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ORIGINAL ARTICLE

An Expressed Sequence Tag Analysis of the Intertidal Brown Seaweeds *Fucus serratus* (L.) and *F. vesiculosus* (L.) (Heterokontophyta, Phaeophyceae) in Response to Abiotic Stressors

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Abstract In order to aid gene discovery and uncover genes responding to abiotic stressors in stress-tolerant brown algae of the genus *Fucus*, expressed sequence tags (ESTs) were studied in two species, *Fucus serratus* and *Fucus vesiculosus*. Clustering of over 12,000 ESTs from three libraries for heat shock/recovery and desiccation/rehydration resulted in identification of 2,503, 1,290, and 2,409 unigenes from heat-shocked *F. serratus*, desiccated *F. serratus*, and desiccated *F. vesiculosus*, respectively. Low overall annotation rates (18–31%) were strongly associated with the presence of long 3′ untranslated regions in *Fucus* transcripts, as shown by analyses of predicted protein-coding sequence in annotated and nonannotated tentative consensus sequences. Posttranslational modification genes were overrepresented in the heat shock/recovery library, including many chaperones, the most abundant of which were a family of small heat shock protein transcripts, Hsp90 and Hsp70 members. Transcripts of LI818-like light-harvesting genes implicated in photoprotection were also expressed during heat shock in high light. The expression of several heat-shock-responsive genes was confirmed by quantitative reverse transcription polymerase chain reaction. However, candidate genes were notably absent from both desiccation/rehydration libraries, while the responses of the two species to desiccation were divergent, perhaps reflecting the species-specific physiological differences in stress tolerance previously established. Desiccation-tolerant *F. vesiculosus* overexpressed at least 17 ribosomal protein genes and two ubiquitin-ribosomal protein fusion genes, suggesting that ribosome function and/or biogenesis are important during cycles of rapid desiccation and rehydration in the intertidal zone and possibly indicate parallels with other poikilohydric organisms such as desiccation-tolerant bryophytes.

Keywords Abiotic stress response · Brown alga · Desiccation · EST library · Ecological genomics · *Fucus serratus* · *Fucus vesiculosus* · Gene expression profile · Heat shock

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Introduction

Brown algae (Heterokontophyta) of the genus *Fucus* and related genera dominate the biomass of temperate rocky intertidal shores in the northern hemisphere. They play key ecological roles, both in primary production and as the main habitat-forming component of the ecosystem. Fucoid algae exist at the interface between marine and terrestrial habitats, where periods of immersion in seawater alternate with aerial exposure as tides rise and fall, posing severe challenges particularly for large sessile photoautotrophic organisms (Davison and Pearson 1996). In common with, e.g., many bryophytes, fucoids are poikilohydric (i.e., they lack physiological or structural adaptations to prevent water loss), and much physiological research has focused on desiccation, a major abiotic stressor in the intertidal zone (Schonbeck and Norton 1978; Dring and Brown 1982; Lipkin et al. 1993), although temperature variation (heat shock and freezing), osmotic stress, and oxidative stress arising from excess light are also significant during low-tide periods (Henley et al. 1992; Pearson and Davison 1993, 1994; Chapman 1995; Davison and Pearson 1996; Collén and Davison 1999a, b; Pearson et al. 2009).

Heterokont algae are not only important foundation species in littoral ecosystems (kelps and fucoid macrophytes), but unicellular planktonic members (diatoms) are major players in open oceanic and coastal marine systems and therefore in global carbon and biogeochemical cycles. Complete genome sequences for diatoms have recently become available (Armbrust et al. 2004; Bowler et al. 2008) and demonstrate both the unique nature of their genomes and the enormous diversity that exists even within the diatom branch of the heterokont lineage. However, despite the anticipated availability of a brown algal genome (Peters et al. 2004) and the publication of a few brown algal expressed sequence tag (EST) projects (Crépineau et al. 2000; Roeder et al. 2005; Wong et al. 2007), multicellular heterokonts remain highly underrepresented in sequence databases, and genomic studies in brown algae remain in the gene discovery phase.

Several research groups use *Fucus* spp. as models for ecological and evolutionary genomic studies, including analyses of abiotic stress resilience at the interspecific and intraspecific level (Pearson et al. 2000, 2009), mating system evolution and hybridization (Coyer et al. 2002a, b; Billard et al. 2005b; Engel et al. 2005; Perrin et al. 2007), and population genetics and phylogeography (Coyer et al. 2003; Billard et al. 2005a; Hoarau et al. 2007). A number of questions concerning the microevolutionary and macroevolutionary history within and between members of this recently evolved (Serrão et al. 1999; Coyer et al. 2006) genus remain, the answers to which will require tools developed from extensive gene sequence and polymorphism data. EST libraries are a widely used and convenient entry point for generating the genomic data necessary for such goals, by simultaneously allowing for gene discovery, providing an overall picture of gene expression, and (by using a pooled sample of individuals for library construction) allowing the identification of polymorphic genetic markers present in, or closely linked to, specific gene loci, such as EST-linked microsatellites (Coyer et al. 2008) and single-nucleotide polymorphisms (SNPs).

In this study, we report the generation and analysis of >12,000 EST sequences from two *Fucus* species: *Fucus vesiculosus* undergoing desiccation stress and rehydration, and *Fucus serratus* during a heat shock (HS) treatment and subsequent recovery. The responses of *Fucus* spp. to the two stressors were very different; a rapid transcriptional response to HS contrasted sharply with the response to desiccation/rehydration. Our data also suggest that similar levels of desiccation stress result in quite different responses in desiccation-susceptible *F. serratus* and desiccation-tolerant *F. vesiculosus*.

Methods

Culture Conditions and Stress Treatments

Fucus vesiculosus (L.) was collected from the Ria Formosa coastal lagoon, Portugal (37° 00′ 40″ N, 7° 59′ 18″ W). *F. serratus* (L.) was collected from the intertidal zone at Widemouth Bay, Cornwall, UK (50° 47′ 12″ N, 4° 33′ 40″ W). Adult individuals (≥20) were transported to the laboratory in coolers and placed into culture in filtered natural seawater within hours (*F. vesiculosus*) or 2 days (*F. serratus*). Apical tips (approximately 5 cm) were cut from the algae and allowed to acclimate for approximately 1 month immersed at 15°C, 14:10 h L:D cycle, and 50 – 100-µmol photons per square meter per second. Following acclimation, the algae were exposed to either heat shock (*F. serratus*) or desiccation (*F. serratus* and *F. vesiculosus*). Heat shock treatments consisted of a transfer of apices to SW at 30°C for a period of 4 h, both at culture irradiance and increased irradiance (approximately 350-µmol photons per square meter per second). Samples were taken for RNA extraction after 30 min and 4 h (stress samples). The remaining tissue was returned to acclimation conditions and further samples were taken after 30 min and 2 h (recovery samples). For desiccation treatments, apices were allowed to desiccate in air at 25°C, 350 µmol photons per square meter per second for 3 h and samples for RNA extraction were taken after 10, 30 min, 1, and 3 h (stress samples). The remaining tissue was transferred back to acclimation conditions and a

further sample was taken after 1 h (recovery sample). The HS and desiccation conditions impose sublethal physiological stresses on these species, as reported (Pearson et al. 2009). All samples for both species/stresses were immediately frozen in liquid nitrogen and stored at −80°C, before being lyophilized in preparation for extraction (Pearson et al. 2006).

cDNA Library Construction, Sequencing, and Assembly

Total RNA was isolated from the lyophilized tissue following Pearson et al. (2006). RNA quality was verified by agarose gel electrophoresis. Equal quantities of total RNA from the heat shock (stress and recovery; *F. serratus*) and the desiccation (stress and recovery; both species) treatments were combined to provide starting RNA pools for the three EST libraries.

Poly-A messenger RNA (mRNA) was isolated from total RNA (0.9–1.3 mg) using the GenElute mRNA Miniprep Kit (Sigma). The purification step was repeated a second time to improve mRNA enrichment and yields of 5–6 μg of poly-A mRNA were obtained.

Directionally cloned complementary DNA (cDNA) libraries were synthesized using the SMART cDNA Library Construction Kit (Clontech) according to the manufacturer's instructions. Poly-A^+ mRNA (approximately 1 μg per library) was used for first-strand synthesis with the CDS III/30 polymerase chain reaction (PCR) primer and SMART IV oligonucleotide (Clontech, Palo Alto, CA, USA). Double-stranded cDNA synthesis was performed by long-distance PCR with a PTC100 thermocycler (MJ Research) using the following amplification profile: denaturation at 95°C for 1 min, followed by 20 cycles at 95°C (15 s) and 68°C (6 min); primer adaptors specific for the 5′ and 3′ ends introduced asymmetrical *Sfi*I restriction sites during cDNA synthesis, which after digestion facilitated directional cloning. Digested cDNA was size-fractionated using Chroma Spin-400 columns (Clontech). The size fractionation was conservative, targeting insert sizes of >500 bp. Only the three fractions containing the largest cDNAs were selected for subsequent ligation. Ligation in pDNR-LIB vector was performed overnight at 16°C. Recombinant plasmids were transformed by electroporation into DH5α electromax *Escherichia coli* (Invitrogen) and the bacterial suspension was plated onto 22×22-cm Luria Bertani agar plates containing 30 μl/ml of chloramphenicol. Plates were incubated for 12 h at 37°C and clones were robotically transferred into 384-well plates using a robot at the Max Planck Institute for Molecular Genetics, Berlin, Germany. The cloned cDNAs were 5′-end-sequenced using Big Dye 3.1 chemistry and ABI 3130XL capillary sequencers after plasmid preparation.

EST Analysis

After screening for high-quality reads (Phred 13), a total of 12,115 sequence reads were produced from three projects: 5,571 for *F. serratus* heat shock/recovery (FsHS); 2,048 for *F. serratus* desiccation/rehydration (FsD); 4,496 for *F. vesiculosus* desiccation/rehydration (FvD). The ESTs were clustered and assembled into tentative consensus sequences (TCs), using the TIGR clustering algorithm and cap3, respectively, at the bioinformatics platform of the University of Bielefeld, Germany, using the TIGR default clustering parameters (minimum overlap length 40 bp, identity 0.95, maximum unmatched overhang 20). After clustering, the redundancy of the libraries was calculated from:

$$(1 - (\text{singletons}/\text{reads})) \times 100$$

TCs and singletons were annotated automatically (E values$\leq 10^{-5}$) by comparison with sequences in the databases (KEGG, KOG/COG, SwissProt, "algal" EST collections, GenBank nonredundant accessions, the *Thalassiosira pseudonana* (diatom) genome, *Ectocarpus siliculosus* (brown alga) ESTs, and a 7× genome coverage of the brown alga *E. siliculosus*). Each *Fucus* library was also compared by Blastn to each of the others to identify homologous sequences. Vector-screened and quality-clipped EST sequences were submitted to dbEST within GenBank (accession numbers GH694677–GH700252, GH700253–GH702300, and GH702301–GH706794 for FsHS, FsD, and FvD, respectively).

