TABLE 2.8 Reassessment of the 96 candidate LGT cases identified in the original genome publication

Acc.[a]	RefSeq Acc.[b]	EhL[b]	Top prokaryotic BLAST hit	PL[c]	% ID[d]	Top eukaryotic BLAST hit	EL[c]	%ID[d]	PE-score[e]	EE-score[f]	P/E ratio[g]
41 LGT cases that remain strongly supported according to our criteria											
EAL43201	XP_648590.1	487	*Treponema denticola*	507	57	*Trichomonas vaginalis*	398	43	1.00E-167	5.00E-88	2.00E-80
EAL43619	XP_649008.1	621	*Vibrio vulnificus*	673	41	*Saccharomyces cerevisiae*	664	40	1.00E-132	1.00E-125	1.00E-07
EAL43678	XP_649067.1	538	*Fusobacterium nucleatum*	562	47	*Trichomonas vaginalis*	477	34	1.00E-135	2.00E-60	5.00E-76
EAL43850	XP_649240.1	880	*Mannheimia succiniciproducens*	898	63	*Mastigamoeba balamuthi*	882	45	0	0	N/A
EAL44182	XP_649570.1	260	*Bacteroides thetaiotaomicron*	273	34	*Yarrowia lipolytica*	298	29	2.00E-35	4.00E-10	5.00E-26
EAL44226	XP_649612.1	262	*Bacteroides thetaiotaomicron*	267	28	*Tetrahymena thermophila*	1476	30	2.00E-25	0.11	1.82E-24
EAL44778	XP_650165.1	188	*Bacteroides thetaiotaomicron*	188	43	*Neurospora crassa*	546	34	8.00E-41	1.8	4.44E-41
EAL45076	XP_650453.1	358	*Bacteroides fragilis*	362	46	*Trichomonas vaginalis*	562	22	1.00E-87	0.24	4.17E-87
EAL45145	XP_650531.1	825	*Staphylococcus aureus*	1036	30	*Trichomonas vaginalis*	2468	20	3.00E-59	0.016	1.88E-57
EAL45220	XP_650606.1	479	*Clostridium tetani*	471	45	*Arabidopsis thaliana*	581	31	1.00E-114	1.00E-54	1.00E-60
EAL44744	XP_650131.1	160	*Bacteroides fragilis*	424	41	*Yarrowia lipolytica*	169	31	3.00E-24	7.00E-11	4.29E-14
EAL46110	XP_651498.1	157	*Bacteroides fragilis*	166	49	*Arabidopsis thaliana*	627	35	5.00E-35	3.2	1.56E-35

(*continued*)

TABLE 2.8 *(continued)*

Acc.[a]	RefSeq Acc.[b]	EhL[b]	Top prokaryotic BLAST hit	PL[c]	% ID[d]	Top eukaryotic BLAST hit	EL[c]	%ID[d]	PE-score[e]	EE-score[f]	P/E ratio[g]
EAL45378	XP_650765.1	311	*Haloarcula marismortui*	299	43	*Leishmania major*	411	43	3.00E-54	1.00E-32	3.00E-22
EAL45618	XP_651004.1	159	*Bacteroides thetaiotaomicron*	157	46	*Plasmodium vivax*	1275	33	2.00E-28	0.69	2.90E-28
EAL46311	XP_651697.1	248	*Synechococcus elongates*	270	36	*Trichomonas vaginalis*	3075	18	1.00E-30	0.38	2.63E-30
EAL46679	XP_652065.1	218	*Methanosarcina mazei*	230	37	*Candida glabrata*	461	24	8.00E-31	0.079	1.01E-29
EAL46975	XP_652361.1	370	*Bordetella bronchiseptica*	368	46	*Cryptococcus neoformans*	372	40	8.00E-83	3.00E-71	2.67E-12
EAL47525	XP_652912.1	380	*Clostridium perfringens*	296	23	*Plasmodium falciparum*	390	34	2.00E-13	1.3	1.54E-13
EAL47905	XP_653291.1	227	*Clostridium perfringens*	259	33	*Tetrahymena thermophila*	1425	24	4.00E-19	0.32	1.25E-18
EAL48587	XP_653973.1	425	*Desulfovibrio vulgaris*	442	60	*Yarrowia lipolytica*	572	37	1.00E-149	9.00E-57	1.11E-93
EAL48979	XP_654365.1	732	*Thermotoga neapolitana*	740	40	*Cryptococcus neoformans*	735	28	1.00E-135	3.00E-64	3.33E-72
EAL49084	XP_654474.1	350	*Methanococcus jannaschii*	241	29	*Anopheles gambiae*	784	40	1.00E-24	5.00E-06	2.00E-19
EAL49209	XP_654596.1	247	*Bacteroides fragilis*	243	38	*Thalassiosira pseudonana*	269	22	7.00E-43	0.0002	3.50E-39
EAL49277	XP_654665.1	737	*Bacteroides thetaiotaomicron*	781	31	*Cryptococcus neoformans*	935	24	1.00E-111	6.00E-44	1.67E-68
EAL49613	XP_654999.1	168	*Sulfolobus solfataricus*	237	34	*Tetrahymena thermophila*	487	38	1.00E-16	6.00E-06	1.67E-11
EAL49813	XP_655200.1	186	*Escherichia coli*	200	31	*P. brasiliensis*	257	26	2.00E-13	0.47	4.26E-13

