retrieved from GenBank.

A list of EST clones and their extracted tags was used for mapping of the SAGE tags generated in the course of this project. A complete list of the tags generated by this project, as well as the annotated EST clones to which they were mapped, is available online.

ACKNOWLEDGMENTS

This article is dedicated to the memory of Jean Sage, who passed away shortly after retiring from research activities. We thank Q. Abdali, B. Anderson, and J. Sage for technical assistance in template preparation and sequencing; and M. Kielland-Brandt for critically reading the manuscript.

LITERATURE CITED

- Altman, D. G. 1991. Pages 162-163 in: Practical Statistics for Medical Research. Chapman-Hall, London.
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403-410.
- Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: a New generation of protein database search programs. *Nucleic Acids Res.* 25:3389-3402.
- Andrews, G. K. 2000. Regulation of metallothionein gene expression by oxidative stress and metal ions. *Biochem. Pharmacol.* 59:95-104.
- Audic, S., and Claverie, J.-M. 1997. The significance of digital gene expression profiles. *Genome Res.* 7:986-995.
- Chomczynski, P., and Sacchi, N. 1987. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156-159.
- Dean, R. A. 1997. Signal pathways and appressorium morphogenesis. *Annu. Rev. Phytopathol.* 35:211-234.
- de Jong, J. C., McCormack, B. J., Smirnov, N., and Talbot, N. J. 1997. Glycerol generates turgor in rice blast. *Nature* 389:244-245.
- d'Enfert, C. 1997. Fungal spore germination: Insights from the molecular genetics of *Aspergillus nidulans* and *Neurospora crassa*. *Fungal Genet. Biol.* 21:163-172.
- Eckert, S., Both, M., DeBruijn, A., Mueller, E., and Spanu, P. 2001. Sequence analysis and expression studies of *Erysiphe graminis* cDNAs from epiphytic mycelium. *Fungal Genet. Newsl.* 48 (Suppl.):95.
- Hall A. A., Bindslev L., Rouster J., Rasmussen S. W., Oliver, R. P., and Gurr, S. J. 1999. Involvement of cAMP and protein kinase A in conidial differentiation by *Erysiphe graminis* f. sp. *hordei*. *Mol. Plant-Microbe Interact.* 12:960-968.
- Hamer, J. E., and Talbot, N. J. 1998. Infection-related development in the rice blast fungus *Magnaporthe grisea*. *Curr. Opin. Microbiol.* 1:693-697.
- Howard R. J., Ferarri M. A., Roach D. H., and Money N. P. 1991. Penetration of hard surfaces by a fungus employing enormous turgor pressures. *Proc. Natl. Acad. Sci. U.S.A.* 88:11281-11284.
- Howard, R. J., and Valent, B. 1996. Breaking and entering: Host penetration by the fungal rice blast pathogen *Magnaporthe grisea*. *Annu. Rev. Microbiol.* 50:491-512.
- Hwang, C., and Kolattukudy, P. E. 1995. Isolation and characterization of genes expressed uniquely during appressorium formation by *Colletotrichum gloeosporioides* conidia induced by the host surface wax. *Mol. Gen. Genet.* 247:282-294.
- Ishii, M., Hashimoto, S., Tsutsumi, S., Wada, Y., Matsushima, K., Kodama, T., and Aburatani, H. 2000. Direct comparison of genechip and SAGE on the quantitative accuracy in transcript profiling analysis. *Genomics* 68:136-143.
- Jedd, G., and Chua, N. 2000. A new self-assembled peroxisomal vesicle required for efficient resealing of the plasma membrane. *Nat. Cell Biol.* 2:226-231.
- Justesen A., Somerville, S., Christiansen, S., and Giese, H. 1996. Isolation and characterization of two novel genes expressed in germinating conidia of the obligate biotroph *Erysiphe graminis* f. sp. *hordei*. *Gene* 170:131-135.
- Kal, A. J., van Zonneveld, A. J., Benes, V., van den Berg, M., Groot Koerkamp, M., Albermann, K., Strack, N., Ruitjer, J. M., Richter, A., Dujon, B., Ansoorge, W., and Tabak, H. F. 1999. Dynamics of gene expression revealed by comparison of serial analysis of gene expression transcript profiles from yeast grown on two different carbon sources. *Mol. Biol. Cell* 10:1859-1872.
- Kamakura T., Xiao, J.-Z., Choi, W.-B., Kochi, T., Yamaguchi, S., Teraoka, T., and Yamaguchi, I. 1999. cDNA subtractive cloning of genes expressed during early stage of appressorium formation by *Magnaporthe grisea*. *Biosci. Biotechnol. Biochem.* 63:1407-1413.

