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A signal peptide secretion screen in *Fucus distichus* embryos reveals expression of glucanase, EGF domain-containing, and LRR receptor kinase-like polypeptides during asymmetric cell growth

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Abstract Zygotes of the brown alga *Fucus distichus* (L.) Powell develop polarity prior to the first embryonic cell division and retain a pattern of asymmetric growth during early embryogenesis. In order to identify *F. distichus* polypeptides secreted during asymmetric cell growth, we used a functional assay in *Saccharomyces cerevisiae* to screen a cDNA library generated from asymmetrically growing *Fucus* embryos for sequences encoding polypeptides that function as signal peptides for secretion. We isolated and sequenced 222 plasmids containing *Fucus* cDNAs encoding signal peptide activity. The cDNA inserts from these plasmids were translated *in silico* into 244

potential polypeptide sequences, 169 of which are predicted to contain signal peptides. BlastP analysis of the *Fucus* sequences revealed similarity between many *Fucus* proteins and cell surface proteins that function in development in other eukaryotes, including epidermal growth factor (EGF)-like repeat-containing proteins, plant leucine-rich repeat (LRR)-receptor kinases, and algal β -1, 3-exoglucanase. However, most of the isolated *Fucus* polypeptides lack similarity to known proteins. The isolation of cDNAs encoding secreted *Fucus* proteins provides an important step toward characterizing cell surface proteins important for asymmetric organization and growth in fucoid embryos.

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Abbreviations ECM: extracellular matrix · EGF: epidermal growth factor · ER: endoplasmic reticulum · LRR: leucine-rich repeat · SST: signal sequence trap · WAK: wall-associated kinase

Introduction

Polar cells exhibit different morphological and/or molecular characteristics at opposing ends, with these distinct ends aligned along an axis of symmetry (Grebe et al. 2001). Polar organization allows distinct regions—and distinct surfaces—of a single cell to have markedly different activities. Cell surface molecules play important roles in generating and retaining cellular polarity, including sensing the extracellular environment, adhering cells to adjacent surfaces, and linking the internal contents of the cell physically to the extracellular environment (Quatrano 1997; Doe and Bowerman 2001). Because secreted polypeptides are involved in cell signaling and structural functions in polar cells, the identification of novel cell-surface proteins is important for understanding

the molecular basis of the generation and maintenance of cellular asymmetry. Surface molecules fulfilling these functions serve important roles in the generation and maintenance of polarity in many diverse cell types, including prokaryotes, fungi, protists, metazoans, and plants (reviewed in Drubin and Nelson 1996; Cove 2000).

The fucoid brown algae, including *Fucus* and *Pelvetia* species, provide model organisms for studying the role of cell surface molecules in the generation of cell polarity and subsequent asymmetric cell growth. Fucoid algae exhibit an early developmental pattern in which zygotes generated from symmetric eggs undergo the formation—and eventual fixation—of a polar axis in response to environmental cues, including unilateral light (reviewed in Quatrano 1997; Brownlee et al. 2001). Fucoid zygotes develop cortical and cell surface asymmetries as polar development progresses. While the initial secretion of extracellular material is symmetric (Quatrano 1982; Hable and Kropf 1998), a polar axis forms within a few hours of fertilization (Hable and Kropf 2000). Associated with the formation of this polar axis is an asymmetric targeting of molecules, creating a localized accumulation of specific polypeptides (Wagner et al. 1992; Shaw and Quatrano 1996; Pu et al. 2000) and carbohydrates (Quatrano and Crayton 1973; Novotny and Forman 1974; Brawley and Quatrano 1979; Hable and Kropf 1998) at the cell surface prior to the visible morphological changes associated with asymmetric cell growth (Quatrano and Shaw 1997). The polarized secretion and deposition of these cell surface molecules is essential for fixation of the embryonic axis and for subsequent asymmetric growth (reviewed in Belanger and Quatrano 2000a; Brownlee et al. 2001). Thus, determining the identity of polypeptides localized to the surface of polarized embryos is critically important to our understanding of the mechanisms of axis formation and asymmetric cell growth.

The vast majority of proteins expressed at the cell surface of eukaryotic cells are targeted to the plasma membrane via vesicle-mediated secretion (reviewed in Vitale and Denecke 1999). The cotranslational translocation of a secreted protein into the endoplasmic reticulum (ER) is dependent upon the presence of an endogenous signal peptide within the secreted polypeptide (Johnson and van Waes 1999). This signal peptide is a degenerate, highly variable stretch of 15–50 amino acids containing a hydrophobic α -helical core of 6–15 residues which is flanked at both the amino and carboxyl ends by polar residues (Nielsen et al. 1997; Martoglio and Dobberstein 1998). After translocation into the ER, proteins are secreted to the cell surface through the ER to Golgi to exocytotic vesicle transport pathway, unless they contain an additional sorting signal targeting them to an intracellular compartment within the endomembrane system (Vitale and Denecke 1999).

Despite evidence linking cell surface molecules to axis formation and asymmetric cell growth in fucoid embryos, specific secreted molecules that regulate or promote polar orientation and/or growth have yet to be

identified. Mutations in secreted proteins from the yeast *Saccharomyces cerevisiae* have frequently been used to examine the molecular mechanisms of secretion in eukaryotes (Kaiser et al. 1987; Ngsee et al. 1989). In an effort to identify cell surface proteins that are potentially important for development of asymmetry and subsequent asymmetric cell growth in fucoid embryos, we have used a signal sequence trap (SST; Klein et al. 1996; Jacobs et al. 1997) to screen a *Fucus distichus* cDNA library for polypeptides that function as signal peptides in yeast. We isolated 222 cDNA sequences, of which 169 are predicted to encode a polypeptide containing an amino-terminal signal peptide. BlastP analysis (Altschul et al. 1990) revealed that a significant fraction of these *Fucus* polypeptides exhibits regions of similarity to cell surface proteins that were previously identified in other organisms, including leucine-rich repeat (LRR) receptor-like kinases, metazoan cadherin-like proteins, and β -1,3-exoglucanase. However, most of the polypeptides identified in this screen appear to be novel. We discuss the potential roles in asymmetric growth of several putative proteins isolated in this screen.

Materials and methods

Construction of *Fucus* libraries and SST control plasmids

A synchronous population of *Fucus distichus* (L.) Powell embryos was obtained as previously described (Belanger and Quatrano 2000b). mRNA was isolated from asymmetrically growing 18-h embryos by hexadecyltrimethylammonium bromide (CTAB) extraction and precipitated using LiCl (Apt et al. 1995). A cDNA library was generated using the λ ZAPII random-prime library kit (Stratagene, La Jolla, CA, USA). Plasmid DNA was excised from the λ ZAPII phage using standard protocols, and *Fucus* cDNA inserts were isolated from the phagemids using restriction endonucleases *EcoRI* and *XhoI*. The cDNA inserts were purified by agarose gel electrophoresis and ligated directionally into the *EcoRI* and *XhoI* sites of the SST vector pSUC2T7M13ORI (Jacobs et al. 1997). Electrocompetent DH10B *Escherichia coli* were transformed with the library and resulting transformants utilized without amplification for plasmid DNA isolation.

The Wak2-79::SST fusion was constructed by digesting Wak2-cDNA (He et al. 1999) with *EcoRI* and *XcmI* and inserting the resulting fragment encoding the amino-terminal 79 amino acids of Wak2 into the *SacII* site of the pSUC2T7M13ORI SST vector by blunt end ligation. Wak2-222::SST was synthesized by digesting Wak2-cDNA with *EcoRI* and *StuI* and ligating the resulting fragment into *EcoRI* and *Scal* sites of the SST vector. The Act2-52::SST fusion was generated by digesting Act2 cDNA (An et al. 1996) with *HindIII* and *NsiI* and cloning the excised Act2 fragment into the SST vector at *EcoRI* and *StuI* sites. Act2-359 was cloned similarly, by excising Act2 using *HindIII* and *StyI* and inserting the resulting fragment into SST vector *EcoRI* and *StuI* sites. DNA sequencing of each plasmid was performed to confirm in-frame fusion between the Wak2 or Act2 insert and the *SUC2* invertase gene located on the SST plasmid.

Yeast transformation and selection

Library plasmids were transformed into yeast strain YTK12 (*suc2 Δ 9 trp1 Δ ade2-101 ura3-52*; Jacobs et al. 1997) by lithium acetate transformation (Ito et al. 1983). Forty-eight thousand Trp⁺ transformants were obtained by selection on complete

minimal media lacking tryptophan (Ausubel et al. 1995) and containing 0.1% dextrose and 2% sucrose. Transformants were incubated at 30 °C for 2–3 days, and then replicated onto YEP media containing 2% raffinose (YP-Raf) as the sole carbon source. After incubation at 30 °C for 3 days, viable colonies were restreaked to YP-Raf and grown at 30 °C for 4–6 days. Plasmids were isolated from viable colonies by glass bead lysis, transformed into DH10B *E. coli*, and isolated by miniprep procedure. Plasmids were re-transformed into YTK12, and transformants were treated as described for the screen above. Two hundred and sixty nine plasmids that conferred growth on YTK12 yeast on sucrose and on raffinose were retained for sequencing.

Plasmid sequencing and analysis

The plasmids were sent to the University of North Carolina-Chapel Hill (Chapel Hill, NC) Automated DNA Sequencing Facility for Taq FS Dye Terminator sequencing of the *Fucus* cDNA inserts using the primer 5'-CTG CAC AAT ATT TCA AGC TAT ACC AAG C -3'. The 222 resulting sequences were analyzed visually using Abi-Prism EditView (Perkin Elmer) and cDNA inserts translated *in silico* using ExPasy translation tool (Appel et al. 1994). Polypeptide sequences were compared with sequences in Genbank using BlastP (Altschul et al. 1990) and examined for signal peptides using SignalP (Nielsen et al. 1997) and TargetP (Emanuelsson et al. 1999).

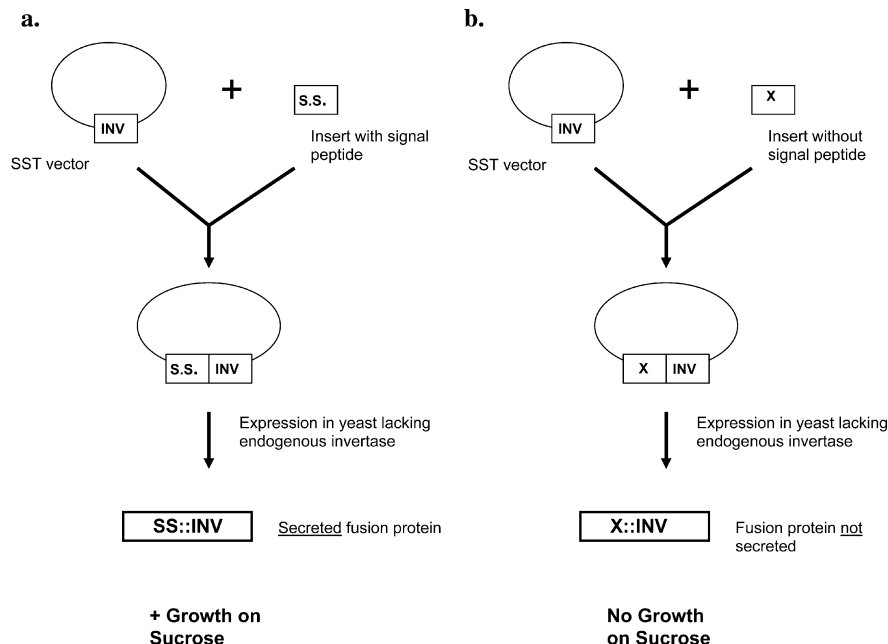
Results

The SST screen we have employed to identify secreted proteins in *Fucus distichus* utilizes a mutant invertase lacking a signal peptide to identify exogenous sequences that can target the mutant enzyme to the cell surface (Jacobs et al. 1997; see Fig. 1). While this signal peptide selection screen had previously been used to identify signal peptides from human and rat libraries (Klein et al. 1996; Jacobs et al. 1997) and from plants (Goo et al. 1999), the ability of this system to recognize signal peptides from brown algae had not yet been determined. In order to initially confirm the ability a plant signal

peptide to mediate detectable secretion from yeast using the mutant invertase vector in the SST system, we cloned sequences encoding the amino-terminal region of the secreted plant wall-associated kinase WAK2 (He et al. 1999) in-frame with the mutant invertase on an SST vector (Jacobs et al. 1997). We then assayed yeast expressing the WAK2::invertase fusion for growth on sucrose (Fig. 2). WAK2::invertase fusions containing the amino-terminal 79 and 222 amino acids of WAK2 allow a yeast strain otherwise lacking invertase to grow on sucrose, indicating that these WAK2 sequences do function as signal peptides in yeast and that these plant sequences can generate a positive result in this assay. Fusions expressing the first 52 or 359 amino acids of *Arabidopsis* actin, which lacks a signal peptide, do not confer growth on sucrose when fused to invertase. These data confirm that signal peptides from vascular plants can function in the SST selection. *Fucus* proteins containing signal peptides had not been previously identified, and thus could not be used as controls prior to initiating our screen for secreted *Fucus* polypeptides.

We utilized the SST selection to identify secreted proteins that may play a role in polar axis formation and asymmetric cell growth during early furoid development. We generated a cDNA library using mRNA isolated from a synchronous population of *Fucus* embryos

Fig. 1a, b Use of the signal sequence trap (SST; Jacobs et al. 1997) to identify eukaryotic signal peptides. **a** Cloning of a nucleotide sequence encoding a signal peptide in-frame with an invertase mutant lacking its endogenous signal peptide generates a signal peptide::invertase (SS::INV) fusion. If the signal sequence is functional, the fusion protein is secreted when expressed in yeast. The resulting extracellular invertase activity confers viability on yeast cells cultured on media containing a disaccharide carbon source such as sucrose. **b** Expression of a fusion protein lacking a signal peptide (*X::INV*) precludes invertase secretion. Yeast cells failing to secrete invertase fail to grow on sucrose



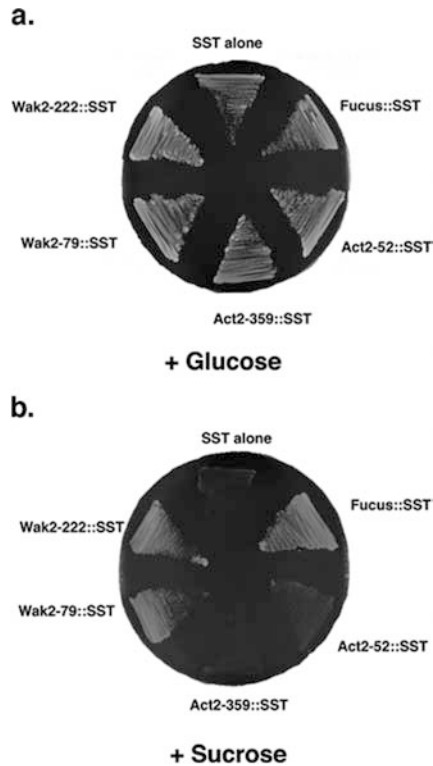


Fig. 2a, b The signal sequence trap can be used to identify secreted polypeptides from *Arabidopsis thaliana* and *Fucus distichus*. SST vectors expressing fusions of yeast invertase with the amino-terminal 79 amino acid residues (*Wak2-79::SST*) and 222 residues (*Wak2-222::SST*) of the secreted *Arabidopsis* Wak2 protein are viable on both glucose (a) and sucrose (b). Growth on sucrose requires extracellular invertase activity, confirming that Wak2 contains a signal peptide. Fusions of the amino-terminus of *Arabidopsis* actin Act2 (*Act2-52::SST*, *Act2-359::SST*) are not viable on sucrose, confirming the absence of a signal peptide in actin and the selectivity of the SST screen. A *Fucus distichus* cDNA insert fused to invertase as an isolate from the SST screen confers growth on sucrose (*Fucus::SST*), while the empty SST vector lacking an insert does not (*SST alone*)

18 h after fertilization. At this developmental stage the two-celled embryos are undergoing rapid asymmetric growth at the presumptive rhizoid tip (Quatrano 1997) and should be expressing genes encoding proteins important for polar growth. In order to identify which of these genes encode signal peptides, we subcloned this cDNA library upstream of the invertase gene in the SST plasmid, and assayed for those *Fucus* sequences that could target invertase for secretion. In order to identify the polypeptides encoded by the secretion-positive *Fucus* clones, we isolated and sequenced each of the positive plasmids and translated the resulting sequence *in silico*. We were able to obtain readable sequence containing at least one detectable open reading frame from 222 plasmids. For some plasmids, the cDNA fragment encoded more than one open reading frame beginning with a methionine; thus, we examined more than one putative polypeptide sequence from some cDNAs. This analysis generated 244 different polypeptide sequences, which were then further characterized.

