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Proteomic study of the brackish water mussel *Mytilopsis leucophaeata*

Feico MAH Schuurmans Stekhoven[1*], Gerard van der Velde[1,4], Tsung-Han Lee[2] and Andrew R Bottrill[3]

Abstract

Background: We encountered the opportunity to study proteochemically a brackish water invertebrate animal, *Mytilopsis leucophaeata*, belonging to the bivalves which stem from the second half of the Cambrian Period (about 510 million years ago). This way, we were able to compare it with the vertebrate animal, the frilled shark (*Chlamydoselachus anguineus*) that stems from a much later period of geologic time (Permian: 245–286 MYA).

Results: The mussel contains a well-adapted system of protein synthesis on the ER, protein folding on the ER, protein trafficking via COPI or clathrin-coated vesicles from endoplasmic reticulum (ER) to Golgi and plasmalemma, an equally well-developed system of actin filaments that with myosin forms the transport system for vesicular proteins and tubulin, which is also involved in ATP-driven vesicular protein transport via microtubules or transport of chromosomes in mitosis and meiosis. A few of the systems that we could not detect in *M. leucophaeata* in comparison with *C. anguineus* are the synaptic vesicle cycle components as synaptobrevin, cellubrevin (v-snare) and synaptosomal associated protein 25-A (t-snare), although one component: Ras-related protein (O-Rab1) could be involved in synaptic vesicle traffic. Another component that we did not find in *M. leucophaeata* was Rab11 that is involved in the tubulovesicular recycling process of H^+/K^+-ATPase in *C. anguineus*. We have not been able to trace the H^+/K^+-ATPase of *M. leucophaeata*, but Na^+/K^+-ATPase was present. Furthermore, we have studied the increase of percent protein expression between 1,070 MYA (the generation of the Amoeba *Dictyostelium discoideum*) and present (the generation of the mammal *Sus scrofa* = wild boar). In this time span, three proteomic uprises did occur: 600 to 500 MYA, 47.5 to 4.75 MYA, and 1.4 to 0 MYA. The first uprise covers the generation of bivalves, the second covers gold fish, chicken, brine shrimp, house mouse, rabbit, Japanese medaka and *Rattus norvegicus*, and the third covers cow, chimpanzee, *Homo sapiens*, dog, goat, *Puccinia graminis* and wild boar. We hypothesise that the latter two uprises are related to geological and climate changes and their compensation in protein function expression.

Conclusions: The proteomic and evolutionary data demonstrate that *M. leucophaeata* is a highly educatioanal animal to study.

Keywords: *Mytilopsis leucophaeata*; Proteomics; Localisation; Function and adaptation periods

Background

Mytilopsis leucophaeata or the brackish water mussel, belonging to the Dreissenidae or bivalve mussels, originated from Europe more than 60 million years ago (Paleocene, Verween et al. 2010). Subsequently, it disappeared to Central America and returned to Europe (harbour of Antwerp) in 1835. Since then, it is a stable inhabitant of European brackish waters.

In the period of September 2009 to September 2010, two of my colleagues studied the influence of a number of parameters (depth, temperature, salinity and illumination) on size, growth condition, diet and attachment via development of byssal threads (Grutters and Verhofstad 2010). Bivalves have survived a long history from about 510 million years ago to present. This means that it has been adapted to geologically and climate-changing conditions, which might be reflected in the evolution of their proteome. For this reason, the present study was started in order to see whether the presence of certain proteins might unveil certain metabolic systems in this aquatic animal. Almost simultaneously, an article from

* Correspondence: F.Stekhoven@gmail.com
[1]Department of Animal Ecology and Ecophysiology, Faculty of Science, Radboud University Nijmegen, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands
Full list of author information is available at the end of the article

Riva et al. (2012) did so in relation to the effect of a pollutant (triclosan) on the metabolism of *Dreissena polymorpha* with emphasis on gills. In the same year, Fields et al. (2012) and Tomanek et al. (2012) published a study on the effect of temperature and hyposalinity on protein expression in the gills of the Mytilidae *Mytilus galloprovincialis* and *Mytilus trossulus*. In addition, we were interested in comparing amino acid sequences of our mussel with animals stemming from later periods of life in order to cheque the phylogenetic developments that had taken place meanwhile.

Methods

Forty individuals of the brackish water mussel, caught from a branch of the North Sea Channel to Amsterdam harbour, were taken by scalpel knives and tweezers from their shells, yielding a total wet weight of 1.8 g, sufficient for further analysis. The body parts were taken up in 5 ml triethanolamine HCl, pH 7.0 in 25% glycerol in the presence of 0.5 mM phenylmethylsulfonyl fluoride (PMSF) to prevent autolysis (Schuurmans Stekhoven et al. 2003). Further procedures, such as Potter-Elvehjem homogenisation; fractionated centrifugation to fractions F1, F2 and F3; delipidation of fractions prior to electrophoresis; electrophoretic separation of proteins in the fractions; staining and destaining of the gels; determination of the apparent molecular weights of the protein bands on gel; excision of the protein bands; transport to the mass spectrometric analysis laboratory in Leicester; as well as the mass spectrometric analysis itself, is given in full detail in our previous publication (Schuurmans Stekhoven et al. 2010).

Information as to the cellular localisation and function of the analysed proteins stem from handbooks like Biochemistry of Hubert Stryer, Google (Scholar), Pubmed.com, BLAST and UniProtKB/Swiss-Prot Protein Knowledgebase and literature referred to therein. The absorption spectrum of the brownish coloured F1 fraction (325 to 750 nm) was made with a Zeiss M4QIII spectrophotometer at 20- to 50-nm intervals. A 100 μl of the F1 fraction was dissolved in 1 ml 2% SDS, subsequently centrifuged for 5 min at 5,000 rpm in a table top centrifuge, and the supernatant scanned.

Results

Homogenisation and fractional centrifugation

Potter-Elvehjem homogenisation of the mussels required very harsh and frequent pottering, yielding a brownish homogenate. Subsequent centrifugation at 1,200, 9,000, and 100,000 g yielded the F1 to F3 fractions. Total protein (mg) of the fractions amounted to 78.3 for F1, 7.03 for F2 and 6.2 for F3, hence ratio F1:F2; F3 = 12.6:1.13:1.0. This ratio brought about association with the kidney (21.5:11.2:1.0) and colon (19.4:3.9:1.0) of the frilled shark *Chlamydoselachus anguineus* (Schuurmans Stekhoven et al. 2012) in which particular proteins (L-plastin, moesin, filamin A and α-actinin) are serving as additional construct in linking filaments (microtubules) to the plasma membrane. However, in the mussel case, in particular in relation to the brown colour of F1, and less so of F2, we had to think more in terms of byssal threads, the biopolymers by which mussels attach themselves to their substrate like rocks or even ship walls. The brown colour is based on an aqueous solution of pheomelanin (Napolitano et al. 2008) of which the almost exponential absorption curve (down to 325 nm) fits to our curve of *M. leucophaeata* F1 (Figure 1). Byssal threads apparently are high MW biopolymers as F1 did not demonstrate any entrance of protein into the gel. This started only in the lightly brown F2 and came to full expression by the light yellow F3, which demonstrated proteins in the apparent molecular weight range of 14.1 to 240 kDa (Tables 1, 2, 3 and 4). The tables are subdivided into prokaryotic and eucaryotic ribosomal subunits (Table 1), proteins from the ER, Golgi network and plasma membrane (Table 2), proteins of the cytoskeleton and muscle (Table 3) and cellular vacuoles, vaults, nuclei and mitochondria (Table 4). From all these proteins, the prokaryotic or eucaryotic origin is mentioned as well as the function and cellular localisation as could be found in literature, including data banks. All proteins are accompanied by their accession numbers from [UniProtKB/SwissProt] between square brackets. Confusion between capital O and the number zero is excluded since capital O is only present at the first position and number zero in any position from 2 to 6 of the accession series. Translation of the accession data to protein easily occurs by using the programme PubMed (www.ncbi.nlm.nih.gov/pubmed) by choosing the term protein.

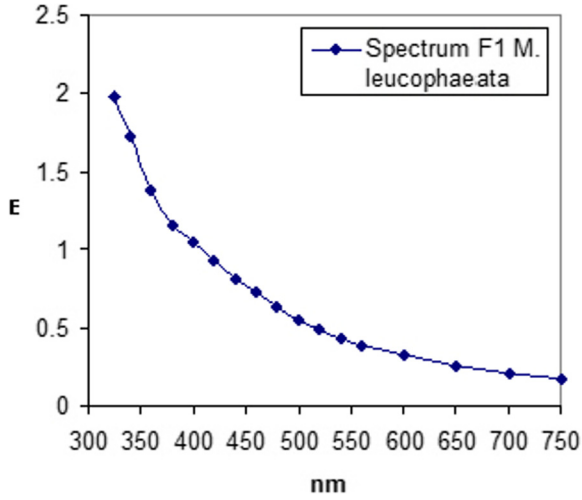

Figure 1 Absorption spectrum of SDS-solubelised F1 fraction from *M. leucophaeata* from 325–750 nm. Data points are indicated by black spots that have been line connected.

Table 1 Ribosomal composition of the F3 fraction (14.1 to 240 kDa) of *M. leucophaeata* in the prokaryotic and eucaryotic range

Ribosomal subunit	Pro-/eucaryote	Function	Localisation
30S-S1 [Q9HZ71]	*Pseudomonas aeruginosa* PAO1	Protein synthesis	Protoplasma
30S-S2 [C3K5E6]	*Pseudomonas fluorescens* SBW25	Idem	Idem
30S-S3 [Q3K5Z4]	*Pseudomonas fluorescens* PfO-1	Idem	Idem
30S-S4 [Q3K611]	*Pseudomonas fluorescens* PfO-1	Idem	Idem
30S-S5 [C3K2V9]	*Pseudomonas fluorescens* SBW25	Idem	Idem
30S-S6 [A4XPZ7]	*Pseudomonas mendocina* ymp	Idem	Idem
30S-S7 [C3K2Y0]	*Pseudomonas fluorescens* SBW25	Idem	Idem
30S-S7 [A1AGM8]	*Escherichia coli* APEC O1	Idem	Idem
30S-S8 [A4VHP4]	*Pseudomonas stutzeri* A1501	Idem	Idem
30S-S9 [C3K6E2]	*Pseudomonas fluorescens* SBW25	Idem	Idem
30S-S10 [A4VHM9]	*Pseudomonas stutzeri* A1501	Idem	Idem
30S-S11 [A4VHQ3]	*Pseudomonas stutzeri* A1501	Idem	Idem
30S-S13 [C3K2V4]	*Pseudomonas fluorescens* SBW25	Idem	Idem
30S-S14 [Q48D49]	*Pseudomonas syringae* pv. phaseolicola 1448A	Idem	Idem
50S-L1 [C3K246]	*Pseudomonas fluorescens* SBW25	Idem	Idem
50S-L1 [Q889Y1]	*Pseudomonas syringae* pv. tomato str. DC3000	Idem	Idem
50S-L2 [C3K2X3]	*Pseudomonas fluorescens* SBW25	Idem	Idem
50S-L3 [C3K2X6]	*Pseudomonas fluorescens* SBW25	Idem	Idem
50S-L4 [C3K2X5]	*Pseudomonas fluorescens* SBW25	Idem	Idem
50S-L5 [C3K2W4]	*Pseudomonas fluorescens* SBW25	Idem	Idem
50S-L6 [B1JAJ7]	*Pseudomonas putida* W619	Idem	Idem
50S-L9 [C3KE70]	*Pseudomonas fluorescens* SBW25	Idem	Idem
50S-L9 [A1AJA7]	*Escherichia coli* APEC O1	Idem	Idem
50S-L10 [C3K2Y5]	*Pseudomonas fluorescens* SBW25	Idem	Idem
50S-L11 [C3K2Y7]	*Pseudomonas fluorescens* SBW25	Idem	Idem
50S-L13 [C3K6E1]	*Pseudomonas fluorescens* SBW25	Idem	Idem
50S-L14 [A4VHP0]	*Pseudomonas stutzeri* A1501	Idem	Idem
50S-L15 [C3K2V7]	*Pseudomonas fluorescens* SBW25	Idem	Idem
50S-L16 [C3K2W9]	*Pseudomonas fluorescens* SBW25	Idem	Idem
50S-L17 [A4VHQ6]	*Pseudomonas stutzeri* A1501	Idem	Idem
50S-L18 [C3K2W0]	*Pseudomonas fluorescens* SBW25	Idem	Idem
50S-L19 [C3K1G8]	*Pseudomonas fluorescens* SBW25	Idem	Idem
50S-L22 [A4XZ85]	*Pseudomonas mendocina* ymp	Idem	Idem
50S-L24 [C3K2W5]	*Pseudomonas fluorescens* SBW25	Idem	Idem
50S-L25 [Q3K6W3]	*Pseudomonas fluorescens* Pf 0-1	Idem	Idem
40S-SA [A3RLT6]	*Pinctada fucata* (pearl oyster)	Protein synthesis	ER
40S-SA [P38981]	*Urechis caupo* (spoon worm)	Assembly and/or stabilisation of the 40S ribosomal subunit	Localisation in adhesion complexes (Willett et al. 2010)
40S-S2 [O18789]	*Bos taurus* (cattle)	Protein synthesis	ER
40S-S3 [P23396]	*Homo sapiens* (human)	Idem	Idem
40S-S3a [A7S3J7]	*Nematostella vectensis* (starlet sea anemone)	Idem	Idem
40S-S4 [Q4GXU6]	*Carabus granulatus* (beetle)	Idem	Idem
40S-S5 [P46782]	*Homo sapiens* (human)	Idem	Idem

