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Short Communication

High Temperature, Differentiation, and Endoplasmic Reticulum Stress Decrease but Epigenetic and Antioxidative Agents Increase *Aspergillus* Ribosomal Protein Gene Expression

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Abstract

Genome-wide gene expression assays using next-generation sequencing techniques have allowed the identification of transcriptomes in many species. Transcript abundance of ribosomal protein (RP) genes can serve as a proxy for the capacity of general transcription and synthesis of cellular proteins that provide molecular functions. We analyzed and compared numbers and expression levels of RP genes of four RNA-Seq datasets. These included studies on effects of temperature, developmental stages, and epigenetic and antioxidative agents on *A. flavus*, and culture type and endoplasmic reticulum (ER) stress on *A. oryzae* RP gene expression. Under normal growth conditions, regardless of medium composition, about 55 to 65% of total *Aspergillus* RP genes were highly expressed (defined as among the top 2% of the total genes). Stress factors such as high temperature and hyperoxidant state (differentiation) decreased RP gene expression levels and, to a much lesser extent, the expressed RP gene populations. In contrast, factors that relieve oxidative stress increased the expression levels. ER stress greatly decreased the expression level of individual RP genes, but barely changed the population. Transcriptomic studies can provide new insights into how abiotic and biotic factors affect RP gene expression.

Keywords: Transcriptome; Ribosomal protein; Endoplasmic reticulum stress; *Aspergillus*; Oxidative stress

Abbreviations

RP: Ribosomal Protein; ER: Endoplasmic Reticulum; rRNA: Ribosomal RNA; PDA: Potato Dextrose Agar; PDB: Potato Dextrose Broth; 5AC: 5-azacytidine; GA: Gallic Acid; CD: Czapek-Dox Medium; DTT: Dithiothreitol; GEO: Gene Expression Omnibus; SRA: Sequence Read Archive; CNT: Control; RPKM: Reads per Kilobase Exon Model Per Million Mapped Reads; FPKM: Fragments Per Kilobase of Transcript Per Million Mapped Reads; ROS: Reactive Oxygen Species; T: Temperature; Myc: Mycelia; Scl: Sclerotia; SC: Solid-state Culture; LC: Liquid-state Culture

Introduction

Ribosomes, the translation machinery, are ribonucleoprotein particles that catalyze all cellular protein synthesis using transfer RNAs and elongation factors. Bacterial ribosomes like those of *Echerichia coli* have served as a basis for elucidating mechanisms of protein synthesis. For *E. coli*, two-dimensional gel electrophoresis has been used to separate the 21 proteins in the 30 small subunit and the 34 proteins in the 50S large subunit [1]. In contrast to the bacterial counterparts, eukaryotic ribosomes including those of fungi are more complex and much larger. They are fundamentally different in many ways and contain additional ribosomal RNA (rRNA) called expansion segment and many other ribosomal proteins [2]. Fungal ribosomes are 80S ribosomes, which consist of two subunits of 40S and 60S. The small 40S subunit contains an 18S rRNA while the large 60S subunit contains a 26S rRNA. Both subunits as inferred from their eukaryotic counterparts likely contain a total of about 80 ribosomal proteins [3]. Ribosome biogenesis requires that rRNAs and ribosomal proteins in precise stoichiometric balance. The expression of ribosomal protein (RP) genes is coordinately regulated to ensure that equimolar amounts of RP are available for ribosome assembly [4].

Ribosomal proteins are synthesized preferentially in cells growing actively. Increasing evidence indicates that individual ribosomal proteins and changes in amounts can modulate a wide array of activities that are associated with cell growth and death [5,6]. In the post-genomics era, RNA-Seq has been developed as the standard approach for profiling transcriptomes [7]. Research using this technique has generated vast amounts of data and analyses of sequence reads have revolutionized current views on the complexity of transcriptome and on the context of gene expression regulation [8]. Transcriptomic studies on fungi have been mostly focused on characterizing all transcript species [9], determining cellular protein genes differentially expressed [10], and elucidating pathogenicity or virulence [11,12]. In a recent analysis of the human transcriptome, the molar ratio of transcripts among 80%-90% of the RP genes, with little tissue specificity, was found to vary less than three-fold [13]. Up to now, no systematic analyses on the expression levels of the entire

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population of RP genes in fungi have been attempted.