Annotation Rate Versus Coding Sequence and 3′ UTR Length

For each TC in the three species- and stress-specific assemblies, we used the high-throughput open reading frame (ORF) analysis tool Diogenes (Crow and Retzel 2005) to identify ORF candidates by conceptual translation in all six reading frames. The best prediction in the forward (+) frames was selected and used to generate a probability distribution for the directionally cloned inserts. These data were generated for two classes of TCs: those returning significant BLASTx hits (E values$\leq 10^{-5}$) against protein databases (KEGG, COG, SP, and NCBI nr.; "annotated") and those that did not ("nonannotated"). Since only P values of ≤0.0099 are reported by the Diogenes software, a conservative P value of 0.01 was assigned to all TCs that did not return a significant value. A Kruskal–Wallis rank sum test was then used to test the probability that the P values from the 2 TC classes come from the same distribution for each of the three libraries.

We then tested the hypotheses that nonannotated TCs contain shorter potential ORFs and longer 3′ untranslated regions (UTRs) than annotated TCs. The top 100 largest TCs from each assembly were rebuilt locally (CodonCode Aligner software, CodonCode Corporation) and examined to determine the relative contribution of coding sequence and 3′ UTR to each. First, poly-A tails were identified by examining chromatogram files at the 3′ end of the TC. In the subset of poly-A-containing TCs, protein-coding regions were identified based either on annotations against protein databases and location of the potential start and stop codons or, in cases where no annotation was available, the longest ORF in the three forward frames was used as a conservative measure using ORF Finder (www.ncbi.nlm.nih.gov/gorf/gorf.html) and manually checking for 5′ truncated ORFs. Kruskal–Wallis rank sum tests were used to compare total TC lengths, ORF lengths, and 3′-UTR lengths between annotated and nonannotated TC classes.

Identification of Potential Expression Differences Between Libraries

In order to extract semiquantitative estimates of gene expression from the TCs, the frequencies of reads from the 50 largest TCs for each library were compared by reciprocal local BLASTn (E values$\leq 10^{-10}$) to identify potential orthologs. These were then tested for differential expression based on the number of reads/TC using the method of Audic and Claverie (1997), implemented in the IDEG6 software (Romualdi et al. 2003) with correction for multiple tests using the false discovery rate adjustment $((m+1)/2m)\cdot\alpha$. The analysis assumes that the equal quantities of total RNA/treatment that were used to produce the libraries reflect equal mRNA quantities.

Validation of Gene Expression Patterns by Quantitative PCR

Apical tissue of *F. vesiculosus* from Viana do Castelo, Portugal, was exposed for a total of 3 h to either desiccation (in air at 20–21°C, 300 μmol $m^{-2}s^{-1}$) or heat shock (seawater at 28°C, 300 μmol $m^{-2}s^{-1}$); following exposure, tissues were returned to culture conditions (15°C, 50 μmol $m^{-2}s^{-1}$). Samples were taken after 15-, 30-, 60-, and 180-min exposure to the respective stress and after 1-h rehydration (desiccation) or recovery (heat shock). Treated algae were compared to unstressed control samples from culture conditions. Total RNA was extracted as described above for cDNA library construction. After purification (Qiagen RNeasy cleanup with DNase treatment), the RNA was quantified by UV-spectrophotometry and integrity was checked by denaturing agarose gel electrophoresis. Total RNA (2 μg) was reverse-transcribed by using SuperScript III (Invitrogen) in two parallel 20-μl reactions and pooled. Primers for candidate genes were designed using the Primer3 web application (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi), with a Tm of 68–70°C and an amplicon size between 100 and 150 bp. PCR reactions were performed in a total volume of 20 μl using SYBR-green-based detection (Bio-Rad), a 1:10 dilution of cDNA template, and 0.5 μM of primers. The amplification efficiency of each primer pair was calculated from dilution curves, using a pooled cDNA mix from all treatment conditions as template. Quantitative PCR (qPCR) reactions were amplified in triplicate using an iCycler iQ Detection System (Bio-Rad). Cycle parameters were 95°C for 2 min and then 50 cycles at 95°C for 10 s and 68°C for 30 s. Relative expression was analyzed with iQ5 2.0 software (Bio-Rad Laboratories), using the $\Delta\Delta C_T$ method corrected for amplification efficiency (Pfaffl et al. 2002) and implementing the models developed by Vandesompele et al. (2002) for multiple reference (housekeeping) genes: β-actin and α-tubulin. Unstressed treatments were used as the reference condition.

Results and Discussion

EST Sequencing and Cluster Analysis

Four 5′-end-sequenced EST libraries produced a total of 12,121 reads distributed as follows: FsHS=5,577 (3,448+2,129 from two pooled libraries); FvD=4,496; FsD=2,048, resulting in 2,503, 2,409, and 1,290 nonredundant sequences or unigenes, respectively (Table 1). The redundancy of the three libraries was 66.9%, 58.5%, and 49.3% for FsHS, FvD, and FsD, respectively. The redundancies were broadly similar and, as expected, declined with library size.

The distribution of ESTs per TC in the three libraries was broadly similar; the largest TCs contained 150 and 63 reads in FsHS and FvD, respectively, with two TCs of 33 reads in FsD, which has less than half the total number of ESTs of the first two. The mean length (Table 1) varied between 913 bp (FsHS) and 771 bp (FvD), and the number of TCs of length less than 500 bp was also the greatest for FvD, at nearly 17%, compared with 4–6% for the *F. serratus* libraries.

Annotation Success and Presence of Coding Sequences

Although the cDNA libraries were constructed using a method that selects full-length mRNA (using SMART® technology) and clones were sequenced from the 5′ end, the average length of unigenes (TCs and singletons) was only approximately 500–650 bp (slightly greater for *F. serratus*). We further observed that a large number of TCs appeared to

Table 1 Number and average length of ESTs after removal of contaminating vector sequences, low-quality reads, and poly-A+ sequences for three *Fucus* libraries

Library	Category	Nº of sequences	Average length (bp)	Annotated (%)
FsHS	Total ESTs	5577	541	
	TCs	658	913	45.0
	Singletons	1845	540	20.7
	Nonredundant	2503	638	27.1
FsD	Total ESTs	2048	498	
	TCs	252	771	49.6
	Singletons	1038	438	26.4
	Nonredundant	1290	503	30.9
FvD	Total ESTs	4496	457	
	TCs	542	796	34.5
	Singletons	1867	492	13.2
	Nonredundant	2409	560	18.0

The number of sequences with hits against protein databases (Blastx against NCBI nr, SP, KEGG, and KOG; $E \leq 10^{-5}$) are indicated

FsHS F. serratus heat shock/recovery (two pooled libraries), *FsD F. serratus* desiccation/rehydration, *FvD F. vesiculosus* desiccation/rehydration

contain a poly-A tail. Therefore, we tested whether overall low annotation rates were due mainly to the presence of unique proteins with no homologs in the databases or to the lack of coding sequence and long 3′ UTR sequences previously noted in brown algae (Apt et al. 1995).

We analyzed two groups of TCs classified as either "annotated" or "nonannotated" using an ORF prediction algorithm (Crow and Retzel 2005). The probability distributions obtained were significantly different in each case (Kruskal–Wallis tests, $df=1$, $P<0.0001$ for all libraries). The FsHS library contained 299 annotated and 357 nonannotated TCs, of which 69.2% and 8.1%, respectively, had a "good" probability of containing an ORF (using a cutoff P value of 10^{-6} as suggested by Crow and Retzel 2005). The values for the other two libraries followed a similar pattern: FsD contained 126 annotated and 126 nonannotated TCs, with ORF prediction rates of 72.2% and 10.3%, and FvD contained 196 annotated and 346 nonannotated TCs, with ORF prediction rates of 64.3% and 2.3%, respectively. The results confirmed that the large majority of nonannotated TCs did not contain significant protein-coding regions rather than encoding unknown proteins.

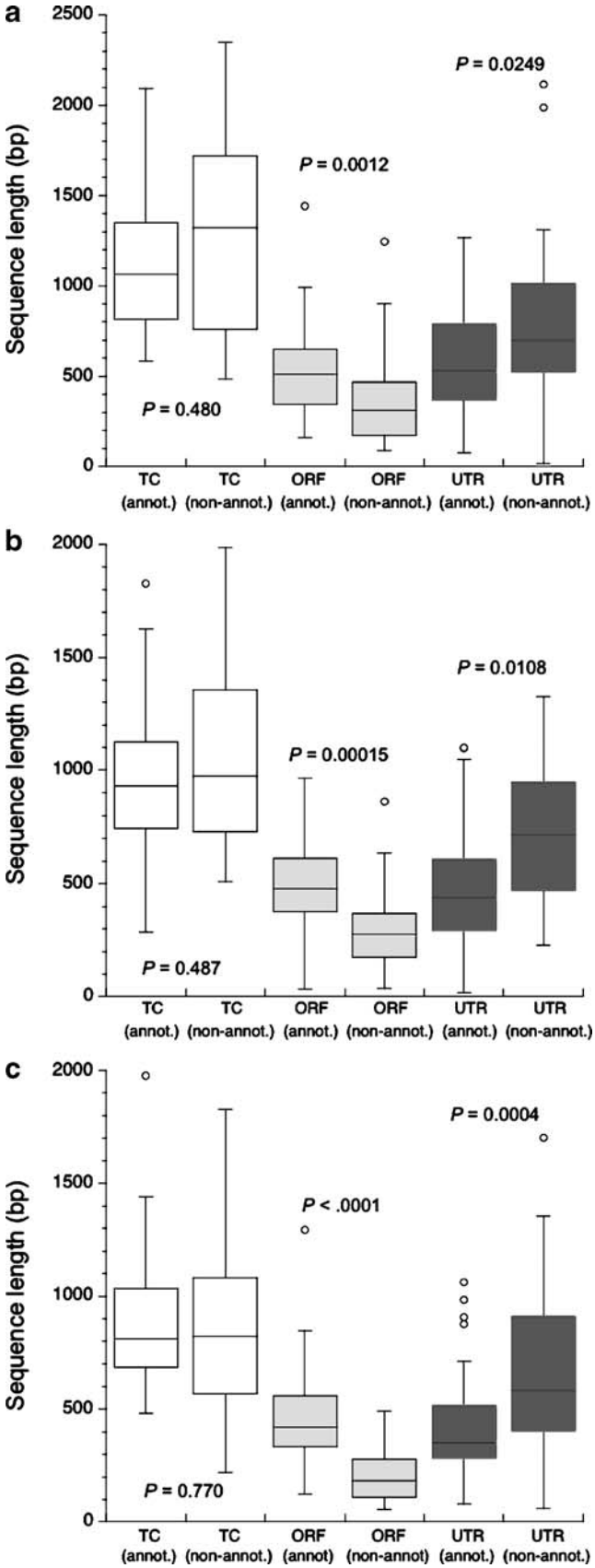
The proportion of the largest 100 TCs containing a 3′ poly-A region for FsHS, FsD, and FvD was 93%, 58%, and 93%, respectively. Total TC lengths were not significantly different between the two groups for any library (Fig. 1). However, the 3′ UTRs were significantly longer in nonannotated TCs, indicating that more of the total length in this category was noncoding sequence. In particular, the predicted ORF lengths were significantly shorter for nonannotated TCs, further supporting the conclusion that annotation failure is primarily linked with a lack of sufficient coding sequence whose transcription is hindered by long 3′ UTR sequences.