Table 1 Ribosomal composition of the F3 fraction (14.1 to 240 kDa) of *M. leucophaeata* in the prokaryotic and eucaryotic range *(Continued)*

40S-S6 [Q90YR8]	*Ictalurus punctatus* (channel catfish)	Idem	Idem
40S-S7 [A6H769]	*Bos taurus* (cow)	rRNA maturation	Together with NEK6 (serine/threonine kinase) in centrosome (microtubule organising centre, MTOC)
40S-S9 [A6QLG5]	*Bos taurus* (cow)	Protein synthesis	ER
40S-S13 [P49393]	*Xenopus tropicalis* (western clawed frog)	Idem	Idem
40S-S14 [P14130]	*Drosophila melanogaster* (fruit fly)	Idem	Idem
40S-S16 [P14131]	*Mus musculus* (house mouse)	Idem	Idem
40S-S17 [A5PK63]	*Bos taurus* (cattle)	Idem	Idem
40S-S18 [A5JST6]	*Capra hircus* (goat)	Idem	Idem
40S-S18 [Q8IT98]	*Argopecten irradians* (bay scallop)	Idem	Idem
40S-S19 [Q94613]	*Mya arenaria* (soft-shell clam)	Idem	Idem
40S-S24 [O42387]	*Takifugu rubripes* (tiger puffer)	Idem	Idem
40S-S26 [P27085]	*Octopus vulgaris*	Idem	Idem
40S-S27-like [P24051]	*Rattus norvegicus* (Norway rat)	Idem	Idem
60S-L4 [P50878]	*Rattus norvegicus* (Norway rat)	Idem	Idem
60S-L4-B [P02385]	*Xenopus laevis* (African clawed frog)	Idem	Idem
60S-L5 [P09895]	*Rattus norvegicus* (Norway rat)	Idem	Idem
60S-L5 [O76190]	*Bombyx mori* (silk worm)	Idem	Idem
60S-L7a [Q90YW2]	*Ictalurus punctatus* (channel catfish)	Idem	Idem
60S L7c[O60143]	*Schizosaccharomyces pombe* 972 h-	Idem	Idem
60S-L8 [P41569]	*Aedes albopictus* (Asian tiger mosquito)	Idem	Idem
60S-L12 [E2RR58]	*Canis lupus familiaris* (dog)	Idem	Idem
60S-L16a [P26784]	*Saccharomyces cerevisiae* S288c (baker's yeast)	Idem	Idem
60S-L17 [A0NGY0]	*Anopheles gambiae* (African malaria mosquito)	Idem	Idem
60S-L23a [P62750]	*Homo sapiens* (human)	Idem	Idem
60S-L26 [P12749]	*Rattus norvegicus* (Norway rat)	Idem	Idem

With names of the animals to whom the proteins are related + function and cellular localisation of these proteins.

Table 1 shows that 52% of the ribosomal subunits is of bacterial origin with a decreasing order in percentage for *Pseudomonas fluorescens* (32.8%), *Pseudomonas stutzeri* (7.5%), *Pseudomonas mendocina* (3%), *Pseudomonas syringae* (3%), *Escherichia coli* (3%), *Pseudomonas putida* (1.5%) and *Pseudomonas aeruginosa* (1.5%). The other half of the components is occupied by eucaryotes, ranging from pearl oyster to Norway rat. Possible causes and impacts of the bacterial contamination will be handled under 'Discussion' section.

Outside Table 1, only very few bacterial proteins have been identified, except peptidoglycan-associated lipoprotein in the plasma membrane of *P. putida* (Table 2), outer membrane porin F from *P. fluorescens* (Table 2) and 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase from *Rhodospirillum centenum* SW (Table 2). The latter photosynthetic bacterium is housing in marine and brackish water and so can be easily caught by the mussel valves. Still another intruder in the list of proteomics is ribulose bisphosphate carboxylase from *Agrostis stolonifera* (creeping bent grass) as this reaction takes place in chloroplasts. The habitat of creeping bent grass is on wetlands with tolerance to flooding (Garry Oak Ecosystems Recovery team: www.goert.ca/documents/A.stolonifera.pdf) or inundation of riparian zones which may have brought the plants in contact with the mussels.

Major intracellular activities, presented in Table 2, are protein folding on endoplasmic reticulum (ER) (endoplasmin, peptidyl-prolyl cis-trans isomerase C = cyclophilin C), assembly of multimeric protein complexes inside the ER (heat-shock 70 kDa protein cognate 3, 78 kDa glucose-regulated protein) and protein translocon formation across the ER (dolichyl-diphosphooligosaccharide protein glycosyltransferase). In addition, we found a number of transport processes, such as cargo transport from trans-Golgi to plasma membrane (guanine nucleotide-binding

Table 2 Proteins from ER, Golgi network and plasma membrane

Protein	Pro-/Eucaryote	Function	Localisation
Endoplasmin [O18750]	*Oryctolagus cuniculus* (rabbit)	Ca^{2+}-binding protein, possibly involved in protein folding (Rowling et al. 1994)	ER
Guanine nucleotide-binding protein subunit β [Q5GIS3]	*Pinctada fucata* (Japanese pearl oyster)	Cargo transport from trans-Golgi network to plasma membrane	Golgi network and plasmalemma (Irannejad and Wedegaertner 2010)
AP-1 complex, subunit β-1 [O35643]	*Mus musculus* (mouse)	Subunit of adaptor protein complex-1, involved in protein sorting, mediating the recruitment of clathrin to the membrane and recognition of sorting signals within the cytoplasmic tails of transmembrane cargo molecules	Trans-Golgi network and/or clathrin-coated vesicles (UniProt KB/Swiss- Prot:O35643.2, cf. Robinson and Bonifacino 2001)
Clathrin heavy chain 1 [P11442]	*Rattus norvegicus*	Involved in cargo sorting (cf. adaptor protein complex-1 of *Mus musculus*)	Clathrin-coated vesicles at the plasma membrane or trans-Golgi network
α-Amylase [P04745]	*Homo sapiens* (human)	Formation of maltose, maltotriose and α-dextrin from starch	In oyster (*Crassostrea gigas)* preferentially in digestive tract (Huvet et al. 2003), RER, Golgi, cisternae, condensing vacuoles, and secretory granules (Geuze et al. 1979)
Glyceraldehyde-3-phosphate dehydrogenase 2 [Q9ESV6]	*Rattus norvegicus*	Conversion of glyceraldehyde-3P to 1,3-bisphosphoglycerate; + Rab2 and protein kinase Ci driven tubulovesicular recycling of proteins from the Golgi to the ER (Tisdale et al. 2009)	Vesicular tubular clusters
Transitional endoplasmic reticulum ATPase (TERA) [P03974]	*Sus scrofa* (wild boar)	Involved in fragmentation of Golgi stacks during mitosis and reassembly after mitosis; further is TERA involved in the formation of tER (transitional ER) (UniProt KB/Swiss-Prot information)	Golgi and ER
Guanine nucleotide-binding protein G(o), subunit α [O15976]	*Mizuhopecten yessoensis* (Yesso scallop)	Major neural signalling GTPase. Reacts on food deprivation (Hofler and Koelle 2011)	
Idem G(q), subunit α [P38411]	*Lymnaea stagnalis* (great pond snail)		
Guanine nucleotide-binding protein subunit β-2-like 1, Rack1 = receptor for activated c kinase-1 [Q93134]	*Biomphalaria glabrata* (blood fluke planorb = gastropod)	Involved in integrin signalling at adhesions, e.g. in a complex with kindlin-3	Plasmalemma (Feng et al. 2012)
Peptidyl-prolyl cis-trans isomerase C (cyclophilin C) [Q08E11]	*Bos taurus* (cow)	Cyclophilin is a protein folding catalyst	ER (Wang and Heitman 2005)
Actin, cytoplasmic 2 (from fibroblastic and epithelial cells) [A2BDB0]	*Xenopus laevis* (African clawed frog)	Probably involved in contractile ring formation (Dugina et al. 2009)	Colocalisation with myosin 2a in stress fibres and with VASP (vasodilator-stimulated phosphoprotein) in lamellipodia and focal adhesions (Dugina et al. 2009)
ADP-ribosylation factor 1 (Arf 1) [P36579]	*Schizosacharomyces pombe* 972 h-	Protein trafficking via COPI or clathrin-coated vesicles from ER to Golgi and plasmalemma	Cis/trans-Golgi and plasmalemma (Chavrier and Goud 1999; D'Souza-Schorey and Chavrier 2006)
Peptidoglycan-associated lipoprotein [P0A138]	*Pseudomonas putida* KT2440	Presence in bivalves may be due to ingestion by the host (Wood 2011)	Cell outer membrane
Sarcoplasmic/endoplasmic reticulum calcium ATPase 3 [Q9YGL9]	*Gallus gallus* (chicken)	Involved in muscle contraction	Localisation is in the name
Outer membrane porin F [P37726]	*Pseudomonas fluorescens*	Stabilisation of plasmalemma (multipass membrane protein)	Cell outer membrane (cf. VDAC in *C. anguineus*, Schuurmans Stekhoven et al. 2012)
Ribulose bisphosphate carboxylase large chain [A1EA16]	*Agrostis stolonifera* (creeping bent grass)	Ribulose 1,5-bisphosphate + CO_2 + H_2O ⟶ 2 3-P-glycerate + $2H^+$, and 3-P-glycerate + 2-P-glycolate ⟶ ribulose 1,5-bisP + O_2	Reactions take place in chloroplasts; presence in mollusks indicates contamination by plants
Glycogen phosphorylase from liver [P06737]	*Homo sapiens* (human)	Glycogen (n) + Pi↔glucose-1P + glycogen(n-1)	Microsomal fraction (ER or glycogen particles) (Tata 1964, Margolis et al. 1979)