EAL49869	XP_655257.1	390	*Campylobacter jejuni*	407	56	*Ashbya gossypii*	490	39	1.00E-124	8.00E-73	1.25E-52
EAL50263	XP_655646.1	390	*Porphyromonas gingivalis*	408	48	*Yarrowia lipolytica*	428	38	1.00E-98	3.00E-60	3.33E-39
EAL50440	XP_655826.1	344	*Bacillus anthracis*	491	54	*Rhizopus oryzae*	510	40	1.00E-101	2.00E-67	5.00E-35
EAL50508	XP_655888.1	348	*Wolinella succinogenes*	340	55	*Mus musculus*	168	40	1.00E-106	2.00E-18	5.00E-89
EAL50603	XP_655988.1	567	*Bacteroides thetaiotaomicron*	622	45	*Trichomonas vaginalis*	632	39	1.00E-141	2.00E-99	5.00E-43
EAL50801	XP_656185.1	499	*Bacteroides thetaiotaomicron*	513	52	*Trichomonas vaginalis*	514	28	1.00E-145	3.00E-40	3.33E-106
EAL50992	XP_656375.1	140	*Archaeoglobus fulgidus*	184	40	*Trichomonas vaginalis*	195	46	1.00E-27	0.018	5.56E-26
EAL50997	XP_656380.1	656	*Bacteroides thetaiotaomicron*	718	53	*Cryptococcus neoformans*	770	32	0	2.00E-69	0.00E + 00
EAL51149	XP_656535.1	343	*Bacteroides fragilis*	359	43	*Pichia ofunaensis*	378	34	8.00E-84	1.00E-53	8.00E-31
EAL51236	XP_656622.1	259	*Symbiobacterium thermophilum*	274	45	*Oryza sativa*	315	21	3.00E-51	0.003	1.00E-48
EAL51348	XP_656749.1	171	*Methanopyrus kandleri*	204	37	*Tetrahymena thermophila*	2872	22	3.00E-21	0.1	3.00E-20
EAL51525	XP_656903.1	316	*Bacteroides thetaiotaomicron*	300	29	*Candida boidinii*	314	32	8.00E-27	0.0007	1.14E-23
EAL51565	XP_656946.1	415	*Clostridium perfringens*	900	43	*Trichomonas vaginalis*	897	40	1.00E-89	5.00E-81	2.00E-09
EAL51925	XP_657304.1	448	*T. tengcongensis*	481	43	*Giardia lamblia*	937	33	3.00E-96	2.00E-60	1.50E-36
EAL52001	XP_657387.1	303	*Oceanobacillus iheyensis*	306	27					2.00E-15	0.00E + 00

(continued)

TABLE 2.8 (*continued*)

Acc.[a]	RefSeq Acc.[b]	EhL[b]	Top prokaryotic BLAST hit	PL[c]	% ID[d]	Top eukaryotic BLAST hit	EL[c]	%ID[d]	PE-score[e]	EE-score[f]	P/E ratio[g]
27 LGT cases that are more weakly supported than before according to our criteria											
EAL45152	XP_650539.1	122	*Shewanella oneidensis*	132	34	*Trypanosoma bruzeii*	385	24	5.00E-10	6.6	7.58E-11
EAL43347	XP_648734.1	848	*Burkholderia pseudomallei*	779	38	*Plasmodium falciparum*	2463	32	1.00E-136	4.00E-44	2.50E-93
EAL44257	XP_649643.1	407	*Clostridium acetobutylicum*	406	25	*Homo sapiens*	468	24	6.00E-23	1.00E-14	6.00E-09
EAL45586	XP_650972.1	460	*Clostridium tetani*	476	47	*Xenopus laevis*	513	38	1.00E-116	5.00E-84	2.00E-33
EAL46313	XP_651699.1	118	*Prochlorococcus marinus*	163	42	*Hordeum vulgare*	223	22	2.00E-21	1.4	1.43E-21
EAL46399	XP_651785.1	218	*Clostridium perfringens*	235	65	*Trypanosoma bruzeii*	295	52	3.00E-73	9.00E-54	3.33E-20
EAL46421	XP_651808.1	205	*Clostridium acetobutylicum*	230	40	*Arabidopsis thaliana*	241	33	7.00E-34	6.00E-12	1.17E-22
EAL46701	XP_652087.1	294	*Bacteroides fragilis*	308	45	*Thalassiosira pseudonana*	348	27	4.00E-63	1.00E-14	4.00E-49
EAL46757	XP_652143.1	95	*Lactococcus lactis*	103	31	*Tetrahymena thermophila*	112	32	3.00E-09	1.00E-07	3.00E-02
EAL46858	XP_652245.1	192	*Pseudomonas aeruginosa*	195	41	*Caenorhabditis briggsae*	229	40	6.00E-36	2.00E-17	3.00E-19
EAL47026	XP_652397.1	164	*Bacillus subtilis*	181	30	*Trichomonas vaginalis*	182	26	3.00E-10	2.00E-08	1.50E-02
EAL47464	XP_652839.1	504	*Treponema denticola*	509	39	*Piromyces* sp.	555	27	5.00E-88	2.00E-30	2.50E-58
EAL47648	XP_653034.1	259	*Methanosarcina mazei*	272	36	*Arabidopsis thaliana*	345	25	2.00E-39	3.00E-11	6.67E-29

