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Heme biosynthesis and regulation in the filamentous fungus *Aspergillus niger*

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supplementary data

Supplementary Table 2.1: BlastP results of reported proteins involved in synthesis of heme against *Aspergillus niger* CBS 513.88 (taxid: 425011). N.P. = not present, * = no significant blast & no bidirectional match. Mito: mitochondria; Cyto: cytoplasm; Nucl: nucleus; O : Any other location; mTP: mitochondrial targeting peptide; SP: signal peptide; Loc: Prediction of localization; RC: Reliability class; Tplen: Predicted presequence length;. Prediction tools used: WoLF PSORT, MitoProt II and TargetP (available on www.expasy.org/tools/ and <http://wolfpsort.org/>).

	protein name	Protein (accession version)	Protein (accession version)	identifier (AnXXgXXXXX)	Bit score	E-value	Bit score	E-value
		<i>E. coli</i>	<i>S. cerevisiae</i>		<i>E. coli</i>		<i>S. cerevisiae</i>	
heme biosynthesis	aminolevulinic acid synthase (5-aminolevulinatase synthase)	N.P.	hem1p (CAA88511.1)	An17g01480			582	7,00E-167
	5-aminolevulinic acid dehydratase (porphobilinogen synthase, 5-aminolevulinic acid dehydrase)	hemB (CAQ30840.1)	hem2p (NP_011475.1)	An08g00010	211	2E-55	454	2,00E-128
	Porphobilinogen deaminase (hydroxymethylbilane synthase, Uro I synthase)	hemC (ABD20330.1)	hem3p (NP_010076.1)	An18g02970	190	4E-49	286	5,00E-78
				An01g08640	177	4E-45	251	2,00E-67
	Uroporphyrinogen III synthase (Uro III synthase, Uro III Cosynthase)	hemD; no bidirectional hit	hem4p (NP_014921.1)	An08g10680			101	3,00E-22
	Uroporphyrinogen III decarboxylase (Uro D, Uroporphyrinogen decarboxylase)	hemE (CAQ34344.1)	hem12p (NP_010332.1)	An01g04250	279	1E-75	471	2,00E-133
	Coproporphyrinogen III oxidase (coproporphyrinogenase)	hemF (CAQ32815.1)	hem13p (NP_010329.1)	An07g10040	251	2E-67	357	3,00E-99
	protoporphyrinogen oxidase Ferrochelatase (ferrolyase, protoheme ferrolyase, heme synthase)	hemG, anaerobe PPO hemH (CAQ30948.1)	hem 14p (NP_010930.1) hem15p (NP_014819.1)	An11g01580 An15g02690	90.1	8E-19	407	4,00E-114
Siroheme synthesis		cysG (CAQ33688.1)	met1p (NP_012995.1)	An11g09700	156	1E-38	408	2,00E-114
			met8p (NP_009772.1)	An07g02200	69.3	2,00E-12	78,4	4E-18

Supplementary Table 2.1 (continued)

similarity to:	MitoProt II: probability of export to mitochondria	MitoProt II predicted cleavage site	TargetP 1.1						predicted localization WoLFPSORT prediction
			mTP	SP	other	Loc	RC	Tplen	
strong similarity to 5-aminolevulinic acid synthase <i>hemA</i> - <i>Aspergillus nidulans</i>	0.9989	89	0.741	0.035	0.365	M	4	65	mito: 25.5, cyto_mito: 14.0
strong similarity to porphobilinogen synthase <i>hem2</i> <i>Candida glabrata</i>	0.4983	not predictable	0.349	0.030	0.761	-	3	-	cyto: 10.5, cysk: 8.0, cyto_nucl: 7.0, mito: 3.0, extr: 3.0
strong similarity to hydroxymethylbilane synthase <i>Hem3</i> - <i>Saccharomyces cerevisiae</i>	0.2069	not predictable	0.484	0.081	0.408	M	5	66	mito: 23.0, cyto: 2.5, cyto_nucl: 2.5
strong similarity to hydroxymethylbilane synthase <i>Hem3</i> - <i>Saccharomyces cerevisiae</i>	0.1054	15	0.295	0.045	0.753	O	3	-	mito: 21.0, cyto: 5.5, cyto_nucl: 3.5
similarity to uroporphyrinogen-III synthase <i>Hem4</i> - <i>Saccharomyces cerevisiae</i>	0.0071	not predictable	0.085	0.119	0.889	O	2	-	cyto_nucl: 10.5, nucl: 9.5, cyto: 8.5, mito: 4.0, extr: 2.0
strong similarity to uroporphyrinogen decarboxylase from patent WO9925839-A1 - <i>Thielavia terrestris</i> ; cytoplasm	0.0775	not predictable	0.171	0.049	0.760	O	3	-	cyto: 14.5, cyto_nucl: 9.0, mito: 6.0, plas: 3.0
strong similarity to coproporphyrinogen oxidase III <i>Hem13</i> - <i>Saccharomyces cerevisiae</i>	0.9853	36	0.925	0.082	0.053	M	1	35	mito: 17.0, nucl: 5.0, cyto: 4.0
similarity to protoporphyrinogen oxidase PPO - <i>Mus musculus</i>	0.9980	56	0.995	0.016	0.072	M	1	83	mito: 25.0
strong similarity to ferrochelatase <i>fch</i> - <i>Danio rerio</i>	0.8411	17	0.928	0.017	0.115	M	1	74	mito: 25.0
similarity to siroheme synthase <i>cysG</i> - <i>Escherichia coli</i>	0.0826	not predictable	0.051	0.169	0.846	O	2	-	cyto: 21.0, nucl: 2.0, mito: 1.0, extr: 1.0
similarity to siroheme synthase <i>cysG</i> - <i>Escherichia coli</i>	0.0905	not predictable	0.221	0.180	430	O	4	-	cyto: 17.5, cyto_pero: 9.5, mito: 6.0