While the screen was designed to isolate polypeptides containing signal peptides, we could not easily confirm which of our polypeptides harbored a signal peptide. Due to the degenerate nature of signal peptides, a true signal peptide consensus sequence does not exist. However, several algorithms have been independently developed to predict the presence of a signal sequence in a novel polypeptide based on similarities in patterns of hydrophobicity, charge, polarity, and amino acid sequence among members of a large data set of confirmed signal peptide sequences (von Heijne 1986; Nakai and Kanehisa 1992; Nielsen et al. 1997). We utilized the SignalP signal sequence prediction program (Nielsen et al. 1997) to examine each *Fucus* polypeptide for a potential amino-terminal signal peptide. Of the 244 putative polypeptides (Tables 1, 2) potentially encoded by *Fucus* inserts isolated from the SST screen, 169 (69.3%) were predicted by SignalP to have an amino-terminal signal peptide (Table 1). If we take into account the inclusion in our analysis of multiple reading frames from some cDNA clones, the percent of isolated cDNAs encoding at least one signal peptide increases to 76.1% (169 of 222). Thirty-five different sequences were isolated more than one time (Table 3), and 30 of those 35 are predicted by SignalP to contain an amino-terminal signal peptide. Importantly, we have isolated 87 unique cDNAs that are expressed during asymmetric embryonic growth in *Fucus* embryos and encode a predicted signal peptide for targeting to the secretory pathway.

In order to gain potential insights into the function of the polypeptides encoded by these cDNAs, we performed a BlastP homology search (Altschul et al. 1990) on each predicted amino acid sequence against the GenBank sequence database (Tables 1, 2). The BlastP results revealed similarity between a significant portion of the *Fucus* sequences and previously identified proteins entered into GenBank. Importantly, several *Fucus* polypeptides exhibit conserved sequences with metazoan and plant proteins with well-characterized roles in polarized cell growth. Three *Fucus* polypeptides (*FucusSST036*, 139, and 172) show similarity to epidermal growth factor (EGF)-like repeat-containing proteins from metazoans (Fig. 3a), while three others (*FucusSST052*, 067, and 102) exhibit similarity to leucine-rich repeat (LRR) receptor-like kinases from higher plants (Fig. 3b). In both cases, the similarity is confined to a defined region of the *Fucus* polypeptide. Six *Fucus* polypeptides exhibit a high degree of similarity to β -1,3-exoglucanase from *Neurospora crassa* and other fungi (Fig. 3c). LRR receptor kinases, proteins containing EGF-like repeats, and proteins regulating glucan synthesis perform essential functions in mediating asymmetric growth and development in a broad range of organisms (see Discussion). BlastP also revealed similarity between *Fucus* sequences and calmodulin, a calmodulin-like protein, and a Rac-GTPase, all of which have also been proposed to play a role in polarized cell growth in other organisms (see Tables 1 and 2).

Table 1 *Fucus distichus* sequences predicted to contain signal peptides. The signal peptidase cleavage site for each polypeptide, as predicted by SignalP (Nielson et al. 1997), is located immediately after the *underlined* amino acid residue

Fucus SST No.	Predicted amino acid sequence of amino-terminal polypeptide region ^a	Mean S-score ^b	GenBank Access. No.	Polypeptides exhibiting similarity ^c
1	MARFALVTLACAVIGAAAGDLEVLVLP	0.925	BU037996	–
2	MEMMPRSVIAAIMAITIICGINRATFAVLDEGVIRASSASTLFSSVDFTELPNCFTSLASQRSI WASEDDSCNKLPFNLWIIMKAEGTANDPPDAKDVEEQCIQILEVNLLEVLVLP	0.794	BU037997	–
3	MARFALVTLACAVIGAAAGDLEVLVLP	0.925	BU037998	–
4	MEFLRRSMRALPAMALVASTSAQVAFEKIAMVYDIYPKLRIMGSGFDGLDPSSVKFSFAPKVSDDKDYSI DITSPTIITLSLEEGKKWIQLEAGSPTKLYLSAAKNKGDNTLLEDSVQVATVLPPTVEAFSKEYMSATPK VNGTVLEVLVLP	0.749	BU037999	–
5	MTAALLVFLRAAVLSAMAPLAFGFITPFTFSVRTSARSQAQVTKMGPVAVKEKFDWVPILEVLVLP	0.901	BU038000	–
6	MKSAVIAACVAGAQAFVPSAFNGAALITSAKSSAMKMSFESEIGAQPPLGFWDPLGLLNDAD QERFDRURYVEIKHGRISMLAVLGHIVQQNVRPLGMLSTADISFADMPNGVAALSKIPPLGL AQVAFVGFLEAVMKNVEGSPFGDFGTNGNPFEGSWESEETKGVETRDRAQQRRRRAN GHSSHDGAREIFNQPLEGFPY	0.613	BU038001	Fucoxanthin (<i>Laminaria saccharina</i>)
7	MALFSIFVATFITVGLAAGATIRITSPDSASAYHAGDITVAVQWSYDGDGGEKFSIYLKRSGEQ VADLCAEEADGVCFDLTQDQTVLLPDSGLEVLVLP	0.959	BU038002	–
8	MARSAAFAMALTPAALGFLSDNLAAATNVVQSQTADAEYTELGCYEDSQKRFSEGTKRLDD LLETSCAAICEDFPYFGLQYGNWCWGDDYTSIEDSDSESDGDMTCTGSDSANGGNYAMRIF TRAMTDDDAIGCYGDFTRVFSDKARRSVKDE	0.92	BU038003	β -1,3-Exoglucanase (<i>Neurospora crassa</i>)
9	MRLSAGTVGVWISVDVSSFAFAPLQAPPSSANVARVTSHGQALVQRVRRPRGLAAIIMSVAE DELWRGEPWDEAETSQVMTKPKRSELDDIMSEVAGRNEPSPRIKADPIPMDAD DVPKGIQVDIDITLVKQKDLLEVLVLP	0.62	BU038004	–
10	MWSTQIFVMLGLLMAVSNALVCSNGIAGVEGSDACCVAECCGCGGCGSRARAASLSSGDC CIGPQRDANVFCDDSDAAPCINIVEETCSSNGLPIEGAGGCCAAEFCPCGCGGCGSALSGLTSS DCCVGPITSAGVFCDDFEMAPCHIGEDPSEVMCSDGITGLEVLVLP	0.939	BU038005	–
11	MVSTHMFLAATACTIASSAFVAPMAIRSMRSSTLKMESGASAYVNTLPGAPFGDGKIFDPLGL SDGAAPNDIKKWRDAEIKHGRVAMLAALGVLVAEEYHPLFMGPDYIGPAVDHFQEITARFP AFWAIALTGMGHIETNLEVLVLP	0.744	BU038006	Fucoxanthin (<i>Chlamydomonas reinhardtii</i>)
12	MARFAFVTLACTIVIGTVGALEVLVLP	0.938	BU038007	–
13	MARFALVTLACAVIGAAAGDLEVLVLP	0.925	BU038008	–
14	MARLSFAAILALSASAATAFVPLAPSFSGGRAVSATSSSTRMMPSPVPPPTAESLKQMAESCQKEG CSVEAVNTLLDQLKAKKTELEVLVLP	0.897	BU038009	–
15	MKQSTLRVLGALFIAAGVAATAYPADRHFVSHKELPPQHEELKRHVGGVGVDAERSLLHPL PHYLFGLFLEWSEVLVLP	0.882	BU038010	–
16	MTRSAATAVALLALALVPLJAAKGRVDQPHLSGESQASGRIVRRRLSTLDNDDEASSSILAGDYVC SNGLFGIESDGSVCCVCEGQGGEGCSTVAAPDYGASDCCAEERDLLWHVMQFHHGGTLRY MVATQMFVGTAAASLIASSAFVAPMAVRSLSPPSSSLKMQSAGSAYDITLPGAPFGDGKIFDPLGLSDGA	0.949	BU038011	–
17	SPKDIKWRREAEIKHGRVCMALALGHRHEEPKLNFDPPWAPNTYTLTVVDENQLRDLDLVEGSMWNKL PGALMIKGINVGDVLSNGLKVAITILEVSL	0.752	BU038012	Fucoxanthin (<i>C. reinhardtii</i>)
18	MTAALLVFLRAAVLSAMAPLAFGFITPFTFSVRTSARSQAQVTKMGPVAVKEKFDWVPILEVLVLP	0.901	BU038013	–
19	MTSIRKAGLALLTLGLVWRWAWGQVTTCCDDLKSSADGETLELGSDFTCSEIISVDEGEVETIDGADFTL SIDADLLYDEEGTNVAVYKGSLLTGLTINVTDDTAGNKFRAYINEGDLTVNSCTFNGLNLGPDPLDD GGVIYSESTGTVSIENSDFTSNGCGLKGGAIHAADEQLTITDCEFSNDNEAGTTGKLEVSL MKSAVIAACVAGAQAFVPSAFNGAALITSAKSSAMKMSFESEIGAQPPLGFWDPLGLLNDADQOERFD RLRYVEIKHGRISMLAVLGHIVQQNVRPLGMLSTADISFADMPKRCGGPLQDPPPARPAQIVAVGFLE LAVMKNVGEFSFPGLHQTAGTPSRVGRSSRKRPRRSARSSSTTVARRKWA	0.898	BU038014	Calmodulin-like protein (<i>Arabidopsis thaliana</i>)
20	MLASMWRFGALLALACAGVRANDPEPMGTIVGIDLGTTSYCVGFKNRGRVEIANDQGNRITPSYVSWD SNGERLIGDAAKNQATVNPETTVDFVEAFHRPSIQ	0.613	BU038015	Fucoxanthin (<i>L. saccharina</i>)
21	MPQNTSRYFRWTVAAASVALSLVAPSAAEEDKGLTFGPGEATERFLAIDNAITHLEDGETARRLQSGDW CNTISATETYCCGFGGQASCIARGIDYSGPDSPEVILTSDNGTMYCEGIDFDSGNLIDICDCEADFF TDISGYGPSYRRPVIDPADATMSPGETEMPATLEVLVLP	0.904	BU038016	BiP luminal chaperone (<i>A. thaliana</i>)
22		0.74	BU038017	–

Table 1 (Contd.)

Fucus SST No.	Predicted amino acid sequence of amino-terminal polypeptide region ^a	Mean S-score ^b	GenBank Access. No.	Polypeptides exhibiting similarity ^c
23	MYLVITRIQVKALFLVRWTRCGP	0.78	BU038018	-
24	MRFSFGSSAWVAMICVFLGDA PARDWPPGSRPVVKFRAQYCTV TEDRGNWTPDNLDELQORDRWMDKSWREDMVMG HIPOVNHFTAYLERLYAIINEKQV GIGESTCGAIWFRPLSDDCNNA TPCLTFSSILRVFP MRTPTATRPLAFIWFALVQSV AEIVDERRRSGFLAKPHSGSNHRV LVSSSDSITAPIVGVGFVSSA DITSGDELPTTAPPEEELVEDLE VLP	0.88	BU038019	-
25	MARSAFAMALTPAALGFLSDN LAAAATNVVQTFADAEYTELG CYEDSQKRIFSEGTKRLDD LLETSCAAICEDFPYFGLQY GNECWCW MRTPTATRPLAFIWFALVQSV AEIVDERRRSGFLAKPHSGSNHRV LVSSSDSITAPIVGVGFVLS ADITSGDELPTTAPPEEELVED LEVLVLP	0.829	BU038020	-
26	MARSAFAMALTPAALGFLSDN LAAAATNVVQTFADAEYTELG CYEDSQKRIFSEGTKRLDD LLETSCAAICEDFPYFGLQY GNECWCW	0.92	BU038021	β -1,3-Exoglucanase (<i>N. crassa</i>)
27	MRTPTATRPLAFIWFALVQSV AEIVDERRRSGFLAKPHSGSNHRV LVSSSDSITAPIVGVGFVLS ADITSGDELPTTAPPEEELVED LEVLVLP	0.829	BU038022	-
28	MVRSDAFAMASSMLPVALG SLSNLAIAENVVQNFVAQY EELGCYKDYQRGRIFTEVTK FLDELLETSCAAICEDFPYFGLQY GSEWCWGDYTSAINRYIESN DCMMCTGDSKANCGGCYAM RIFTRITDDDDAIGCYEDF FTRVFSDMIVNTLGMISGG L	0.5	BU038023	-
29	MKVTAATAAVASLGMTSA FMAPAFRPTSSSLKATTT SSMSEGGQIGVSNVQTFAD AEYTELGCEYEDSQKRIF SEGTKRLDD	0.662	BU038024	-
30	LHKINPDVNPHELEVLVLP	0.926	BU038025	-
31	MLHLRGPGRSSMCLFWTT SWVLVILPI	0.704	BU038026	-
32	MSTIPWLDILRLNVAF AWPMSIFDVPLDYQLGSR DLTY	0.901	BU038027	-
33	MTAALLVRLAAVLSA MAPLAFGFTPTFSVRTS VARSAQVTKGPPAVKEK FDWVPILEVLVLP	0.865	BU038028	-
34	MRTDVATVFFGLVICG VASAPTYSCFNPLAEGQ TNPFGAGLDHLVEAGC CEALTDESILAERLV VWDPEFCNYGPSETPI AEALFQAHDTDGRFCH LCEGLELIPCNEIFFR DSQAYLSSCWR	0.859	BU038030	-
35	MRNISARFLVLTAT ATGASGRITANEGRV TSKEDNYEAVRHMA GSEEFARRLGD DAGTVTPCSANRFLVDR VCCPECGTTCGGV GSSRSQSECTSI EISLGPCKGDS KAAPCHMDG SYKTSETSSGG DNPCLNGGSC TSDGYYQCS CALYGGTDC GTDFTDFT GNNLRSSL	0.801	BU038031	EGF-like repeats
36	MIRLTCVLLARQAV AFTPNLSSIF PVDGMGNRLT TVGSGATRA STLFAASGGK MTAENVL KSPKWPDKW PFYDIDD FNRMDESP SLEVLVLP	0.824	BU038032	-
37	MHGIASLCV SFVAITALQ QAFASNREL ADAELGMV GLSGDIN AAA GSSNNRAL RTRRSY FYIDV TFTNLLG VPKGYK FDIPIH GSSGLI GITLI APARGK TKKFN FCLPAK GQKRAK PSETM DDDTVL IVDIEP DVCPG VKACSD PHLQ LRLQ LGRG HIEWT GVDG GWYAL VADK EDDL QIMSP NDL AIDFP	0.894	BU038033	-
38	MARFAFVTFACVI IGTAGALEVLVLP	0.937	BU038034	-
39	MKTFGVA VLLGLL AANS FAQNIC AGAEQ SQAC APAC SNQIV NDDV NGTP NAGK FNV GGGN	0.911	BU038035	-
40	QGDGNDGF NNVGS FNIG SNK GNCAIT FRLH PAAH CDP GNN AGQI VLQ VLAR VLP KQ PHLPA	0.613	BU038036	Rac-GTPase (<i>Dictyostelium discoideum</i>)
41	IPDTLPEFSQ SQDQLP AVV ASCS AWMKS AVIAA ACV AGA QAFV PPSA FNG AAL TTS AKSS SAM KMS FESE IG AQ PPL GF WD PL GL LND AD	0.613	BU038037	Rac-GTPase (<i>D. discoideum</i>)
42	QERFDR LRY VEIK HGRIS MLAV LGHIV QQN VRL PGL M LTS AD IS FAD MP NG V AALS KIP PL GLA QIV AFV GFL E LAV MK NVE GSP FD TNG G NPE G S WE V	0.92	BU038038	β -1,3-Exoglucanase (<i>N. crassa</i>)
43	HDGAPD LQQA LEVSLMKS AVIAA ACV AGA QAFV PPSA FNG AAL TTS AKSS SAM KMS FESE IG AQ PPL GF WD PL GL LND AD	0.92	BU038039	β -1,3-Exoglucanase (<i>N. crassa</i>)
44	DOERFDR LRY VEIK HGRIS MLAV LGHIV QQN VRL PGL M LTS AD IS FAD MP NG V AALS KIP PL GLA QIV AFV GFL E LAV MK NVE GSP FD TNG G NPE G S WE V	0.92	BU038039	β -1,3-Exoglucanase (<i>N. crassa</i>)