Table 2 Proteins from ER, Golgi network and plasma membrane *(Continued)*

Heat shock 70 kDa protein [P08106]	*Gallus gallus* (chicken)	Conservation of protein shape and anti-stress protectant (De Maio 1999)	Cytosol, plasma membrane and endosomes + lysosomes (Nylandsted et al. 2004)
Heat shock 70 kDa protein cognate 3 [P29844]	*Drosophila melanogaster* (fruit fly)	Probably plays a role in facilitating the assembly of multimeric protein complexes inside the ER	ER (for additional locations and actions see above)
78 kDa glucose-regulated protein [Q16956]	*Aplysia californica* (California sea hare = mollusc)	Belongs to the heat shock protein 70 family with function as above	ER
Dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit STT3A [P46977]	*Homo sapiens* (human)	Transfer of a high mannose oligosaccharide to Asn-X-Ser/Thr in nascent polypeptide chains. The complex associates with Sec61 at the channel forming translocon complex for protein translocation across the ER	ER (expressed at high levels in the placenta, liver, muscle, pancreas; low in the brain, lung, and kidney)
Calcium transporting ATPase [P22700]	*Drosophila melanogaster* (fruit fly)	Reversible Ca^{2+} transport	Via ER and plasmalemma
Trypsin [P00761]	*Sus scrofa* (wild boar)	Serine protease	In digestive tract (synthesised in pancreas), localised in plasmalemma (Takeuchi et al. 2000)
Na^+/K^+-ATPase, α-subunit [P05025]	*Torpedo californica* (Pacific electric ray)	$3Na^+/2\ K^+$ exchange	Plasmalemma
Gelsolin-like protein 1 [Q7JQD3]	*Lumbricus terrestris* (common earth worm)	Regulator of actin filament assembly and disassembly	Plasma and intracellular membranes, including ER, cortical vesicles and mitochondria, plus short actin filaments adhering to the plasma membrane (Hartwig et al. 1989) renal brush border membranes
Malate dehydrogenase cytoplasmic [Q6PAB3]	*Xenopus laevis* (African clawed frog)	Oxidation of malate to oxaloacetate; confers selectivity of the nucleic acid-conducting channel in renal brush border membranes (Hanss et al. 2002)	Renal brush border membranes
Guanine nucleotide- binding protein subunit β-1 [P17343]	*Caenorhabditis elegans*	Involved in Gαβγ activation of phospholipase c activity in a cellular signalling process (Yan et al. 2007)	Localisation not disclosed
Ras-related protein O-Rab1 [P22125]	*Discopyge ommata* (ocellated electric ray)	Probably involved in vesicular traffic	O-Rab1 was found largely in the synaptic vesicle fraction (Ngsee et al. 1991)
Enolase [O02654]	*Loligo pealei* (longfin inshore squid)	Conversion of 2-phosphoglycerate ↔ phosphenolpyruvate + H_2O	Cytoplasm and plasma membrane of synaptosomes (Ueta et al. 2004)
2,3-bisphosphoglycerate-dependent phosphoglycerate mutase [B6IYD3]	*Rhodospirillum centenum* SW	Catalyses the interconversion of 2-phosphoglycerate and 3-phosphoglycerate	
Ras-related protein Rap-1b [A5A6J7]	*Pan troglodytes* (chimpanzee)	Ca^{2+} ATPase effector (Lacabaratz-Porret et al. 1998)	Plasmalemma (Marridonneau-Parini and de Gunzburg 1992; Mollinedo et al. 1993)

protein), protein sorting at trans-Golgi network and recruitment of clathrin to the membrane (AP-1 complex, clathrin heavy chain 1), protein trafficking via COPI or clathrin-coated vesicles from ER to Golgi and plasmalemma (ADP-ribosilation factor 1 = Arf 1), tubulovesicular recycling of protein from Golgi → ER (glyceraldehyde-3-phosphate dehydrogenase 2) and synaptic vesicle traffic (O-Rab1). Further, a few constructional processes are involved like fragmentation and reassembly of Golgi stacks during and after mitosis + formation of tER (transitional endoplasmic reticulum ATPase), contractile ring formation (actin, cytoplasmic 2) and regulation of actin filament assembly + disassembly (gelsolin-like protein 1). Subsequently, we record a number of cellular signalling components like neural signalling GTPase (guanine nucleotide-binding protein G (o) or G(q), subunit α), integrin signalling at adhesions (Rack 1) and Gαβγ activation of phospholipase c in a cellular signalling process (guanine nucleotide-binding protein subunit β-1). Another constructional component is heat-shock 70 kDa protein, yielding conservation of protein shape and protection against stress. In addition, we noticed a number of metabolic enzymes (α-amylase, glycogen phosphorylase, trypsin, enolase) and cation-activated enzymes (sarcoplasmic/endoplasmic reticulum calcium ATPase as modulated by Rap-1b, calcium-transporting ATPase, Na^+/K^+-ATPase) and the selectivity conferring protein in renal brush border nucleic acid conducting channel (cytoplasmic malate dehydrogenase).

The contribution of cellular signalling components appears to be modest in number but has been encountered

Table 3 Proteins of cytoskeleton and muscle

Protein	Eucaryote	Function	Localisation
Cytoplasmic actin [Q93129]	*Branchiostoma Belcheri* (Belcher's lancelet)	Transport track for myosin + support of cell stability (Pollard and Cooper 2009)	Cytoskeleton
Actin, cytoplasmic 1 (β-actin) [P79818]	*Oryzias latipes* (Japanese medaka)	Idem	Idem
Actin [O16808]	*Maietiola destructor* (Hessian fly or barley midge)	Cell support + providing trafficking routes for myosin in signal transduction (Pollard and Cooper)	cytoskeleton, microfilaments
Actin, cytoskeletal 1A [P53472]	*Strongylocentrotus purpuratus* (purple sea urchin)	Idem	Idem
Actin, non-muscle 6.2 [P17126]	*Hydra vulgaris* (fresh water polyp)	Idem	Idem
Actin-3 [P53457]	*Diphyllobothrium dentricum* (flatworm)	Idem in metazoan muscle cells actin forms a scaffold in which myosin generates force to support muscle contraction	Idem
Actin-3 [P41113]	*Podocoryne carnea* (jellyfish)	As for cytoplasmic actin	Idem
Actin-18 [P07828]	*Dictyostelium discoideum* (amoeba)	Idem	Idem
Actin [O17320]	*Crassostrea gigas* (Pacific oyster)	Idem	Idem
Actin [P50138]	*Puccinia graminis*(mould)	Idem	Idem
Actin, clone 205 [P18600]	*Artemia* sp. (crustacean)	Idem	Idem
Plastin-1 [P19179]	*Gallus gallus* (chicken)	Actin bundling protein	Cytoskeleton
Spectrin α-chain, non-erythrocytic 1 [P07751]	*Gallus gallus*	Playing an important role in membrane organisation	Cytoplasm, cytoskeleton and cell cortex
Spectrin β-chain [Q00963]	*Drosophila melanogaster* (fruit fly)	Spectrin links the actin cytoskeleton to the plasma membrane, thus forming a flexible scaffold in the cell cortex (Djinovic-Carugo et al. 2002)	Plasmalemma + cytoskeleton
Heat shock cognate protein HSP90-β [Q04619]	*Gallus gallus* (chicken)	Early embryonic development, germ cell maturation, cytoskeletal stabilisation, cellular transformation, signal transduction, long-term cell adaptation (Sreedhar et al. 2004)	Cytoplasm + cytoskeleton (microtubules and actin filaments, Cambiazo et al. 1999)
Radixin [P26043]	*Mus musculus* (mouse)	Participates in signal transduction and regulates cell migration and intercellular adhesion via Rac 1 (Valderrama et al. 2012)	Linking plasmalemma to actin filaments
Ubiquitin [Q86WD4]	*Encephalitozoon cuniculi* (protozoan)	Involved in the ubiquitin proteasome pathway (Hegde 2010)	Plasmalemma and cytoskeleton (microtubules) (Murti et al. 1988, Hicke and Dunn 2003)
T-complex protein 1 subunit α [P50157]	*Ambystoma mexicanum* (axolotl, salamander)	TCP-1 is chaperonin, involved in protein folding, e.g. of actin and tubulin (Souès et al. 2003; Yam et al. 2008)	Nucleus, cytoskeleton (microtubule organising centre) and cytoplasm (Souès et al. 2003)
Tubulin β-chain [P11833]	*Paracentrotus lividus* (sea urchin)	Mitosis, intracellular vesicle transport (www.buzzle.com/articles/microtubules-function. html)	Part of cytoskeleton: microtubules
Tubulin β-2 chain [P52275]	*Caenorhabditis elegans*	Part of transport track for ATP-driven vesicle movement or chromosomes in mitosis and meiosis	Cytoskeleton: microtubules
Tubulin α-1A chain [A5A6J1]	*Pan troglodytes* (chimpanzee)	Involved in supporting the cell shape and transport of vesicles	Idem
Tubulin α-3 chain [P05214]	*Mus musculus* (house mouse)	cf. Tubulin β-chains	Idem
Myosin-9 [P35579]	*Homo sapiens* (human)	Cytokinesis: vesicle transport via actin filaments, cell shape, secretion and capping	Cytoskeleton, cell cortex together with actin filaments at lamellipodia and at the leading edge of migrating cells

Table 3 Proteins of cytoskeleton and muscle *(Continued)*

Elongation factor 1α [P02993]	*Artemia salina* (brine shrimp)	Promoting the GTP-dependent binding of aminoacyl-tRNA to the A-site of ribosomes during protein biosynthesis	Cytoskeleton (actin filaments) (Liu et al. 1996)
Eucaryotic initiation factor 4A-I [A5A6N4]	*Pan troglodytes* (chimpanzee)	ATP-dependent RNA helicase, involved in mRNA binding to the ribosome	Cytoskeleton (Ziegler et al. 2012)
Myosin-11 [P10587]	*Gallus gallus* (chicken)	Involved in contraction	Smooth muscle
Myosin catalytic light chain LC-1 [P05945]	*Todarus pacificus* (Japanese flying squid = cephalopod)	Is dependent on Ca^{2+} binding for muscle contraction; in molluscan muscle Ca^{2+} regulation is associated with myosin rather than with actin	Mantle muscle
Myosin essential adductor muscle light chain [P07290]	*Mizuhopecten yessoensis* (Yesso scallop, bivalve)	Idem	Idem
Paramyosin [O96064]	*Mytilus galloprovincialis* (Mediterranean mussel)	Attachment to the substrate	Mytilus anterior byssus retractor paramyosin (thick filaments at the myofibrils)
Adductor muscle actin (precursor) [Q26065]	*Placopecten magellanicus* (sea scallop)	Involved in muscle contraction	Location is in the name
Actin, muscle (precursor) [P12431]	*Strongylocentrotus purpuratus* (purple sea urchin)	Is involved in muscle contraction	Idem
Tropomyosin [Q9GZ71]	*Haliotis diversicolor* (variously coloured abalone)	In association with the troponin complex plays a central role in the Ca^{2+}-dependent regulation of muscle contraction	Idem
α-Actinin, sarcomeric [P18091]	*Drosophila melanogaster* (fruit fly)	F-actin cross-linking protein, anchoring actin to a variety of intracellular structures. By allowing insertion of thick filaments (myosin) the thin filaments (actin) are led to contraction (Stryer 1995a)	
Actin, larval muscle [P02574]	*Drosophila melanogaster* (fruit fly)	Actin, together with myosin, is involved in muscle contraction	
Myosin heavy chain, striated muscle [P24733]	*Argopecten irradians* (baby scallop)	Involved in muscle contraction (Nyitray et al. 1991)	

before in a proteomic analysis of *Mytilus galloprovincialis* and *Mytilus trossulus*: three to four signalling components in a total of 47 to 61 proteins, i.e. 6.4% to 6.5% (Tomanek and Zuzow 2010).