EAL47787	XP_653173.1	546	*Spirochaeta thermophila*	571	56	*Solanum tuberosum*	552	46	1.00E-175	1.00E-135	1.00E-40
EAL48186	XP_653572.1	232	*Bacillus cereus*	279	34	*Thalassiosira pseudonana*	271	32	2.00E-10	2.00E-08	1.00E-02
EAL49309	XP_654698.1	358	*Methanosarcina mazei*	379	42	*Leishmania major*	373	31	5.00E-77	9.00E-44	5.56E-34
EAL48568	XP_653954.1	113	*Chlamydia pneumoniae*	271	38	*Debaryomyces hansenii*	699	38	5.00E-14	7.00E-16	7.14E + 01
EAL48767	XP_654156.1	165	*Bacteroides fragilis*	177	40	*Trichomonas vaginalis*	189	28	7.00E-28	2.00E-05	3.50E-23
EAL48783	XP_654172.1	217	*Pseudomonas putida*	225	46	*Giardia lamblia*	239	35	2.00E-43	7.00E-24	2.86E-20
EAL49703	XP_655090.1	396	*Clostridium acetobutylicum*	398	34	*Tetrahymena thermophila*	445	29	4.00E-64	3.00E-44	1.33E-20
EAL49996	XP_655383.1	358	*Bacteroides thetaiotaomicron*	368	60	*Brachydanio rerio*	367	43	1.00E-121	5.00E-76	2.00E-46
EAL50325	XP_655711.1	447	*Clostridium tetani*	448	30	*Trichomonas vaginalis*	871	29	4.00E-46	1.00E-37	4.00E-09
EAL50521	XP_655905.1	285	*Streptococcus agalactiae*	323	29	*Leishmania major*	452	24	2.00E-22	3.00E-06	6.67E-17
EAL50620	XP_656005.1	261	*Wolinella succinogenes*	655	27	*Trichomonas vaginalis*	261	28	6.00E-21	1.00E-06	6.00E-15
EAL50838	XP_656225.1	299	*Anabaena* sp.	287	27	*Trichomonas vaginalis*	336	29	4.00E-15	0.0009	4.44E-12
EAL50986	XP_656369.1	219	*Bacteroides thetaiotaomicron*	240	31	*Xenopus laevis*	309	29	2.00E-20	1.00E-12	2.00E-08
EAL52121	XP_657511.1	220	*T. tengcongensis*	222	36	*Caenorhabditis elegans*	255	26	1.00E-30	1.00E-07	1.00E-23

(continued)

TABLE 2.8 *(continued)*

Acc.[a]	RefSeq Acc.[b]	EhL[b]	Top prokaryotic BLAST hit	PL[c]	% ID[d]	Top eukaryotic BLAST hit	EL[c]	%ID[d]	PE-score[e]	EE-score[f]	P/E ratio[g]
14 cases where increased sampling has weakened that case for LGT											
EAL42539	XP_647925.1	213	*Bacteroides thetaiotaomicron*	319	47	*Entodinium caudatum*	411	43	3.00E-53	1.00E-32	3.00E-21
EAL42738	XP_648124.1	313	*Campylobacter jejuni*	324	40	*Trichomonas vaginalis*	313	36	1.00E-63	4.00E-42	2.50E-22
EAL44270	XP_649657.1	179	*Methanococcus maripaludis*	193	37	*Anopheles gambiae*	186	21	2.00E-27	2.00E-09	1.00E-18
EAL44593	XP_649979.1	220	*Vibrio vulnificus*	244	24	*Trichomonas vaginalis*	238	21	0.0002	2.6	7.69E-05
EAL45320	XP_650707.1	154	*Geobacillus kaustophilus*	183	53	*Thalassiosira pseudonana*	182	43	8.00E-38	2.00E-32	4.00E-06
EAL45332	XP_650718.1	392	*Methanosarcina acetivorans*	420	48	*Trichomonas vaginalis*	396	47	8.00E-99	2.00E-93	4.00E-06
EAL45528	XP_650913.1	349	*Sulfolobus acidocaldarius*	343	28	*Cyanophora paradoxa*	313	27	1.00E-24	5.00E-17	2.00E-08
EAL45907	XP_651293.1	380	*Streptomyces coelicolor*	603	32	*Dictyostelium discoideum*	457	30	2.00E-39	2.00E-35	1.00E-04
EAL46026	XP_651412.1	176	*Bacteroides fragilis*	184	51	*Tetrahymena thermophila*	323	32	2.00E-44	8.00E-08	2.50E-37
EAL46116	XP_651488.1	662	*Bacillus clausii*	684	48	*Solanum tuberosum*	761	48	0	1.00E-172	0.00E + 00
EAL46656	XP_652044.1	419	*Dictyoglomus thermophilum*	579	30	*S. pombe*	493	41	2.00E-35	2.00E-19	1.00E-16
EAL50605	XP_655990.1	392	*Thermotoga maritima*	417	38	*Cryptococcus neoformans*	445	30	2.00E-69	1.00E-33	2.00E-36
EAL51270	XP_656656.1	251	*Porphyromonas gingivalis*	261	50	*Anopheles gambiae*	272	39	6.00E-53	1.00E-35	6.00E-18