The overall annotation rate for brown algal EST collections is generally <50% (Roeder et al. 2005; Wong et al. 2007) and was even lower in the current study for *Fucus* (Table 1). This is generally attributed to the evolutionary distance between heterokonts and other taxa used for database comparisons. However, given these results and the fact that our average sequencing read lengths were comparable with previous studies, we suggest that extensive 3′ UTR length in brown algae generally (e.g., Apt et al. 1995) contributed to low annotation rates. This may continue to present a significant challenge to EST-based gene identification in this group in future.

Functional Annotation Against Protein Databases

Functional annotation of the TCs and singleton reads was achieved by Blastn and Blastx searches against nucleotide and protein sequence databases. Comparison of the EST sequences against the COG database (Tatusov et al. 2003) resulted in tentative annotation ($E \leq 10^{-5}$) of 1,042 ESTs in FsHS (18.7%), 260 in FsD (12.7%), and 574 in FvD (12.8%), which clustered into 221, 85, and 154 TCs, respectively. A graphical representation of the distribution of ESTs and TCs into functional categories is presented in Fig. 2. Overall, the largest category for all three libraries was for genes involved in translation (J), mainly encoding ribosomal protein genes (data not shown). The proportion was larger in FvD (67.4%) than for FsHS (40.5%) or FsD (33.8%). However, when all ESTs were considered (i.e., annotated and nonannotated), the proportion in the translation category was more constant across libraries at 7.6%, 4.3%, and 8.6% in FsHS, FsD, ad FvD, respectively. This was also reflected in the proportion of annotated TCs (see Fig. 5; small pie charts). These differences may have more to do with differential annotation success in other categories than to expression differences between the libraries (see below).

The most striking difference between the three libraries was with respect to the stress imposed, rather than species;



◀ **Fig. 1** *Box plots* showing the distributions of TC (*open boxes*), predicted ORF (*gray boxes*), and 3′ UTR (*dark boxes*) lengths for the largest 100 TCs, classified as "annotated" or "nonannotated" (see "Methods") from **a** *F. serratus* heat shock/recovery, **b** *F. serratus* desiccation/rehydration, and **c** *F. vesiculosus* desiccation/rehydration EST libraries. *Boxes* enclose the 25% to 75% quartiles, with the median (*horizontal bar*); *vertical bars* show the upper and lower range of the data; outliers represented as open circles. Where present, *P* values show significant differences between annotated and non-annotated sequence length distributions (Kruskal–Wallis rank sum tests)

the posttranslational modification category (O) was over-represented in FsHS annotated ESTs (30.4%) and comprised 5.7% of the total library. In contrast, the proportion of ESTs was only 8.5% and 7.5% in the FsD and FvD annotated set, and only 2.05% and 1.0% of the total ESTs, respectively.

Heat Shock Protein Genes

Numerically, the most important COG posttranslational category genes are the chaperone genes, in particular the alpha crystallin class of small heat shock proteins (sHsps), which contribute disproportionately to the difference between stress treatments, with 12 TCs in FsHS, comprising 328 EST reads, compared with a single EST from FvD and none from FsD (Table 2). Other Hsps were either uniquely or overrepresented in the HS library: three Hsp90 genes and the Hsp70 gene family members, dnaK (plastid encoded) and BiP (luminal-binding protein). Interestingly, the Hsp70 cochaperones, such as DnaJ/Hsp40 and GrpE, were poorly represented in the FsHS library, with a single TC (containing three reads) for STI (Hsc70/Hsp90 organizing protein), while a singleton for GrpE (organellar Hsp70 cochaperone) was found in each of the desiccation libraries (FsD and FvD). This suggests that, unlike Hsp70/Hsp90 family members, these cochaperones are not under strong transcriptional regulation by HS in *Fucus*, although confirmation of this requires further investigation.

sHsps are ubiquitously expressed in both prokaryotes and eukaryotes in response to heat stress (Sun et al. 2002) and, despite low sequence similarities between classes, share a conserved 90-amino-acid carboxyl terminal domain (the α-crystallin domain), originally identified in the lens of the vertebrate eye. There was no evidence for transcriptional regulation of sHsps by desiccation or rehydration in either *F. serratus* or *F. vesiculosus*, although in plants, where diversity and function are greatest, sHsps are induced by a variety of stresses in addition to heat stress, e.g., drought, cold, osmotic stress, at different developmental stages (Sun et al. 2002; Wang et al. 2004), and accumulate in desiccation-tolerant tissues such as seeds (Wehmeyer and Vierling 2000). Plant sHsps are classified into six gene families based on their cellular localization (Wang et al.

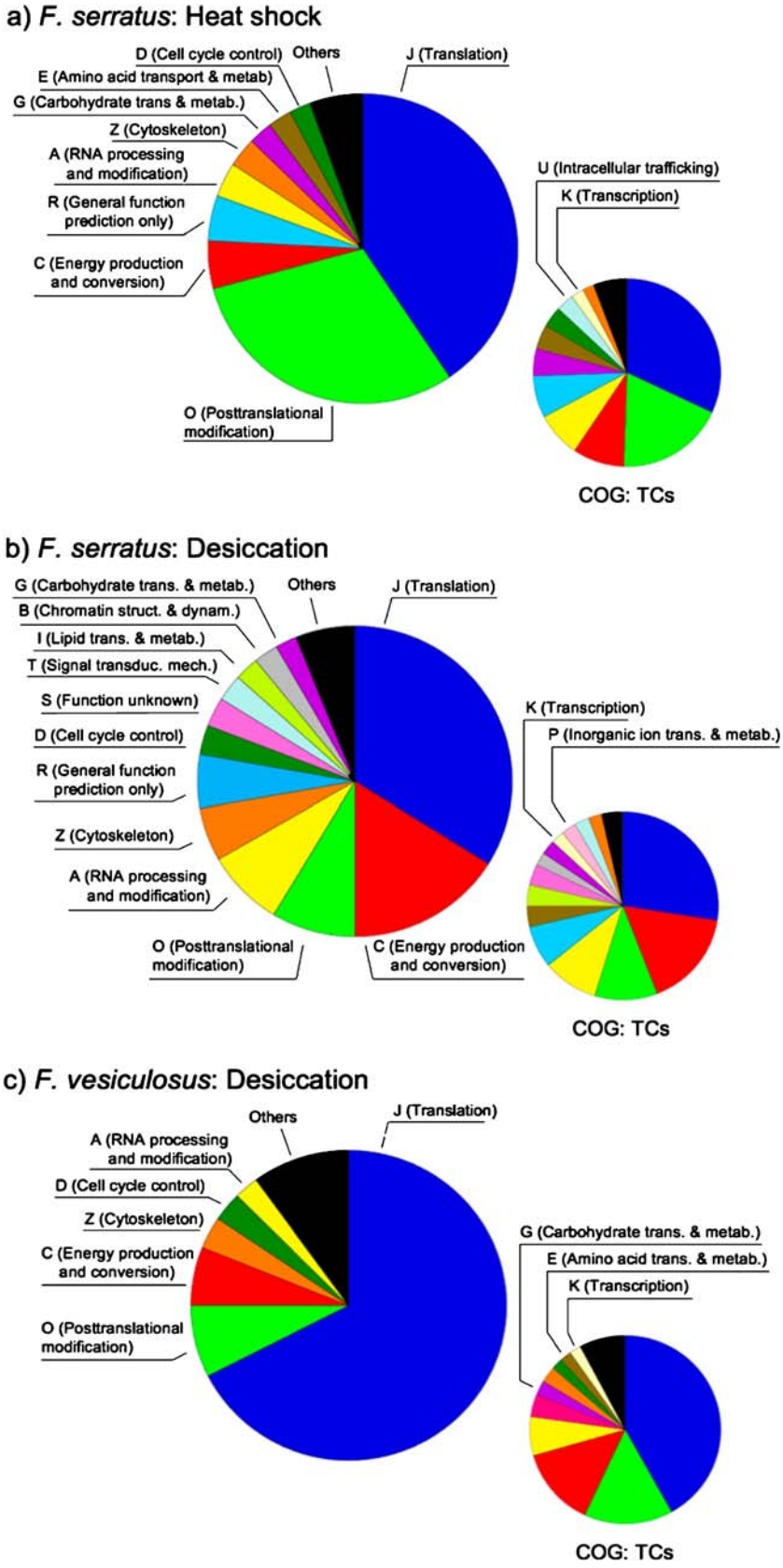


Fig. 2 *Pie charts* showing major functional categories (COGs) for the ESTs (*large circles*) and TCs (*small circles*) from **a** *F. serratus* heat shock/recovery, **b** *F. serratus* desiccation/rehydration, **c** *F. vesiculosus* desiccation/rehydration libraries

Table 2 Posttranslational modification genes (COG category O) identified from the three EST libraries