The cytoskeletal and muscle components (Table 3) with their numbers between parentheses can be summarised as follows: actin non-muscle (11), cytoskeleton (microfilaments) = transport track for myosin; linkers of actin to plasma membrane: plastin, radixin, spectrin α and β chains (4); myosin-9, involved in vesicle transport via actin filaments (1); tubulin α and β chains (microtubules, involved in ATP-driven vesicle transport or transport of chromosomes in mitosis and meiosis) (4), myosin muscle, involved in contraction: myosin-11 (smooth muscle) (1), myosin LC-1 + heavy chain + adductor muscle light chain (3), paramyosin (byssus retractor muscle) (1), adductor muscle actin (precursor) → contraction (1), actin, muscle precursor, tropomyosin, α-actinin, actin larval muscle (4).

Additional cytoskeletal organised components are: the chaperonin TCP-1, heat-shock cognate protein HSP90-β (with a plurality of functions), ubiquitin (involved in proteolysis), elongation factor 1α (involved in protein synthesis) and eucaryotic initiation factor 4A-I (involved in mRNA binding to the ribosome).

After corrections for bacterial and plant contaminations in Tables 1 and 2, we come to a total of *M. leucophaeata*-related analyses of 112. The analyses, related to the cytoskeletal and muscle components (1st paragraph of Table 3) amount to 30, i.e. 26.8% of all analyses. Similar results have been scored by Tomanek and Zuzow (2010) for *M. trossulus* and *M. galloprovincialis*: 16.4% and 25.5%, respectively. In a later study (Fields et al. 2012), they even scored 39.4% and 52.8%, respectively. These numbers underline the importance for these water-bound animals of a sturdy built body with solid protection against predators. Intracellular stability by the cytoskeleton via linking of actin filaments to the cell membrane, presence of adductor muscles for closure of the shell halves provide additional support for the above ideas, and of course, the presence of paramyosin is essential for binding of the animal to the substrate, including stones and ship walls that bring them to the harbours.

In this last part of our analyses (Table 4), we find only transport vesicles (V-type H^+-ATPase, major vault protein); histones H2A, H2AV, H2B, H3 and H4; proteasome

Table 4 Proteins from cellular vacuoles, ribonucleoprotein particles (vaults), nuclei and mitochondria

Protein	Eucaryote	Function	Localisation
V-type proton ATPase catalytic subunit A [P314400]	*Manduca sexta* (tobacco horn worm)	Involved in cellular trafficking, exocytosis and endocytosis, and interaction with the cytoskeleton (Marshansky and Futai 2008)	Cellular vacuoles
Major vault protein [Q5EAJ7]	*Strongylocentrotus purpuratus* (purple sea urchin)	Signal pathway regulation and immune defence (Berger et al. 2009)	Ribonucleoprotein particles ($41 \times 41 \times 71.5$ nm) or vaults
Histone H2A.V [P02272]	*Gallus gallus* (chicken)	Histones play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability	Part of nucleosome core (nuclei)
Histone H2A [P02268]	*Sepia officinalis* (common cuttlefish)	See above and below	See above and below
Histone H2B, gonadal [P02284]	*Patella granatina* (sand paper limpet)	See above; further, histones and histone fragments circulate via transporters through the cytoplasm and so may be transferred to the plasma membrane (Schuurmans Stekhoven et al. 2004) from where they execute their anti-microbial activity (Seo et al. 2011)	Nuclei, cytoplasm, and plasmalemma
Histone H3 [P02299]	*Drosophila melanogaster* (fruit fly)	See above	See over
Histone H4 [P35059]	*Acropora formosa* (stony corals)	See above	See over
Proteasome subunit α type-2 [Q73672]	*Carassius auratus* (gold fish)	Cleavage of peptide bonds with very broad specificity	Cytoplasm and nucleus
Proteasome subunit α type-5-A [O81149]	*Arabidopsis thaliana* (thale cress)	Brake down of damaged or redundant proteins	Cytosol and nucleus
Proteasome subunit α type-7 [O13268]	*Gallus gallus* (chicken)	ATP dependent cleavage of peptide bonds with broad specificity	Cytoplasm and nucleus
14-3-3 protein ε [P92177]	*Drosophila melanogaster* (fruit fly)	Multifunctional: regulation of enzymatic activity, regulation of subcellular localisation, inhibition of protein-protein or protein-DNA interaction, protection against dephosphorylation or proteolytic degradation, stabilisation of multiprotein complexes (Obsil and Obsilova 2011)	Plasmalemma, mitochondrion, nucleus
ATP synthase, subunit α [Q9XXK1]	*Caenorhabditis elegans* (round worm)	ATP synthesis	Mitochondrion
ATP synthase, subunit β precursor [Q5ZLC5]	*Gallus gallus* (chicken)	ATP synthesis	Mitochondrion
Succinate dehydrogenase [Q28ED0]	*Xenopus tropicalis* (Western clawed frog)	Transfer of electrons to coenzyme Q	Mitochondrion
ADP/ATP carrier protein 3 [O49447]	*Arabidopsis thaliana* (thale cress)	Exchange of ADP and ATP across the mitochondrial inner membrane	Idem
Probable malate dehydrogenase 3 [Q54VM2]	*Dictyostelium discoideum* (amoeba)	Oxidation of malate to oxaloacetate	Cytoplasm and mitochondria (Danis and Farkas 2009; Gietl 1992)
Phosphoenolpyruvate carboxykinase [Q05893]	*Ascaris suum* (pig roundworm)	Conversion of oxaloacetate to phosphoenolpyruvate and vice versa	Mitochondria (Stryer 1995b)

subunit α type-2, −5A and −7; the 14-3-3 protein ε; ATP synthase subunit α- + β-precursor; the citric acid cycle enzymes succinate dehydrogenase; probable malate dehydrogenase 3; and phosphoenolopyruvate carboxykinase that is responsible for the conversion of oxaloacetate to phosphoenolpyruvate in the gluconeogenesis pathway (Stryer 1995b); and the mitochondrial ADP/ATP exchanger.

This gives a fair picture of what is going on in the subcellular compartments indicated, but some of the animals (or plants) of comparison (second column) require some criticism with regard to their comparability as will be brought forward in the discussion of Tables 3 and 4.

As mentioned in the 'Introduction' section, we were also interested in the protein chemical developments that had taken place in the mussel's long history. For that reason, we have registered the generation time for all vertebrates and invertebrates that had provided sequences that led to the identification of proteins that have been presented in Tables 1, 2, 3 and 4. Proteins that could be considered as intestinal contaminants: bacterial proteins, but also a single plant, living in flooded wetlands, have been omitted. Figure 2A,B provides the graphical result of MYA from 1,100 to zero vs. the sum of the vertebrate + invertebrate contribution. It can be seen that the

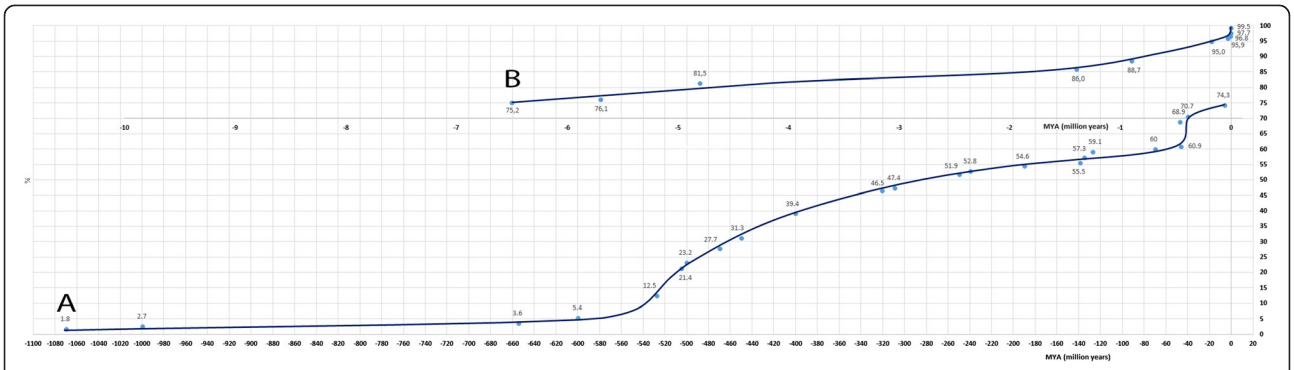

Figure 2 A + B. Graphical expression of pro- and eucaryotes from which proteins were found in the mussel's proteome. The *x*-axis indicates the time of generation in MYA (million years ago), and the *y*-axis indicates the accumulated expression in percent of total (100%). Point for point has been connected graphically for the period from 1,070 to 6.5 MYA in part A and from 6.5 to 0 MYA in part B. A complete survey of the data is shown in Table 5.

graph curves upward from 600 to 500 MYA, thereby covering the generation of bivalves. From 320 to 50 MYA, the line is linear, covering the insect *Drosophila melanogaster* (321 MYA) teleosts *Takifugu rubripes* and goldfish (70 to 48 MYA). Thereupon follows a second uprise until 4.75 MYA in which period Birds (Phasianidae), Decapoda (brine shrimp), Muridae (rat and house mouse), rabbit and the Japanese medaka participate. After this period, there is some levelling off, but is followed by a third uprise, lasting from 1.4 to 0 MYA (Figure 2B). Animals and fungi, involved in this period, are: cow (5), chimpanzee (3), *Homo sapiens* (7), dog (1), goat (1), stemrust (1) and wild boar (2) (Table 5). From the total of 20 items, 10 (50%) belong to Table 1 and are part of ribosomes that are involved in the protein synthesis machinery. The only cow that was missing in Table 1 can be found in Table 2 as a provider of cyclophilin C, a protein folding catalyst on the ER. Chimpanzee can be found in Table 2 at Ras-related protein Rap-1b and in Table 3 at tubulin α-1 A chain and eucaryotic initiation factor 4A-I. Rap-1b is a cellular signalling component in neuronal cells (Sahyoun et al. 1991) and lymphocytes (Awasthi et al. 2010). Tubulin α-1 A chain is part of the cytoskeleton (microtubules), which supports the cell shape and serves as transport track for vesicles. Eucaryotic initiation factor 4A-I is involved in binding of the messenger RNA to the ribosome. *H. sapiens* (human) is for about half categorised under the ribosomes at Table 1. Further, humans can be found in Table 2 at α-amylase, glycogen phosphorylase, and dolichyl-diphosphooligosaccharide protein glycosyltransferase and in Table 3 at myosin-9. Functions are: provision of glucose from its polymers, protein transport across the ER and transport of vesicles along actin filaments. The only dog is related to protein synthesis (Table 1); stemrust (*Puccinia graminis*) is also related to the cytoskeletal actin (Table 3) and wild boar to transitional ER ATPase (TERA) and the serine protease trypsin (Table 2). The first is involved in breakdown and repair of Golgi stacks during and after mitosis, respectively, plus formation of the transitional ER. The second is involved in proteolysis.

Summarising all processes involved in the third proteochemical uprise (covering the Pleistocene and Holocene) shows a composition of protein synthesis, breakdown, folding and transportation across the ER. In addition, binding of messenger RNA to the ribosome, cellular signalling, microtubular vesicle transport, breakdown and repair of Golgi stacks during and after mitosis and formation of the transitional ER are also involved. Glucose supply is provided by α-amylase and glycogen phosphorylase; in the latter case yielding glucose-1P. All these processes are essential for survival of the animal, and during its evolution, it may have been necessary to adapt to the changing environmental conditions.