In this short communication, using limitedly available transcriptomic datasets we collectively determined the overall expression profiles of RP genes in *Aspergillus flavus* and the closely related *Aspergillus oryzae* under common growth conditions. We evaluated how temperature, developmental stages, and epigenetic and antioxidative agents affected the RP gene expression of *A. flavus* as well as how culture type and endoplasmic reticulum (ER) stress affected the RP gene expression of *A. oryzae*. The analyses showed that the portion of RP genes normally expressed at high levels was fairly constant, in the range of 55 to 65%. Abiotic and biotic stress factors and relief of the factors were related to changes in the overall RP gene expression. The information provides a possible means to use culturing practices in combination with transcriptomic analyses to control general transcription, thus cellular activities in fungi.

Materials and Methods

Fungal strains and culturing conditions

Two *Aspergillus flavus* strains, NRRL3357 and CA43, the latter differs from the former in that it produces sparse conidia but abundant small sclerotia (S strain), and one *Aspergillus oryzae* strain, RIB40, were used. Fungal cultures were routinely maintained on potato dextrose agar (PDA) plates. For the experiment examining temperature effects, cultures of NRRL3357 were harvested after they were grown at 30°C and 37°C in liquid glucose minimal salts (GMS) media for 24 h [14]. For production of mycelia and sclerotia, cultures of CA43 were grown on PDA plates, each overlaid with a layer of cellophane, and incubated at 30°C in the dark. The mycelia were collected after 48 h and sclerotia after 7 days [15]. For the experiments studying chemical effects, cultures of NRRL3357 grown in potato dextrose broth (PDB) containing 1 mM 5-azacytidine (5AC, treatment 1), 1% (w/v) gallic acid (GA, treatment 2) or none (control) were prepared [16]. These cultures were incubated at 30°C in the dark for 72 h. For experiments investigating the effect of culture type RIB40 was grown on solid or in liquid glucose-based Czapek-Dox (CD) medium for 40 h. For ER stress treatment the resulting solid culture along with the cellophane was transferred to a stack of 2-cm thick filter paper soaked in 20 ml CD supplemented with 20 mM dithiothreitol (DTT) and the liquid culture used also was supplemented with 20 mM DDT [9].

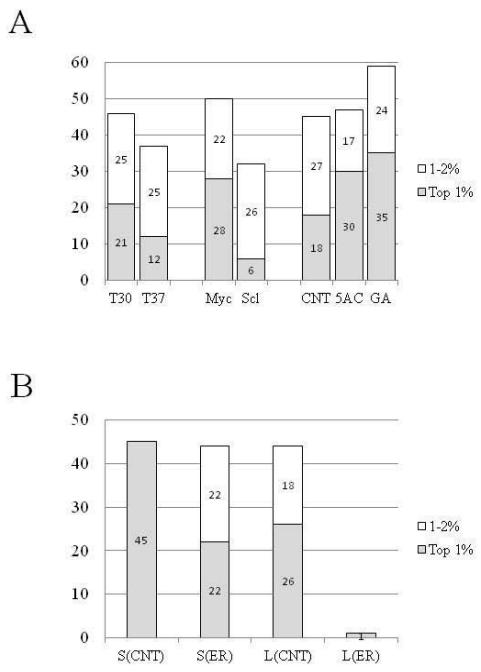

Figure 1: Numbers of RP genes highly expressed under various growth conditions. (A) *A. flavus* at different growth temperatures, at mycelia growth and sclerotial differentiation stages, and treated with epigenetic modulator 5AC and antioxidant GA. (B) *A. oryzae* on solid or in liquid medium without or under the endoplasmic reticulum (ER) stress condition caused by the treatment with DTT.