45	MIPLLARLIGTTKATAVAVATLLLAGSSEARTVEAGTIVTVVTDAPLYDSTADDNG CDPTGCVGELTRDGDLLTPDSRWSCRPSLGPDDGTSIKYSLADVATIEAINIALYKG DERTRTVDIYVDDVFEASWTSSGNTAELETNLDVGTQEVMLVGLDDSEWLSVK EVEILVDDGTDPTVEAGTLGTIVGVAALYDTRALADNGCDPSSGC MLRSIIVLLSCGVGYFVYAPHPCHYDQREVDVERDLTFCSDMDYAPKGYCCTEAEETALEETFNA VGDLTAEADYYKQMLCGVCGTYSGHLRYERLADDLGTENGLSMKSEFNALHLSFVAAAAGI ETVRVRAIRAAASHLNPAPKVSVHRETVVGPAPERGVIIQKGFPLHDLLEVLV MEFLRRSMRALPALALVASTSAQVAFEEKIAMVYDIYPKLRIMGSGFDGLDPSVK FSFAPKVSDDKYSIDITSPTLEVLV MVSTHMFLAATACTIASSAFVAPMAIRSMRSSSTLKMSEGSAYVNTLPGAPFGDGKIFDPLGL SDGAAPNDIKKWRDAEIKHGRVAMLAALGVLVAEEYHPLFMGPDYIGPAVDHFQETARFPAP WAIALTGMGIETNTLLEVLV	0.812	BU038040	-	
46	MRTDYATVFFGLVICGVAASAPTYSCFNPPLAEGQTNPFAGLDHLVEAGCEALTDESILAERL VVWDPFGCNYGPSETPIAEFLCOAHDTDGCRFCHLSEGLEGLCIPCNEIFSATASPTSVQVAG AIGAIETSAITTSPTSVRAGTTEASFATA MFARYAVKTVVAVAFI GAVTATPHPECEPCAATPVNLDALTIAGKYLCCDLSQODAPVAGCGIFG LPLVCKACYDGDPTPAYDDPIDEEGASPTPAPVEASTVVPAPYEESMTVTVPSTPAQLEVLV MKFTAIFLAWMCASAFIAPSAVVKSRRFVTPVRSSTVAPKTTMNMGLQIVDMVPMVPTFA HDQVLEVLV	0.794	BU038041	-	
47	MRTDYATVFFGLVICGVAASAPTYSCFNPPLAEGQTNPFAGLDHLVEAGCEALTDESILAERL VVWDPFGCNYGPSETPIAEFLCOAHDTDGCRFCHLSEGLEGLCIPCNEIFSATASPTSVQVAG AIGAIETSAITTSPTSVRAGTTEASFATA MFARYAVKTVVAVAFI GAVTATPHPECEPCAATPVNLDALTIAGKYLCCDLSQODAPVAGCGIFG LPLVCKACYDGDPTPAYDDPIDEEGASPTPAPVEASTVVPAPYEESMTVTVPSTPAQLEVLV MKFTAIFLAWMCASAFIAPSAVVKSRRFVTPVRSSTVAPKTTMNMGLQIVDMVPMVPTFA HDQVLEVLV	0.802	BU038042	-	
48	MRTDYATVFFGLVICGVAASAPTYSCFNPPLAEGQTNPFAGLDHLVEAGCEALTDESILAERL VVWDPFGCNYGPSETPIAEFLCOAHDTDGCRFCHLSEGLEGLCIPCNEIFSATASPTSVQVAG AIGAIETSAITTSPTSVRAGTTEASFATA MFARYAVKTVVAVAFI GAVTATPHPECEPCAATPVNLDALTIAGKYLCCDLSQODAPVAGCGIFG LPLVCKACYDGDPTPAYDDPIDEEGASPTPAPVEASTVVPAPYEESMTVTVPSTPAQLEVLV MKFTAIFLAWMCASAFIAPSAVVKSRRFVTPVRSSTVAPKTTMNMGLQIVDMVPMVPTFA HDQVLEVLV	0.757	BU038043	Fucoxanthin (<i>C. reinhardtii</i>)	
49	MRTDYATVFFGLVICGVAASAPTYSCFNPPLAEGQTNPFAGLDHLVEAGCEALTDESILAERL VVWDPFGCNYGPSETPIAEFLCOAHDTDGCRFCHLSEGLEGLCIPCNEIFSATASPTSVQVAG AIGAIETSAITTSPTSVRAGTTEASFATA MFARYAVKTVVAVAFI GAVTATPHPECEPCAATPVNLDALTIAGKYLCCDLSQODAPVAGCGIFG LPLVCKACYDGDPTPAYDDPIDEEGASPTPAPVEASTVVPAPYEESMTVTVPSTPAQLEVLV MKFTAIFLAWMCASAFIAPSAVVKSRRFVTPVRSSTVAPKTTMNMGLQIVDMVPMVPTFA HDQVLEVLV	0.859	BU038044	-	
50	MRTDYATVFFGLVICGVAASAPTYSCFNPPLAEGQTNPFAGLDHLVEAGCEALTDESILAERL VVWDPFGCNYGPSETPIAEFLCOAHDTDGCRFCHLSEGLEGLCIPCNEIFSATASPTSVQVAG AIGAIETSAITTSPTSVRAGTTEASFATA MFARYAVKTVVAVAFI GAVTATPHPECEPCAATPVNLDALTIAGKYLCCDLSQODAPVAGCGIFG LPLVCKACYDGDPTPAYDDPIDEEGASPTPAPVEASTVVPAPYEESMTVTVPSTPAQLEVLV MKFTAIFLAWMCASAFIAPSAVVKSRRFVTPVRSSTVAPKTTMNMGLQIVDMVPMVPTFA HDQVLEVLV	0.801	BU038045	Insect intestinal mucin (<i>Trichoplusia ni</i>)	
51	MRTDYATVFFGLVICGVAASAPTYSCFNPPLAEGQTNPFAGLDHLVEAGCEALTDESILAERL VVWDPFGCNYGPSETPIAEFLCOAHDTDGCRFCHLSEGLEGLCIPCNEIFSATASPTSVQVAG AIGAIETSAITTSPTSVRAGTTEASFATA MFARYAVKTVVAVAFI GAVTATPHPECEPCAATPVNLDALTIAGKYLCCDLSQODAPVAGCGIFG LPLVCKACYDGDPTPAYDDPIDEEGASPTPAPVEASTVVPAPYEESMTVTVPSTPAQLEVLV MKFTAIFLAWMCASAFIAPSAVVKSRRFVTPVRSSTVAPKTTMNMGLQIVDMVPMVPTFA HDQVLEVLV	0.967	BU038046	-	
52	MILFRSTLVSFLSTDAFVAPLAVKGAASAAARRASTRWGDHKGALCMSVEEPWFQEAATV LLDVDELDRASDMKGDKPKKSVADLADAELEVLV MKFIAACALAGVASSQAFMAQPTMKTTGSPDATSRADF AKIVTGA VAAASFVAAAPA VAKSGTA AKQNYFGVLGADQNLGGGMSNYFAESETYSPYGPDPKALYNEADPFMIKIKVDVLK DSQKQLQAVPAFIETKKEWEEIRLLTNKASSLREAMNTL MTAALLVFRLAAVLSAMAPLAFGFTTPTFSVRTSVARSAQVTKMGPVAVKEKFDWVPILEVLV MEMPRSVVIAIMAITLLCGINRATFVLDDEGVIRASSASTLFSVDFTLPCNFTSLASQRSIW ASEDDSCNQKLPNLWIIMKAEGTANDPDAKDVEEQIQILEVNLLEGLP MRTPTATPLAFIWFALVGSVAEIVERRSGFSLAKPHSGSNHRLVLSVSSDSITAPIVGVGFVSSAD TTSQDELPTTAPPEEELVEDLEVLV	0.841	BU038047	LRR-receptor like kinase (<i>A. thaliana</i> et al.)	
53	MILFRSTLVSFLSTDAFVAPLAVKGAASAAARRASTRWGDHKGALCMSVEEPWFQEAATV LLDVDELDRASDMKGDKPKKSVADLADAELEVLV MKFIAACALAGVASSQAFMAQPTMKTTGSPDATSRADF AKIVTGA VAAASFVAAAPA VAKSGTA AKQNYFGVLGADQNLGGGMSNYFAESETYSPYGPDPKALYNEADPFMIKIKVDVLK DSQKQLQAVPAFIETKKEWEEIRLLTNKASSLREAMNTL MTAALLVFRLAAVLSAMAPLAFGFTTPTFSVRTSVARSAQVTKMGPVAVKEKFDWVPILEVLV MEMPRSVVIAIMAITLLCGINRATFVLDDEGVIRASSASTLFSVDFTLPCNFTSLASQRSIW ASEDDSCNQKLPNLWIIMKAEGTANDPDAKDVEEQIQILEVNLLEGLP MRTPTATPLAFIWFALVGSVAEIVERRSGFSLAKPHSGSNHRLVLSVSSDSITAPIVGVGFVSSAD TTSQDELPTTAPPEEELVEDLEVLV	0.766	BU038048	-	
54	MILFRSTLVSFLSTDAFVAPLAVKGAASAAARRASTRWGDHKGALCMSVEEPWFQEAATV LLDVDELDRASDMKGDKPKKSVADLADAELEVLV MKFIAACALAGVASSQAFMAQPTMKTTGSPDATSRADF AKIVTGA VAAASFVAAAPA VAKSGTA AKQNYFGVLGADQNLGGGMSNYFAESETYSPYGPDPKALYNEADPFMIKIKVDVLK DSQKQLQAVPAFIETKKEWEEIRLLTNKASSLREAMNTL MTAALLVFRLAAVLSAMAPLAFGFTTPTFSVRTSVARSAQVTKMGPVAVKEKFDWVPILEVLV MEMPRSVVIAIMAITLLCGINRATFVLDDEGVIRASSASTLFSVDFTLPCNFTSLASQRSIW ASEDDSCNQKLPNLWIIMKAEGTANDPDAKDVEEQIQILEVNLLEGLP MRTPTATPLAFIWFALVGSVAEIVERRSGFSLAKPHSGSNHRLVLSVSSDSITAPIVGVGFVSSAD TTSQDELPTTAPPEEELVEDLEVLV	0.901	BU038049	-	
55	MILFRSTLVSFLSTDAFVAPLAVKGAASAAARRASTRWGDHKGALCMSVEEPWFQEAATV LLDVDELDRASDMKGDKPKKSVADLADAELEVLV MKFIAACALAGVASSQAFMAQPTMKTTGSPDATSRADF AKIVTGA VAAASFVAAAPA VAKSGTA AKQNYFGVLGADQNLGGGMSNYFAESETYSPYGPDPKALYNEADPFMIKIKVDVLK DSQKQLQAVPAFIETKKEWEEIRLLTNKASSLREAMNTL MTAALLVFRLAAVLSAMAPLAFGFTTPTFSVRTSVARSAQVTKMGPVAVKEKFDWVPILEVLV MEMPRSVVIAIMAITLLCGINRATFVLDDEGVIRASSASTLFSVDFTLPCNFTSLASQRSIW ASEDDSCNQKLPNLWIIMKAEGTANDPDAKDVEEQIQILEVNLLEGLP MRTPTATPLAFIWFALVGSVAEIVERRSGFSLAKPHSGSNHRLVLSVSSDSITAPIVGVGFVSSAD TTSQDELPTTAPPEEELVEDLEVLV	0.741	BU038050	-	
56	MILFRSTLVSFLSTDAFVAPLAVKGAASAAARRASTRWGDHKGALCMSVEEPWFQEAATV LLDVDELDRASDMKGDKPKKSVADLADAELEVLV MKFIAACALAGVASSQAFMAQPTMKTTGSPDATSRADF AKIVTGA VAAASFVAAAPA VAKSGTA AKQNYFGVLGADQNLGGGMSNYFAESETYSPYGPDPKALYNEADPFMIKIKVDVLK DSQKQLQAVPAFIETKKEWEEIRLLTNKASSLREAMNTL MTAALLVFRLAAVLSAMAPLAFGFTTPTFSVRTSVARSAQVTKMGPVAVKEKFDWVPILEVLV MEMPRSVVIAIMAITLLCGINRATFVLDDEGVIRASSASTLFSVDFTLPCNFTSLASQRSIW ASEDDSCNQKLPNLWIIMKAEGTANDPDAKDVEEQIQILEVNLLEGLP MRTPTATPLAFIWFALVGSVAEIVERRSGFSLAKPHSGSNHRLVLSVSSDSITAPIVGVGFVSSAD TTSQDELPTTAPPEEELVEDLEVLV	0.82	BU038051	-	
57	MILFRSTLVSFLSTDAFVAPLAVKGAASAAARRASTRWGDHKGALCMSVEEPWFQEAATV LLDVDELDRASDMKGDKPKKSVADLADAELEVLV MKFIAACALAGVASSQAFMAQPTMKTTGSPDATSRADF AKIVTGA VAAASFVAAAPA VAKSGTA AKQNYFGVLGADQNLGGGMSNYFAESETYSPYGPDPKALYNEADPFMIKIKVDVLK DSQKQLQAVPAFIETKKEWEEIRLLTNKASSLREAMNTL MTAALLVFRLAAVLSAMAPLAFGFTTPTFSVRTSVARSAQVTKMGPVAVKEKFDWVPILEVLV MEMPRSVVIAIMAITLLCGINRATFVLDDEGVIRASSASTLFSVDFTLPCNFTSLASQRSIW ASEDDSCNQKLPNLWIIMKAEGTANDPDAKDVEEQIQILEVNLLEGLP MRTPTATPLAFIWFALVGSVAEIVERRSGFSLAKPHSGSNHRLVLSVSSDSITAPIVGVGFVSSAD TTSQDELPTTAPPEEELVEDLEVLV	0.629	BU038052	Glyceraldehyde-3-phosphate dehydrogenase (<i>A. thaliana</i>)	
58	MTAALLVFRLAAVLSAMAPLAFGFTTPTFSVRTSVARSAQVTKMGPVAVKEKFDWVPILEVLV MKSAVIAAACVAGAQAFVPPSAFNGAALTTSAKSSAMKMSFESEIGAQPLGFWDPLGLLNDAD QERFDRRLRYVEIKHGRISMLAVLGHIVQNVRLPGMLSTADISFADMPNGVAALSKIPPLGLA QVAFVGFLELAVMKNVEGSPGDF TNGGNPFEGSWESEETKESKRAIELNNGRCGANG HSSHDGAREISPSFEVLV	0.901	BU038053	-	
59	MTAALLVFRLAAVLSAMAPLAFGFTTPTFSVRTSVARSAQVTKMGPVAVKEKFDWVPILEVLV MKSAVIAAACVAGAQAFVPPSAFNGAALTTSAKSSAMKMSFESEIGAQPLGFWDPLGLLNDAD QERFDRRLRYVEIKHGRISMLAVLGHIVQNVRLPGMLSTADISFADMPNGVAALSKIPPLGLA QVAFVGFLELAVMKNVEGSPGDF TNGGNPFEGSWESEETKESKRAIELNNGRCGANG HSSHDGAREISPSFEVLV	0.613	BU038054	Fucoxanthin (<i>L. saccharina</i>)	
60	MKSAVIAAACVAGAQAFVPPSAFNGAALTTSAKSSAMKMSFESEIGAQPLGFWDPLGLLNDAD QERFDRRLRYVEIKHGRISMLAVLGHIVQNVRLPGMLSTADISFADMPNGVAALSKIPPLGLA QVAFVGFLELAVMKNVEGSPGDF TNGGNPFEGSWESEETKESKRAIELNNGRCGANG HSSHDGAREISPSFEVLV	0.613	BU038055	Fucoxanthin (<i>L. saccharina</i>)	
61	MKSAVIAAACVAGAQAFVPPSAFNGAALTTSAKSSAMKMSFESEIGAQPLGFWDPLGLLNDAD QERFDRRLRYVEIKHGRISMLAVLGHIVQNVRLPGMLSTADISFADMPNGVAALSKIPPLGLA QVAFVGFLELAVMKNVEGSPGDF TNGGNPFEGSWESEETKESKRAIELNNGRCGANG HSSHDGAREISPSFEVLV	0.886	BU038056	Cysteine protease (<i>Caenorhabditis elegans</i> , <i>A. thaliana</i> et al.)	
62	MKSAVIAAACVAGAQAFVPPSAFNGAALTTSAKSSAMKMSFESEIGAQPLGFWDPLGLLNDAD QERFDRRLRYVEIKHGRISMLAVLGHIVQNVRLPGMLSTADISFADMPNGVAALSKIPPLGLA QVAFVGFLELAVMKNVEGSPGDF TNGGNPFEGSWESEETKESKRAIELNNGRCGANG HSSHDGAREISPSFEVLV	0.612	BU038057	-	
63	MKSAVIAAACVAGAQAFVPPSAFNGAALTTSAKSSAMKMSFESEIGAQPLGFWDPLGLLNDAD QERFDRRLRYVEIKHGRISMLAVLGHIVQNVRLPGMLSTADISFADMPNGVAALSKIPPLGLA QVAFVGFLELAVMKNVEGSPGDF TNGGNPFEGSWESEETKESKRAIELNNGRCGANG HSSHDGAREISPSFEVLV	0.761	BU038058	-	