FsHS	FsD	FvD	Description
33	–	1[a]	Q84Q72—17.4-kDa class I heat shock protein
20	–	–	Q84Q72—17.4-kDa class I heat shock protein
8	–	–	Q84J50—17.4-kDa class I heat shock protein
21	–	–	Q84J50—17.4-kDa class I heat shock protein
35	–	–	P27396—17.8-kDa class I heat shock protein
40	–	–	Q84Q72—17.4-kDa class I heat shock protein
55	–	–	Q84Q72—17.4-kDa class I heat shock protein
4	–	–	Q84J50—17.4-kDa class I heat shock protein
53	–	–	Q84Q72—17.4-kDa class I heat shock protein
35	–	–	Q84J50—17.4-kDa class I heat shock protein
22	–	–	Q84Q77—17.4-kDa class I heat shock protein
2	–	–	Q84J50—17.4-kDa class I heat shock protein
51	7	2	Q69QQ6—heat shock protein 90
6	–	–	P06660—heat-shock-like 85-kDa protein
2	–	–	P06660—heat-shock-like 85-kDa protein
58	–	–	Q8DI58—heat shock 70-kDa protein 2 (dnaK)[b]
5	–	3[a]	P41753—heat shock 70-kDa protein
7	–	–	Q03683—luminal-binding protein 3 (BiP 3)
29	5[a]	17[a]	P34790—peptidyl-prolyl *cis–trans* isomerase CYP18-3
5	6	8	O42993—peptidyl-prolyl *cis–trans* isomerase (FKBP)
8[a]	2	4	P52009—peptidyl-prolyl *cis–trans* isomerase 1
2	2[a]	1[a]	P31106—peptidyl-prolyl *cis–trans* isomerase
2	–	1	P62937—peptidyl-prolyl *cis–trans* isomerase A
8	–	–	P21569—peptidyl-prolyl *cis–trans* isomerase
–	–	2	Q9LEK8—peptidyl-prolyl *cis–trans* isomerase 1
11		2[a]	P27773—protein disulfide-isomerase A3
9	2[a]	3[a]	P43156—thiol protease SEN102
–	2	–	Q8H166—thiol protease aleurain
7	2	1[a]	Q9TM05—ATP-dependent Clp protease ATP-binding subunit clpA
–	–	2	Q9L4P4—putative ATP-dependent Clp protease proteolytic subunit like
2	1[a]	8	P25249—cysteine proteinase EP-B 1
3	–	2	P46437—glutathione *S*-transferase
2	–	–	O16116—glutathione *S*-transferase 3
2	–	1[a]	Q16772—glutathione *S*-transferase A3
3	–	8	P55143—glutaredoxin
14[a]	1[a]	2	P55142—glutaredoxin-C6
2	–	–	Q8LBK6—monothiol glutaredoxin-S15
6[a]	2[a]	6	P73728—putative peroxiredoxin sll1621
–	–	3	P0A4L1—thioredoxin-1
1	–	2	P28627—mitochondrial inner membrane protease subunit 1
1[a]	1[a]	5	P55956—aspartic protease 3
–	–	2	Q9NZS9—bifunctional apoptosis regulator (RING finger protein)
3[a]	2[a]	4	P22589—ubiquitin
3[a]	2[a]	6[a]	P22589—ubiquitin
1[a]	1[a]	2	P22589—ubiquitin
7	2	1[a]	P55857—ubiquitin-like protein SMT3
2	–	–	P50623—SUMO-conjugating enzyme UBC9
–	2	–	Q9P6I1—ubiquitin-conjugating enzyme E2
1[a]	–	2	Q6DCZ9—ubiquitin-conjugating enzyme E2 (NEDD8 protein ligase)
3	–	–	O24362—proteasome subunit alpha type 3
3	–	–	P42742—proteasome subunit beta type 1
2	–	–	Q7DLR9—proteasome subunit beta type 4
–	2	–	P34120—proteasome subunit alpha type 7
3[a]	–	4	P42742—proteasome subunit beta type 6
1[a]	–	3	O65084—proteasome subunit beta type-3
1[a]	1[a]	2	Q8LD27—proteasome subunit beta type 6
2	–	–	Q6IQT4—COP9 signalosome complex subunit 2
–	–	2	Q9FVU9—COP9 signalosome complex subunit 5a
5	–	8	Q39757—14-3-3-like protein
2	–	–	Q39757—14-3-3-like protein
3	–	–	O94777—dolichol phosphate-mannose biosynthesis regulatory protein

The first three columns show the number of EST reads from each library in the TC; UniProt accession numbers of the top Blastx hit and a brief gene description are provided

– no orthologous sequences were detected by pairwise BLASTn between the libraries

[a] Putative ortholog identified by pairwise BLASTn between libraries (i.e., annotated gene in one library used to identify a homolog in which the coding sequence was truncated and/or using the 3′ UTR)

[b] Organelle-encoded transcript (plastid)

2004), and the best hits (SwissProt) for all the *Fucus* sHsps suggested that they are class I (cytosolic) members. A search for signal peptides in five putatively full-length *Fucus* representatives using SignalP (http://www.cbs.dtu.dk/services/SignalP-3.0/) revealed no evidence of organellar targeting signals, supporting a cytosolic localization. However, when submitted to the DAS Transmembrane Prediction server (Cserzo et al. 1997; http://www.sbc.su.se/~miklos/DAS/), a single 11-amino-acid transmembrane domain was found 43 residues from the putative start site (PGSSV/IXL/VVSAV/I; where X indicates any amino

acid), which is not present in class I proteins from plants and which may indicate a membrane association.

A phylogenetic analysis of five putatively full- or nearly-full-length *F. serratus* sHSPs was performed after alignment using MUSCLE (Edgar 2004) of the conceptual translation products, together with a dataset of 49 protein neighbors (top ten Blastx hits) and other more distant sequences obtained from the protein database. The dataset contains plant, animal, fungal, protist, and bacterial representatives, and a tree (Fig. 3) was produced using PHYML (Guindon and Gascuel 2003), with the LG protein model (Le and Gascuel 2008), estimated gamma distribution parameter, and four substitution rate categories, with 100 bootstrap replicates. The deeper relationships between major taxonomic groups on the tree were unresolved, presumably reflecting the ancient origin and conserved nature of these proteins. However, *F. serratus* sHSP sequences formed a tight cluster of closely related proteins with high bootstrap support (Fig. 3). Two diatom sHsp sequences from *Phaeodactylum tricornutum* were more divergent than and did not cluster together with the *Fucus* sequences, showing that sHSPs in *Fucus* form a gene family that arose from a single ancestor following the divergence between the two lineages. The heterokont sequences (*Fucus* and diatom) were no more closely related than those at much larger phylogenetic distance, e.g., plant and red algal sHsps (Collén et al. 2006). The latter also formed distinct and well-supported clades, as did some sHsps from bacterial groups such as Chlorobi and Thermotogae.

Other Stress-Related Genes

The TCs and singletons for each library were searched on the SAMS database (https://www.cebitec.uni-bielefeld.de/groups/brf/software/sams/cgi-bin/sams_login.cgi?cookie_test=1) for annotation terms related to stress (heat, desiccation/dehydration, oxidative stress) and those identified were checked against a list of stress-responsive genes in UniProtKB/SwissProt. The list of genes found and corresponding TC sequences can be found for each species in the supplementary data (Table S1, TC_fasta S2). The

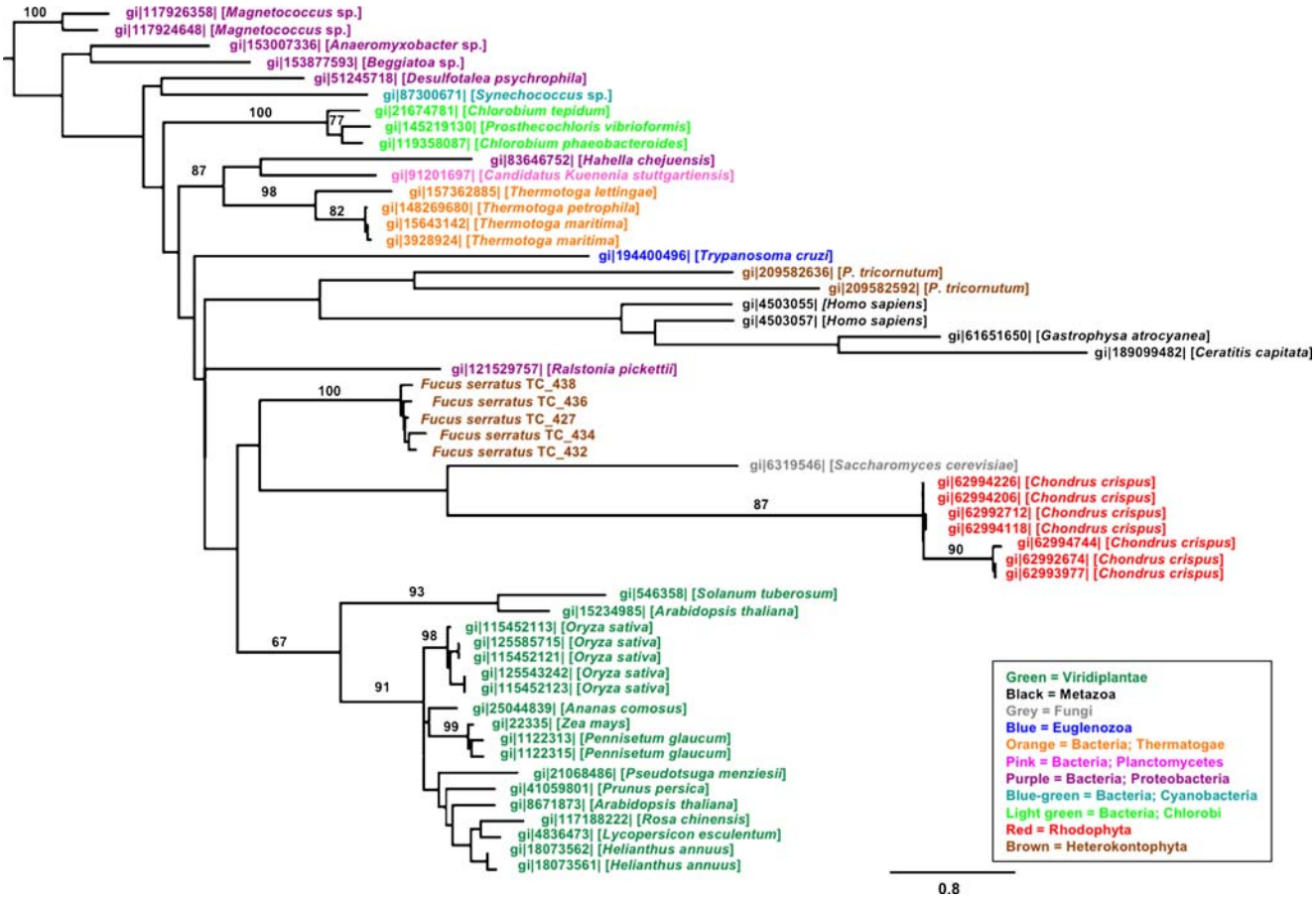


Fig. 3 An ML phylogenetic tree for sHsp protein sequences based on an alignment of 54 proteins across several eukaryotic and prokaryotic groupings. Protein accession numbers are given together with taxon names or the TC in the case of *F. serratus* (FsHS library). The tree was obtained using PHYML, the LG model, a discrete gamma distribution with four rate categories, and 100 bootstrap resamplings. Bootstrap values >50 are shown on the *branches*