Preparation of total RNA and isolation of mRNA

Mycelia or sclerotia collected were ground to a fine powder in liquid nitrogen. Total RNA was extracted using TRIzol® Reagent (Invitrogen, USA), RNAiso™ Plus (TaKaRa, Japan) or the hot acid phenol method. All total RNA samples were treated with RNase-free DNase I. The integrity and concentration of the resulting total RNA were determined by an Agilent Technologies 2100 Bioanalyzer. All samples had a RNA integrity number value greater than six. Poly (A) RNAs were isolated from total RNA samples using magnetic oligo(dT) beads.

Construction of cDNA libraries and sequencing

Samples of poly (A) RNA (0.2-1.0 μg) were used for cDNA library construction following the Illumina protocol (http://www.illumina.com). The routine procedures involved fragmentation of mRNA into smaller pieces (200-500 bp), first strand cDNA synthesis, second strand cDNA synthesis, end repair, ligation of adapters, purification of ligated products, and PCR amplification to enrich cDNA templates. All cDNA libraries were sequenced using the Illumina Genome Analyzer II or the HiSeq2000 platform.

Sequence reads deposition into databases, and mapping

Raw sequence data were processed and filtered using the Illumina pipeline (http://www.illumina.com) to generate fastq files. Raw sequence data of the temperature effect on *A. flavus* NRRL3357 were deposited in the NCBI's Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) under the accession number of GSE30031. Data of mycelia and sclerotia of *A. flavus* CA43 transcriptome were deposited in Sequence Read Archive (SRA; http://www.ncbi.nlm.nih.gov/sra/) under the accession number of SRP018670. Data of the effect of 5-azacytidine or gallic acid on *A. flavus* NRRL3357 were deposited in GEO under the accession number of GSE40202. Data of the effect of endoplasmic reticulum stress on *A. oryzae* RIB40 grown on solid and in liquid media were deposited in GEO under the accession number of GSE18851. Good sequence reads were mapped to the reference genome of NRRL3357 or RIB40 using CLC Genomic Workbench (http://www.clcbio.com), Cufflinks [17] (http://cufflinks.cbcb.umd.edu/), or SOAP [18]. All reads were mapped to coding sequences. The expression values for every gene in the RPKM (Reads Per Kilobase exon model per Million mapped reads) unit or, in the experiments of 5AC and GA treatment, as its equivalent of FPKM (Fragments Per Kilobase of transcript per Million mapped reads) [19] were calculated. These determinations are normalized values, which allows for cross-sample comparisons in an experiment.

Results and Discussion

Despite having similar genome sizes of about 37 Mb, *A. flavus*

Table 1: Relative ratios of highly expressed RP genes in *A. flavus* and *A. oryzae* transcriptomes from various culture conditions.

A. flavus	T30	T37	Mycelia	Sclerotia	CNT	5AC	GA
	1.00	0.57[a]	1.00	0.45	1.00	1.55	1.86

A. oryzae	Solid (CNT)	Solid (ER)	Liquid (CNT)[b]	Liquid (ER)[c]
	1.00	0.30	1.00	0.03

a: The RPKM counts of the RP genes among the top 2% of total genes are compared to those of the respective controls.
b: The ratio of Liquid (CNT) to Solid (CNT) is 0.37.
c: The ratio of Liquid (ER) to Solid (CNT) is 0.01.