Table 1 (Contd.)

Fucus SST No.	Predicted amino acid sequence of amino-terminal polypeptide region ^a	Mean S-score ^b	GenBank Access. No.	Polypeptides exhibiting similarity ^c
64	MVSTHMF ^{LA} ATACTASSAFVAPMAIRMSRSSSTLKMESGSA ^{YV} N ^{TL} PGAPFGDGKIF DPLGSDGAA ^{PN} DIKK ^{WR} DAEIKHGRVAMLAALGVLVAEEYHPLFMGPDYIGPAVDH FOEITARFPAFWAIALTGMGIETNLEVLV	0.744	BU038059	Fucoanthin (<i>C. reinhardtii</i>)
65	MKFIACALAGVASSQAFMAQPTMKTTGSPDATSRADF ^{AK} IVTGA ^{VA} ASFA ^{VA} APA ^{VA} AKSGTAAK QNYFVGLGADQNLGGGMSNYFAESETYSPY ^{GT} DPKALYNEAD ^{PF} MIKIKVDVLKDSQKKL QAPAFIETK ^W EIRSLTNKAYSLREAMN ^{TL} AKDKPNAN ^{MG} KV ^{FY} RDIEQLTGT ^{LA} KNPE MAPVINA ^{AM} AFASAFASAFVAP ^{SA} FN ^{GA} ALSTNS ^{AS} TKLSMA ^{TD} CGING ^{FR} IGRLV ^{AR} SMI KNPETNLK ^{IN} TGAAP ^{EY} MAYQ ^{FY} DI ^{VH} GF ^{GG} TV ^{VD} GM ^{DL} ILD ^Q R ^{VT} SH ^{TR} NPEE IPFVATGAEYVCESTGAFLTEEKVQPHLKAGAKKIVFSAPAKDDSP TM VMGVNAE ^{TY} ESSMN LVLCASCTTNGLPREGINATCDQ ^{GA} LMTRAR	0.766	BU038060	–
66	MILFRSTCLVSVLSTSDAFVAPLVKGAASAAASRRASTRWGDHK ^{GA} LCMSVEEPWFQEA KATLLD ^{VD} DELDRAS ^{DM} KGDKPKKSVADLADAELEVLV	0.629	BU038061	Glyceraldehyde-3-phosphate dehydrogenase (<i>A. thaliana</i>)
67	MSKVA ^{AW} LV ^{CL} AVALSMD ^{SR} ADIERAPAGSANE ^{QQ} PLA ^V IVPAY RD DLQQA ^V SSLER ^{RP} IV CSSLTOV ^{NA} D ^{LV} LYA ^{ED} E ^{DE} GH ^{QD} ITS ^V LESISQ ^{GA} GRCFAR ^{TR} TLEVLV	0.841	BU038062	LRR-receptor like kinase (<i>A. thaliana</i> et al.)
68	MVSTHMF ^{LA} ATACTASSAFVAPMAIRMSRSSSTLKMESGSA ^{YV} N ^{TL} PGAPFGDGKIFD PLGSDGAA ^{PN} DIKK ^{WR} DAEIKHGRVAMLAALGVLVAEEYHPLFMGPDYIGPAVDHFQEIT GRFP ^{AF} WAIALTGMVH ^{ET} N ^{TK} LEVLV	0.929	BU038063	–
69	MWSIRGGY ^W DTVLVLIWLSGSA ^{VT} GLDALNQRFLK ^{SD} MVSGH ^{FE} GEDALSQ ^{EN} DDDLFV NSADDSV ^{CL} DKWGS ^W PSANTQ ^{AE} LEVLV	0.744	BU038064	Fucoanthin (<i>C. reinhardtii</i>)
70	MRLSAGVTGVG ^W ISV ^{VD} V ^{SS} EAF ^{AP} LQAPSSANV ^{AR} VTSH ^Q ALV ^R QRV ^{RP} RGLAAISM ^{SA} VEDELWRGEP ^{DE} ADETSSQ ^{VM} TKPKRSELDDIMSEV ^{AG} RNEP ^{VI} PR ^S AGIGLPP ^{GR} IRI	0.91	BU038065	–
71	KADPIPGMD ^{AD} DDVPGKI ^Q VD ^{IT} LVK ^Q KDLEVLV	0.612	BU038066	–
72	MARSA ^{AF} AMAL ^{TL} PAALG ^{FL} SDN ^{LA} AA ^{TN} V ^{SO} TF ^{DA} EY ^{TEL} GCY ^{ED} SD ^{SQ} KR ^{IF} SE ^{GT} KR ^{LD} DD L ^{TE} TS ^{CA} ICED ^{PF} Y ^{GL} QY ^{GN} EC ^{WG} DY ^{TE} SID ^{SD} SE ^{SD} GD ^{CM} TCTG ^{SD} DAN ^{CG} GY ^{AM} RIF ^{TR} AM ^T DD ^{AI} GCY ^{GD} STDRV ^{FS} DKL ^{VDR} SGM ^{SP} EV ^{CF} DHC ^N SE ^{AP} Y ^{TT} EP ^{NG} VR ^{NV} GA D ^{TR} KIR ^{PI} GSALRS	0.92	BU038067	β -1,3-Exoglucanase (<i>N. crassa</i>)
73	MRLSAGVTGVG ^W ISV ^{VD} V ^{SS} EAF ^{AP} LQAPSSANV ^{AR} VTSH ^Q ALV ^R QRV ^{RP} RGLAAISM ^{SA} EDELWRGEP ^{DE} ADETSSQ ^{VM} TKPKRSELDDIMSEV ^{AG} RNEP ^{VI} PR ^S AGIGLPP ^{GR} IRI DPIPGMD ^{AD} DDVPGKI ^Q VD ^{IT} LVK ^Q KDLEVLV	0.612	BU038068	–
74	MALIGAPAS ^{VT} QSFICAP ^{QQ} QQ ^{QQ} Q ^Q GAH ^{GT} S ^{FL} T ^{LN} G ^{NG} S ^{FG} GG ^{VM} PR ^{GG} GG ^{MG} SL ^{RS} G	0.521	BU038069	–
75	MARFA ^{FT} LACTVIGTVGALEVLV	0.938	BU038070	–
76	MHGIASL ^{CV} FSV ^{AI} TALGQ ^{AF} AASN ^{RE} LAD ^{VEL} GMV ^{GL} SGD ^{IN} AAA ^G SSN ^{NR} AL ^{RT} SR ^{IR} S ^{FY} IDV ^{TF} INLLGV ^{PK} GY ^K FDI ^{PI} GSS ^{GL} I ^{GT} LEVLV	0.895	BU038071	–
77	MVSTHMF ^{LA} ATACTASSAFVAPMAIRMSRSSSTLKMESGSA ^{YV} N ^{TL} PGAPFGDGKIFDP LGLSDGAA ^{PN} DIKK ^{WR} DA ^Y ILSTGCV	0.744	BU038072	Fucoanthin (<i>C. reinhardtii</i>)
78	MKTPSAGGAI ^{CA} AALIPV ST MEKERQ ^{PS} RRLLQ ^{DA} PTV ^G IG ^{FT} TST ^{AT} SAD ^{AS} VSS ^{SI} E ^{DE} LV ^E A ^E LEVLV	0.636	BU038073	–
79	MEWPSTL ^{AA} ALAFATFSATSAA ^{VC} DEVANALD ^{LD} NIV ^{PL} GGID ^T TIELASSIT ^{LE} CPAN ^{EED} EELGEIL ^V SGG ^{KL} TISS ND TV ^{RV} N ^{RV} FT ^{VE} DGAEL ^{IF} DM ^{PK} TK ^{FG} PN ^{DG} YSEN ^{AP} GY ^{MLN} VTEGGSAT ^{FM} G ^{FG} W ^Q VEN ^{VR} SM ^{FY} NG ^S IE ^{FG} KN ^{AN} FQSN ^{RE} RFSE ^{QR} GT ^{LK} FA ^{GR} PGSRTLY ^L LSTTAR ^{LR} FR ^{NG} TF ^{KK} RG	0.895	BU038074	–
80	MTAALLV ^{RL} AAVLSAM ^{AP} LAF ^{GF} IT ^{PT} FS ^{RV} TSV ^{ARS} AQ ^{VT} MK ^{GPP} AV ^{KE} K ^{FD} W ^{VP} ILE ^{VLP}	0.901	BU038075	–
81	MNRLMS ^{LV} LLSSAT ^{SE} K ^M VED ^{NI} PT ^{GW} R ^{MQ} DR ^{FG} H ^{FR} FEAK ^{LG} N ^{NC} OAY ^{AQE} AV ^{TA} ADELAC ^{FG} W ^{VQ} W ^{TS} NTV ^{AG} ERC ^{SK} AT ^{GS} Q ^{ML} D ^{QL} R ^D GP ^{GA} CA ^{VD} TF ^{DA} K ^D Y ^{ED} TK ^{IKL} HF ^{SH} FK ^{IL} DEAR ^K TC ^{FR} DP ^{PH} QC ^{PE} LAT ^{IN} TID ^{SK} ME ^{QA} CA ^{OD} TTE ^{LE} VLV	0.792	BU038076	–
82	MFARY ^{AV} K ^V Y ^{VA} A ^{AF} IGA ^{VT} AT ^{PH} PE ^{CE} PCA ^{AIT} PN ^{LD} AL ^{TI} AG ^{KY} L ^{CC} DL ^{SC} Q ^D AP ^V AG ^{CG} I FGL ^Y V ^{CK} AC ^{YD} G ^{DD} T ^{PAY} DD ^{PP} IDE ^E GA ^{SP} TP ^{AP} VE ^{AS} TV ^{AS} T ^{PA} YE ^{ES} MT ^{VT} VP ST PAP ^{IV} ATV ^{AS} T ^{PA} VE ^{AS} TV ^{AS} T ^{PA} PK ^{LE} V ^{SL}	0.801	BU038077	Insect intestinal mucin (<i>T. ni</i>)