results (best Blastx hit $E \leq 10^{-5}$) provide further illustration of the difference between the stress conditions imposed: a total of 80 genes were identified in *F. serratus* subjected to heat shock/recovery, whereas only 31 and 37 genes were identified in *F. serratus* and *F. vesiculosus*, respectively, subjected to desiccation/rehydration. In addition to the Hsps discussed above, genes representing other major classes of chaperone or cochaperone protein families were also either uniquely present or overrepresented in FsHS, e.g., Hsp60/GroEL, STI (mediating interactions between Hsp70 and Hsp90), and Hsp100 (ClpB). Several peptidyl-prolyl *cis–trans* isomerases were found in all libraries and encoded cochaperones that assist in protein folding or refolding. Redox regulation and/or oxidative stress genes were found in all three libraries in similar numbers and abundances, e.g., peroxiredoxins, glutaredoxins, and superoxide dismutase. An exception appears to be glutathione *S*-transferases, which were represented by seven TCs in FsHS and only two in FvD (none were identified in FsD).

The FsHS library also contained a highly abundant transcript that does not contain a well-supported ORF (FsHS tc_00425), a carbonic anhydrase (inorganic carbon acquisition for photosynthesis), and PsbU, which is involved in stabilizing the PSII oxygen-evolving complex during heat stress and which appears to be restricted to Cyanobacteria and eukaryotes containing red-lineage chloroplasts, including red algae and diatoms. Transposable elements (TE) of the retroviral *Pol* or *Copia*-type were identified as several distinct TCs (FsHS) or singletons (FvD), and these were more abundant overall in heat-shocked than desiccated tissues (Table 3, see also Table 4). Retrotransposons are common features in heterokonts, accounting for 2–6% of the genome in diatoms (Armbrust et al. 2004; Bowler et al. 2008) and up to 90% of the genome in some plants (Wessler 2006). Transcription of TE is known to increase under a variety of stress conditions in several systems (Wessler 1996; Grandbastien 1998; Capy et al. 2000; Grandbastien et al. 2005; Wessler 2006; Ramallo et al. 2008) and may be an important source of genetic novelty through gene and/or intron duplication or loss. Our data suggest that they may be more transcriptionally active during HS and recovery than during desiccation/rehydration in *Fucus* spp.

TCP-1 subunit genes were found only in desiccation/rehydration libraries (as singletons, Table S1). TCP-1 forms part of cytosolic type II chaperonin containing t-complex polypeptide 1 that has an important role in the folding of actin and tubulin (Llorca et al. 2000; Grantham et al. 2002) and therefore in cytoskeletal integrity and control. Cytoskeletal (particularly microtubule) dynamics plays an important role in maintaining cellular integrity during desiccation/rehydration cycles, which involve large changes in cell volume (Bagniewska-Zadworna 2008).

Relative Quantitation of Gene Expression in Large TCs

Relative expression differences were investigated for the 50 largest TCs from the three libraries, which after reciprocal

Table 3 Retroviral-like pol- and/or copia-related sequences in *Fucus* EST libraries

Contig/singleton	TC size	Length (bp)	Description	*E* value	Observations
F. serratus					
FsHS_tc_00483	15	2,113	P10978: retrovirus-related Pol polyprotein (reverse-transcriptase region)	$5\times E^{-48}$	poly-A
FsHS_tc_00686	3	1,072	P10978: retrovirus-related Pol polyprotein (integrase core domain)	$2\times E^{-29}$	poly-A
FsHS_tc_00818	2	1,178	P04146: copia protein (reverse-transcriptase region)	$3\times E^{-20}$	poly-A
FsHS_tc_00933	2	1003	P04146: copia protein (reverse-transcriptase region)	$5\times E^{-35}$	poly-A on antisense strand
FsHS_tc_00960	2	869	P04146: copia protein (3′ region, no conserved domain)	$1\times E^{-17}$	poly-A
FsD_tc_00251	2	605	P04146: copia protein (3′ region, no conserved domain)	$5\times E^{-3}$	no poly-A
F. vesiculosus					
g5P03O10	1	482	P04146: copia protein (3′ region, no conserved domain)	$9\times E^{-18}$	no poly-A
g5P04B14	1	615	P10978: retrovirus-related Pol polyprotein (reverse-transcriptase region)	$6\times E^{-14}$	no poly-A
g5P05H16	1	722	P10978: Retrovirus-related Pol polyprotein (reverse transcriptase region)	$2\times E^{-8}$	no poly-A
g5P11H06	1	711	P10978: Retrovirus-related Pol polyprotein (reverse transcriptase region)	$2\times E^{-32}$	poly-A

Table 4 Differential gene expression

TC	Description	(1) Fser_HS (norm)	(2) Fser_D (norm)	(3) Fves_D (norm)	1 vs. 2	1 vs. 3	2 vs. 3	Relative expression	
								Up	Down
FsD_tc_00016	Unknown	10.8	43.9	60.1	0.004	0.000	ns	FsD, FvD	
FsD_tc_00042	Unknown	1.8	24.4	60.1	0.005	0.000	0.008	FsD, FvD	
FsD_tc_00024	Fucoxanthin–chlorophyll a–c binding protein	3.6	34.2	11.1	0.001	ns	0.011	FsD	
FsD_tc_00028	GTP-binding regulatory protein beta chain	7.2	34.2	11.1	0.007	ns	0.011	FsD	
FsHS_tc_00440	Metallothionein	113.1	161.1	120.1	0.017	ns	0.012	FsD	
FsD_tc_00072	Profilin	5.4	24.4	0.0	0.022	ns	0.001	FsD	
FsD_tc_00027	Unknown	7.2	34.2	8.9	0.007	ns	0.007	FsD	
FsD_tc_00033	Unknown	7.2	29.3	8.9	0.017	ns	0.014	FsD	
FsD_tc_00039	Unknown	3.6	24.4	11.1	0.012	ns	ns	FsD	
FsD_tc_00044	Unknown	5.4	24.4	0.0	0.022	ns	0.001	FsD	
FsD_tc_00049	Unknown	1.8	24.4	6.7	0.005	ns	0.017	FsD	
FsD_tc_00029	ATP synthase gamma chain	12.6	34.2	4.4	ns	ns	0.002	FsD?	
FsD_tc_00046	Ferredoxin-NADP reductase	8.1	24.4	4.4	ns	ns	0.009	FsD?	
FsD_tc_00030	Unknown	12.6	29.3	6.7	ns	ns	0.008	FsD?	
FsD_tc_00036	Unknown	7.2	24.4	4.4	ns	ns	0.009	FsD?	
FsD_tc_00048	Unknown	7.2	24.4	0.0	ns	ns	0.001	FsD?	
FsD_tc_00026	Similar to tubulin, beta 2	17.1	34.2	0.0	ns	0.001	0.000	FsD?	FvD
FsHS_tc_00445	Chlorophyll a–b binding protein	28.7	73.2	0.0	0.004	0.000	0.000	FsD	FvD
FsHS_tc_00446	Chlorophyll a–b binding protein	68.2	112.3	0.0	0.014	0.000	0.000	FsD	FvD
FsHS_tc_00502	Unknown	25.1	102.5	2.2	0.000	0.001	0.000	FsD	FvD
FsHS_tc_00454	Unknown	62.8	161.1	0.0	0.000	0.000	0.000	FsD	FvD
FsHS_tc_00469	Unknown	41.3	122.1	0.0	0.000	0.000	0.000	FsD	FvD
FvD_tc_00022	40S ribosomal protein S11	16.2	24.4	46.7	ns	0.002	0.023	FvD	
FvD_tc_00031	40S ribosomal protein S17	12.6	4.9	35.6	ns	0.005	0.004	FvD	
FvD_tc_00042	40S ribosomal protein S28	10.8	9.8	33.4	ns	0.005	0.015	FvD	
FsD_tc_00038	40S ribosomal protein S3	8.1	24.4	57.8	ns	0.000	0.009	FvD	
FvD_tc_00016	40S ribosomal protein S3	21.5	4.9	44.5	ns	0.010	0.001	FvD	
FvD_tc_00035	40S ribosomal protein S5	16.2	4.9	33.4	ns	0.020	0.006	FvD	
FsD_tc_00041	40S ribosomal protein S7	19.7	24.4	53.4	ns	0.001	0.014	FvD	
FvD_tc_00014	40S ribosomal protein S8	19.7	9.8	42.3	ns	0.010	0.005	FvD	
FvD_tc_00043	60S ribosomal protein L11	7.2	9.8	31.1	ns	0.002	0.019	FvD	
FvD_tc_00032	60S ribosomal protein L13	12.6	9.8	35.6	ns	0.005	0.012	FvD	
FsHS_tc_00477	60S ribosomal protein L18	26.9	19.5	44.5	ns	0.025	0.018	FvD	
FvD_tc_00052	60S ribosomal protein L23	8.1	9.8	31.1	ns	0.004	0.019	FvD	
FvD_tc_00010	60S ribosomal protein L31	10.8	0.0	62.3	ns	0.000	0.000	FvD	
FvD_tc_00040	60S ribosomal protein L32	10.8	9.8	28.9	ns	0.012	0.024	FvD	
FvD_tc_00057	60S ribosomal protein L7	8.1	4.9	28.9	ns	0.007	0.010	FvD	
FsHS_tc_00459	Eukaryotic translation elongation factor 1a	43.1	29.3	64.5	ns	0.021	0.009	FvD	
FvD_tc_00041	Ribosomal protein (*Phytophthora infestans*)	10.8	0.0	28.9	ns	0.012	0.002	FvD	
FvD_tc_00047	Ribosomal protein (Phytophthora infestans)	10.8	4.9	26.7	ns	0.019	0.014	FvD	
FvD_tc_00034	Ubiquitin/40S ribosomal protein S27 fusion	8.1	4.9	33.4	ns	0.002	0.006	FvD	
FvD_tc_00055	Ubiquitin/60S ribosomal protein L40 fusion	7.2	4.9	24.5	ns	0.010	0.019	FvD	