In addition, we liked to analyse also the second proteochemical uprise (48 to 4.75 MYA, corresponding with half Eocene to second half of Pliocene). Animals belonging to this time range are chicken (9), brine shrimp (2), house mouse (4), rabbit (1), Japanese medaka (1), and *Rattus norvegicus* (6), with the number of recorded protein components between parentheses. Summation yields a number of 23, slightly more than the third uprise with 20 components, but taking an appreciably longer time span: 43 vs. 1.4 million years.

Processes involved in this second period of development of the mussel are: transcription regulation, DNA repair and replication + chromosome stabilisation (chicken histone H2A. V: 1 item), protein synthesis at the ER by ribosomes from Muridae (*Rattus* and *Mus musculus*: 5 items), binding of aminoacyl-tRNA to the ribosomes in protein synthesis (elongation factor 1α, brine shrimp: 1 item), protein folding at the ER (endoplasmin, rabbit: 1 item), cargo sorting at the plasma membrane and trans-Golgi network

Table 5 Geologic time table of animal, plants, amoebae and fungi's contribution to the proteomics of *M. leucophaeata*

Component with proteins	Time of generation (MYA)	Contribution (%)	Sum (%)
Amoeba (Dictyosteliidae): actin 18; probable malate dehydrogenase.	1070 (Wegener Parfrey et al. 2011)	1.8	1.8
Encephalitozoon cuniculi (protozoon parasite): ubiquitin.	1000 (Hedges 2002)	0.9	2.7
Starlet sea anemone (polyp): ribosomal subunit 40S-S3a.	655 (Wegener Parfrey et al. 2011)	0.9	3.6
Fission yeast (*S. pombe*): ribosomal subunit 60S-L7c; ADP-ribosylation factor 1 (Arf1).	600 (Wegener Parfrey et al. 2011)	1.8	5.4
Lancelet & worms: ribosomal subunit 40S-SA; cytoplasmic actin; actin 3; gelsolin-like protein 1; guanine nucleotide-binding protein; tubulin β-2 chain; ATP synthase, subunit α; phosphoenolpyruvate carboxykinase.	551 to 505 (Bagley 2013)	7.1	12.5
Bivalves: ribosomal subunit 40S-SA; ribosomal subunit 40S-S18; ribosomal subunit 40S-S19; guanine nucleotide-binding protein, subunit β; guanine nucleotide-binding protein G(o), subunit α; actin; myosin essential adductor muscle light chain; paramyosin; adductor muscle actin (precursor); myosin heavy chain.	approximately 510 (Kansas Geological Survey 2008)	8.9	21.4
Hydrozoa (*Hydra vulgaris*; *Podocoryne carnea*): actin, non-muscle; actin 3.	approximately 505 (Cartwright et al. 2007)	1.8	23.2
Gastropoda: *Lymnaea stagnalis*: guanine nucleotide-binding protein G(q), subunit α; 2005) *Biomphalaria glabrata*; guanine nucleotide-binding protein, subunit β-2-like, Rack 1; *Aplysia californica*: 78 kDa glucose-regulated protein; *Haliotis diversicolor*: tropomyosin; *Patella granatina*: histone H2B, gonadal.	500 (Kansas Geological Survey 2005)	4.5	27.7
Cephalopoda: ribosomal subunit 40S-S26; enolase; myosin catalytic light chain LC-1; histone H2A.	500 to 440 ([PPT] Life and Geologic Time)	3.6	31.3
Echinodermata (*Strongylocentrotus purpuratus*): actin, cytoskeletal 1A; actin, muscle (precursor); major vault protein.	approximately 450 (Smith 1984)	2.7	34.0
Insects (Arthropoda, Hexopoda): ribosomal subunit 40S-S4; ribosomal subunit 60S-L5; ribosomal subunit 60S-L8; ribosomal subunit 60S-L17; actin; V-type proton ATPase catalytic subunit A.	400 (Grimaldi and Engel 2005)	5.4	39.4
Fruit fly (*Drosophila melanogaster*): ribosomal subunit 40S-S14; heat-shock 70 kDa protein cognate 3; Ca^{2+} transporting ATPase; spectrin β-chain; α-actinin, sarcomeric; actin, larval muscle; histone H3; 14-3-3 protein ε.	321 (Wegener Parfrey et al. 2011)	7.1	46.5
Fungi (*Saccharomyces cerevisiae*): ribosomal subunit 60S-L16a.	309 (Wegener Parfrey et al. 2011)	0.9	47.4
Amphibians (*Xenopus tropicalis*, *Xenopus laevis*): ribosomal subunit 40S-S13; ribosomal subunit 60S-L4b; actin, cytoplasmic 2; malate dehydrogenase, cytoplasmic; succinate dehydrogenase.	250 (Zhang et al. 2005a, b)	4.5	51.9
(Anthozoa, stony corals): histone H4.	240 (Waggoner 2000)	0.9	52.8
Elasmobranchii (rays): Na^{+}/K^{+}-ATPase; Ras-related protein O-Rab1.	190 (prehistoric sharks - megalodon, fossil teeth, shark attacks) (Pre-Historic Sharks)	1.8	54.6
Amphibians (salamander): T-complex protein 1, subunit α.	139 (Zhang et al. 2005a)	0.9	55.5
Teleosts (channel catfish): ribosomal subunit 40S-S6; ribosomal subunit 60S-L7a.	160 to 110 (Volff 2005)	1.8	57.3
Plantae (cruciferae, thale cress): proteasome subunit α type-5-A; ADP/ATP carrier protein 3.	127 (Wegener Parfrey et al. 2011)	1.8	59.1
Teleosts (*Takifugu rubripes*): ribosomal subunit 40S-S24.	80 to 60 (Volff 2005)	0.9	60.0
Goldfish: proteasome subunit α type-2.	49 to 46 (Wang et al. 2007)	0.9	60.9
Birds (chicken, Galliformes, Phasianidae): sarcoplasmic/endoplasmic reticulum ATPase 3; heat-shock 70 kDa protein; plastin-1; spectrin α chain, non-erythrocytic 1; heat-shock cognate protein HSP90-β; myosin-11; histone H2A.V; proteasome subunit α type-7; ATP synthase, subunit β precursor.	58 to 37 (Mlikovský 1989)	8.0	68.9
Decapoda (brine shrimp): actin, clone 205; elongation factor 1α.	40 (Baxeranis et al. 2006)	1.8	70.7
Mammals (house mouse): ribosomal subunit 40S-S16; AP-1 complex, subunit β-1; radixin; tubulin α-3 chain.	6.5 (Veyrunes et al. 2006)	3.6	74.3
Rabbit: endoplasmin.	6.5 (Branco et al. 2000)	0.9	75.2
Teleosts (Japanese medaka); actin, cytoplasmic 1 (β-actin).	6.0 to 5.4 (Takehana et al. 2003)	0.9	76.1

Table 5 Geologic time table of animal, plants, amoebae and fungi's contribution to the proteomics of *M. leucophaeata* (Continued)

Rattus norvegicus: ribosomal subunit 40S-S27-like; ribosomal subunit 60S-L4; ribosomal subunit 60S-L5; ribosomal subunit 60S-L26; clathrin heavy chain 1; glyceraldehyde-3-phosphate dehydrogenase 2.	6 to 3.5 (Furano and Usdin 1995; Verneau et al. 1998)	5.4	81.5
Cow: ribosomal subunit 40S-S2; ribosomal subunit 40S-S7; ribosomal subunit 40S-S9; ribosomal subunit 40S-S17; peptidyl-prolyl cis-trans isomerase C (cyclophilin C).	1.4 (Mac Hugh et al. 1997)	4.5	86.0
Chimpanzee: Ras-related protein Rap-1b; tubulin-1A chain; eucaryotic initiation factor 4A-I.	0.9 to 0.86 (Won and Hey 2005)	2.7	88.7
Homo sapiens: ribosomal subunit 40S-S3; ribosomal subunit 40S-S5; ribosomal subunit 60S-L23a; α-amylase; glycogen phosphorylase; dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit STT3A; myosin-9.	0.2 to 015 (Reid and Hetherington 2010)	6.3	95.0
Dog (*Canis lupus familiaris*): ribosomal subunit 60S-L12.	0.032 (Germonpré et al. 2009)	0.9	95.9
Goat (*Capra hircus*): ribosomal subunit 40S-S18.	0.006 to 0.007 (www.ansi.okstate.edu/breeds/goats/)	0.9	96.8
Fungi (*Puccinia graminis*: stemrust): actin.	0.0033 (Kislev 1982)	0.9	97.7
Wild boar: transitional endoplasmic reticulum ATPase (TERA); trypsin.	0.005 to 0 (Hardjasasmita 1987)	1.8	99.5

Time is expressed in MYA (million years ago), and contribution in percent of total (112 items), excluding bacterial contamination and vegetable contribution from wetlands.

(clathrin heavy chain 1, *R. norvegicus*: 1 item, and AP-1 complex, subunit β-1, *M. musculus*: 1 item), also including endocytosis.

Besides protein synthesis, folding and sorting, there is also the recycling from Golgi to ER (glyceraldehyde-3 phosphate dehydrogenase 2, *R. norvegicus*: 1 item). Not only protein synthesis is involved, but also protein cleavage (proteasome subunit α type-7, chicken: 1 item). Protein is transcellularly transported via phosphorylation/dephosphorylation of myosin on the cytoskeletal actin tracks. Components of this system are provided by brine shrimp (actin, clone 205: 1 item) and Japanese medaka (cytoplasmic actin: 1 item). Intracellular stability is given by linking of actin filaments to each other (plastin-1, chicken: 1 item) or to the plasma membrane (spectrin α chain, chicken: 1 item, and radixin, house mouse: 1 item). Intracellular vesicle transport is provided by tubulin α-3 chain, which is also involved in mitosis (house mouse: 1 item). Heat-shock proteins: 70 kDa and cognate protein HSP 90-β are involved in conservation of protein shape (anti-stress protectant) and cytoskeletal stabilisation + signal transduction (chicken: 2 items). Last, but not least, muscle contraction for closing and opening the valves is effected by sarcoplasmic/endoplasmic reticulum calcium ATPase 3 and myosin-11 (chicken: 2 items). Not to forget is ATP synthase (chicken: 1 item), which will make transportation and muscle contraction, besides many other processes, possible via its formation of ATP.

Comparison of the processes, represented by the proteins of the second and third uprise, shows similarities and differences. For instance: protein synthesis via ribosomes at the ER counts for 50% in uprise 3, but only for half as much (26%) in uprise 2. Uprise 2 covers transcription regulation, DNA repair and replication + chromosome stabilisation (1 item), whereas uprise 3 does not. Protein folding at ER (1 item) occurs in both periods, but cargo sorting at the plasma membrane and trans-Golgi network (2 items) is only in period 2. The same holds for recycling from Golgi → ER (1 item), whereas protein transport across the ER (1 item) belongs only to period 3. On the other hand, protein cleavage (either by proteasome subunit α type-7 or trypsin) occurs in both periods. The same holds true for actin tracks (2 and 1 item, respectively), but not for actin linkers (3 in period 2 only). Both periods contain vesicle transport (1 and 2 items in periods 2 and 3, respectively). Further activities that relate only to period 2 are protein shape conservation + cytoskeletal stabilisation + signal transduction by heat-shock proteins (2 items). Activities that are related to closing and opening the valves, plus the enzyme that this facilitates (ATP synthase) (3 items), only occur in the second uprise, but cellular signalling by Rab-1b and enzymes involved in the hydrolysis of polysaccharides are confined to uprise 3 (4 items).