and *A. oryzae* are predicted to have 13,485 and 12,074 protein-coding genes, respectively [20,21]. To define highly expressed genes in all experiments, we first sorted the normalized expression values and then arbitrarily set the top 2% as the cutoff criterion. The glycerol-3-phosphate dehydrogenase gene, highly expressed in eukaryotic microorganisms and its promoter used by many researchers to overexpress genes of interest [22], also was in this range. The determination of the total number of RP genes (in parentheses) expressed in cultures grown under commonly used conditions from all experiments revealed the following: (i) *A. flavus* at 30°C in GMS for 24h (46), at 30°C in PDB for 72h (45), and at 30°C on PDA for 48h (50) and (ii) *A. oryzae* at 30°C for 40 h on solid CD (45) or liquid CD (44) (Figures 1A and 1B). Assuming that *Aspergillus* like other eukaryotes has 80 RP genes, the results showed that about 55 to 65% of RP genes were highly expressed under normal culturing conditions. We further dissected the top 2% into two tiers, that is, top 1% and 1-2% to determine any difference in the RP gene expression pattern. Regardless of medium type 40 to 60% of the highly expressed RP genes of *A. flavus* were in the top 1%. Although in liquid medium the RP gene expression pattern of *A. oryzae* was similar to that of *A. flavus*, on solid medium all highly expressed RP genes of *A. oryzae* were in the top 1%. Exposing cultures to air such as on plates did not significantly affect the overall highly expressed RP gene number but substantially increased the number in the top1% as seen from *A. flavus* Myc vs. T30 and CNT (Figure 1A), and in particular *A. oryzae* S(CNT) vs. L(CNT) (Figure 1B). Although *A. flavus* and *A. oryzae* are phylogenetically closely related, they are classified as separate species because of food safety and economic concerns [23]. The analysis showed that 60% more top 1% RP genes were in *A. oryzae* than in *A. flavus* when both were grown on solid medium, that is, S(CNT) vs. Myc. Taken together, these results suggest that *A. oryzae* on solid medium has a higher capacity for making cellular proteins than in liquid medium [24]. Thousands-of-years domestication of its nonaflatoxigenic *A. flavus* ancestor by solid-state fermentation and selection for strains as solid-state cultures that grow fast and produce high activities of amylases and proteases to degrade macromolecules in rice, wheat bran, and soybean [25] may in part have shaped the distinct pattern of RP gene expression in current *A. oryzae*.

The transcriptomic data obtained from different growth temperature, developmental stages and under (relief of) stress conditions allow us to assess how these factors affect the expression of RP genes. The number of RP genes highly expressed at 37°C compared to that at 30°C decreased about 20% (Figure 1A), but the expression level decreased greater than 40% (Table 1). High temperature is known to represses general transcription and translation. Adaptation

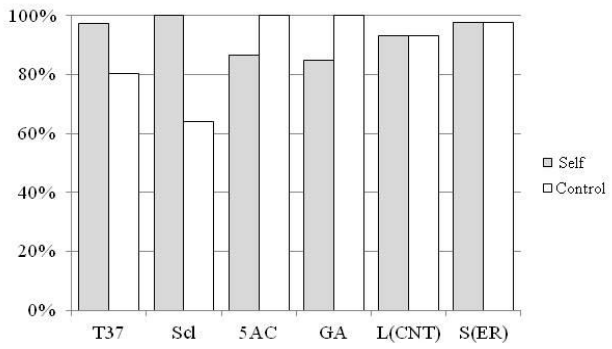

Figure 2: RP genes highly expressed at designated conditions relative to their own RP populations and common in the respective control sets. The control sets are T30 for T37, Myc for Scl, CNT for 5AC and GA, and S(CNT) for L(CNT) and S(ER). See Tables S1 and S2 for the total numbers of highly expressed RP genes at these specific growth conditions. See Figure 1 for the total numbers of highly expression RP genes for the control sets. For example, all RP genes expressed in the control set were also expressed in the 5AC and GA sets, but the common genes represent 87% and 85% of those in the population of 5AC and GA, respectively.

to elevated growth temperatures involves coordination of stress responses and signaling pathways. Cells subjected to temperature elevation (40°C) have been shown to induce either a partial or the full ER stress pathway [26]. Reduced translation in *Saccharomyces cerevisiae* also has been implicated to protect the yeast from ER stress [27]. Notably, the RP genes missing in the aforementioned 20% decrease at 37°C were all those highly expressed at 30°C, which included genes for L14, L23, L27e, L32, L35, S10a, S19, S22, S25 and S26. At 37°C the decreased expression levels varied from 43 to 84%. Only the expression of the new RP gene for L4 was elevated at 37°C; the increase was greater than two-fold (Figure 2; also see Table S1 for RP gene populations). Changes in numbers and levels