83	MWSIRGGYWWDTVLVLWLSGSAVTLGLDALNORFLKKSMDVSGHFEGEDALSQDE NDDLFFVNSADDSVCLDKWGSWPSANTQALEVLP	0.982	BU038078	–
84	MWYIMVATLAISLLQIQPASCFFGTSLSKLRGSIIPRRNASAVWPPSGHVDASAPIVRRRAA APLRGRHSSWLRKGYDTLEEAFARAQARKEPASASEGSIKDSGTDSDSKGRGDAVLEVLV MKVTAATAVASLGMTSAFMAPAARPTSSSLKATTTSSMMEGQIGVSNKGVFDPGLGLAELH KINPDVNPHELEVLV	0.844	BU038079	–
85	MIRLTCVLLARQA VAFTPNLSIFFVDMGNRLTTVGSGATRPASTLFAASGGKMTAENVLKSPK WPKDKWPFYDDDFNRMDESPSLEVLV	0.707	BU038080	–
86	MIRLTCVLLARQA VAFTPNLSIFFVDMGNRLTTVGSGATRPASTLFAASGGKMTAENVLKSPK WPKDKWPFYDDDFNRMDESPSLEVLV	0.824	BU038081	–
87	MIRNISRFLVLAITATGASGRITANEGRVTSKEDNYEAVRHMNAGSEEEFARRLGGDDAGT VTPCSNGLPVELRDVCCPECGTGGVGCSSRSGGASECCTSYIESLGPCKCGSKAAPCHMD GSYKETSQSDVTRTLASMDLAQLLVMDIMLLSLGYGGTDCGQTHFRFTGK	0.797	BU038082	–
88	MARFAAFAMALTLPAALGFLSDNLAATNVVSOQTFADAEBYTELGCYEDSQKRIFSEGTKRLD DLTETSCAAICEDFPYFGLQYGNECWGDYTSSEIDSDSESDGDMTCTGDSANCGGNYA MIRIFRAMITDDDAIGCYDSTDRVFDKLVDRSRMSPEVCFDHCNSESALYYGT	0.925	BU038083	–
89	MARSHRCRVLVITMVAVALVSLVKADYDKIAGYTPVSDVTEHSELDDMQEIEENADLQ TEAGFSAAWTAYSEGGNSEKTSIRTRGFSTDAEAVLGGEPWVVIYKDYWGEPTYADLF TTAACNGTDDDFDGAEMATRSEAWTKGAQYQNVWVYVHLEVLV	0.92	BU038084	β -1,3-Exoglucanase (<i>N. crassa</i>)
90	MARSHRCRVLVITMVAVALVSLVKADYDKIAGYTPVSDVTEHSELDDMQEIEENADLQ TEAGFSAAWTAYSEGGNSEKTSIRTRGFSTDAEAVLGGEPWVVIYKDYWGEPTYADLF TTAACNGTDDDFDGAEMATRSEAWTKGAQYQNVWVYVHLEVLV	0.859	BU038085	–
91	MRTPTATRPLAFIWFALVQSVAEIVDERRSFSLAKPHSGSNHRVLYSSSDSITAPIVGVGF VSSADTTSGDELPTTAPPEEELVEDLEVLV	0.829	BU038086	–
92	MWSTOWIFVLGLLTVISNNAQVCSNGIAGIENDDICCVAFECQCGRRRCGRKARRVGLTS SDCCTGQITSAGVSCNDSGMAPCVDSVCGPALTILEWSELNDDLAVTDPDGTFFG	0.949	BU038087	–
93	MARSHRCRVLVITMVAVALVSLVKADYDKIAGYTPVSDVTEHSELDDMQEIEENADLQ DLQTEAGFSAAWTAYSEGGNSEKTSIRTRGFSTDAEAVLGGEPWVVIYKDYWGEPTY ADLFTTAAACNGTDDDFDGAEMATRSEAWTKGAQYQNVWVYVHLEVLV	0.859	BU038088	–
94	MRTDVAIVFGLVIGVASAAPTYSFCNPLAEGQTPFAGLDHLVEAGCEALTTDESILAE RLVWVDFPGCNYGPSETFAEALFCAQHDITDGCRCFCHLSCGLEGLCIPCNEIFSATASPTSVQV AGAIGAIETLFAITSPTSRGRVAGTEAPSRLOQYSRVVTGTEVF	0.859	BU038089	–
95	MRAVCLLAVAIGVAAAFVNPSTTTTLRSARLQHRPSTTSPELNQRLARLARRELSASAAVPTT ERGLRMDGSTETLGVIVCDHSGRRRENANDMLFEAERYRAFAGFDIVEAAHMELAQPS IEQAFDRCVAAGAHKIILHPFLSPGRHVTFDIPGLIAAAALLESV	0.93	BU038090	Hypothetical protein 93341 (<i>A. thaliana</i>)
96	MAFSNMILVASASRIVHCALLAVLLGGSEYSVACSDDDVECYGVNVCSSSEKCAAPADCTASNIQ DKDDHIEIRGNCRVITMEAIRVALPDAVTKEGDEYVLQKVVWRDGCVLEIHGSSASSDA AVSLKLLKSDSSSAPIVAYHGKISIMNTPRSYDESIDGPDRLVSL	0.794	BU038091	–
97	MRTPTATRPLAFIWFALVQSVAEIVDERRSFSLAKPHSGSNHRVLYSSSDSITAPIVGVGVSS ADTTSGDELPTTAPPEEELVEDLEVLV	0.829	BU038092	–
98	MPQNTSRYFRWTVAAASVALSLVAPSAAAEDKGLTFPGPEATERFLAIDNAITHLEDGETARRL QSGDW/CNTISATETYCCGFGGQASCIRGIDYSGPDPCEVILTTSDNGTMYCEGIDFDSGNL DICDCEEADLLPIPLPGPVTVVPLSD	0.74	BU038093	–
99	MTKLASFVLLATLIAVTDAASTDAADVAVIAEAPLYDTRLSDAGGDPAGCSGDLTRDDN IATOGSRWSCPELGSVGTCSITYNLLDLTHIEELNIAMYKGDERTRTVDIYVDDVLITSW TSSGTFGLDRDRPWRDRQQLNFVCAAR	0.943	BU038094	Protein phosphatase 2C (<i>A. thaliana</i> , <i>D. sativa</i> , et al.)
100	MSSVRALCAILALMASGSLAWDDSSLHSHKSHYVQFFRMMRQHKVQFGTKGEFERRLQ IFAENSDLIEVHNSASDEAMYTLGHNEFSLHSEEFKDTHFGYNQRPPQEPAAEVKPSRRS RAVTSRRRLTPVAELPDEVWEDK	0.886	BU038095	Cysteine protease (<i>A. thaliana</i> et al.)
101	MRTPTATRPLAFIWFALVQSVAEIVDERRSFSLAKPHSGSNHRVLYSSSDSITAPIVGVGVGF VSSADTTSGDELPTTAPPEEELVED	0.832	BU038096	–
102	MILFRSTCLVSVLSTSDAFVAPLAVKGAASAAAARRASTRWGDHKGALCMSVEEP WFQEAKATLLDVEDELDRASDAMKGDPKKSVADLADAE	0.841	BU038097	LRR-receptor like kinase (<i>A. thaliana</i> et al.)
103	MTAALLVRLAAVLSAMAPLAFGFITPFTSVRTSARSAQVTMKGPPAVKKEKFDWVPI MKVTAATAVASLGMTSAFMAPAARPTSSSLKATTTSSMMEGQIGVSNKGVFDPGLGLAELH KINPDVNPHELEVLV	0.901	BU038098	–
104	MKVTAAAAVASLGMTSAFMAPAARPTSSSLKATTTSSMMEGQIGVSNKGVFDPGLGLAELH KINPDVNPHELEVLV	0.707	BU038099	–

Table 1 (Contd.)

Fucus SST No.	Predicted amino acid sequence of amino-terminal polypeptide region ^a	Mean S-score ^b	GenBank Access. No.	Polypeptides exhibiting similarity ^c
105	MWRSSVIAVCGLLSSTSVMAIMDVGDHAHACATVGTVLK CWGNNERGQLGRGDKNTT GDEPEMGGDHLVPVDLGTGEVADEIAGKEHSCVLLSSGDVVKCFGYNDKGQLGQGG DPCLAGNYRTSWVMSCQPSILAPVYSRFKVTVGCDDHTCLLARHDS	0.808	BU038100	RCC1 (<i>H. sapiens</i>), hypothetical protein AC021640 (<i>A. thaliana</i>)
106	MRAVCLLAIVAIGVAAAFVNPSTTRILSARLQHRPSTTSPSELNQRRLARLARLRELS ASAAVPTTERGLRMDGSTETLGVVCDHGSRRRENANDMLFEVAERYAFAGFDIV EAAHMELAQPSIEQAFDFRCVAAGAHKIILHPFLSPGRHVTFDIPGLIAAALS MRYLSEGGLCPRDCRRRLPLSITAMFRLGIGLSLLPLARAFIVAGNGAGLLGGTS LTRGDGRMVLRRGATTMNSSPAPASSAQYAQHLLGHTQGR	0.93	BU038101	Hypothetical protein 93341 (<i>A. thaliana</i>)
107	MRTDVATVFFGLVICGVAASASTYSCFNPLAEGQTNPFGAGLDHLVEAGCCEALTDSDILA ERLVVWDPFGCNYGPSETPIAEAFLCQAHDRRLPGSVICHAKAWKGLVHLSLQRGYSSRTA MARFAFATLACTVIGTVGALEVL	0.528	BU038102	–
108	MARRGLSKNKLLVLCWIRSGCMAKSLNFWQAMEEVGEVIPKEENCLPCDSIFSKL	0.865	BU038103	–
109	MARFAFATLACTVIGTVGALEVL	0.907	BU038104	–
110	MARRGLSKNKLLVLCWIRSGCMAKSLNFWQAMEEVGEVIPKEENCLPCDSIFSKL	0.803	BU038105	Glutathione peroxidase (<i>A. thaliana</i>)
111	MARFAFATLACTVIGTVGALEVL	0.927	BU038106	–
112	MKGSMFVAVMQLALIGMASLVIAEGSTNTFKSVRGVEDOYELKGAEHNLFMQMGDN QDAKNRYHSRTDSEAR	0.882	BU038107	–
113	MGCFHSWRGAFVLSASASAMGAYLGALECDPQYAHQINGNGYCDMGNTACGYDGGD CCGCTCQDSENFCCGNGY ^N CLDPDTACADLVEIETARDLMEASFTTMTYPVQPPTS ^R RFFL	0.909	BU038108	–
114	MARFAFVTFACVIIGTAGALEVLP	0.937	BU038109	–
115	MKSAVIAAACAAGVAFVPPSAFNGAALTSKSSAMKMSFESEIGAQPPLGFWDPLGLLN DADQERFDRLRVYKIHGRISMLAVLGHIVQAERAPARHAVHFGGHFLR	0.613	BU038110	Fucoxanthin (<i>L. saccharina</i>)
116	MKTPSAGAILCAAALIPVSWAMEKERQPGTSRLLQDAPTVGIGFTTSTATSADASVSSIE DELVEALEVLP	0.742	BU038111	–
117	MRTPATRPLAFIWFALVQSVAEIVDERRSFSLAKPHSGSNHRVLVSSSDSITAPI	0.829	BU038112	–
118	MKSAVIAACVAGAQALVPPSAFNGAALTSKSTSAMKMSLSPTARRSPRIGFWDPLVLP	0.719	BU038113	Fucoxanthin (<i>L. saccharina</i>)
119	MQRLLTDAFLIVASISILKEGLSEFTVKDGRDGFPLSKYDKVPAVLIVNVAASYCG YTDHYRELOQLRAEYDESKLAIVAFPCNQFGAQEPGTWEEIAKFVDTQYGVTFPLM DKVNVNGDQSDPLLEVLPI	0.796	BU038114	–
120	MARRGLSKNKLLVLCWIRSGCMAKSLNFWQAMEEVGEVIPKEENCLPCDSIFSKL	0.803	BU038115	Glutathione peroxidase (<i>A. thaliana</i>)
121	MARFAFATLACTVIGTVGALEVLP	0.927	BU038116	–
122	MFARYAVKTVVAFAFIGAVTATPHPECEPCAAITPVNLDALTIAGKYLCCDLSCQDAPVA GCGIFGLPLVCKACYDGGDDTPAYDDPPIDEEGAFPTAPAVEASTVVA ^S TTPPMKNHDGNCA IDFSPATRGSPI	0.801	BU038117	Insect intestinal mucin (<i>T. ni</i>)
123	MKAYVFFVLAFLACAWSFVVPQGS ^S SLKLSRPARLSDAAAAA ^S VSAGGGGAARAPS PRARRVSGISM ^K RK ^G QNSNIQQRGSYNQMLQ ^Q ELQYKEQKAMDTGMPAFQLY ARTKVNMMWYPCGTMMDNNAKATVDGMMGGFLSGVSKYSLEVLP	0.964	BU038118	F12A21.16-encoded hypothetical protein (<i>A. thaliana</i>)
124	MKAYVFFVLAFLACAWSFVVPQGS ^S SLKLSRPARLSDAAAAA ^S VSAGGGGAARA PSPRARRVSGISM ^K RK ^G QNSNIQQRGSYNQMLQ ^Q ELQYKEQKAMDTGMPAFQLY ARTKVNMMWYPCGTMMDNNAKATVDGMMGGFLSGVSKYSLEVLP	0.964	BU038119	F12A21.16-encoded hypothetical protein (<i>A. thaliana</i>)
125	MTRSFIVACSM ^L TVGARALEVITPSEGLTVIADRTYVTEWTDGDSGRFEIDL ^Y YCGSFC MEDECGDWV ^T ALCPY ^G STGCPDNESDYD ^I VIA	0.851	BU038120	–
126	MRHAVNALHLSLLAVVSVSGC ^P FLADHQDDGT ^E LPIGGGHRLLGRKQNDK ^K DR ^S NGP DFSAKTYNALR ^K DDIV ^M LD ^S SKDY ^W PA ^D FGNYAP ^F MIRLAW ^H CAG ^S YR ^S VRM VEVAATVDRDP	0.888	BU038121	Catalase (<i>Bacillus stearomphila</i>)
127	MGRKKRLLP ^F LL ^L QV ^K ER ^I Y ^P AP ^R SL ^P L ^L RI ^L AK ^I WT ^L DI ^V IG ^I V ^K SM ^F LA ^S LF ^L LLCDDSSD ^Q KK ^S GG ^V RL ^G GG	0.597	BU038122	–