Table 4 (continued)

TC	Description	(1) Fser_HS (norm)	(2) Fser_D (norm)	(3) Fves_D (norm)	1 vs. 2	1 vs. 3	2 vs. 3	Relative expression	
								Up	Down
FvD_tc_00056	Unknown	7.2	0.0	26.7	ns	0.006	0.003	FvD	
FsHS_tc_00493	Unknown	43.1	53.7	140.1	ns	0.000	0.000	FvD	
FvD_tc_00073	Unknown	5.4	4.9	33.4	ns	0.000	0.006	FvD	
FsHS_tc_00470	Translationally controlled tumor protein	28.7	53.7	53.4	ns	0.011	ns	FvD?	
FvD_tc_00018	Unknown	7.2	14.6	28.9	ns	0.003	ns	FvD?	
FvD_tc_00008	60S ribosomal protein L26	25.1	0.0	48.9	0.009	0.011	0.000	FvD	FsD
FsHS_tc_00460	40S ribosomal protein S11	34.1	24.4	46.7	ns	ns	0.023	FvD?	FsD?
FvD_tc_00038	40S ribosomal protein S15	14.4	0.0	28.9	ns	ns	0.002	FvD?	FsD?
FvD_tc_00059	40S ribosomal protein S25	17.1	4.9	26.7	ns	ns	0.014	FvD?	FsD?
FvD_tc_00039	40S ribosomal protein S30	14.4	4.9	24.5	ns	ns	0.019	FvD?	FsD?
FsHS_tc_00463	40S ribosomal protein S9	35.9	24.4	46.7	ns	ns	0.023	FvD?	FsD?
FvD_tc_00049	60S ribosomal protein L27	19.7	4.9	28.9	ns	ns	0.010	FvD?	FsD?
FvD_tc_00036	Unknown	17.1	4.9	33.4	ns	ns	0.006	FvD?	FsD?
FsHS_tc_00474	40S ribosomal protein S27	26.9	0.0	35.6	0.007	ns	0.001		FsD
FsHS_tc_00475	60S ribosomal protein L10	26.9	0.0	24.5	0.007	ns	0.005		FsD
FsHS_tc_00482	Chlorophyll a–b binding protein	25.1	0.0	22.2	0.009	ns	0.007		FsD
FsHS_tc_00452	Peptidyl-prolyl *cis–trans* isomerase, cyclophilin type	52.1	9.8	37.8	0.003	ns	0.009		FsD
FsHS_tc_00451	60S ribosomal protein L5	57.4	53.7	22.2	ns	0.002	0.007		FvD
FsHS_tc_00464	Actin-depolymerizing factor	34.1	24.4	2.2	ns	0.000	0.004		FvD
FsHS_tc_00444	Chlorophyll a–b binding protein	70.0	87.9	2.2	ns	0.000	0.000		FvD
FsHS_tc_00473	EsV-1-163 [*Ectocarpus siliculosus* virus 1]	28.7	43.9	2.2	ns	0.000	0.000		FvD
FsHS_tc_00507	Fucoxanthin–chlorophyll a–c binding protein	25.1	34.2	6.7	ns	0.008	0.004		FvD
FsD_tc_00025	Peroxisomal membrane protein MPV17/PMP22	17.1	34.2	4.4	ns	0.020	0.002		FvD
FsD_tc_00017	Unknown	23.3	43.9	0.0	ns	0.000	0.000		FvD
FsHS_tc_00453	Unknown	71.8	83.0	35.6	ns	0.003	0.002		FvD
FsHS_tc_00466	Carbonic anhydrase	32.3	14.6	13.3	ns	0.014	ns	FsHS	
FsHS_tc_00426	Heat shock protein Hsp20 (a-crystallin family)	59.2	0.0	2.2	0.000	0.000	ns	FsHS	
FsHS_tc_00427	Heat shock protein Hsp20 (a-crystallin family)	35.9	0.0	0.0	0.001	0.000	NA	FsHS	
FsHS_tc_00429	Heat shock protein Hsp20 (a-crystallin family)	37.7	0.0	0.0	0.001	0.000	NA	FsHS	
FsHS_tc_00430	Heat shock protein Hsp20 (a-crystallin family)	62.8	0.0	0.0	0.000	0.000	NA	FsHS	
FsHS_tc_00432	Heat shock protein Hsp20 (a-crystallin family)	71.8	0.0	0.0	0.000	0.000	NA	FsHS	
FsHS_tc_00434	Heat shock protein Hsp20 (a-crystallin family)	98.7	0.0	0.0	0.000	0.000	NA	FsHS	
FsHS_tc_00436	Heat shock protein Hsp20 (a-crystallin family)	95.1	0.0	0.0	0.000	0.000	NA	FsHS	
FsHS_tc_00437	Heat shock protein Hsp20 (a-crystallin family)	62.8	0.0	0.0	0.000	0.000	NA	FsHS	
FsHS_tc_00438	Heat shock protein Hsp20 (a-crystallin family)	39.5	0.0	0.0	0.001	0.000	NA	FsHS	
FsHS_tc_00443	Heat shock protein Hsp90	91.5	34.2	4.4	0.003	0.000	0.002	FsHS	
FsHS_tc_00483	Retrovirus-related Pol polyprotein	25.1	0.0	0.0	0.009	0.000	NA	FsHS	
FsD_tc_00018	Unknown	93.3	43.9	40.0	0.008	0.000	ns	FsHS	

Table 4 (continued)

TC	Description	(1) Fser_HS (norm)	(2) Fser_D (norm)	(3) Fves_D (norm)	1 vs. 2	1 vs. 3	2 vs. 3	Relative expression	
								Up	Down
FsHS_tc_00425	Unknown	269.3	68.4	80.1	0.000	0.000	ns	FsHS	
FsHS_tc_00450	Unknown	66.4	24.4	33.4	0.008	0.004	ns	FsHS	
FsHS_tc_00471	Unknown	28.7	14.6	11.1	ns	0.015	ns	FsHS	
FsHS_tc_00538	Unknown	26.9	0.0	2.2	0.007	0.001	ns	FsHS	
FsHS_tc_00480	Photosystem II 12-kDa extrinsic protein, PsbU	26.9	9.8	11.1	ns	0.021	ns	FsHS?	FvD?
FsHS_tc_00449	Late-embryogenesis-abundant-related protein	62.8	43.9	31.1	ns	0.005	ns	FsHS?	FvD?

List of TCs drawn from the 50 largest EST clusters from each of the three libraries that differ significantly after testing with the method of Audic and Claverie (1997). Gene descriptions are given where annotation information is available (see text for details). Normalized sequence counts for each TC/library are shown; Significant *P* values (adjusted for false discovery rate; $P=0.02508$) are shown. The two columns on the right indicate putative upregulation and/or downregulation, assuming common "basal" expression levels between species in the absence of stress

FsHS F. serratus heat shock/recovery, *FsD F. serratus* desiccation/rehydration, *FvD F. vesiculosus* desiccation/rehydration, *ns* nonsignificant, *NA* test not applicable (no counts available for comparison)

Blastn resulted in 100 unique sequences (Table 4). After normalization of read numbers to account for different sizes of the libraries and *P* value correction for false discovery rate, 86 genes with putative differential expression were found. One striking result is the lack of concordance between the two desiccation/rehydration libraries (Table 4). Of the 55 TCs significantly upregulated in one of these two libraries, only two showed a common pattern in both (both nonannotated). Twenty TCs were significantly and uniquely upregulated in FsD relative to either of the other two libraries, including 11 unknown proteins, three light-harvesting genes, a GTP-binding regulatory subunit (transmembrane signal transduction), profilin (actin-binding), a tubulin gene, ferredoxin-NADP reductase, and adenosine triphosphate synthase gamma chain (thylakoid electron transport), as well as a metallothionein that was relatively abundant in all three libraries. Two of the LHC genes and three of the unknown proteins were downregulated (or not detected) in FvD. In contrast, 33 genes were uniquely upregulated in FvD, of which 17 were ribosomal proteins. The presence of ubiquitin and two ubiquitin fusion proteins suggests that the protein targeting and degradation pathway via the 26S proteasome was upregulated in FvD (O'Mahony and Oliver 1999). A translationally controlled tumor protein homolog involved in microtubule stabilization was also putatively upregulated, as well as five unknown proteins. In particular, the coordinated transcription of many ribosomal protein genes, as well as the increased abundance of eukaryotic translation initiation factor 1α (which also interacts with the proteasome and actin cytoskeleton) strongly suggests a role for de novo translation during desiccation or more likely rehydration in *F. vesiculosus*, but not in *F. serratus*, following a similar stress exposure.

The situation in *F. vesiculosus* is reminiscent of the translational control of the recovery/repair during rehydration of desiccation-tolerant bryophytes (Wood and Oliver 1999; Wood et al. 2000), in which certain mRNAs are selectively bound in ribonucleoprotein particles in desiccating tissue, allowing their rapid translation during rehydration. Nevertheless, the clear differences in transcript functional category between desiccation/rehydration libraries in *F. serratus* and *F. vesiculosus* are initially puzzling: transcripts for light-harvesting and electron transport genes increase in *F. serratus*, while translation and protein degradation pathways appear to dominate in *F. vesiculosus*. This could be explained by species-specific differences in desiccation tolerance: photosynthetic efficiency in *F. vesiculosus* recovers fully from 3-h desiccation (see Pearson et al. 2000), while *F. serratus* does not (Pearson et al. 2009). Therefore, our working hypothesis is that the expression patterns observed might reflect fundamental differences at the organismal level and underpin the physiological tolerance of *F. vesiculosus*. Despite clues to the desiccation/rehydration process, we did not identify any typical "desiccation" genes in either species' library, such as the late-embryogenesis-abundant (LEA) proteins found in desiccation-tolerant plant and rehydrating bryophyte tissues (Velten and Oliver 2001; Oliver et al. 2004; Bartels 2005). The only transcript with homology to LEA proteins was abundant in all three libraries and significantly more abundant in FsHS compared with FvD (Table 3). A future priority will be to identify, by rapid amplification of cDNA ends PCR or other techniques, the nonannotated transcripts that are candidates for desiccation/rehydration-responsive genes in *F. vesiculosus* (Table 3). Confirmation of the possible

importance of translation and degradation pathway genes also awaits further analysis.