Table S1: RP gene expression in *A. flavus* under various growth conditions

A. Temperature

T30				T37			
Top 1%		1-2%		Top 1%		1-2%	
Protein	Expression	Protein	Expression	Protein	Expression	Protein	Expression
S3Ae	2787	S6	866	S3Ae (1)[a]	1619	L12 (3)	691
L1	1763	L9	845	S8e (9)	1170	L16a (18)	680
L12	1647	S17	835	L3 (16)	1011	S17 (24)	660
L18	1587	L18ae	822	L8 (7)	995	S13 (45)	640
S5	1541	L27e	802	L21 (11)	910	L18ae (25)	633
S11	1481	L35	755	L6 (10)	883	S11 (6)	624
L8	1402	L7	736	L13 (41)	869	S13p/S18e (12)	604
L15	1344	L27a	728	L15 (8)	865	L18 (4)	597
S8e	1288	S22	725	S5 (5)	857	S3 (44)	592
L6	1287	S10a	693	S6 (22)	834	L5 (38)	592
L21	1285	L19	692	S7e (14)	831	L7A (34)	580
S13p/S18e	1242	L7A	688	S0 (19)	782	S4 (15)	569
S9	1172	S25	683			L4	542
S7e	1105	L32	652			S23 (S12) (21)	540
S4	1096	L11	650			L1 (2)	518
L3	1026	L5	639			L26 (39)	515
L23	958	L26	631			L27a (30)	510
L16a	949	L17	623			L19 (33)	492
S0	894	L13	615			S15 (20)	486
S15	892	L14	586			L17 (40)	483
S23 (S12)	884	S26	531			L7 (29)	466
		S3	529			S9 (13)	453
		S13	525			L9 (23)	408
		S19	509			L11 (37)	401

a: The number in the parentheses correcponds to the rank in the control set, T30.

B. Mycelia and sclerotia

Mycelia				Sclerotia			
Top 1%		1-2%		Top 1%		1-2%	
Protein	Expression	Protein	Expression	Protein	Expression	Protein	Expression
L30	1298	L16a	578	L32 (5)[a]	794	L37a (6)	568
S10b	1198	L8	572	S25 (4)	698	L23 (11)	554
L19	1186	L34	566	L19 (3)	669	L35Ae (10)	554
S25	1112	S24	565	S13p/S18e	639	S8e (17)	510
L32	1108	L18ae	538	S13 (8)	634	L35 (16)	510
L37a	1107	L6	535	L30 (1)	610	S10b (2)	498
S13p/S18e	1082	L5	535			S3Ae (22)	497
S13	985	S9	533			S17 (15)	487
L18	944	S19	529			L9 (20)	474
L35Ae	901	S5	527			S11 (13)	468
L23	896	L14	520			L3 (27)	459
S26	848	L26	472			S26 (12)	456
S11	835	L27a	460			L21 (14)	456
L21	825	S15	438			L18 (9)	441
S17	825	S4	436			S6 (26)	401
L35	817	L7A	409			L12 (24)	399
S8e	805	L28	395			S7e (21)	396
L27e	804	S3	372			S10a (23)	394
S5	783	L15	371			L6 (34)	386
L9	724	L4	368			L27e (18)	374
S7e	676	L13	354			L7 (25)	374
S3Ae	652	L37	333			S15 (42)	368
S10a	648					S5 (38)	348
L12	647					S19 (37)	341
L7	632					L18ae (33)	334
S6	622					L4 (48)	331
L3	612						
L1	594						

a: The number in the parentheses correcponds to the rank in the control set, Mycelia.