Discussion

Table 1 showed an abundance of 52% in bacterial ribosomal subunits. Although the presence of bacteria in the intestine of a eucaryote is a common phenomenon, the capacity of the present bacteria to break down and thereby detoxicate organic pollutants raises the possibility that these bacteria have been added on purpose to the canal inhabited by the mussel. The following detoxifying properties have been ascribed to some of the indicated strains: *P. fluorescens* is beneficial for plants in terms of suppressing pathogens, aiding nutrient absorption

and degrading environmental pollutants (www.buzzle.com/articles/pseudomonas-fluorescens.html). *P. putida* is a versatile environmental isolate that is capable of growth on several aromatic hydrocarbons, including benzene, toluene, ethylbenzene and p-cymene. Its broad substrate toluene dioxygenase has been widely utilised in biocatalytic synthesis of chiral chemicals, as well as in the metabolism and detoxification of trichloroethylene (TCE). *P. putida* F1 is known to be chemotactic to aromatic hydrocarbons and chlorinated aliphatic compounds and has the potential for use in biomediation applications (genome.jgi-psf.org/psepu/psepu.home.html) (site of DOE Joint Genome Institute, University of California). On the other hand, the strain W619 that showed up in our analyses is more competent with regard to heavy metal resistances and beneficial effects on plants (Wu et al. 2011).

P. mendocina DSWY0601 and ymp extrude a polyhydroxybutyrate (PHB) depolymerase that can degrade PHB plastic (Yan et al. 2012). *P. stutzeri* strain A1501 is equally beneficial to plants by denitrification of NO_3^-, converting it to N_2 and fixation of $N_2 \rightarrow 2\ NH_3$. Subsequently, NH_4^+ is coupled to α-ketoglutarate under formation of glutamate (Stryer 1995c; Lalucat et al. 2006).

In contrast to the above positive descriptions, the list of bacteria also contains some negatively acting contributors: *P. aeruginosa*, *P. syringae* and *E. coli. P. aeruginosa*, despite its positive contribution in oil degradation in the presence of glycerol or the biosurfactant rhamnolipid (Zhang et al. 2005b), also excretes toxins that are deleterious for the pulmonary system (Roy-Burman et al. 2001). *P. syringae* is a plant-pathogenic bacterium, infecting bean to tomato, causing bacterial speck to bacterial cancer (*P. syringae* Genome Resources home page: Pseudomonas-Plant Interaction (PPI) from Cornell University: Department of Plant Pathology: www.pseudomonas-syringae.org). *E. coli* APEC01 is a deleterious avian pathogenic bacterium causing epidemic colibacillosis in the poultry industry (Kabir 2010).

Some data of similarity with *C. anguineus* (Schuurmans Stekhoven et al. 2012) are: synaptic vesicle traffic (Ngsee et al. 1991) and enolase in the plasma membrane of synaptosomes (Ueta et al. 2004). The first reminded us of the neurotransmitter cycle that we found in the brain of *C. anguineus* via its modulator α-synuclein and v- and t-snares VAMP1/2 and SNAP-25 + syntaxin 1. However, although bivalves contain a nervous system (Encyclopaedia Brittanica: www.brittannica.com/EBchecked/topic/67293/bivalve/35745/The-shell), we have not been able to find the abovementioned v- or t-snares for bivalves. In partial contrast to this are the results obtained for *M. galloprovincialis* (Venier et al. 2009) in which results for three t-snares in the Mediterranean mussel have been obtained via transcribed sequences: SNAP-25A [Accession No. Q5TZ66], SNAP-type protein [Accession No. Q25391] and SNAP-47 [Accession No. Q0P4A7]. Yet, v-snares have not been detected either in this case. Presence of enolase in the plasma membrane appears to have an endangering effect via its complex formation with plasminogen that by subsequent activation to plasmin can break down the extracellular matrix and so can allow invasion of pathogens, viruses and metastatic cancer cells (Liu and Shih 2007; Díaz-Ramos et al. 2012). Normally, plasmin is used to dissolve fibrin blood clots but upon generation on the cell surface might cause the above effects. However, in our analyses, neither plasminogen nor plasmin (MW 81 and 75.4 kDa, Barlow et al. 1969) or plasminogen activator (tPA, MW 72 kDa, Manosroi et al. 2001) has been traced. On the other hand, in the transcribed sequences of *M. galloprovincialis*, two sequences were found that matched plasminogen [Accession No's. Q01177 and Q6PBA6] (Venier et al. 2009). Furthermore, despite the clear presence of Na^+/K^+-ATPase in our analyses, we have been unable to find the presence of phospholemman (FXYD1), known as a modulator of Na^+/K^+-ATPase (Mahmmoud et al. 2000), even though our analyses covered a wide range of molecular weights (14.1 to 240 kDa). Since phosphorylation of Na^+/K^+-ATPase causes dissociation of phospholemman, this may have led to its absence in the analyses. In addition, salinity may also decrease the FXYD content relative to the Na^+/K^+-ATPase content (Wang et al. 2008). In another report (Horisberger 2006), it has been indicated that no FXYD protein can be found in arthropods or any nonvertebrate animals. We think that the only way that is left to trace the absence or presence of FXYD in bivalves is to analyse their DNA.

One of the fungi that have entered the list of comparative sequences is *P. graminis* at actin in Table 3. This mould spreads its occurrence by spore formation via two different hosts, thereby causing the so-called stemrust, especially in wheat and barley (Schumann and Leonard 2000). Although contamination of the brackish water mussel with infected wheat and/or barley from freight ships in the harbour cannot be excluded, another possibility is indistinguishable peptides formed by trypsin treatment (cf. Schuurmans Stekhoven et al. 2010) as used in the analysis of actin from *Crassostrea gigas* [Accession No. O17320] or *Puccinia graminis* [Accession No. P50138]. A few of the possibilities are a^{20}gfagddapr^{29}, h^{41}qgvmvgmgqk^{51} and y^{70}piehgivtnwddmek^{85} for *Crassostrea gigas* and the same sequences for *Puccinia graminis*, but with a numbering of a^{19}-r^{28}, h^{40}-k^{50} and y^{69}-k^{84}. It appears that the sequences are quite conserved since they are also found in β-actin of the mammal *M. musculus* [Accession No. ABL01512].

Another subject of criticism is the possibly hereditary plant sequences in the genome of the mussel. An example could be the occurrence of Proteasome subunit α

type-5A and ADP/ATP carrier protein from *Arabidopsis thaliana* (thale cress). Thale cress grows on edges of agricultural fields, stone walls alongside tracks and roads and Mediterranean scrublands with scattered holm oaks but is no inhabitant of wet lands (Picó et al. 2008). Therefore, we have looked for comparable sequences in MytiBase: a knowledgebase of mussel (*M. galloprovincialis*) with 3,275 transcribed sequences (Venier et al. 2009). To our surprise: also in this large list of transcribed proteins, a few examples of plant heritage were met: first the occurrence of 14-3-3-like protein b of *Oryza sativa* (India Group) [Accession No. ABR25888] together with 14-3-3 C1 protein from *Oncorhynchus mykiss* [Accession No. Q6UFZ7]. Identical sequences, found for *Oryza sativa* with those for *O. mykiss* (the latter between parentheses), are e^{16}-e^{34} (e^{113}-e^{131}), p^{65}-f^{80} (p^{162}-f^{177}), l^{94}-d^{100} (l^{191}-d^{197}) and s^{113}-d^{134} (s^{210}-d^{231}). It is evident that the sequence for *Oryza sativa* is 97 amino acids less than that of *O. mykiss*, due to incomplete DNA. Yet, we can calculate an identity that must be minimally 25% the same. Since the sequences in case of *M. galloprovincialis* have been determined via DNA, we have to accept a genetic link between animals and plants, and so the link with proteasome subunit α type 5A, ADP/ATP carrier protein 3 and *Arabidopsis thaliana* may be genuine and not artificial.

A second example of plant heritage by *M. galloprovincialis* is found in the presence of probable ATPase from the chloroplast of *Oenothera organensis* (organ Mountains evening primrose) [Accession No. Q0H0T1] which grows in the mountains of New Mexico, far away from the mussel of the Mediterranean Sea. Hence, there has been a time that they were neighbours. This hypothesis is built on three assumptions:

1. Some DNA of consumed food can be taken up in cells and incorporated into the DNA of the consumer if it displays some similarity with DNA of that consumer.
2. Since bivalves do not inhabit the mainland, the plant consumers could be snails (Gastropods), which form a sister clade with bivalves, forming the Pleistomollusca (Kokot et al. 2011).
3. If geological conditions, like flooding, would force gastropods to evolve to bivalves, it is not unthinkable that bivalves would contain land plant sequences in their genome as has been shown by Venier et al. (2009). Before accepting this hypothesis, it will be necessary to trace the snail's genome or RNA for plant resemblances.

In the generation of the brackish water mussel *M. leucophaeata* in the period of 1,070 to 0 MYA, two additional genetic uprises occurred beyond the uprise caused by the generation of the bivalves per se (approximately 510 MYA). The question arises why this has to be achieved by acceleration in a developmental uprise: the second in the period of 48 to 4.75 MYA and the third in the period of 1.4 to 0 MYA. In the 48 to 4.75 MYA period (Eocene-Pliocene), earth was in motion with formation of mountains and separation or collision of geological plates, volcano formation, followed by climate cooling (Pidwirny 2012). In the later period (1.4 to 0 MYA: Pleistocene + Holocene), earth was subject to freezing (Pleistocene Ice Age) with extinction of many species (Pidwirny 2012), which may have forced the mussel to a counterreaction by speeding up its adaptation of DNA to that of modern species (cow, chimpanzee, human, dog, stemrust and wild boar, Table 5).

Evidence for climate change-induced effects on adaptation of the genome of animals and plants has been recently published (Reusch and Wood 2007; Buckley et al. 2012; Franks and Hoffmann 2012). Failure to adapt to the changes may eventually lead to extinction. In this respect, *M. leucophaeata* did not fail, otherwise it could not have been able to survive for 500 million years until present (Figure 2). Therefore, it is remarkable that it has only a limited range of salt concentration: 6.7 to 7.4 ppt ($^0/_{00}$) to provide for an optimal condition. At higher salinities, the condition index is reduced to 50 at a salinity of 11 ppt (Grutters and Verhofstad 2010). Seawater usually has a salinity of 35 ppt (Office of Naval Research: www.onr.navy.mil/focus/ocean/water/salinity 1.htm). Therefore, one may question how *M. leucophaeata* can survive the trip via the ocean to the brackish North Sea Channel. Transportation of larvae and postlarvae with tolerance to salinity of 32 ppt in ballast water makes the trip possible (Verween et al. 2010). The salinity of the North Sea Channel varies from 1.7 to 9.2 ppt (Van der Velde et al. 1998), which *M. leucophaeata* can enter with confidence.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

FSS carried out the homogenisation and fractional centrifugation of the bivalve homogenate, electrophoresis of the fractions, staining and destaining of the gels and conveying the gel strips to the analytical laboratory of ARB in Leicester. Further, he interpreted the data and wrote this article. THL offered his contribution in formatting of the text and provided his data (included in this paper) on the effect of salinity change on the expression of FXYD, a modulator of Na^+/K^+-ATPase. GvdV, the malacologist of our department, provided essential information on the anatomy and physiology of mollusks, and ARB, together with Lady S. Ibrahim, analysed the gel strips and sent me the proteomic data together with their accession numbers in the data banks. All authors read and approved the final manuscript.