EAL52102	XP_657492.1	345	*Bacteroides thetaiotaomicron*	358	54	*Thalassiosira pseudonana*	354	47	1.00E-105	7.00E-86	1.43E-20
Nine cases where *Entamoeba* is now recovered with a recently sequenced gene from another microbial eukaryote											
EAL44213	XP_649600.1	710	*Bdellovibrio bacteriovorus*	698	37	*Trichomonas vaginalis*	713	35	1.00E-127	1.00E-127	1.00E + 00
EAL44435	XP_649823.1	250	*Bacteroides fragilis*	395	40	*Trichomonas vaginalis*	395	33	1.00E-43	3.00E-35	3.33E-09
EAL44766	XP_650152.1	401	*Porphyromonas gingivalis*	419	36	*Trichomonas vaginalis*	445	32	3.00E-65	1.00E-51	3.00E-14
EAL47785	XP_653171.1	234	*Bacillus anthracis*	242	32	*Trichomonas vaginalis*	256	39	2.00E-30	3.00E-33	6.67E + 02
EAL47859	XP_653246.1	337	*Clostridium acetobutylicum*	322	50	*C. reinhardtii*	352	44	9.00E-74	0	N/A
EAL49158	XP_654544.1	397	*T. tengcongensis*	412	49	*Trichomonas vaginalis*	416	46	1.00E-100	4.00E-99	2.50E-02
EAL49488	XP_654874.1	320	*Geobacter sulfurreducens*	336	34	*Leishmania major*	357	31	1.00E-38	4.00E-30	2.50E-09
EAL49791	XP_655177.1	164	*Oceanobacillus iheyensis*	177	42	*Thalassiosira pseudonana*	96	38	8.00E-30	6.00E-09	1.33E-21
EAL50404	XP_655790.1	718	*T. tengcongensis*	717	37	*Trichomonas vaginalis*	721	34	1.00E-139	1.00E-118	1.00E-21
Five cases where vertical inheritance is now the simplest explanation for the new tree											
EAL44346	XP_649732.1	314	*Oceanobacillus iheyensis*	239	47	*Dictyostelium discoideum*	278	65	1.00E-52	3.00E-95	3.33E + 42

(*continued*)

TABLE 2.8 (*continued*)

Acc.[a]	RefSeq Acc.[b]	EhL[b]	Top prokaryotic BLAST hit	PL[c]	% ID[d]	Top eukaryotic BLAST hit	EL[c]	%ID[d]	PE-score[e]	EE-score[f]	P/E ratio[g]
EAL45466	XP_650849.1	209	*Agrobacterium tumefaciens*	254	31	*Thalassiosira pseudonana*	227	35	3.00E-23	1.00E-27	3.00E + 04
EAL45548	XP_650934.1	259	*Bacillus cereus (strain ZK)*	233	29	*Candida glabrata*	270	30	7.00E-06	5.00E-05	1.40E-01
EAL45595	XP_650981.1	284	*Pyrobaculum aerophilum*	293	27	*Ashbya gossypii*	343	27	1.00E-23	7.00E-16	1.43E-08
EAL50185	XP_655571.1	186	*Aeropyrum pernix*	192	31	*Thalassiosira pseudonana*	149	30	4.00E-13	5.00E-06	8.00E-08

Abbreviated taxon names: *Chlamydomonas reinhardtii: C. reinhardtii; Paracoccidioides brasiliensis: P. brasiliensis; Schizosaccharomyces pombe: S. pombe; Thermoanaerobacter tengcongensis: T. tengcongensis.*

Note: All 96 trees reanalysed here can be downloaded (in pdf format) from the following web site: http://www.ncl.ac.uk/microbial_eukaryotes/

[a] GenBank accession numbers and RefSeq accession numbers, respectively, for the 96 original candidates LGT identified by phylogenetic analysis (Loftus *et al.*, 2005).

[b] EhL, the length of the *E. histolytica* protein.

[c] PL/EL, the protein length of the prokaryotic or eukaryotic top BlastP hit, respectively.

[d] %ID, the percent identity between the *E. histolytica* protein and the top prokaryotic or eukaryotic protein in BlastP alignments (in respective columns).

[e] PE-score, the e-score of the top, prokaryotic hit.

[f] EE-score, the e-score of the top eukaryotic hit.

[g] P/E ratio, the e-score ratio between the top prokaryotic hit and top eukaryotic hit.