C. Treatments with 5-azacytidine (5AC) and gallic acid (GA)

Control				5AC				GA			
Top 1%		1-2%		Top 1%		1-2%		Top 1%		1-2%	
Protein	Expression	Protein	Expression	Protein	Expression	Protein	Expression	Protein	Expression	Protein	Expression
L35Ae	9051	S26	1483	L35Ae (1)[a]	10851	S5 (37)	1683	L35Ae (1)[a]	8928	L38	1550
S25	5586	L23	1451	S25 (2)	7831	S8e (42)	1654	L44 (3)	7723	L9 (32)	1547
L44	4581	L19	1436	S28e (4)	5633	S9 (36)	1649	S25 (2)	7703	L16a (35)	1543
S28e	4155	S17	1378	L44 (3)	5270	L7 (45)	1563	S21 (6)	5452	S9 (36)	1529
L36	3610	L31e	1358	L37 (7)	4559	L11 (40)	1546	S28e (4)	5120	S13 (26)	1462
S21	3466	L24a	1306	S21 (6)	4475	S22 (38)	1544	L36 (5)	4870	S8e (42)	1448
L37	3140	L22	1303	L18 (8)	4349	L3 (39)	1486	L37 (7)	4132	L15 (27)	1386
L18	2696	S13	1278	L36 (5)	4149	S6	1473	L18 (8)	3595	S4 (33)	1355
L12	2401	L15	1252	L8 (11)	3424	S19 (43)	1465	L8 (11)	2891	L11 (40)	1352
L30	2391	L27e	1243	L12 (9)	3353	S4 (33)	1424	L37a (12)	2872	S5 (37)	1305
L8	2251	L21	1205	S10b (14)	3334	L28 (41)	1393	S12 (16)	2649	L3 (39)	1304
L37a	1984	S15	1186	L34 (13)	3081	S7e	1296	L12 (9)	2618	S10a	1266
L34	1882	S11	1146	L35 (15)	2926	L18ae	1096	L34 (13)	2557	S22 (38)	1233
S10b	1743	L9	1104	L37a (12)	2761	L38	1087	L30 (10)	2557	L28 (41)	1228
L35	1610	S4	1088	L27a (18)	2698	L13	1052	S10b (14)	2524	L1 (44)	1216
S12	1603	L6	1079	L30 (10)	2479	L17	1026	L35 (15)	2331	L7 (45)	1202
S3Ae	1601	L16a	1062	S3Ae (17)	2448	S10a	937	L31e (23)	2327	S6	1187
L27a	1596	S9	1029	L27e (28)	2350			S17 (22)	2206	S19 (43)	1157
		S5	1008	L24a (24)	2323			S26 (19)	2180	S7e	1069
		S22	999	S12 (16)	2264			L27a (18)	2148	L13	997
		L3	966	L19 (21)	2215			S3Ae (17)	2134	L18ae	880
		L11	954	S17 (22)	2211			L22 (25)	2002	L17	852
		L28	945	S26 (19)	2180			L23 (20)	1890	S3	840
		S8e	940	L16a (35)	2016			L19 (21)	1855		
		S19	936	L22 (25)	1994			L6 (34)	1854		
		L1	851	L23 (20)	1981			L21 (29)	1791		
		L7	849	S15 (30)	1946			L24a (24)	1735		
				L1 (44)	1941			S15 (30)	1717		
				S11 (31)	1925			L27e (28)	1714		
				S13 (26)	1860			S11 (31)	1600		
				L6 (34)	1858						
				L21 (29)	1856						
				L31e (23)	1782						
				L15 (27)	1764						
				L9 (32)	1725						

a: The number in the parentheses correcponds to the rank in the untreated control set.

of the expressed RP genes in turn can affect translation capacity of the fungus including the synthesis of hundreds of cellular proteins involved in ribosome biogenesis [28], which further decreases general transcription and translation.

Vegetative growth and sclerotial formation represent distinct stages in the life cycle of S-stain *A. spergillus flavus*. Cell differentiation in eukaryotic microorganisms is a response to oxidative stress [29]. The hyperoxidant state is a state that the production of reactive oxygen species (ROS) exceeds the cell's capacity to neutralize these damaging molecules. Fungal mycelia in early growth stages maintain minimal ROS levels. Increasing levels of ROS at later developmental stages are a major determinant for sclerotial biogenesis, which acts as a defense mechanism against oxidative stress [30]. ER stress and oxidative stress are closely linked events; activation of the unfolded protein response, an intracellular signaling pathway, on exposure to oxidative stress serves as a mechanism to preserve cell function and survival [31]. The number of highly expressed RP genes in sclerotia compared to that in mycelia decreased about 36% (Figure 1A), which included genes for L1, L5, L7A, L8, L13, L14, L15, L16a, L26, L27a, L28, L34, L37, S3, S4, S5, S9 and S24 (Table S1). The overall expression level decreased 55% (Table 1), which corresponded to a decreased expression in the respective genes from 27 to 52% (data not shown). These results suggest that oxidative stress is able to cause ER stress and manifests its effect in the RP gene expression. The number of RP genes expressed in sclerotia was decreased but no new RP genes compared to those in mycelia were expressed (Figure 2). Alternatively, sclerotia being in a resting state and metabolic inactive likely have decreased general transcription and translation. Whether this resting state has bearing on ER stress is unknown.