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Author details
[1]Department of Animal Ecology and Ecophysiology, Faculty of Science, Radboud University Nijmegen, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands. [2]Department of Life Sciences, National Chung-Hsing University, Taichung 402, Taiwan. [3]Protein and Nucleic Acid Chemistry Laboratory, Proteomics Facility, University of Leicester, Lancaster Road, Leicester LE1 9HN, UK. [4]Naturalis Biodiversity Center, P.O. Box 9517,2300RA Leiden, The Netherlands.

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References

Awasthi A, Samarakoon A, Chu H, Kamalakannan R, Quiliam LA, Chrzanowska-Wodnicka M, White GC II, Malarkannan S (2010) Rap-1b facilitates NK cell functions via IQGAP1-mediated signalosomes. J Exp Med 207:1923–1938

Bagley N (2013) Cambrian Period: Facts & Information. www.livescience.com/28098-cambrian-period.html

Barlow GH, Summaria L, Robbins KC (1969) Molecular weight studies on human plasminogen and plasmin at the microgram level. J Biol Chem 244:1138–1141

Baxeranis AD, Kappas I, Abatzopoulos TJ (2006) Molecular phylogenetics and asexuality in the brine shrimp *Artemia*. Mol Phyl Evol 40:724–738

Berger W, Steiner E, Grusch M, Elbling L, Micksche M (2009) Vaults and the major vault protein: novel roles in signal pathway regulation and immunity. Cell Mol Life Sci 66:43–61

Branco M, Ferrand N, Monnerot M (2000) Phylogeography of the European rabbit (*Orectolagus cuniculus*) in the Iberian Peninsula inferred from RFLP analysis of the cytochrome b gene. Heredity 85:307–317

Buckley J, Butlin RK, Bridle JR (2012) Evidence for evolutionary change associated with the recent range expansion of the British butterfly, *Aricia agestis*, in response to climate change. Mol Ecol 21:267–280

Cambiazo V, González M, Isamit C, Maceoni RB (1999) The β-isoform of heat shock protein hsp-90 is structurally related with human microtubule-interacting protein Mip-90. FEBS Lett 457:343–347

Cartwright P, Halgedahl SL, Hendricks JR, Jarrad RD, Marques AC, Collins AG, Lieberman BS (2007) Exceptionally preserved jellyfishes from the Middle Cambrian. PLoS One 2(10):e1121

Chavrier P, Goud B (1999) The role of ARF and Rab GTPases in membrane transport. Curr Opin Cell Biol 11:466–475

D'Souza-Schorey C, Chavrier P (2006) ARF proteins: roles in membrane traffic and beyond. Nature Rev Cell Biol 7:347–358

Danis P, Farkas R (2009) Hormone-dependent and hormone-independent control of metabolic and developmental functions of malate dehydrogenase. Review Endocrine Reg 43:39–52

De Maio A (1999) Heat shock proteins: facts, thoughts, and dreams. Shock 11:1–12

Díaz-Ramos À, Roig-Borellas A, Garcia-Melero A, López-Alemany R (2012) α-Enolase a multifunctional protein: its role on pathophysiological situations. J Biomed Biotech doi:10.1155/2012/156795

Djinovic-Carugo K, Gautel M, Ylänne J, Young P (2002) The spectrin repeat: a structural platform for cytoskeletal assemblies. FEBS Lett 513:119–123

Dugina V, Zwaenepoel I, Gabbiani G, Clément S, Chaponnier C (2009) β- and γ-cytoplasmic actins display distinct distribution and functional diversity. J Cell Sci 122:2980–2988

Feng C, Li Y-F, Yan Y-H, Lee H-S, Tang X-Y, Xue Z-H, Zhou Y-C, Lim W-M, Cornvik TC, Ruedl C, Schochat SG, Tan S-M (2012) Kindlin-3 mediates integrin αLβ2 outside-in signaling, and it interacts with scaffold protein receptor for activated-c kinase 1 (Rack1). J Biol Chem 287:10714–10726

Fields PA, Zuzow MJ, Tomanek L (2012) Proteomic responses of blue mussel (*Mytilus*) congeners to temperature acclimation. J Exp Biol 215:1106–1116

Franks SJ, Hoffmann AA (2012) Genetics of climate change adaptation. Ann Rev Genet 46:185–208

Furano AV, Usdin K (1995) DNA "fossils" and phylogenetic analysis, using L1 (Line-1, long interspersed repeated) DNA to determine the evolutionary history of mammals. J Biol Chem 270:25301–25304

Germonpré M, Sablin MV, Stevens RE, Hedges REM, Hofreiter M, Stiller M, Després VR (2009) Fossil dogs and wolves from Paleolithic sites in Belgium, the Ukraine and Russia: ostometry, ancient DNA and stable isotopes. J Archaeol Sci 36:473–490

Geuze JJ, Slot JW, Tokuyasu KT, Goedemans WEM, Griffith JM (1979) Immunochemical localization of amylase and chymotrypsinogen in the exocrine pancreatic cell with special attention to the Golgi complex. J Cell Biol 82:697–707

Gietl C (1992) Malate dehydrogenase isoenzymes: cellular locations and role in the flow of metabolites between the cytoplasm and cell organelles. Biochim Biophys Acta 1100:217–234

Grimaldi D, Engel MS (2005) Evolution of the insects, Part of Cambridge Evolution Series. Cambridge University Press, Cambridge

Grutters BMC, Verhofstad MJJM (2010) Assessing and comparing three invasive bivalves: *Dreissena polymorpha*, *Dreissena rostriformis bugensis* and *Mytilopsis leucophaeata*. Master thesis Department of Ecology and Ecophysiology, Radboud University, Nijmegen

Hanss B, Leal-Pinto E, Teixeira A, Christian RE, Shabanowitz J, Hunt DF, Klotman PE (2002) Cytosolic malate dehydrogenase confers selectivity of the nucleic acid conducting channel. Proc Natl Acad Sci U S A 99:1707–1712

Hardjasasmita HS (1987) Taxonomy and phylogeny of the Suidae (Mammalia) in Indonesia. Scripta Geol 85:1–68

Hartwig JH, Chambers KA, Stossel TP (1989) Association of gelsolin with actin filaments and cell membranes of macrophages and platelets. J Cell Biol 108:467–479

Hedges SB (2002) The origin and evolution of model organisms. Nat Rev Genet 3:838–849

Hegde AN (2010) The ubiquitin-proteasome pathway and synaptic plasticity. Learn Mem 17:314–327

Hicke L, Dunn R (2003) Regulation of membrane protein transport by ubiquitin and ubiquitin-binding protein. Ann Rev Cell Dev Biol 19:141–172

Hofler C, Koelle MR (2011) AGS-3 alters *Caenorhabditis elegans* behavior after food deprivation via Ric-8 activation of the neural G protein $G\alpha_o$. J Neurosci 31:11553–11562

Horisberger J-D (2006) Evolution of Na-K-ATPases. Plenary Lecture LT_1.02. 99, Annual Meeting of the German Zoological Society, September. 16-20. Abstractband, Münster, p 9

Huvet A, Daniel J-Y, Quéré C, Dubois S, Prudence M, van Wormhoudt A, Sellos D, Samain J-F, Moal J (2003) Tissue expression of two α-amylase genes in the pacific oyster *Crassostrea gigas:* Effects of two different food rations. Aquaculture 228:321–333

Irannejad R, Wedegaertner PB (2010) Regulation of constitutive cargo transport from the trans-Golgi network to plasma membrane by Golgi-localized G protein βγ subunits. J Biol Chem 285:32393–32404

Kabir SML (2010) Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. Int J Environ Res Public Health 7:89–114

Kansas Geological Survey (2005) www.kgs.ku.edu/Extension/fossils/gastropod.html

Kansas Geological Survey (2008) www.kgs.ku.edu/Extension/fossils/bivalve.html

Kislev ME (1982) Stem rust of wheat 3300 years old found in Israel. Science 216:993–994

Kokot KM, Cannon JT, Todt C, Citarella MR, Kohn AB, Meyer A, Santos SR, Schander C, Moroz LL, Lieb B, Halanych KM (2011) Phylogenomics reveals deep molluscan relationships. Nature 477:452–457

Lacabaratz-Porret C, Corvazier E, Kovàcs T, Bobe R, Bredoux R, Launay S, Papp B, Enouf J (1998) Platelet sarco/endoplasmatic reticulum Ca^{2+} ATPase isoform 3b and rap 1b: interaction and regulation in physiopathology. Biochem J 332:173–181

Lalucat J, Bennasar A, Bosch R, Garcia-Valdés E, Palleroni NJ (2006) Biology of *Pseudomonas stutzeri*. Microbiol Mol Biol Rev 70(2):510–547

Liu K-J, Shih N-Y (2007) The role of enolase in tissue invasion and metastasis of pathogens and tumor cells. J Cancer Mol 3:45–48

Liu G, Tang J, Edmonds BT, Murray J, Levin S, Condeels J (1996) F-actin sequesters elongation factor 1α from interaction with aminoacyl-tRNA in a pH-dependent reaction. J Cell Biol 135:953–963

Mac Hugh DE, Shriver MD, Loftus RT, Cunningham P, Bradley DG (1997) Microsatellite DNA variation and the evolution, domestication and phylogeography of Taurine and Zebu cattle (*Bos taurus* and *Bos indicus*). Genetics 146:1071–1086

Mahmmoud YA, Vorum H, Cornelius F (2000) Identification of a phospholemman-like protein from shark rectal glands. Evidence for indirect regulation of Na, K-ATPase by protein kinase C via a novel member of the FXYD family. J Biol Chem 275:35969–35977