Supplementary Table 2.2: BlastP results of reported proteins involved in regulation of heme against *Aspergillus niger* CBS 513.88 (taxid: 425011). N.P. = not present, * = no significant blast & no bidirectional match. Mito: mitochondria; Cyto: cytoplasm; Nucl: nucleus; O: Any other location; mTP: mitochondrial targeting peptide; SP: signal peptide; Loc: Prediction of localization; RC: Reliability class; Tplen: Predicted presequence length. Prediction tools used: WoLF PSORT, MitoProt II and TargetP (available on www.expasy.org/tools/ and <http://wolfsort.org/>).

protein name	Protein (accession version)	Protein (accession version)	identifier (AnXXgXXXXX)	Bit score	E-value
	<i>Aspergillus sp</i>	<i>S. cerevisiae</i>			
heme activator protein 1		hap1p (NP_013357.1)	An16g07460	62,8	9,00E-10
heme activator protein 2		hap2p (NP_011277.1)	An15g03650	120	6,00E-28
heme activator protein 3		hap3p (NP_009532.1)	An01g02620	155	6,00E-39
heme activator protein 4		hap4p (NP_012813.1)	An01g02090	30,4	1,50E+00
heme activator protein 5		hap5p (NP_015003.1)	HapE [<i>Aspergillus niger</i>]	169	6,00E-43
Rox1		rox1p (NP_015390.1)	An04g05820	92	3,00E-19
Mot3		mot3p (NP_013786.1)	An04g09290	57,8	9,00E-09
mga2		mga2p (NP_012299.1)	An08g05200	161	1,00E-39
Aft1		aft1p (NP_011444.1)	An02g13160	31,2	1,30
Aft2		aft2p (NP_015122.1)	Unnamed protein product	28,9	3,20
hapX	<i>A. fumigatus hapX</i> (EAL85914.1)		An09g06280	603	5,00E-173
sreA	<i>A. nidulans sreA</i> (AAD25328.1)		An01g02370	664	0,00E+00

Supplementary Table 2.2 (continued)