The epigenetic modifier, 5-azacytidine (5AC) is a DNA methylation inhibitor; it can turn on expression of silent genes [32]. 5AC induces in fungi on solid media a "fluffy" phenotype that simulates a prolonged vegetative state [33]. *A. flavus* treated with 5AC even in PDB would correspond to a low ROS status as in an early growth stage. Likewise, the treatment with the antioxidant, gallic acid (GA), can lower the intracellular ROS level. Compared to the control *A. flavus* treated by 5AC or GA increased the total number of highly expressed RP genes. Also, the increase in the expression level for 5AC was 55% and GA 86% (Table 1). A further comparison of the top 1% showed that the increase for 5AC was 98% and GA 136% as evidenced by a nearly a two-fold increase in the number of RP genes from 18 to 30 and 35, respectively (Figure 1A). In addition to having all common RP genes expressed in the control set, both 5AC and GA treatments induced a few new highly expressed RP genes. Interestingly, all seven new highly expressed RP genes for L13, L17, L18ae, L38, S6, S7e and S10a in the 5AC set were identical to seven of the eight genes newly included in the highly expressed RP genes in the GA set (Table S1). The increase in the expression of these RP genes, except the one for L38 in the GA treated sample, was in general less than two-fold (data not shown). This finding of nearly identical RP genes in the 5AC and GA sets suggests that changes in intracellular redox status, such as a decreased in oxidative stress, do not greatly influence the relative expression ratios among most RP genes, but instead elevate the expression levels of individual gene comparably. The broad increase in the RP gene levels likely raise the ranking of some of the RP genes to the arbitrarily set top 2% range.

A marked difference was observed for RP genes expressed by *A. oryzae* solid- (SC) and liquid-state (LC) cultures. The overall expression level based on the total transcript count of the highly expressed RP genes in SC was about 2.7-fold that of in LC (Table S2). Studies have shown that expression levels of many protein folding related genes are higher in SC than in LC [9, 34]. Elevated amounts of folding proteins can lead to higher production of stable cellular and ribosomal proteins, which may further increase general

Table S2. Culture state and endoplasmic reticulum (ER) stress on RP gene expression in A. oryzae