128	MKAYVFFVLAFLACAWSFVFNPGSSSLKLSRRARLSDAAAAAASVSAAGGGAA RAPSRRARVSGISMKRKQKQNSNIQQRSSYNQMLQELQYKEQQKAMDTGM PAFLYARTKVNMMWYPCGTMGDNNAKATVDGMGGFLSGVSKYSL MKTPSAGGAILCAAALILPVSWAMEKERQPGTSSLQDAPTVGIGFTTSTATSADALV SSLDRRTRGGSGSPY	0.964	BU038123	F12A21.16-encoded hypothetical protein (<i>A. thaliana</i>)
129	MKTAMIAAACTGTQAFVAPSAFNGAALTSAPSSMKMSFESEIGAQPPLGFWDPPL GLLNDADQERFDRRYVEIKHGRISMLAVLGHIVQQNVRLLPGLMLSTSSNLSFADMPN GVAALS KIPPLGLAQVAFVGFLELAVMKNV	0.742	BU037980	-
130	MARFALVTLACAVIGAAGDLEVLV MARLSFAIALSASAATAFVTLAPSFSGGRAVSA TSSSTTRMMSVPEPPPTAESLKQMA ESCKEGCSVEAVNTLDDQLKAKKTELEVLV MPDSIVEHLQVYASMAVOLFLIGLTPVIHCTGIRGPDVACERLVKGPVVVYNGIALSPN KERLLVADAGTMQVFNRSDDTGETLLKQVELLDFGQPRADVVYRLLEW MVLEAMQGPVVALAVAGAFATVIVALFRFCCKPTATYAVNHGLDEEEMAFKKS MEQHQGDEIDELFNFSGHE:ELDFDINDLDFEMLEVLV	0.533	BU037981	Fucoxanthin (<i>L. saccharina</i>)
131	MARFALVTLACAVIGAAGDLEVLV	0.925	BU037982	-
132	MARLSFAIALSASAATAFVTLAPSFSGGRAVSA TSSSTTRMMSVPEPPPTAESLKQMA ESCKEGCSVEAVNTLDDQLKAKKTELEVLV MPDSIVEHLQVYASMAVOLFLIGLTPVIHCTGIRGPDVACERLVKGPVVVYNGIALSPN KERLLVADAGTMQVFNRSDDTGETLLKQVELLDFGQPRADVVYRLLEW MVLEAMQGPVVALAVAGAFATVIVALFRFCCKPTATYAVNHGLDEEEMAFKKS MEQHQGDEIDELFNFSGHE:ELDFDINDLDFEMLEVLV	0.897	BU037983	-
133	MARFALVTLACAVIGAAGDLEVLV	0.482	BU037984	-
134	MVLEAMQGPVVALAVAGAFATVIVALFRFCCKPTATYAVNHGLDEEEMAFKKS MEQHQGDEIDELFNFSGHE:ELDFDINDLDFEMLEVLV	0.614	BU037985	-
135	MTSIRKAGLALLTLGLVWRWQVTTCDLKSIIADGKTLGLSDFTCSEIISVDE GVEVTIDGADFTLSDADLLYDEEGTNAVINYKGSLLTGLTINVTDDTAGNKFRAI YNEGDLTVNSCTFNGLNLPDPSMTEG	0.898	BU037986	Calmodulin-like protein (<i>A. thaliana</i>)
136	MARLSFAIALSASAATAFVTLAPSFSGGRAVSA TSSSTTRMMSVPEPPPTAESLKQMA AESCKEGCSVEAVNTLDDQLKAKKTELEVLV	0.897	BU037987	-
137	MTSIRKAGLALLTLGLVWRWQVTTCDLKSIIADGKTLGLSDFTCSEIISVDE GVEVTIDGADFTLSDADLLYDEEGTNAVINYKGSLLTGLTINVTDDTAGNKFRAIYNEG DLTVNSCTFNGLNLPDPSMTEG MRLSAGVTYGVGWSVVDVSSFAFA PLQAPSSANVARVTSHGQAL VRQVRPRGLAAISMSAVED ^{EL} WRGEPWDEADETSQV MTKPKRSELDIMSEVAGRGNEP VIVPRSAGIPLPPGRKADPIPQMD ADDPVKIQVDITLVKQKQDLEVLV MLLHFRSRLLLCAAGLVALILDAAASAALA EVAGVSAANGWDTRVDGDGCSPSG CVPSNVLDSGSEDDASRWCSAE VSDGDA CELTLEGLP MQLMKISLAVAFVTAAGSAASTSRVSRPSPAPLAH RAQQHTDDHLELVYRAQACARLARLGGAGADNSAERIDGPGCIGIDLGTTYSCVAV WQNGRVEICANDQGNRNLEVLV LRFELSGRARGRVRHFLVY	0.898	BU037988	Calmodulin-like protein (<i>A. thaliana</i>)
138	MRLSAGVTYGVGWSVVDVSSFAFA PLQAPSSANVARVTSHGQAL VRQVRPRGLAAISMSAVED ^{EL} WRGEPWDEADETSQV MTKPKRSELDIMSEVAGRGNEP VIVPRSAGIPLPPGRKADPIPQMD ADDPVKIQVDITLVKQKQDLEVLV MLLHFRSRLLLCAAGLVALILDAAASAALA EVAGVSAANGWDTRVDGDGCSPSG CVPSNVLDSGSEDDASRWCSAE VSDGDA CELTLEGLP MQLMKISLAVAFVTAAGSAASTSRVSRPSPAPLAH RAQQHTDDHLELVYRAQACARLARLGGAGADNSAERIDGPGCIGIDLGTTYSCVAV WQNGRVEICANDQGNRNLEVLV	0.612	BU037989	-
139	MLLHFRSRLLLCAAGLVALILDAAASAALA EVAGVSAANGWDTRVDGDGCSPSG CVPSNVLDSGSEDDASRWCSAE VSDGDA CELTLEGLP MQLMKISLAVAFVTAAGSAASTSRVSRPSPAPLAH RAQQHTDDHLELVYRAQACARLARLGGAGADNSAERIDGPGCIGIDLGTTYSCVAV WQNGRVEICANDQGNRNLEVLV	0.945	BU037990	EGF-like repeat
140	MQLMKISLAVAFVTAAGSAASTSRVSRPSPAPLAH RAQQHTDDHLELVYRAQACARLARLGGAGADNSAERIDGPGCIGIDLGTTYSCVAV WQNGRVEICANDQGNRNLEVLV	0.814	BU037991	hsp70 (<i>A. thaliana</i> , <i>Zea mays</i>)
141	MFARYAVKTAVAVAAFIAGTATPHPECEPCSAITPVNLDALTIAGKYCV LRFELSGRARGRVRHFLVY	0.704	BU037992	-
142	MKTAMIAAACTGTQAFVAPSAFNGAALTSAPSSMKMSFESEIGAQPPLGFWDPPL GLLNDADQERFDRRYVEIKHGRISMLAVLGHIVQQNVRLLPGLMLSTSSNLSFADMPN GVAALS KIPPLGLAQVAFVGFLELAVMKNV	0.528	BU037993	Fucoxanthin (<i>L. saccharina</i>)
143	MVSTHMF LAATACTASSAFVAPMAIRMSRSSSTLTKMESEGSAYVNTLPGAPFGDGKIFDPLGLS DGAAPNDIKKWRDAEIKHGRVAMLAALGVLVAAEYHPLFMGPDYIGPAVDHFQEITARFPA FWAIALTGMGHIETNQLLEVLV	0.744	BU037994	Fucoxanthin (<i>C. reinhardtii</i>)
144	MARLSFAIALSASAATAFVTLAPSFSGGRAVSA TSSSTTRMMSVPEPPPTAESLKQMA ESCKEGCSVEAVNTLDDQLKAKKTELEVLV	0.897	BU037995	-
145	MSAIFPSPAKATCCLHITAKCLSAI SPHSWSPRLRRPSSPHLPSASLRRPRRPPA MSLGAIASLMTVITSTAAFI VTPSVRFIASAASPSLKMQSEGFNILDGPA GCSVSV TAKIFYPHCGFSGDGAVFSEIKKMG	0.482	BU038124	-
146	MSLGAIASLMTVITSTAAFI VTPSVRFIASAASPSLKMQSEGFNILDGPA GCSVSV TAKIFYPHCGFSGDGAVFSEIKKMG	0.853	BU038125	-
147	MFARYAVKTVVAVAAFIAGTATPHPECEPCSAITPVNLDALTIAGKYCV AGCGIFGLPVCKACYDGDPTPAYDDPDI DEEASRRQPRKPLRLWLRPQPMKNP MFTYSSVFLCALVFAGRDGGFPQSSRQMMASADELPPGVQNRDLDDDKKGGIMDTLEKF GQDVMAPFTGGKKTAKAEADAADAADTKTASDAATDAADVTTDAADFDTKKTASSA ATDTADSASDAVKDAASDTKETAFNPPKTPPDSVAKPPRKRNR	0.801	BU038126	Insect intestinal mucin (<i>T. ni</i>)
148	MFTYSSVFLCALVFAGRDGGFPQSSRQMMASADELPPGVQNRDLDDDKKGGIMDTLEKF GQDVMAPFTGGKKTAKAEADAADAADTKTASDAATDAADVTTDAADFDTKKTASSA ATDTADSASDAVKDAASDTKETAFNPPKTPPDSVAKPPRKRNR	0.827	BU038127	-

Table 1 (Contd.)

Fucus SST No.	Predicted amino acid sequence of amino-terminal polypeptide region ^a	Mean S-score ^b	GenBank Access. No.	Polypeptides exhibiting similarity ^c
149	MGFTCNTAIFIFHIGILLTGASAFIAFTAFMGTTRAGGQQEGCVAGVSMVASTGLDKGVMEH PLIAEDVTQLIGKTPVKNLNRVTEGCEATILAKLE	0.821	BU038128	Cysteine protease (<i>A. thaliana</i> et al.)
150	MTRSAATAVFALLALALPLIAAKGRVDQPHLSGESQASGRIVPRR LSTLDNDEASSILAGDYVCSNGLFGIES SDGSVCCVCEGCGGEGSTVAAPDYGASDCCANEIAFFGMSNCNSTMA APCVIDKLGSTTTTFNELSVDSVGIFTSPHVGSTLRP	0.949	BU038129	–
151	MVPLVVRGALAVAAAVLGEAFDFDSSCTAKTAEQCRGRVEEWSGYPCWSCKAIPSECLGS TIAKKLPKAVFTCDVQERTRSECAGESVADQKTMIAFKA WKEQHGVSYPPDAEEEE RRLRTFRETLEMVTAHNGRQDARLGSREGGYTLEV	0.814	BU038130	–
152	MFRLIGLSLPLARAFIVAGNGAGLLGGTSLTRGDGRMVLR	0.937	BU038131	–
153	MKSAVIAACVAGAQAFVPPSAFNGAALTTSKSSAMKMSFESEIGA OPPLGFWDPLGLLNDADOERFDRRLRYVEIKH	0.613	BU038132	Fucoanthin (<i>L. saccharina</i>)
154	GRISMLAVLGHVQNVRLPGMLSTSDISFADMPNGVAALFKIPPLGLAQIVAFVGFLELY MRLSAGVTGVGWISVVDVSSFAPLQAPPSSANVARVTSHGQAL	0.612	BU038133	–
155	VRQRVPRGLAISMSAVEDELWRGEPWDEADETSQVMTKPKRSELD DIMSEVAGRNEPVI PRSAGIGPLPPGRIKAVYSRHRGLTTFPKDPGGYLTLP MDICRRRFRFRPFASITTVVSKRCKGHQPRPSPCKAARSAARAGCDIYV CRGRRAVARRAVGRGG	0.51	BU038134	–
156	MLLHFRSRLLLCAAGLVALILDAAASAALAEVAGVSA SANGWDTRVDGDCSPSGCVPSNVLDGSFDDASRWSCSAEVSDDGACELTLEVLTP MRTDVAIVFGLVICGVASAAPTYSFCNPLAEQGTNPFAGLDHL	0.945	BU038135	–
157	VEAGCEALTDSDILAE ^r L ^v VDWDFGCNYGPSETPIAEAF ^r LCQAHDITDG CRFCHLSCEGLEGPCNEIFSATASPTSVQVAGAIETLFA ^r TPTFVRVAGTTEL	0.859	BU038136	–
158	MSLGAIA ^r LVITAA ^r FIDPTPSVRFIA ^r SA ^r APSS ^r LK ^r MHSEGS ^r YLG ^r TLPGA	0.854	BU038137	–
159	MKSAVIAACVAGAQAFVPPSAFNGAALTTSKSSAMKMSFESEIGAQPPLG FWDPLGLLNDADQERFDRRLRYVEIKHGRISMLAVLGHVQNVRLPGMLC TSADISFADMPNGVAALYKIPSLGLAQIVAVRGPFGACCDDEERSRAPSRL HQRSQLRGFVGVVLRGNQRSIARS	0.613	BU038138	Fucoanthin (<i>L. saccharina</i>)
160	MKSIAAAWVIFLARSCFADEVGKASNDCEANR ^r KVFFELLEVL	0.853	BU038139	–
161	MPIRSSAV ^r LVLSLGGV ^r PVQGG ^r TK ^r SPGCE ^r DFD ^r VK ^r YRIAE ^r ATKYCQ ^r G MHGEAA ^r FKLAD ^r V ^r TAK ^r AD ^r V ^r KIHVSD ^r VNG ^r VD ^r LV ^r SKSQGFIS ^r DEID ^r V ^r	0.754	BU038140	–
162	MKLF ^r ALL ^r VLMGV ^r ASAFV ^r TSP ^r SAFT ^r GSAMTEPRASRAGS ^r AVS ^r VP ^r MLD ^r TY WEGKAPPSQ ^r VLGP ^r AL ^r EV ^r LP	0.975	BU038141	–
163	MKCATMIALMVASANAFVPSAPLTKFETR ^r SSSLQ ^r MAAKKGGPPSGPS SMPNAIEATGIVGFDI ^r ESGGV ^r FDPLG ^r FAKNC ^r PPQ ^r MIWY ^r RAAEL KHGRV ^r AMLASLQ ^r IHQ ^r YTH ^r WN ^r DP ^r SGIF ^r DK ^r SNP ^r WEAM ^r QQV ^r YEQ ^r RF ^r AVV ^r QIV ^r LAIF ^r AG	0.819	BU038142	–
164	MPQNTSR ^r YFR ^r WT ^r VA ^r AS ^r VAL ^r SLV ^r APSAA ^r E ^r DK ^r GL ^r TFGP ^r EATER ^r FLAID N ^r AITHLEDGET ^r AR ^r LQ ^r SGD ^r WCNT ^r IS ^r AT ^r EY ^r CG ^r FGG ^r QA ^r SC ^r IA ^r R GIDYSG ^r DPSP ^r CEVIL ^r TTSD ^r NG ^r TY ^r CEGID ^r FD ^r SG ^r NL ^r DIC ^r DCEA ^r DF ^r HRY ^r F RLPRPQ ^r TV ^r AP ^r ST ^r P ^r AD ^r AT ^r MF ^r A	0.74	BU038143	–
165	MQTCFAT ^r PIPR ^r LAS ^r TLLRR ^r HAV ^r TV ^r SPAS ^r RLA ^r CAV ^r RLSAR ^r NA AARAAD ^r PR ^r CLS ^r ALL ^r PA ^r TAA ^r LD ^r PL ^r HR ^r AC ^r SA ^r MT ^r PR ^r W ^r PP ^r NS ^r R ^r F ^r SL	0.576	BU038144	–
166	MWSTQ ^r W ^r FL ^r ML ^r GL ^r LM ^r VAS ^r NNAQ ^r CS ^r NGI ^r AG ^r VEG ^r DAC ^r VAE ^r CG ^r TC ^r GG ^r SG ^r C GSRARA ^r ALSS ^r GD ^r CC ^r IG ^r PIR ^r D ^r AN ^r V ^r CD ^r SD ^r SAP ^r CL ^r IN ^r VEE ^r TC ^r SS ^r NG ^r LP ^r G MGISSY ^r V ^r FV ^r GA ^r VL ^r PL ^r FL ^r STR ^r AYE ^r CR ^r V ^r TRE ^r ST ^r NL ^r G ^r TS ^r IT ^r TA ^r AH ^r ED ^r LT PF ^r PL ^r TEY ^r M ^r GAIS ^r FD ^r ED ^r H ^r CI ^r QM ^r CE ^r Q ^r VE ^r CY ^r V ^r AFD ^r HEE ^r ALC YLKGAT ^r ATH ^r YDF ^r ISE ^r DG ^r Y ^r TS ^r YEM ^r LF ^r REG ^r SE ^r PI ^r LE ^r TE ^r CS ^r ILE ^r V ^r LP	0.929	BU038145	–
167		0.73	BU038146	–

168	MRSLLLTTPLPACRANACKSLPISPKCKYANSTMS	0.716	BU038147	–	FK506 binding protein (<i>A. thaliana</i> et al.)
169	MKHPATLLLVLI ^a ALQHQRCQCD ^b AFLSARPTGFRRCGGGIMRSFRR RSPAVTTCMVATV ^c ASPAVDLSGD ^d GV ^e TREMLQEGK ^f GKGLATGD ^g DIAMVRF TGVVEETGQVFSKGNQYRTTLE ^h DGTMISGWD ⁱ TGLAGLRPGDRAKIRCSRFSL	0.676	BU038148		

^aFor most *Fucus* SST cDNAs, the predicted amino acid sequence extends longer than depicted, up to a maximum of 244 residues for *Fucus* SST044

^bThe S-score is the probability that a given residue is in a signal peptide, as predicted by SignalP (Nielsen et al. 1997). The mean S-score is the mean score of all residues in the predicted signal peptide. A polypeptide containing a mean S-score of greater than 0.48 is predicted to contain a signal peptide (Nielsen et al. 1997)

^c“Similarity” has arbitrarily been defined as an alignment predicted by BlastP (Altschul et al. 1990) in which the E-value is less than 0.001 or in which > 70% similarity is observed over a region of > 10 amino acid residues

Eighteen plasmids representing three different sequences encode polypeptides exhibiting strong similarity to the fucoxanthins, chlorophyll *a/b*-binding proteins that function in harvesting light for photosynthesis. Additional polypeptides isolated more than once exhibit similarity to previously characterized proteins, including glyceraldehyde-3-phosphate dehydrogenase, a cysteine protease, mucins, and several uncharacterized plant open reading frames (ORFs; Table 3). A number of sequences with similarity to known proteins were identified in the screen only a single time, including polypeptides similar to cell-surface and intracellular signaling proteins such as FK506-binding protein (FKBP), mitogen-activated protein (MAP) kinase, and pp2C subunits.