- Kenzelmann, M., and Mühlemann, K. 1999. Substantially enhanced cloning efficiency of SAGE (Serial analysis of gene expression) by adding a heating step to the original protocol. *Nucleic Acids Res.* 27:917-918.
- Kinane, J., Dalvin, S., Bindlev, L., Hall, A., Gurr, S., and Oliver, R. 2000. Evidence that the cAMP pathway controls emergence of both primary and appressorial germ tubes of barley powdery mildew. *Mol. Plant-Microbe Interact.* 13:494-502.
- Kobayashi, I., Tanaka, C., Yamaoka, N., and Kunoh, H. 1991. Morphogenesis of *Erysiphe graminis* conidia on artificial membranes. *Trans. Mycol. Soc. Jpn.* 32:187-198.
- Kupfer, D. M., Reece, C. A., Clifton, S. W., Roe, B. A., and Prade, R. A. 1997. Multicellular ascomycetous fungal genomes contain more than 8000 genes. *Fungal Genet. Biol.* 21:364-372.
- Lash, A. E., Tolstoshev, C. M., Wagner, L., Schuler, G. D., Strausberg, R. L., Riggins, G. J., and Altschul, S. F. 2000. SAGEmap: A public gene expression resource. *Genome Res.* 10:1051-1060.
- Margulies, E. H., and Innis, J. W. 2000. eSAGE: Managing and analyzing data generated with Serial Analysis of Gene Expression (SAGE). *Bioinformatics* 16:650-651.
- McCafferty, H. R. K., and Talbot, N. J. 1998. Identification of three ubiquitin genes of the rice blast fungus *Magnaporthe grisea*, one of which is highly expressed during initial stages of plant colonization. *Curr. Genet.* 33:352-361.
- McKeen, W. E. 1971. Woronin bodies in *Erysiphe graminis* DC. *Can. J. Microbiol.* 17:1557-1560.
- Mengden, K., Hahn, M., and Deising, H. 1996. Morphogenesis and mechanisms of penetration by plant pathogenic fungi. *Annu. Rev. Phytopathol.* 34:367-386.
- Mori, Y., Sato, Y., and Takamatsu, S. 2000. Molecular phylogeny and radiation time of Erysiphales inferred from the nuclear ribosomal DNA sequences. *Mycoscience* 41:437-447.
- Mouyna, I., Fontaine, T., Vai, M., Monod, M., Fonzi, W. A., Diaquin, M., Popolo, L., Hartland, R. P., and Latge, J.-P. 2000. Glycosylphosphatidylinositol-anchored glucanotransferases play an active role in the biosynthesis of the fungal cell wall. *J. Biol. Chem.* 275:14882-14889.
- Paulin, A. E., and Harrington, T. C. 2000. Phylogenetic placement of anamorphic species of *Chalara* among *Ceratocystis* species and other ascomycetes. *Stud. Mycol.* 45:209-222.
- Polyak, K., Xia, Y., Zweier, J. L., Kinzler, K. W., and Vogelstein, B. 1997. A model for p53-induced apoptosis. *Nature* 389:300-305.
- Saenz, G. S., and Taylor, J. W. 1999. Phylogeny of the Erysiphales (powdery mildews) inferred from internal transcribed spacer ribosomal DNA sequences. *Can. J. Bot.* 77:150-168.
- Schena, M., Shalon, D., Davis, R. W., and Brown, P. O. 1995. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 270:467-470.
- Thines, E., Weber, R. W. S., and Talbot, N. J. 2000. MAP kinase and protein kinase A-dependent mobilization of triacylglycerol and glycogen during appressorium turgor generation by *Magnaporthe grisea*. *Plant Cell* 12:1703-1718.
- Thomas, S. W., Rasmussen, S. W., Glaring, M. A., Rouster, J. A., Christiansen, S. K., and Oliver, R. P. 2001. Gene identification in the obligate fungal pathogen *Blumeria graminis* by expressed sequence tag analysis. *Fungal Genet. Biol.* 33:195-211.
- Velculescu, V. E., Zhang, L., Zhou, W., Vogelstein, J., Basrai, M. A., Bassett, D. E., Jr., Hieter, P., Vogelstein, B., and Kinzler, K. W. 1997. Characterization of the yeast transcriptome. *Cell* 88:243-251.
- Velculescu, V. E., Zhang, L., Vogelstein, B., and Kinzler, K. W. 1995. Serial analysis of gene expression. *Science* 270:484-487.
- Xu, J.-R. 2000. MAP kinases in fungal pathogens. *Fungal Genet. Biol.* 31:137-152.
- Yu, J., Zhang, L., Hwang, P. M., Rago, C., Kinzler, K. W. and Vogelstein, B. 1999 Identification and classification of p53-regulated genes. *Proc. Natl. Acad. Sci. U.S.A.* 96:14517-14522.
- Zhang, Z., Hall, A., Perfect, E., and Gurr, S. J. 2000. Differential expression of two *Blumeria graminis* chitin synthase genes. *Mol. Plant Pathol.* 1:125-138.

AUTHOR-RECOMMENDED INTERNET RESOURCES

DNATools sequence handling program: www.dnatools.dk
 Complete list of tags generated by this project: www.crc.dk/phys/blumeria