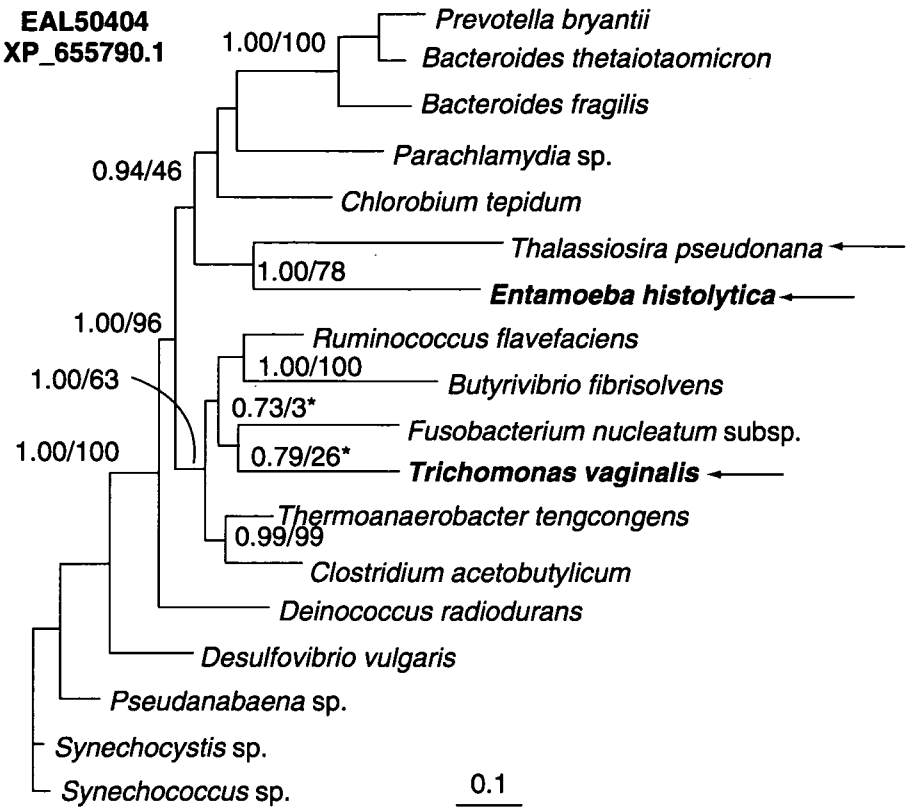

FIGURE 2.12 Phylogenetic relationships of *E. histolytica* glutamine synthase. The gene encoding glutamine synthase (EC 6.3.1.2) is now shared by *E. histolytica* and the diatom *Thalassiosira*. This gene is mainly restricted to prokaryotic genomes (eukaryotes are highlighted by arrows). *T. vaginalis* also contains a homologue but in this case it clusters weakly with *Fusobacterium*. The scale bar represents 10% of inferred sequence divergence. Both the GenBank and RefSeq accession numbers are given for the *E. histolytica* entry. The tree is the consensus Mr Bayes tree with support values corresponding to the posterior probabilities of the Bayesian analysis followed by the bootstrap support value of the equivalent node in maximum likelihood distance analysis. Only a selection of the most relevant support values are shown. A star indicates that the maximum likelihood analysis did not recover the node found in the Bayesian analysis.

environment may share a set of niche-specific genes (Beiko *et al.*, 2005; Mira *et al.*, 2004).

For five trees, the *E. histolytica* gene now appears to be present in eukaryotes from a different taxonomic group and the analysis cannot exclude a common origin for all eukaryotic sequences. Thus, for about 5% of the original 96 cases the simplest explanation is no longer LGT, but vertical inheritance from a common ancestor shared with other eukaryotes.

10.2. Where do the genes come from?

As before, certain prokaryotic groups are favoured as the potential donors of LGT genes in the *E. histolytica* genome (Loftus *et al.*, 2005). In 15 well-resolved trees *E. histolytica* is recovered next to a member of the

Bacteroidetes/Chlorobi group. Bacteroidetes/Chlorobi are abundant members of the intestinal microflora (Shoemaker *et al.*, 2001), providing plenty of opportunities for LGT to occur. Members of the Bacteroidetes/Chlorobi and *Fusobacterium* (one tree) groups are all obligate anaerobes. This bias is consistent with the idea that prokaryotic and eukaryotic cohabitants of the same anaerobic niche are sharing genes (Andersson *et al.*, 2001; Beiko *et al.*, 2005; Lawrence, 2005b). For example, Fig. 2.13 shows an intriguing example where the *T. vaginalis* gene clusters with members of the Bacteroidetes/Chlorobi and *E. histolytica* clusters with *Fusobacterium*.

10.3. What kinds of gene are being transferred?

Most of the 68 laterally transferred genes that can be assigned to a functional category encode enzymes involved in metabolism (Fig. 2.14). This is consistent with the complexity hypothesis, which posits that LGT of genes involved in processing a single substrate are more likely to be transferred than those genes encoding proteins that interact with many other cellular components, such as ribosomal proteins for example (Jain *et al.*, 1999). Mapping the LGT enzymes on the *E. histolytica* metabolic pathway (Loftus *et al.*, 2005) indicates that LGT has affected some important pathways, including iron–sulphur cluster biosynthesis, amino acid metabolism and nucleotide metabolism. Since only 8 of the 68 LGT have obvious homologues in the human genome, the proteins are potentially specific to the parasite and may thus be worth exploring as potential drug targets. The rest of the LGT cases involve hypothetical or unclassified proteins.