Discussion

Secreted polypeptides provide potential links between the intracellular and extracellular environments of cells, often functioning both to provide communication across the plasma membrane and to maintain a physical link that mediates cell/extracellular matrix (ECM) interactions. Klein et al. (1996) and Jacobs et al. (1997) independently developed assays that use *Saccharomyces cerevisiae* to screen large numbers of mammalian sequences for those that encode a functional signal peptide. This method has also recently been used to identify novel signal peptides in plants (Goo et al. 1999). As expected, the proteins identified in these screens included transmembrane receptors, extracellular matrix proteins, and cell adhesion molecules, as well as ion channels, cytokines, chemokines, and proteins of the endomembrane system (Klein et al. 1996; Jacobs et al. 1997; Goo et al. 1999).

We are interested in understanding the mechanism by which zygotes of the furoid algae develop from symmetric, apolar eggs to molecularly and morphologically asymmetric embryos. This asymmetric development involves both the generation of a polar axis within the fertilized egg and the subsequent asymmetric growth that produces the morphologically distinct rhizoid and thallus ends of the embryo. It is likely that the reception and signaling of environmental cues influencing polarity, selection and fixation of a polar axis, and subsequent asymmetric cell growth are dependent, at least in part, on polypeptides expressed at the cell surface or in the extracellular matrix. In an effort to identify proteins potentially involved in these processes, we have used the SST screen to identify polypeptides expressed in *Fucus distichus* zygotes that can function as signal peptides.

We isolated 222 plasmids containing *Fucus* cDNA sequences. Assuming that the open reading frame that encodes a signal peptide is in-frame with invertase in those plasmids that contain multiple reading frames, 76.1% (169/222) of the isolated plasmids were predicted by the SignalP signal peptide prediction program

(Nielsen et al. 1997) to encode a signal peptide. This percentage is comparable to the 85% reported for mammalian sequences (Klein et al. 1996; Jacobs et al. 1997) and the 76% reported for *Arabidopsis* (Goo et al. 1999). A possible explanation for the lower percentage of peptide-containing polypeptides isolated in our screen and the *Arabidopsis* screen is the inherent difficulty of predicting degenerate sequences such as signal peptides. The SignalP algorithm is based on the polypeptide sequence of proteins containing known signal peptides that had previously been entered in GenBank (Nielsen et al. 1997). Because of this, the algorithm is more accurate in predicting signal peptides exhibiting some similarity to those sequences utilized in generating the algorithm (Nielsen et al. 1997). Very few secreted proteins have been identified from *Fucus distichus* or other brown algae, and thus these sequences could not comprise a major portion of the SignalP data set. As a result, sequences derived from brown algae are less likely to be predicted correctly by the SignalP program. In addition, Kaiser et al. (1987) observed that roughly 20% of randomly assembled polypeptides could function as signal sequences using a similar yeast invertase assay. It is possible that *Fucus* expresses a high number of polypeptides that do not have a prototypical signal peptide but do retain a minimal level of "secretory activity," and thus are detected as false positives in the SST screen. Interestingly, most of the false positive sequences isolated in the *Arabidopsis* SST screen were rRNAs (Goo et al. 1999), suggesting that very short hydrophobic sequences may be able to function as signal peptides in this screen. In any case, the presence of a predicted signal peptide in more than 76% of cDNAs isolated in our screen indicates that we have most likely significantly enriched for signal peptide-containing genes expressed during asymmetric cell growth in *Fucus*.

Comparison of the predicted *Fucus* polypeptides against GenBank using BlastP (Altschul et al. 1990) revealed significant sequence similarity between some *Fucus* polypeptides and proteins found in other organisms. Several of these polypeptides, including EGF-like repeat-containing proteins, LRR-receptor kinases, and β -1,3-exoglucanases, perform important functions in asymmetric cell growth and development in other multicellular organisms.

Epidermal growth factor (EGF)-like repeats are cysteine-rich polypeptide domains found in a diverse array of proteins. Although the EGF-like repeat motif was first identified in EGF, a potent soluble peptide stimulator of cell division, other proteins containing these repeat sequences perform a broad range of primarily extracellular functions. These proteins include growth factors, transmembrane receptors, adhesion molecules, soluble and transmembrane ligands, and extracellular matrix proteins (reviewed in Bork et al. 1996, Singson et al. 1998). In metazoans, EGF repeat-containing proteins are involved in cell-cell adhesion, cell and tissue polarity, sperm/egg fusion, asymmetric neuron growth, leg development, metamorphosis, and other functions (Rao

et al. 1995). In plants, the wall-associated kinase (WAK) family of transmembrane receptors also contain EGF-like repeats and thus far have been shown to be involved in cell elongation, seedling shoot and root development, and pathogen response (He et al. 1998, 1999; Lally et al. 2001). We have isolated three different *Fucus* cDNA clones encoding polypeptides containing at least one EGF-like repeat. These predicted polypeptides contain cysteine-rich regions with significant similarity to EGF-like repeat consensus sequences and even higher similarity to specific EGF repeat-containing proteins, including the FAT protocadherin, Notch transmembrane receptors, Caspr proteins (see Fig. 3). All three of these protein types are localized asymmetrically in metazoan cells and have been proposed to have roles in polarized cell organization and asymmetric growth, and exhibit similarity to proteins that organize localized multiprotein complexes and mediate linkage between the cytoskeleton and ECM (Peles and Salzer 2000; Gollan et al. 2002; Strutt et al. 2002). *Fucus* embryos undergoing asymmetric growth require an intact, polarized microfilament network, localized secretion, and an extracellular matrix component in order to maintain oriented expansion at the rhizoid tip (Quatrano 1997). These requirements have led several groups to propose the presence of a protein complex at the site of tip growth that links the actin cytoskeleton and ECM (Quatrano and Shaw 1997; Brownlee and Bouget 1998). Central to this complex is a transmembrane protein (or proteins) that provides an essential link between microfilaments and the ECM, either by physical association, transmembrane signaling, or both. However, such a protein remains to be identified in *Fucus*. The similarity of the novel *Fucus* polypeptides described here to proteins that provide a transmembrane link that assists in defining cell polarity in metazoans may provide clues toward understanding the generation of asymmetry in fucoid embryos.

This screen also resulted in the isolation of three *Fucus* cDNAs (FucusSST052, 067, and 102) that encode secreted polypeptides with a similarity to a 29-amino-acid region of plant leucine-rich repeat receptor kinases. The leucine-rich repeat has been identified in proteins in metazoans, algae, and plants. In higher plants, LRR receptor kinases form a large family of transmembrane receptors (Torii 2000; Shiu and Bleecker 2001), members of which contribute to plant defense, regulate development, and mediate hormone detection (Torii 2000; Li et al. 2002; Nam and Li 2002). The LRR-receptor kinases thus far identified interact extracellularly with steroid or peptide ligands and function as heterodimers or tetramers to transmit their signal intracellularly through Ser/Thr kinase activity (Li and Chory 1997; Jinn et al. 2000; Trotochaud et al. 2000; Li et al. 2002; Nam and Li 2002). In addition to an N-terminal signal peptide, these receptors are comprised of a variable number of extracellular LRR repeats, a single transmembrane domain, and, in most cases, an intracellular Ser/Thr kinase domain (Shiu and Bleecker 2001). The *Fucus* cDNA

Table 2 *Fucus distichus* sequences lacking predicted signal peptides

Fucus SST No.	Predicted amino acid sequence of amino-terminal polypeptide region ^a	GenBank Access. No.	Polypeptides exhibiting similarity ^b
170	MRTSASTASATWRSSTVVSQSRSDTSYKONVRLPGLMSTLADISFADMPNGVAGL	BU38149	–
171	MQPVWGSGARDLGRDRQICHPVVRGDIPSHGQGERQRPPIRPPRGSFY	BU38150	–
172	MVSPNSDAEIRNALVDEITLANAAIEAGVELVDRALNCSATGACGRNGECVDSVLGVLCVCIDEDPPCLF	BU38151	EGF-like repeats
173	MGACLKLSRVERQRRRALCSGRGLTLRVLTPALRLILLGFRFVLT	BU38152	–
174	MTPPQATVSAAGGGGAARAPSPRARRGSGYMKRKGKNSIQQRGSYNQMLQQELOYKEQQKAMDGTGMPA	BU38153	–
175	MGGDAAGVRAAPVGRSDRAGHLRGLKCTATEAQQRNGRGTGFDPLWAQAFPPRRGERVQVLRTRTG	BU38154	–
176	MFSGCKDCQTSADVHDVSEFGLPDAAGGACTNALLLTLVDSEPDVLSLKGMQGLTNEKSYQVPLSA	BU38155	Hypothetical protein F13M23.250 and others (<i>Arabidopsis thaliana</i>)
177	MDTARTTAAWSSWTTGSTPPQTTKAYSTDSIGWSWGRLEQTRLLEVAATRTSRSDSHEVEVR	BU38156	–
178	MMHRAFIRRAVGNAFRLFVYECPRHNGIGELLEILGSIWNGFATPLKKEHVDFLQVCLLSLHTPTSMVNVYHQ	BU38157	pp2A regulatory subunit (<i>A. thaliana</i>)
179	MTERGHLANDEQRNDVELLVGRLEELKEEDQPYNDENMG	BU38158	–
180	MAPWSDSPIWRGALASSTRSERVLAFEIYTFSSLDTDVNVVLGDYKDPKSAFLGPMDTMWT	BU38159	–
181	MAPCIARA STAGTLISATARKRTSFTDISGYPGSPSPRHRPPMPRCSP	BU38160	–
182	MKSAVIAACVAGAQAFVPPSAFNGAALTTSAKSSAMKMSFESEIGAQPPLGFWDPLGLLNDADQERFDRL	BU38161	–
183	MNPALAPLYEYGNLSLYNAEESGAVFGDAITEAEKKAMAIVEAQITGATVNGGRVDGDDGAGAGLAD	BU38162	–
184	MGGSASFSLCAADDSQAPASATPETRWCVDDSAVEEVFVDETRNTFAVVGLEGASVRSVRLDSTVLKML	BU38163	–
185	MSVVPVRRGYWGRKNGQPHITVPNKVTGKCRSVRLLISA	BU38164	–
186	MWEAATRV TAMMASTWMASTAPATRV TALSFGFTRHIA TPATTPARSSFRFLQSGCPSNPTCQFRIP	BU38165	–
187	MSEGDAKAFVQHFSKFTAGQALDQLGALYQPTMSNIQVWGSANIVAKYKTLGNLQFHPDLDVQ	BU38166	–
188	MTRDTRLARNPDYFAA MLRMQPANTVEGEWDGGSSLSASGVGSEGGDGASAGSSSSNGVEFFIDRDP TH	BU38167	–
189	MATINNKYDSLKEINNGDPRSPISRRPIIPE	BU38168	Ribosomal protein L12 (<i>A. thaliana</i> et al.)
190	MFGAGLHICGNAAVAAPIFSGSQPAPSVGDHRVDVTRHLRELTLTAPSRRIEVS L	BU38169	–
191	MTMPPDTTDFRDVYVITNLMESDMDRIIASGQPLTDQHFQYFIYQVLRGLKFIHSANVLHRDMKPSNLLVNA	BU38170	MAP kinase (multiple species)
192	MELLSQP SVTVVPSAEALCREEVEVWDPSALV	BU38171	–
193	MATAITLSAPTEATGMTGGSSMASASIRKPNALENWGNLEERM RDKVVIFSDYDGTLSPIVDEPKAF	BU38172	BcDNA.GH088a60 gene product (<i>Drosophila melanogaster</i>)
194	MACRRRYVVMRSNGESLGRSLPNNCSSIYRIPAVFSLKLFYNVY	BU38173	–
195	MRLSAGVTVGWISVVDVSSFAFAPLQAPPSANVARVTSHGQALVRQRVRRPGLAAISM SA VEDELWRGE	BU38174	–
196	MAAKGTSAGRKS LDRAMSAEAPVLA PPHFQPGASFQENEEGRPGAPSSRFSL	BU38175	–
197	MRRLLGGVHPLQRDLRDSQYLSGGCWRRHRRRDSLRDYQPYLWSGCWHHRGSFATAALLSSGYWHHRGF	BU38176	–
198	MTGPTSSDWNGAKYTLAVSTPVNSRFSL	BU38177	–
199	MGPPPPSQAQYVVLRAIGVEGNTAALAPKLGPLGLSPKKIGEDIQKNTMDWKGLPITV KLTIIINRQATVA	BU38178	–
200	MGRRK.KSTKKIVLKNNTLTDKVKCFPCFNHDKVVECTMNRKEGTARLQCRMCDINYEMGINYLTEPIDVYTD	BU38179	Ykl160Wp (<i>Saccharomyces cerevisiae</i>)
201	MDAPTTARSLDSTA AVPTANTKSADTSPVALRSISSGDSRYPGLRPA APIPTRTSVADISVSPPHLDHLSL	BU38180	–
202	MWQEQTEMETEDGIEEYDEGRDLRNKLDVTSALEQTGNKEAADRRVSWVGLKLRSGRASESIGKVKKEE	BU38181	–
203	MARRQQQQQLLAGGKGLDVPVGVRCAPPWCGPRMQRRKQDDTTPPTAVGQKSAEGPVKKSEAERDDV	BU38182	–
204	MKELNKCDFQVNIHADPCCSNLPSAQRFTDRSMYSRRCALFQPRFVFCMTLSKYEGVAIISCTWTE	BU38183	–
205	MAVGKTNKKGGRRKGNKKKTVDPFTLLKEWYDIKAPSFQVRMPGKTLVTRTKGTKIASEGLKGRVFEFSLGDL	BU38184	Ribosomal protein S3a (<i>A. thaliana</i> et al.)
206	MRSDEPKYCKVFDGIGRRLKSMAFVEDGETLYVVPQRCFVWPTIKIGRKYVTPHVKTLLGGKEVVS RGSPIYI	BU38185	–
207	MRSDEPKYCKVFDGIGRRLKSMAFVEDGETLYVVPQRCFVWPTIKIGRKYVTPHVKTLLGGKEVVS RGSPIYI	BU38186	–
208	MRCFFPREAYGVPTMRWWNNNEVIQTTSIRGHLLHGGNGSLARRGRLRRRRRDARDAPGGQQRDRGRHRHG	BU38187	–

Table 2 (Contd.)