Expression and Diversity of Light-Harvesting Protein Genes

A total of 25 light-harvesting protein (LHC) genes were identified from annotated TCs across all libraries. After pooling and local reassembly, 11 were homologous across species, while 11 and three were unique to *F. serratus* and *F. vesiculosus*, respectively (Table 5). An alignment using conceptual translations of the *Fucus* sequences resulted in 15 predicted full or nearly-full-length proteins. We then added the UniprotKB/SwissProt accessions obtained from the top ten Blastx hits of each TC. A phylogenetic tree was constructed using PHYML as described above for sHSPs, with 100 bootstrap replicates.

The known brown algal sequences all cluster closely in the chlorophyll a–c group of fucoxanthin–chlorophyll c binding proteins, together with FsHS TC_760, TC_536, and the more divergent FsD TC_024 (Fig. 4). Most surprising, however, was a group of eight further TCs that clustered together with a group of red algal/cryptomonad LHC that contained no other brown algal or diatom representatives. Interestingly, these genes were overwhelmingly expressed in *F. serratus* but much less so in *F. vesiculosus*. Expression of seven of eight putative genes was found in *F. serratus* libraries, five uniquely, for a total of 175 ESTs. In contrast, only two of the eight were detected in the *F. vesiculosus* library, for a total of seven ESTs. To our knowledge, this is the first report of genes from this clade (Koziol et al. 2007) being found in heterokont algae, the presence of which implies transfer(s) from the original secondary (red algal) symbiont to the host nuclear genome.

A third set of 4 TCs grouped most closely with the LI818-like LHC genes. This appears to be an ancient group of polypeptides that includes representatives in the haptophyte and diatom (stramenopile/heterokont) lineages (Richard et al. 2000; Koziol et al. 2007). These TCs were also overrepresented in *F. serratus* with two of the four TCs being unique and a total of 32 ESTs to 12 in *F. vesiculosus*. The overall ratio of ESTs for this group of transcripts was 30:2:12 (FsHS/FsD/FvD). In this regard, it is interesting to note that LI818-like proteins have been shown to be overexpressed in HL conditions (Richard et al. 2000; Becker and Rhiel 2006) and are implicated in photoprotection as being responsible for nonphotochemical quenching in the green algae, *Chlamydomonas reinhardtii* (Peers et al. 2007). Therefore, a possibility that remains to be tested is that these genes are upregulated in *Fucus* by HL during heat stress (i.e., under hydrated conditions) but not or less so when tissue is desiccating.

Table 5 TCs encoding light-harvesting proteins from *Fucus* EST libraries

Library	Homologous TCs	# reads	Best hit (SP): gene: description: accession
FsHS	*tc_00445*	16	lhca3: chlorophyll a–b binding protein 3: Q32904
FsD	*tc_00002*	15	
FvD	g5P2A21	1	
FsHS	tc_00507	14	FCPB: fucoxanthin–chlorophyll a–c binding protein B: Q40296
FsD	tc_00022	7	
FvD	tc_00211	3	
FsHS	tc_00553	6	CAB13: chlorophyll a–b binding protein 13: P27489
FsD	tc_00065	4	
FvD	tc_00236	3	
FsHS	tc_00635	2	FCP: fucoxanthin–chlorophyll a–c binding protein: Q39709
FsD	*tc_00024*	7	
FvD	tc_00126	5	
FsHS	tc_00672	4	FCPB: fucoxanthin–chlorophyll a–c binding protein B: Q40296
FsD	tc_00102	3	
FvD	tc_00162	4	
FsHS	*tc_00444*	39	lhca3: chlorophyll a–b binding protein 3: Q32904
FsD	tc_00001	18	
FsHS	tc_00446	38	CAB7: chlorophyll a–b binding protein 7: P10708
FsD	*tc_00003*	22	
FsHS	*tc_00482*	14	L1818: chlorophyll a–b binding protein L1818: Q03965
FvD	tc_00058	10	
FsHS	*tc_00494*	12	LH38: light-harvesting complex I LH38: P08976
FsD	*tc_00105*	4	
FsHS	tc_00501	12	CAB3: chlorophyll a–b binding protein 3: P09756
FvD	tc_00415	2	
FsHS	*tc_00536*	7	FCPB: fucoxanthin–chlorophyll a–c binding protein B: Q40296
FvD	tc_00293	3	
FsHS	tc_00622	4	FCP: fucoxanthin–chlorophyll a–c binding protein: Q39709
FsD	tc_00073	4	
FsHS	*tc_00624*	4	FCP: fucoxanthin–chlorophyll a–c binding protein: Q39709
FsD	tc_00148	2	
FsHS	*tc_00631*	6	LHCB4.2: chlorophyll a–b binding protein CP29.2: Q9XF88
FsD	tc_00171	2	
FsHS	tc_00708	4	FCPA: fucoxanthin–chlorophyll a–c binding protein: Q42395
FvD	tc_00338	2	
FsHS	*tc_00760*	4	FCPB: fucoxanthin–chlorophyll a–c binding protein B: Q40296
FsD	tc_00234	2	
FsHS	tc_00764	5	FCPB: fucoxanthin–chlorophyll a–c binding protein B: Q40296
FsD	tc_00107	4	
FsHS	tc_00945	2	LHCA1: chlorophyll a–b binding protein 1B-21: Q9SDM1
FvD	tc_00094	7	
FsHS	tc_00997	2	FCPB: fucoxanthin–chlorophyll a–c binding protein B: Q40296
FvD	tc_00277	3	
FsHS	*tc_00479*	12	L1818: chlorophyll a–b binding protein L1818: Q03965
FsHS	tc_00636	2	CAB1R: chlorophyll a–b binding protein 1: P12330
FsHS	*tc_00794*	3	L1818: chlorophyll a–b binding protein L1818: Q03965
FvD	*tc_00133*	6	LH38: light-harvesting complex I LH38: P08976

Table 5 (continued)

Library	Homologous TCs	# reads	Best hit (SP): gene: description: accession
FvD	tc_00155	4	FCPD: fucoxanthin–chlorophyll a–c binding protein D: Q40298
FvD	*tc_00511*	2	FCP: fucoxanthin–chlorophyll a–c binding protein: Q39709

Those marked in italics contained sufficient coding sequence information for alignment and were used to construct a PHYML tree

Functional Analysis of Selected Genes by qPCR

A time course analysis of relative gene expression in *F. vesiculosus* was conducted for ten genes selected from a wider screening panel representing mainly chaperone, cochaperone, and other stress-related genes (Fig. 5). As suggested by their representation in the FsHS library, sHsp genes of the α-crystallin family were highly upregulated in *F. vesiculosus* after as little as 15 min HS. In contrast, although desiccation was performed at an elevated temperature, only a slight transcriptional response was observed (Fig. 5a). A similar pattern was found for Hsp90 (Fig. 5b), Hsp70 (Fig. 5c, d), and Hsp100 (Fig. 5e) family