11. MICROARRAY ANALYSIS

Microarray-based analyses can be utilised in conjunction with genome sequencing to assign functional roles to annotated genes and to clarify genomic architecture. A number of groups have utilised DNA microarrays in *E. histolytica* (made from random genomic DNA fragments or long or short oligonucleotides based on annotated genes) to successfully study transcriptional differences between virulent and avirulent *E. histolytica* as well as transcriptional responses to heat shock, collagen and calcium exposure, tissue invasion and cyst development (Debnath *et al.*, 2004; Gilchrist *et al.*, 2006; MacFarlane and Singh, 2006; Weber *et al.*, 2006; Ehrenkaufer *et al.*, 2007). Additionally, using a genomic DNA microarray, comparative genomic hybridisations (CGH) between strains and species of *Entamoeba* have been performed (Shah *et al.*, 2005).

Some interesting aspects of amoebic biology have been uncovered using DNA microarray-based expression profiling. To investigate the hypothesis that virulence determinants will be more highly expressed in

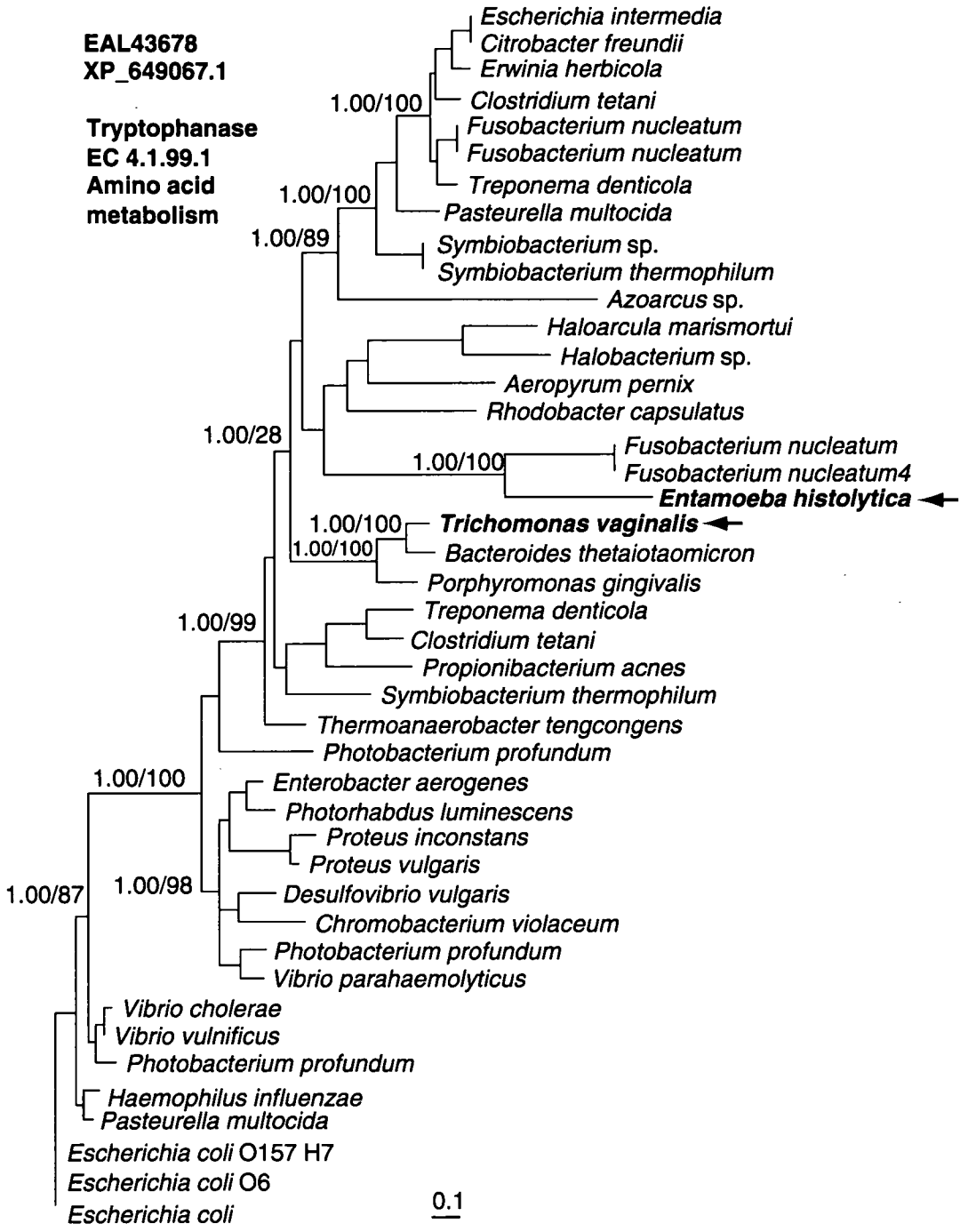

FIGURE 2.13 Phylogenetic relationships of *E. histolytica* tryptophanase. This tree suggests that the *E. histolytica* gene encoding a tryptophanase was acquired by LGT from a relative of the anaerobic bacterium *Fusobacterium*. In contrast, the *T. vaginalis* gene appears to have a separate origin with an LGT from a relative of the anaerobic *Bacteroides* group. The scale bar represents 10% of inferred sequence divergence. Both the GenBank and RefSeq accession numbers are given for the *E. histolytica* entry. The EC number is also shown. Analysis details as for figure 2.12.