SC				SC+ER stress			
Top 1%		1-2%		Top 1%		1-2%	
Protein	Expression	Protein	Expression	Protein	Expression	Protein	Expression
L18	2803	None	None	L10 (21)[a]	1199	L15 (19)	513
S3A	2773			L18 (1)	1044	L32 (18)	510
L11	2507			S3A (2)	932	L14/L17/L23 (24)	508
L23	2506			L11 (3)	799	S24 (38)	485
L26	2444			S18 (7)	781	L24 (35)	478
S17	2444			S17 (6)	768	S4 (22)	457
S18	2441			L23 (4)	729	L13a (29)	455
L10A	2398			S25 (10)	686	S20 (20)	440
L9	2216			L26 (5)	638	L3 (28)	440
S25	2191			L10A (8)	615	L34 (27)	439
S8	2043			S23 (16)	595	L7 (32)	428
L15/L27	2016			S7 (13)	576	S13 (30)	423
S7	1979			L19	568	S4 (22)	402
L27	1950			L6 (25)	563	S16 (26)	387
L22	1905			L15/L27 (12)	559	S6 (41)	382
S23	1900			S8 (11)	558	S14 (36)	340
L30	1892			L13 (37)	553	S15/S22 (39)	329
L32	1845			L30 (17)	547	L28 (31)	315
L15	1833			L22 (15)	540	L22 (34)	313
S20	1833			S7 (13)	539	L5 (42)	293
L10	1790			L9 (9)	533	S2/S5 (44)	292
S4	1735			L27 (14)	532	S3 (45)	289
S12	1727						
L14/L17/L23	1659						
L6	1624						
S16	1622						
L34	1605						
L3	1557						
L13a	1555						
S13	1541						
L28	1532						
L7	1529						
S7	1513						
L22	1507						
L24	1409						
S14	1408						
L13	1362						
S24	1330						
S15/S22	1306						
S4	1292						
S6	1289						
L5	1237						
L7A	1205						
S2/S5	1157						
S3	937						
LC				LC+ER stress			
Top 1%		1-2%		Top 1%		1-2%	
Protein	Expression	Protein	Expression	Protein	Expression	Protein	Expression
L18 (1)[a]	1517	L10A (8)	589	L10 (9)	911	None	None
S3A (2)	1120	S24 (38)	564				
S17 (6)	1063	L13 (37)	557				
L11 (3)	998	L28 (31)	555				
S7 (13)	945	L3 (28)	554				
L10 (21)	888	S20 (20)	538				
L22 (15)	878	S12 (23)	524				
L23 (4)	858	S6 (41)	522				
L9 (9)	834	S4 (40)	516				
S13 (30)	820	L7 (32)	479				
L15/L27 (12)	813	S15/S22 (39)	439				
S18 (7)	811	L7A (43)	428				
L26 (5)	806	L5 (42)	424				
L6 (25)	801	S14 (36)	404				
S23 (16)	770	L34 (27)	400				
L19	731	L22 (15)	393				
S8 (11)	724	S5	378				
L14/L17/L23 (24)	703	S3 (45)	369				
S25 (10)	665						
L24 (35)	652						
L13a (29)	652						
S16 (26)	648						
S7 (13)	635						
L15 (19)	624						
L27 (14)	619						
L32 (18)	607						

a: The number in the parentheses correcponds to the rank in the SC set.

transcription. Heterogeneity of ribosome structure resulting from variations in ribosomal protein composition is of physiological significance in eukaryotes [35]. Different sets of RP genes in yeast have been shown to be associated with various phenotypes such as life span, budding, and drug resistance [27]. RP genes in normal adult human tissues, including brain, liver, muscle, retina, uterus and ovary are also differentially expressed [36]. We found that, for the same experiment, medium composition not the medium type had a major role in determining the expressed RP gene populations. For example, 94% (42/45) of the highly expressed RP genes in LC were common genes expressed in SC, and 98% (44/45) of the RP genes expressed in SC were expressed in SC under the ER stress condition despite a 2 to 3-fold decrease in each expression level (Figure 2 and Table S2). The latter finding also showed that ER stress specifically exerted it effect mainly on individual RP gene expression levels but did not affect the highly expressed RP gene population. This is in striking difference from what were found from the effects of temperature, developmental stages, and treatments by 5AC and GA. Changes in their RP populations apparently were caused by other complex factors if ER stress played a role. It is unclear what the underlying mechanisms are for the general decrease of RP gene expression in LC. Genes differentially expressed in response to oxygen levels are divided into two groups: aerobic genes expressed under normoxic conditions, and hypoxic genes expressed when oxygen is low or absent. Both are regulated by signaling pathways at the level of transcription [37,38]. The analysis showed that lower oxygen tension also was able to decrease the RP gene expression significantly (Table S2). In addition, a striking synergistic effect between hypoxia and ER stress in decreasing the RP gene expression was found (Figure 1B and Table 2).

Conclusion

Slightly more than half of the total RP genes of *A. flavus* and *A. oryzae* are highly expressed under normal growth conditions. This proportion in general is not affected by medium type or composition. The RP gene population and the expression level are decreased by abiotic and biotic stress factors such as elevated growth temperature, differentiation, and hypoxia but elevated by relief of stress factors. ER stress does not change the RP gene population but mainly decreases the expression level of individual genes. Transcriptomic analyses improve our understanding of how RP gene expression profiles in fungi are shaped by their living environments.

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Supporting Information

Table S1: Ribosomal protein gene expression in *A. flavus* under various growth conditions.

Table S2: Culture state and endoplasmic reticulum stress on ribosomal protein gene expression in *A. oryzae*.

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