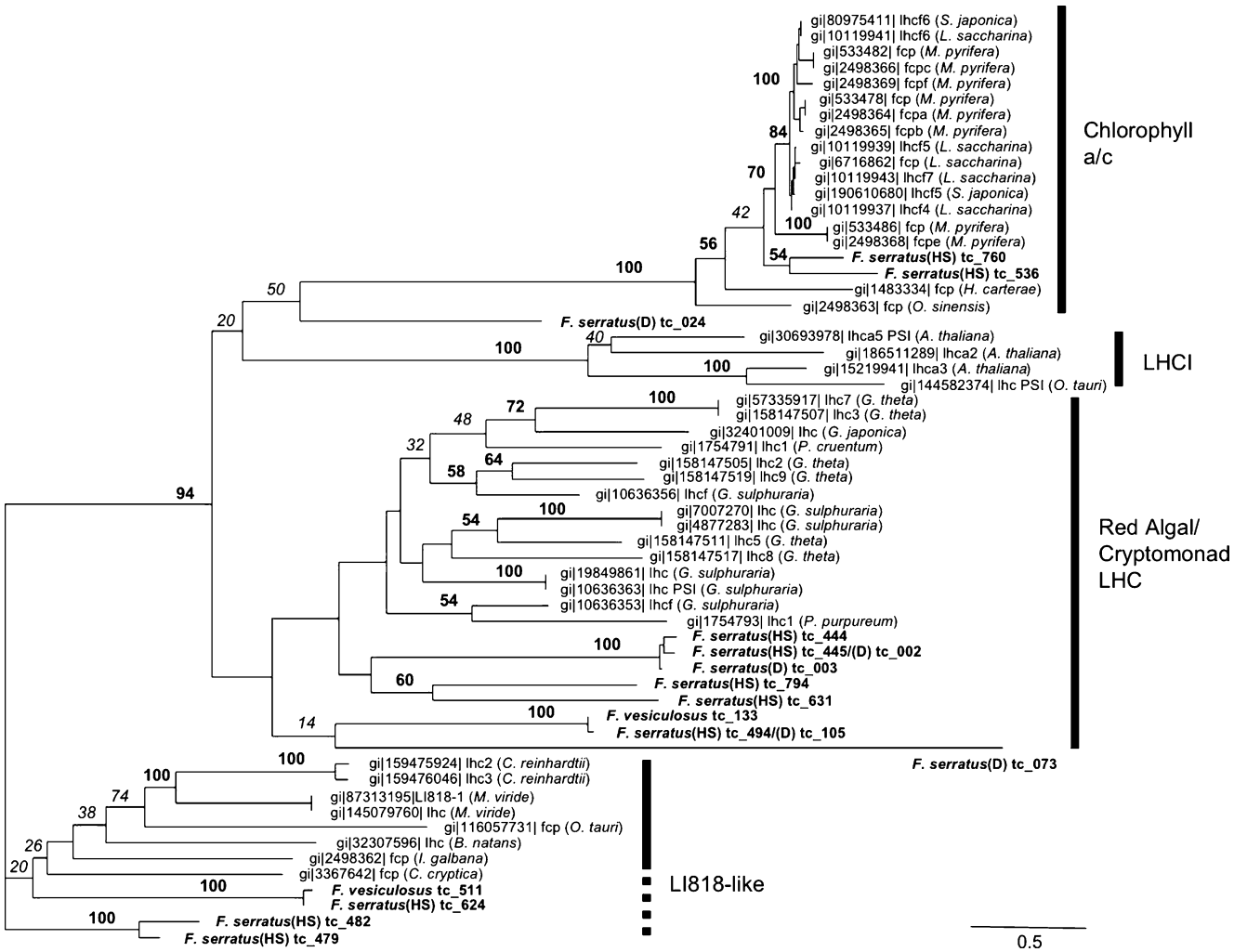


Fig. 4 An ML phylogenetic tree based on an alignment of 59 light-harvesting proteins (*LHC*). The tree was obtained using PHYML, the LG model, a discrete gamma distribution with four rate categories, and 100 bootstrap resamplings. Bootstrap values >50 are shown on the *branches* in *bold type*. The LHC sequences are grouped on the tree following Koziol et al. 2007. Protein accession numbers are given together with taxon names or the TC in the case of *Fucus*. Taxa included on the tree: *S. japonica* = *Saccharina japonica*, *L. saccharina* = *Laminaria saccharina*, *M. pyrifera* = *Macrocystis pyrifera* (Phaeophyceae); *O. sinensis* = *Odontella sinensis*, *C. cryptica* = *Cyclotella cryptica* (Bacillariophyta); *H. carterae* = *Heterosigma carterae* (Raphidophyceae); *I. galbana* = *Isochrysis galbana* (Haptophyceae); *G. theta* = *Guillardia theta* (Cryptophyta); *G. sulphuraria* = *Galdieria sulphuraria*, *P. purpureum* = *Porphyridium purpureum*, *P. cruentum* = *Porphyridium cruentum*, *G. japonica* = *Griffithsia japonica* (Rhodophyta); *C. reinhardtii* = *Chlamydomonas reinhardtii*, *O. tauri* = *Ostreococcus tauri* (Chlorophyta); *A. thaliana* = *Arabidopsis thaliana*, *M. viride* = *Mesostigma viride* (Streptophyta)

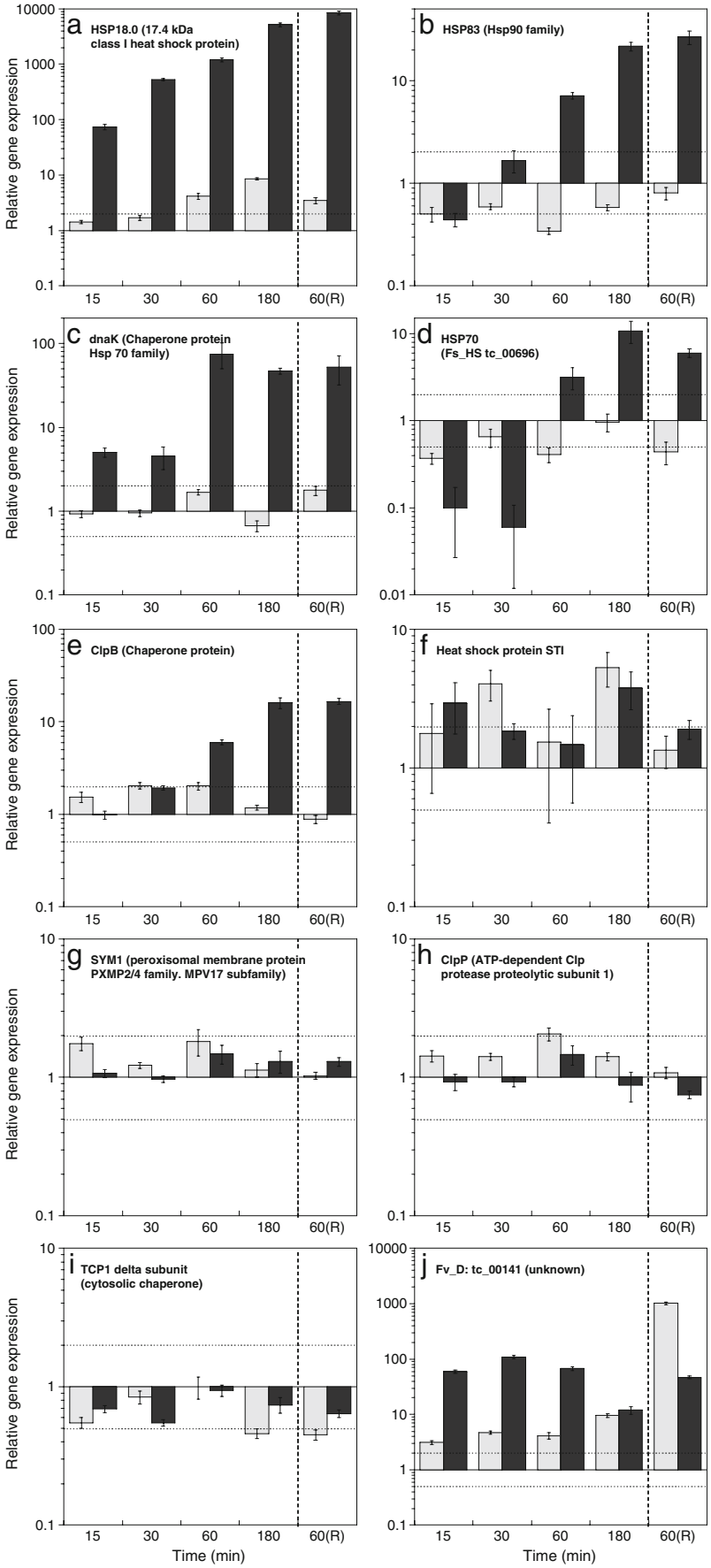


Fig. 5 Quantitative RT-PCR analysis of differential expression during a 3-h time course of desiccation (*light bars*) and heat shock (*dark bars*) in *F. vesiculosus*. Expression following 60-min recovery from both stressors is shown as *60(R)*. Values are means ± SE of three replicate qPCR reactions, expressed as relative gene expression (fold change) compared to controls under acclimation conditions. *Horizontal broken lines* indicate twofold upregulation and downregulation thresholds. See "Methods" for further information

members: HS-induced gene expression was evident within 60 min of stress, while desiccating algae failed to accumulate any of these transcripts. A gene encoding a heat shock protein STI1 homolog (Hsc70/Hsp90 organizing protein), a member of the DnaJ/Hsp40 family and reported to be stress-inducible (Torres et al. 1995), was the only DnaJ family protein found among the ESTs. However, the gene does not appear to be heat shock inducible in *F. vesiculosus* (Fig. 5f). A homolog to the yeast stress-inducible gene SYM1, which encodes a mitochondrial inner membrane protein (Trott and Morano 2004), was also not inducible in *F. vesiculosus* (Fig. 5g) nor was ClpP, a catalytic protease subunit (Fig. 5h). None of the candidate genes so far tested by qPCR (>20, data not shown) has proven to be desiccation or rehydration responsive, with the exception of FvD tc_00141 (Fig. 5j), which was both HS inducible and was massively overexpressed upon rehydration of desiccated algae. Unfortunately, this TC does not contain a predicted ORF and the encoded protein therefore remains unknown.

Concluding Remarks

This study was designed to generate and analyze ESTs to provide an initial picture of gene content and diversity in *Fucus* spp. Many fucoid algae can be regarded as extremophiles, as they exist at the interface between marine and terrestrial environments and are subject to rapid fluctuations in temperature and water stresses (desiccation, freezing, osmotic shock), among others. Sampling the transcriptome of two species from different clades within the genus maximized the usefulness of the data for different research programs, while subjecting these to different stressors was a strategy to increase gene discovery. We also required data that would allow the development of species-specific markers. After initial searches (MsatFinder 2.0.7; Thurston and Field 2005), our unigene sequences yielded 21 polymorphic microsatellite loci in *F. serratus* (Coyer et al. 2008). SNPs initially identified from *Fucus* ESTs using PolyPhred (Nickerson et al. 1997) are currently being confirmed and analyzed via resequencing in order to characterize gene polymorphism and diversity. SNP data are being used to identify genes putatively under positive selection between the two clades (Pearson et al., unpublished data).

Functional analysis was hampered by a low annotation rate, at least in part due to the presence of long 3′ UTR sequences. Nevertheless, we identified several transcriptional responses (and/or lack thereof) of fucoid algae during heat stress and recovery and desiccation/rehydration. In particular, we identified a family of sHsps and several other Hsp genes upregulated by HS, a number of which were confirmed by expression analysis. In common with diatoms (Bowler et al. 2008), retroviral-type transposable elements are present in multiple copies in *F. serratus* and are likely to be transcriptionally activated by HS.

Based on the differential expression of genes between HS and desiccation libraries, there appears to be little evidence for a specific desiccation response in *F. vesiculosus*, although, in the absence of unstressed libraries for the two species, we cannot discount the possibility that some genes are regulated in the same way by both stressors. The general upregulation of genes for the translational machinery suggests parallels with poikilohydric desiccation-tolerant bryophytes, rather than resurrection plants such as *Craterostigma*, where desiccation tolerance is acquired over a period of several hours through abscisic-acid-dependent pathways (Bartels 2005). Posttranscriptional control of desiccation and rehydration responses (by selecting for translation-specific mRNAs already available in the transcript pool) may prove to be a general feature of rapidly desiccating poikilohydric photosynthetic organisms. We were unable to identify potential "rehydrin" genes such as the LEAs present in bryophytes, but several nonannotated candidates are available for future study (Table 3, Fig. 5j).

The genus *Fucus* is of evolutionary as well as ecological interest since it is the most species-rich genus within the intertidal family Fucaceae and contains closely related members with well-established differences in tolerance to abiotic stress, particularly desiccation. This is strongly indicated in this work by the very different EST profiles of the more desiccation-susceptible *F. serratus* compared with desiccation-tolerant *F. vesiculosus*. The occurrence of physiological diversity in congenerics is a potentially powerful tool for the comparative analysis of the regulatory and evolutionary basis of desiccation tolerance. The identification of >3,700 unigenes from *F. serratus* and >2,400 from *F. vesiculosus* should provide the basis from which to further explore the stress biology, ecology, and evolution of fucoid algae and to establish *Fucus* as a model for brown algal ecological genomics.

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