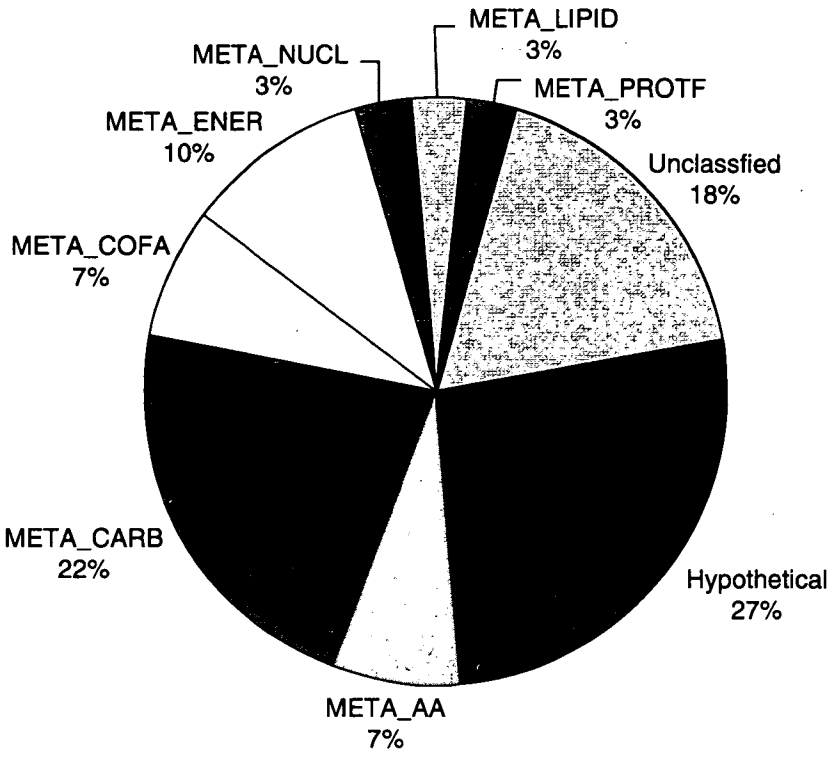

FIGURE 2.14 Pie chart of functional categories for the 68 strongest LGT cases. The cases are those discussed in the text and listed in Table 2.8. Most entries encode metabolic enzymes (KEGG annotation).

virulent strains, the transcriptomes of virulent and avirulent *Entamoeba* species and strains have been studied. It has been confirmed that a number of known virulence determinants have decreased expression in avirulent *Entamoeba* (Davis *et al.*, 2007; MacFarlane and Singh, 2006). A genomic DNA microarray composed of 2,110 genes identified 29 genes with decreased expression in both an attenuated *E. histolytica* strain (Rahman) and the avirulent *E. dispar* (strain SAW760) (MacFarlane and Singh, 2006), while an oligonucleotide microarray composed of 6,242 genes identified 152 genes with a higher level of expression in the virulent *E. histolytica* HM-1:IMSS than in the attenuated Rahman strain (Davis *et al.*, 2007). A majority of these genes are annotated as hypothetical and whether these genes encode novel virulence factors will require genetic analysis of their functions. A peroxiredoxin gene identified as having decreased expression in *E. histolytica* Rahman has been shown to be a virulence factor (Davis *et al.*, 2006), indicating that these comparisons between virulent and avirulent strains are likely to be a fruitful avenue of investigation.

In other microarray-based studies, the large family of transmembrane receptor kinases identified in *E. histolytica* has been found to be differentially expressed under *in vitro* trophozoite culture conditions (Beck *et al.*, 2005). One can easily envision that these kinases may have roles in signalling, allowing the parasite to adapt to its ever-changing

environmental milieu. A substantial transcriptional response to heat shock has been demonstrated (Weber *et al.*, 2006), and interestingly lectin gene family members were identified as being differentially regulated under heat shock conditions.

The most comprehensive microarray data to date used a whole genome short oligonucleotide microarray (based on the Affymetrix platform) to profile the transcriptional changes that occur as the parasite colonises and invades the host colon (Gilchrist *et al.*, 2006). Using a mouse model of colitis, in which the microscopic features replicate human disease and substantial pathology can be seen, the transcriptional response of parasites was assayed soon after colonisation (one day after injection into the caecum) and in a long-term (29 days) disease state. Overall, 326 genes were modulated at day 1 after infection, 109 at 29 days after infection, and 88 at both time points. A number of the well-characterised 'virulence determinants' in *E. histolytica* were highly expressed under all conditions tested and not transcriptionally modulated, although some members of the cysteine protease gene family were highly regulated during tissue invasion. A summary of the genes and gene families that have been identified as being transcriptionally active under the conditions mentioned above is listed in Table 2.9.

The life cycle of *E. histolytica* involves transition between the trophozoite stage, responsible for colonisation as well as invasive disease, and the cyst, responsible for infection transmission. Despite its central role, little is known about cyst development in *E. histolytica*, largely due to our inability to generate *E. histolytica* cysts in axenic culture. Using a whole genome microarray and xenic cultures of recently isolated *E. histolytica* strains that contained spontaneously produced cysts, a cyst transcriptome was developed that identified 1,439 developmentally regulated genes (672 cyst-specific and 767 trophozoite-specific genes; Ehrenkaufer *et al.*, 2007). This first large-scale insight into encystation indicates that ~15% of *E. histolytica* genes are transcriptionally controlled in this developmental pathway. Among the genes identified were a number of stage-specific cysteine proteases, transmembrane kinases, transcriptional regulators and other potential initiators of the developmental cascade. Future characterisation of these genes and pathways will provide important insights into developmental processes in this parasite.