Fucus SST No.	Predicted amino acid sequence of amino-terminal polypeptide region ^a	GenBank Access. No.	Polypeptides exhibiting similarity ^b
209	MVLEAGRSICTTAALSARWTSVVTGLFLFVFMARRGAQTTKAITISSLPPEMTDTSSTSSGYKVRVMDVNDDES	BU38188	—
210	MVQNCRSADIVSWAGSKTTRRTVA TAPTSRQRPTRSEKILLTCSPTTRKTTGLLTLVTHHS	BU38189	—
211	MSNEGGMVMDTLKAPFKSEPKNSDADTAKDAASNTTASKSGFDATNAGDAAKETAIEIAYSSAEKAV	BU38190	—
212	MLPIPKMPPATLPRPLNPSAMPPTLPLTRRKRPRSLTVPPRRPSKPPKPARLPIPLNRPPIRPTLP	BU38191	—
213	MMKPRPTAKTFGPESDAFRSDIQHQDPLDPLGPNVNIHDPRIARSGSTFAGNRRIDTSAEQGLAKLTKKGGKRS	BU38192	Radial spoke protein (<i>Chlamydomonas reinhardtii</i> , <i>H. sapiens</i>)
214	MDGELYIIDENLEVLVLP	BU38193	—
215	MFFGGSPFEHFAGMHGGESGPRRRGPPDVTDEFYQILGLEVLVLP	BU38194	—
216	MAKPADAGKAAATKAKSDKATPNKNEKTKAPNANPAFRWQLGGLIAGVIVAIISSTEPGEQMLRSIGLEVL	BU38195	—
217	MANRLVGRY GALDDPSARQSSPELVGPQSTASLNLDHAGVSESHRGRYSRSLWTVAVISAMAA GTVMT	BU38196	—
218	MTRTRSASTASATWRSSTAASPCWRFLATSNSTTACPVCCPLCRISALLTCRTVWRPYPFRPRLVSPISWR	BU38197	—
219	MKMSFESEIGVQPLGFWDPLGLLNDADQERFYRLRYVEIKHGRISMLAVLGHIEQHNVRLPGLMLSTLSNLC	BU38198	Fucoxanthin (<i>Laminaria</i> <i>saccharina</i>)
220	MKMSFESEIGAQPPLGFWDPLGLLNDADQERFDRRLRYVEIKHGRISMLAVLGHIVQQNVRLPGLMLSTLSADIS	BU38199	Fucoxanthin (<i>L. saccharina</i>)
221	MGEPKVLNKFIPSYFDPKKVPCKMRDPEKQICVGMMPVSLQCNTCGEFMYRGTGPSY	BU38200	—
222	MTRTRSASIAFGYVEIKHGRISMLAVLWYTSLQHNVRLLPGMHV	BU38201	—
223	MKKEIGMIAGGTGTPMLASAAGDLALNPEDLHESHAFVLRQPDPSLRFPP	BU38202	—
224	MEDEPVDKRPDARVIMLCGPPGLKTTLANVVARHAGYRYVEINASDDRSAPVLKQRVLEAMEGNTLLADKKR	BU38203	Replication factor Chl12 (<i>A. thaliana</i> , <i>H. sapiens</i> , <i>S. cerevisiae</i> , et al.)
225	MLRPTPRKPLIRQKHRPIPSRNHQENVRIGADKLPPTMLKTPQDATTK	BU38204	—
226	MIDDRSGEARDEYLAGITAYDQGMRRPFLRRI	BU38205	—
227	MLGDVGLQRILDQSRGRRGEESGRHKGKGRPHLQRRRYRQGTSHCCRRETLQLELRSGVLLHPPQGCI	BU38206	—
228	MGPSTGPRASSMFPNMTPRRPSFLHCRGTLLEVLVLP	BU38207	—
229	MSRAKDLGIVEAHSGHVFWGARVPGRPIRHGSQYGPSSGLVDAQHDPPQTVSPILSTLSRNLRARGSPY	BU38208	—
230	MARLSFAGDIGLSISNCNRVHAACAELRRSCGISDVEHNAHDEPGGATANGGVVEAHGRVMPKGRLLFGGG	BU38209	—
231	MSSPLTKPASPVAVKIAQEGKQDGLPHKIRKQKLVLQGLEPDLIDNVLNGDIDSSGRDQGPLPSPPTLPM	BU38210	—
232	METTSSLKHECKRLNEKATKLEVLVLP	BU38211	—
233	MERASWGVQVSRVEMAACWFCG	BU38212	—
234	MSEGRYNLQESMDIEHNSHAVLNSDEDFENPLLPVPSDGDSDDRGHGEPMYRDKSWAIAFKINVLTVIS	BU38213	—
235	MTRTRSASTASATWRSSTVVSQCSDSYSTRTCACPACCPRLRTPSLTCTVWRPSSRSPRSAPWRSWRS	BU38214	—
236	MDKGPRSIRGARKSTRRLDIFESTPEPSTKPSRELSTSGGDHAGSKQAGHCLQARLTPWRPAMYDSMVRL	BU38215	—
237	MAIAQTQKKKKKKMVRICPAT	BU38216	—
238	MRRLLGVPVLPQRDILRDSQPYLSSGCWRHRRHRDLSRDYQPYLCSGCWWHRA	BU38217	—
239	MTLRKPRRSKEINDCOYDNDKRRRGRFRCTKARLLVGLGVAAMVATFIMTHLAWLSLSDDDDTNWNLSFWDSQQ	BU38218	—
240	MADQLTEEQIAEFKEAFSLFDKDGDDGTTITTKELGTVMRSLGQNPTAEALADNINEVDADGNGTIDFPEFLTM	BU38219	Calmodulin (<i>Macrocystis pyrifera</i> et al.)
241	MADQLTEEQIAEFKEAFSLFDKDGDDGTTITTKELGTVMRSLGQNPTAEALADNINEVDADGNGLSTSRVLDND	BU38220	Calmodulin (<i>M. pyrifera</i> et al.)
242	MTRTRSASTASATWRSSTVVSQCSDSYSTRTCACPACCALRRTFPFSLTCQTVWRPSTRSPRSALPKSWPF	BU38221	—
243	MLMEMCLSVKAKASYPTKLMYHRCRAVQKLLVASPPGKTE	BU38222	—
244	MRPPWPKLRGWVQLGMPPRGRKVVQPSAKRRSFGVNRVLAWSARRKGMGQGRTKTRTMRSHFRYALNRNGRHT	BU38223	—

^aFor most polypeptides, the encoded amino acid sequence extends longer than depicted

^b“Similarity” has arbitrarily been defined as an alignment predicted by a BlastP homology search (Altschul et al. 1990) in which the E-value is less than 0.01

Table 3 *Fucus distichus* polypeptide sequences isolated multiple times from signal peptide screen

FucusSST cDNA numbers	Predicted signal peptide	BLAST similarity
8, 26, 43, 44, 72, 89	+	β -1,3-Exoglucanase (<i>Neurospora crassa</i>)
6, 20, 59, 60, 115, 130, 142, 153, 159	+	Fucoxanthin light-harvesting protein (<i>Laminaria saccharina</i>)
11, 17, 48, 64, 69, 77, 143	+	Fucoxanthin light-harvesting protein (<i>Chlamydomonas reinhardtii</i>)
219, 220	–	Fucoxanthin light-harvesting protein (<i>L. saccharina</i>)
52, 67, 102	+	LRR-receptor-like kinase (<i>Arabidopsis thaliana</i> et al.)
41, 42	+	Rac-GTPases (<i>Dictyostelium discoideum</i>)
57, 66	+	Glyceraldehyde-3-phosphate dehydrogenase (<i>A. thaliana</i>)
61, 100	+	Cysteine protease (<i>A. thaliana</i> and <i>Caenorhabditis elegans</i>)
240, 241	–	Calmodulin (<i>Macrocystis pyrifera</i> and other algae, plants, and animals)
19, 135, 137	+	Calmodulin-like protein (<i>A. thaliana</i>)
123, 124, 128	+	F12A21.16 encoded hypothetical protein (<i>A. thaliana</i>)
33, 34	+	Insect intestinal mucin (<i>Trichoplusia ni</i>)
95, 106	+	Hypothetical protein 93341 (<i>A. thaliana</i>)
9, 62, 71, 73, 138, 154	+	–
25, 27, 56, 91, 97, 101, 117	+	–
1, 3, 12, 13, 39, 75, 88, 109, 111, 114, 121, 131	+	–
5, 18, 32, 54, 58, 80	+	–
33, 35, 49, 94, 108, 157	+	–
14, 132, 136, 144	+	–
50, 82, 122, 147	+	–
29, 85, 104	+	–
22, 98, 164	+	–
16, 150	+	–
139, 156	+	–
38, 76	+	–
37, 86	+	–
53, 65	+	–
36, 87	+	–
90, 93	+	–
146, 158	+	–
110, 120	+	–
116, 129	+	–
197, 238	–	–
206, 207	–	–
170, 235, 242	–	–

sequence we have identified as having similarity with several plant LRR-receptor kinases encodes only the N-terminal 101 amino acids of the *Fucus* protein, including an amino-terminal signal peptide followed by a leucine-rich domain. Interestingly, this leucine-rich domain does not conform to the LRR consensus sequence. Instead, it exhibits greatest similarity to a leucine-containing region found near the C-terminus of a highly similar group of LRR-receptor kinases identified by genetic analysis and genomic sequencing projects (Li and Wurtzel 1998). The function of this domain in our *Fucus* clones and in the related plant sequences remains to be determined.

Six essentially identical *Fucus* polypeptides that are predicted to contain signal peptides also exhibit similarity to β -1, 3-exoglucanase from *Neurospora crassa* and other fungi (Fig. 3). Glucanase proteins from vascular plants are secreted through the ER to Golgi pathway (Pihakaski-Maunsbach et al. 1996). β -1,3-Glucanase expression appears to play a role in plant embryogenesis and germination (Simmons 1994; Cheong et al. 2000; Helleboid et al. 2000), in addition to functioning in plant defense, tissue differentiation, and senescence (reviewed in Simmons 1994). In yeast, regulated β -glucan synthesis at the bud tip is essential for cell

wall expansion during polar growth (Drgonova et al. 1996; Qadota et al 1996). Whether the regulation of β -glucan synthesis and degradation plays a role in asymmetric growth in furoid zygotes remains to be clarified. Hable and Kropf (1998) have observed that the cell wall of furoid algae weakens detectably at the site of presumptive rhizoid outgrowth. Exoglucanase activity may be responsible for partial breakdown of extracellular carbohydrates prior to asymmetric growth, allowing for cell surface expansion at a site of weakened cell wall integrity. However, the role of β -1,3-exoglucanase in this expansion is unclear, as β -1,4-exoglucanase is the primary exoglucanase activity reported in furoid embryos (Stevens and Quatrano 1978). The isolation and characterization of the β -1,3-exoglucanase we have identified will further clarify the role of this polypeptide in cell wall organization and asymmetric cell growth.

Several other signal peptide-containing proteins from *Fucus* were identified one or more times in our screen, including putative cysteine proteases, BiP and Hsp70 chaperones, mucins, and polypeptides with similarity to hypothetical proteins identified in large-scale sequencing projects (see Table 1). Fucoxanthin proteins involved in light harvesting were also isolated multiple times,

a.

SMART00181.5 EGF consensus	ECA-SNG--PCSNGT-CINTPGS-YTCICPPGYTG-DKR	
FucusSST036	141 GCD-SN---PCLNGGSCSTSGDG-YQCSALGYGGTD-CG	175
FucusSST139	49 GCSPS-GCVP-SNVLGDSFDDASRWSCSAEVS-D-G-DACE	84
Mouse Fat1 cadherin	4001 DCSSS---PCQNGGVGNPSPSTGGYCKCNALYVGTFF-CE	4035

b.

FucusSST051/103	66 LLDVDELDRASDASMKGDKPKKSVDLAD	94
	L D+D L + D S+KGD P KS++ AD	
Arab LRR-RK	698 LHDIDLAKMVDPSLKGDPKSLSHFAD	726

c.

FucusSST008	38 AEYTELGCY-EDSQKRIFSEGTRKRLDDLTTETSCAAICE---DFPYFGLQY	83
	A Y T L C E S R + + D + T S C A A C + F Y F G + + Y	
<i>N. crassa</i> β -exogluc	614 AGYTRLRCATEGSGVRALTGASFAYDTMTLESCAANCTAAGNFAYFQVEY	663
FucusSST008	84 GNECWCGDYTSEIDSDSESDGCDMTCTGSDANCGGNAMRIFR 128	
	EC+CG+ + S++ C+M C GD+ CG + ++ +	
<i>N. crassa</i> β -exogluc	664 ARECYCGNSLAASSSEAPDSECNMLCAGDATEYCGAGNRLELYAK	708

Fig. 3a–c Secreted polypeptides expressed in *Fucus* embryos exhibit similarity to protein types important for polar growth. **a** Epidermal growth factor (EGF)-like regions are found in secreted *Fucus* polypeptides. The consensus sequence of the SMART00181.5 EGF-like domain was aligned with *Fucus* predicted polypeptides FucusSST036 and 139. Also included for comparison is an EGF-like region from the mouse protocadherin Fat1. Regions of identity between at least two of the four sequences are indicated in *bold*. **b** Secreted *Fucus* polypeptides contain similarity to leucine-rich repeat (LRR) receptor protein kinases. A 29-amino-acid region from *Fucus* secreted peptides #51 and 103 was aligned with a region of an *Arabidopsis* open reading frame encoding a putative LRR-receptor protein kinase (GenBank accession number 18399502). **c** Alignment of a region of FucusSST008 with a *Neurospora crassa* β -1,3-exoglucanase (GenBank CAC18170). Alignments were performed using BlastP (Altschul et al 1990). *Bold letters* indicate the presence of identical residues, while (+) highlights the location of residues with similar features

potentially due to the similarity between chloroplast targeting sequences and signal peptides (Emanuelsson et al. 1999). Interestingly, analysis of these *Fucus* fucoxanthin sequences by TargetP indicates that the polypeptides can potentially function as chloroplast targeting sequences, but more closely resemble signal peptides (data not shown). Finally, polypeptides lacking a predicted signal sequence include sequences similar to calmodulin, calmodulin-like proteins, a MAP kinase, FK506 binding protein, pp2C phosphatase, pp2A regulatory subunit, and *S. cerevisiae* Yk1160Wp, among others (see Table 2). Interestingly, the vast majority of polypeptides isolated from the screen contain only very limited similarity or lack any predicted similarity to previously identified proteins. This result may be expected, given the small number of algal sequences in GenBank and the distant evolutionary relationship between fucoid algae and organisms in which the most extensive sequencing work has been completed.

Secreted molecules are critical for the generation of polarity and subsequent asymmetric cell growth in fucoid zygotes. However, the exact molecules necessary for these molecular events remain to be identified. The identification of secreted *Fucus* polypeptides with similarity to plant and metazoan proteins mediating asymmetric cell growth provides an important step in determining the proteins important for the establishment

and maintenance of asymmetry. The fucoid algae have long provided a model system for studying the development of polarity because large numbers of free-living, synchronously developing embryos can be obtained and because the polar axis of the embryo population can be environmentally induced and altered during early development (Kropf 1997; Brownlee and Bouget 1998). The ability to perform microinjections of nucleic acids and proteins into living *Fucus* zygotes also makes antibody inhibition, green fluorescent protein (GFP) localization, and RNAi inhibition studies possible avenues for future investigation. The isolation of full-length clones of the gene fragments isolated in this screen will allow for functional analysis of each polypeptide and will shed light on the role of these cell surface proteins in asymmetric cell growth in multicellular organisms.

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