Manosroi J, Tayapiwatana C, Götz F, Werner RG, Manosroi A (2001) Secretion of active recombinant human tissue plasminogen activator derivatives in Escherichia coli. Appl Environ Microbiol 67:2657–2664
Margolis RN, Cardell RR, Curnow RT (1979) Association of glycogen synthase phosphatase and phosphorylase phosphatase activities with membranes of hepatic smooth endoplasmic reticulum. J Cell Biol 83:348–356
Marridonneau-Parini I, de Gunzburg J (1992) Association of rap1 and rap2 proteins with the specific granules of human neutrophils: translocation to the plasma membrane during cell activation. J Biol Chem 267:6396–6402
Marshansky V, Futai M (2008) The V-type H^+-ATPase in vesicular trafficking: targeting, regulation and function. Curr Opin Cell Biol 20:415–426
Mlikovský J (1989) A new guinea fowl (Aves, Phasianidae) from the late Eocene of France. Ann Naturhist Mus Wien 90A:63–66
Mollinedo F, Perez-Sala D, Gajate C, Jimenez B, Rodriguez P, Lacal JC (1993) Localization of rap1 and rap2 proteins in the gelatinase-containing granules of human neutrophils. FEBS Lett 326:209–214
Murti KG, Smith HT, Fried VA (1988) Ubiquitin is a component of the microtubule network. Proc Natl Acad Sci U S A 85:3019–3023
Napolitano A, de Lucia M, Panzella L, d'Ischia M (2008) The "benzothiazine" chromophore of pheomelanins: a reassessment. Photochem Photobiol 84:593–599
Ngsee JK, Elferink LA, Scheller RH (1991) A family of ras-like GTP-binding proteins expressed in electromotor neurons. J Biol Chem 266:2675–2680
Nyitray L, Goodwin EB, Szent-Györgyi AG (1991) Complete primary structure of a scallop striated muscle myosin heavy chain. Sequence comparison with other heavy chains reveals regions that might be critical for regulation. J Biol Chem 266:18469–18476
Nylandsted J, Gyrd-Hansen M, Danielewicz A, Fehrenbacher N, Lademann U, Høyer-Hansen M, Weber E, Multhoff G, Rohde M, Jäättelä M (2004) Heat shock protein 70 promotes cell survival by inhibiting lysosomal membrane permeabilization. J Exp Med 200:425–435
Obsil T, Obsilova V (2011) Structural basis of 14-3-3 protein functions. Semin. Cell Dev Biol doi:10.1016/j.semcdb. 2011.09.001
Oklahoma State University, Department of Animal Science (Ansi): (www.ansi.okstate.edu/breeds/goats/)
Picó FX, Méndez-Vigo B, Martínez-Zapater JM, Alonso-Blanco C (2008) Natural genetic variation of *Arabidopsis thaliana* is geographically structured in the Iberian Peninsula. Genetics 180:1009–1021
Pidwirny M (2012) Geologic time, The Encyclopedia of Earth. Educational site of the Environmental Information Coalition (EIC), Washington D.C, www.eoearth.org
Pollard TD, Cooper JA (2009) Actin, a central player in cell shape and movement. Science 326:1208–1212
Pre-Historic Sharks (2012) *About prehistoric Sharks-Megalodon, fossil teeth*, (http://www.sharkattacks.com/prehistoric.htm)
Reid GBR, Hetherington R (2010) The climate connection: climate change and human evolution. Cambridge University Press, Cambridge, UK, p64.ISBN 0-521-14723-9
Reusch TBH, Wood TE (2007) Molecular ecology of global change. Mol Ecol 16:3973–3992
Riva C, Cristoni S, Binelli A (2012) Effects of triclosan in the freshwater mussel *Dreissena polymorpha*: a proteomic investigation. Aquatic toxicol 118–119:62–71
Robinson MS, Bonifacino JS (2001) Adapter-related proteins. Curr Opin Cell Biol 13:444–453
Rowling PJE, McLaughlin SH, Pollock GS, Freedman RB (1994) A single purification procedure for the major resident proteins of the ER lumen: endoplasmin, BIP, calreticulin and protein disulfide isomerase. Prot Expr Purif 5:331–336
Roy-Burman A, Savel RH, Racine S, Swanson BL, Revadigar NS, Fujimoto J, Sawa T, Frank DW, Wiener-Kronish JP (2001) Type III protein secretion is associated with death in lower respiratory and systemic *Pseudomonas aeruginosa* infections. J Inf Dis 183:1767–1774
Sahyoun N, McDonald OB, Farrell F, Lapetina EG (1991) Phosphorylation of Ras-related GTP-binding protein, Rap-1b, by a neuronal Ca^{2+}/calmodulin-dependent protein kinase, CaM kinase Gr. Proc Natl Acad Sci U S A 88:2643–2647
Schumann GL, Leonard KJ (2000) Stem Rust of Wheat (black rust). The Plant Health Instructor. doi:10.1094/PHI-I-2000-0721-01
Schuurmans Stekhoven FMAH, Grell E, Atsma W, Flik G, Wendelaar Bonga SE (2003) Organ-related distribution of phospholemman in the spiny dogfish *Squalus acanthias*. Biochem Biophys Res Commun 303:1008–1011
Schuurmans Stekhoven FMAH, Wendelaar Bonga SE, Flik G (2004) Extranuclear histones in teleost gills: an evolutionary study. Fish. Physiol Biochem 30:201–211
Schuurmans Stekhoven FMAH, Wendelaar Bonga SE, Lee T-H, Bottrill AR (2010) Presence or absence of the Cl^- channel phospholemman in the rectal gland of sharks: a comparative study. Zool Studies 49:326–334
Schuurmans Stekhoven FMAH, Lee T-H, Wang P-J, Jang-Liaw N-H, Tanaka S, Bottrill AR (2012) Proteomic studies of various organs and tissues of the frilled shark *Chlamydoselachus anguineus*. Zool Studies 51:1248–1269
Seo J-K, Stephenson J, Noga EJ (2011) Multiple antibacterial histone H2B proteins are expressed in tissues of American oyster. Comp Biochem Physiol B 158:223–229
Smith AB (1984) Echinoid Palaeobiology in Special Topics in Palaeontology 1. Allen & Unwin, London
Souès S, Kann M-L, Fouquet J-P, Melki R (2003) The cytosolic chaperonin CCT associates to cytoplasmic microtubular structures during mammalian spermiogenesis and to heterochromatin in germline and somatic cells. Exp Cell Res 288:363–373
Sreedhar AS, Kalmár E, Csermely P, Shen Y-F (2004) Hsp90 isoforms: functions, expression and clinical importance. FEBS Lett 562:11–15
Stryer L (1995a) The Power Stroke in Contraction is Driven by Conformational Changes in the Myosin S1 Head, 4th edn. Biochemistry, WH Freeman and Company, NY, pp 399–400
Stryer L (1995b) Phosphoenolpyruvate Carboxykinase, 4th edn. Biochemistry, WH Freeman and Company, NY, p 571
Stryer L (1995c) Nitrogen Fixation: Microorganisms Use ATP and a Powerful Reductant to Reduce N_2 to NH_3, 4th edn. Biochemistry, WH Freeman and Company, NY, pp 714–717
Takehana Y, Nagai N, Matsuda M, Tsuchiya K, Sakaizumi M (2003) Geographic variation and diversity of the cytochrome b gene in Japanese wild populations of Medaka, *Orizias latipes*. Zool Sci 20:1279–1291
Takeuchi T, Harris JL, Huang W, Yan KW, Coughlin SR, Craik CS (2000) Cellular localization of membrane-type serine protease 1 and identification of protease-activated receptor-2 and single-chain urokinase-type plasminogen activator as substrates. J Biol Chem 275:26333–26342
Tata JR (1964) Subcellular redistribution of liver α-glucan phosphorylase during alterations in glycogen content. Biochem J 90:284–292
Tisdale EJ, Azizi F, Artalejo CR (2009) Rab 2 utilizes glyceraldehyde-3-phosphate dehydrogenase and protein kinase Ci to associate with microtubules and to recruit dynein. J Biol Chem 284:5876–5884
Tomanek L, Zuzow MJ (2010) The proteomic response of the mussel congeners *Mytilus galloprovincialis* and *M. trossulus* to acute heat stress: implications for thermal tolerance limits and metabolic costs of thermal stress. J Exp Biol 213:3559–3574
Tomanek L, Zuzow MJ, Hitt L, Serafini L, Valenzuela JJ (2012) Proteomics of hyposaline stress in blue mussel congeners (genus *Mytilus*): implications for biogeographic range limits in response to climate change. J Exp Biol doi:10.1242/jeb.076448
Ueta H, Nagasawa H, Oyabu-Manabe Y, Toida K, Ishimura K, Hori H (2004) Localisation of enolase in synaptic plasma membrane as an αγ heterodimer in rat brain. Neurosc Res 48:379–386
Valderrama F, Therapala S, Ridley AJ (2012) Radixin regulates cell migration and cell-cell adhesion through Rac 1. J Cell Sci 125:3310–3319
Van der Velde G, van der Gaag M, Rajagopal S, Jenner HA (1998) Where do the exotic mussels *Dreissena polymorpha* and *Mytilopsis leucophaeata* meet in the brackish Noordzeekanaal, The Netherlands? In: 8th International Zebra Mussel and Other Aquatic Nuisance Species Conference, 16–19 March 1998, Sacramento, USA, p. 54
Venier P, de Pittà C, Bernante F, Varotto L, de Nardi B, Bovo G, Roch P, Novoa B, Figueras A, Pallavicini A, Lanfranchi G (2009) MytiBase: a knowledge base of mussel (*M. galloprovincialis*) transcribed sequences. BMC Genomics 10:72, doi:10.1186/1471-2164-10-72
Verneau O, Catzeflis F, Furano AV (1998) Determining and dating recent rodent speciation events by using L1 (Line-1) retrotransposons. Proc Natl Acad Sci U S A 95:11284–11289
Verween A, Vincx M, Degraer S (2010) *Mytilopsis leucophaeata*: the brackish water equivalent of *Dreissena polymorpha*? A review. In: Van der Velde G, Rajagopal S, Bij de Vaate A (eds) The zebra mussel in Europe. Backhuys Publishers, Leiden/ Margraf Publishers, Weikersheim, pp 29–44
Veyrunes F, Dobigny G, Yang F, O'Brien PCM, Catalan J, Robinson TJ, Britton-Davidian J (2006) Phylogenomics of the genus *Mus* (Rodentia, Muridae): extensive genome repatterning is not restricted to the house mouse. Proc R Soc B 273:2925–2934

Volff J-N (2005) Genome evolution and biodiversity in teleost fish. Heredity 94:280–294
Waggoner BM (2000) Anthozoa: Fossil Record. http://www.ucmp.berkeley.edu/cnidaria/anthozoafr.html
Wang P, Heitman J (2005) The cyclophilins. Genome Biol 6:226
Wang X, Junbing L, Shunping H (2007) Molecular evidence for the monophyly of East Asian groups of Cyprinidae (Teleostei, Cypriniformes) derived from the nuclear recombination activating gene 2 sequences. Mol Phyl Evol 42:157–170
Wang P-J, Lin C-H, Hwang H-H, Lee T-H (2008) Branchial FXYD protein expression in response to salinity change and its interaction with Na^+/K^+-ATPase of the euryhaline teleost *Tetraodon nigroviridis*. J Exp Biol 211:3750–3758
Wegener Parfrey L, Lahr DJG, Knoll AH, Katz LA (2011) Estimating the time of early eukaryotic diversification with multigene molecular clocks. Proceed Natl Acad Sci USA doi:10.1073/pnas.1110633108
Willett M, Pollard HJ, Vlasak M, Morley SJ (2010) Localization of ribosomes and translation initiation factors to talin/β3-integrin-enriched adhesion complexes in spreading and migrating mammalian cells. Biol Cell 102:265–276
Won YJ, Hey J (2005) Divergence population genetics of chimpanzees. Mol Biol Evol 22:297–307
Wood RR (2011) Analysis of bacterial communities in the eastern oyster (*Crassostrea virginica*) with emphasis on *Vibrio vulnicus* dynamics under refrigeration. PhD thesis. Auburn University, Alabama
Wu X, Monchy S, Taghavi S, Zhu W, Ramos J, van der Lelie D (2011) Comparative genomics and functional analysis of niche-specific adaptation in *Pseudomonas putida*. FEMS Microbiol Rev 35:299–323
Yam AY, Xia Y, Lin H-TJ, Burlingame A, Gerstein M, Frydman J (2008) Defining the TRIC/CCT interactome links chaperonin function to stabilization of newly-made proteins with complex topologies. Nat Struct Mol Biol 15:1255–1262
Yan C, Sato M, Lanier SM, Smrcka AV (2007) Signaling by a non-dissociated complex of G protein βγ and α subunits stimulated by a receptor-independent activator of G protein signaling AGS8. J Biol Chem 282:19938–19947
Yan W, Fan L, Zhan-Yong W, Dong-Bo L, Hong-Mei X, Ling-Fei L, Shan C (2012) Purification and properties of an extracellular polyhydroxybutyrate depolimerase from *Pseudomonas mendocina* DSWY0601. Chem Res Chin Univ 28(3):459–464
Zhang P, Zhou H, Chen Y-Q, Liu Y-F, Qu L (2005a) Mitogenomic perspectives of the origin and phylogeny of living amphibians. Syst Biol 54:391–400
Zhang G-L, Wu Y-T, Qian X-P, Meng Q (2005b) Biodegradation of crude oil by *Pseudomonas aeruginosa* in the presence of rhamnolipids. J Zhejiang Univ (Sci) 6B(8):725–730
Ziegler ME, Souda P, Jin Y-P, Whitelegge JP, Reed EF (2012) Characterization of the endothelial cell cytoskeleton following HLA class I ligation. Plos ONE 7(1):e29472