The above microarray studies used expression data to identify interesting genes and pathways potentially involved in amoebic pathogenesis or development. In another application of microarrays, CGH identified a number of interesting genomic characteristics of *Entamoeba* (Shah *et al.*, 2005). The *E. histolytica* genome project revealed that a large number of genes are multi-copy or members of highly similar gene families. Due to the repetitive nature of the genome there has been difficulty with genome assembly and thus the large number of gene duplications could have

TABLE 2.9 Examples of microarray-detected transcriptional changes in some gene families and the conditions tested

Gene family	Total number of genes family	Number of genes transcriptionally regulated under condition tested	
		Heat shock[a] (1,131 genes on array)	Host colonisation and invasion[b] (9,435 genes on array)
Cysteine proteases	29[c]	2 up-regulated (CPs 6, 4); 7 down-regulated (CPs 1, 2, 3, 8, 13, 17),	21 genes on array; 4 up-regulated (CPs 1, 9, 4, 6); 1 down-regulated (CP8)
Lectin (heavy, light, and intermediate subunits)	12	1 up-regulated (Hgl-2); 5 down-regulated (Lgl-1 and 3, Igl-1 and -2, Hgl-3)	No change in heavy or intermediate subunits; Light subunit lgl-2 and lgl-3 down-regulated)
Amoebapore	3	1 down-regulated (amoebapore C)	No substantial changes
Transmembrane receptor kinases	>80	NA	6 up-regulated (TMKs 69, 53, 95, 105, 63, 56); 2 down-regulated (TMKs 03 and 17)
AIG-1 (similar to plant antibacterial proteins)	15	NA	5 up-regulated at day 1; 6 down-regulated at day 29 (all non-overlapping)

[a] Adapted from Weber *et al.* (2006).
[b] Adapted from Gilchrist *et al.* (2006).
[c] Number of cysteine protease gene families in genome annotation at time studies were performed.

represented an assembly artefact. The data from CGH confirmed the high copy number of a significant portion (~14%) of the genome and validated the genome assembly. Additionally, genome-wide genetic diversity was demonstrated among strains of *E. histolytica* (Shah *et al.*, 2005), including the

observation that the attenuated *E. histolytica* strain Rahman had a unique genetic pattern suggesting the possibility that a genomic signature may correlate with invasive potential. Since genome sequencing for different *E. histolytica* strains, including clinical isolates, is unlikely the promise of CGH to study genetic diversity and identify genotype-phenotype associations is substantial.

E. dispar, the closely related but avirulent species, had been identified early on as having some genetic divergence from the virulent *E. histolytica*. CGH analysis of *E. histolytica* and *E. dispar* revealed a significant amount of difference between the two species. Whether the genetic drift in these genes is responsible for the non-invasive phenotype of *E. dispar* is not known, but the work has highlighted a number of genes for further functional analyses.

Taken together the DNA microarray analyses of *Entamoeba* have been useful to begin to dissect the genome of this parasite and provide functional context to the genes identified in the genome sequencing effort. Future directions will include analysis of the parasite transcriptome in invasive hepatic disease as well as further characterisation of the developmental conversion to the cyst form. Those data may be useful in the development of novel diagnostic and therapeutic options. Additionally, genetic approaches can now be applied to definitively assign a role for these genes in amoebic biology and pathogenesis.

12. FUTURE PROSPECTS FOR THE *E. HISTOLYTICA* GENOME

Although the genome of *E. histolytica* is not yet complete, it has already revealed much about the biology of the parasite. There appear to be forces acting to compact the genome, leading to a reduction in the coding region and intron length of genes, and resulting in the loss of numerous metabolic pathways. However, there are also opposing evolutionary forces as many gene families have expanded. This applies particularly to genes involved in signalling and trafficking that allow the parasite to sense and respond to its environment, a necessary adaptation for a predatory protist. Unfortunately, it is difficult at present to understand the genome structure on a macro scale due to the fragmented nature of the current assembly. In other parasites, genome structure has been vital to unravelling important biological processes, such as antigenic variation in *T. brucei* and identification of rifin genes in *P. falciparum*. Until the *E. histolytica* genome is complete we will not know what else remains to be uncovered. Efforts are already under way to complete the genome by first generating a HAPPY map (Dear and Cook, 1993). Over 2000 markers are being designed at ~25-kb intervals across all contigs. Using PCR, co-segregation

analysis allows the identification of contigs that are physically linked in the genome. This will allow the ordering and orientation of the contigs and will facilitate gap closure. Shotgun genome sequencing projects of *E. invadens* and *E. dispar* are under way (Loftus and Hall, 2005). At present the *E. invadens* genome appears to assemble with fewer problems than were encountered with that of *E. histolytica*. It is anticipated that an essentially complete *E. invadens* genome sequence will be obtained, enabling extensive comparative analyses to be made, and facilitating the study of pathogenicity, host interaction and the evolutionary forces acting on the genome.

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