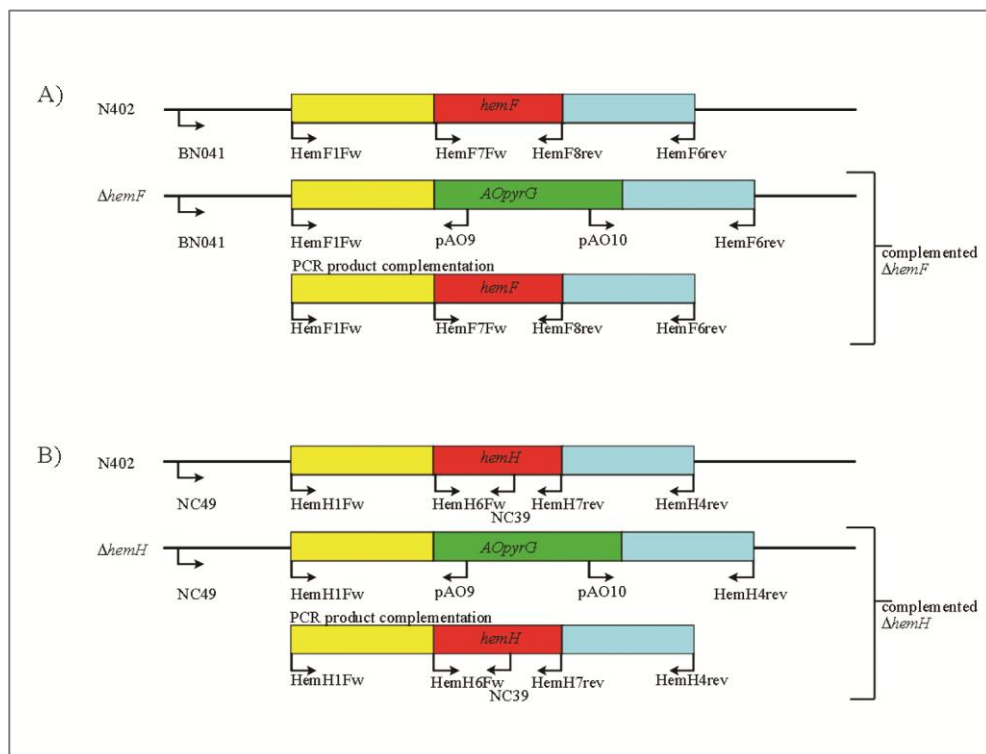
silarity to:	MitoProt II: probability of export to mitochondria	MitoProt II predicted cleavage site	TargetP 1.1						predicted localization WoLFPSORT prediction
			mTP	SP	other	Loc	RC	Tplen	
strong similarity to acetate regulatory DNA binding protein facB - <i>Aspergillus niger</i>	0.4806	49	0.270	0.068	0.669	O	4		nucl: 20.0, mito: 4.0, cyto: 2.0
strong similarity to protein hapB - <i>Aspergillus nidulans</i> ; nucleus	0.0301	202	0.203	0.046	0.877	O	2		nucl: 24.0, cyto_nucl: 14.0
strong similarity to protein hapC - <i>Aspergillus oryzae</i> ; nucleus	0.0215	not predictable	0.056	0.074	0.936	O	1		nucl: 20.0, cyto_nucl: 15.3, cyto: 6.5
similarity to hypothetical protein BX908807_12 - <i>Neurospora crassa</i>	0.0393	not predictable	0.059	0.096	0.927	O	1		nucl: 20.5, cyto_nucl: 13.0, cyto: 4.5
hapE	0.0012	24	0.161	0.050	0.862	O	2		nucl: 14.0, cyto_nucl: 11.5, cyto: 7.0, mito: 3.0
similarity to transcription regulator Rfg1 - <i>Candida albicans</i> * note N- terminal (HMG)	0.0387	not predictable	0.308	0.041	0.769	O	3		nucl: 20.0, cyto_nucl: 14.0, cyto: 6.0
strong similarity to DNA binding regulatory protein amdX - <i>Aspergillus nidulans</i> *	0.0484	not predictable	0.073	0.060	0.924	O	1		nucl: 23.0, cyto: 2.0
strong similarity to transcription suppressor protein Spt23 - <i>Saccharomyces cerevisiae</i> ; nucleus	0.0217	not predictable	0.046	0.084	0.947	O	1		cyto: 7.5, cyto_nucl: 7.0, nucl: 5.5, mito: 5.0, plas: 4.0, golg: 3.0
similarity to ferric reductase CFL1 - <i>Candida albicans</i> ; plasma membrane	0.0017	not predictable	0.107	0.063	0.898	O	2		plas: 24.0, E.R.: 2.0
similarity to hypothetical protein B13M15.020 - <i>Neurospora crassa</i>	0.7739	49	0.305	0.033	0.702	O	4		mito: 14.0, nucl: 8.5, cyto_nucl: 6.5, cyto: 3.5
strong similarity to hypothetical transcription factor BAB47239.1 - <i>Aspergillus oryzae</i>	0.5128	57	0.241	0.115	0.664	O	3		nucl: 24.0, cyto: 2.0
strong similarity to siderophore biosynthesis repressor sreA - <i>Aspergillus nidulans</i>	0.3334	not predictable	0.348	0.059	0.680	O	4		nucl: 26.0

Supplementary Table 2.3: BlastP results of reported proteins involved in transport of heme and intermediates against *Aspergillus niger* CBS 513.88 (taxid: 425011). N.P. = not present, * = no significant blast & no bidirectional match. Mito: mitochondria; Cyto: cytoplasm; Nucl: nucleus; O: Any other location; mTP: mitochondrial targeting peptide; SP: signal peptide; Loc: Prediction of localization; RC: Reliability class; Tplen: Predicted presequence length. Prediction tools used: WoLF PSORT, MitoProt II and TargetP (available on www.expasy.org/tools/ and <http://wolfsort.org/>).

protein name	Protein (accession version)	Protein (accession version)	identifier (AnXXgXXXX)	Bit score	E-value
	<i>Homo Sapiens</i>	<i>S. cerevisiae</i>			
porphyrin uptake gene 1		pug1p (NP_011112.1)	An16g00680	167	3,00E-42
flavin carrier 2		FLC2 (DAA06935.1)	An01g09050	478	5,00E-135
			An01g06610	425	3,00E-119
			An02g14710	164	1,00E-40
flavin carrier 3		FLC3 (DAA07971.1)	An01g09050	440	1,00E-123
			An01g06610	421	7,00E-118
			An02g14710	144	1,00E-34
flavin carrier 1		FLC1 (Q08967.1)	An01g09050	443	8,00E-125
			An01g06610	419	3,00E-117
			An02g14710	146	2,00E-35
ABCB6	AAH43423.1		An07g07500	325	9,00E-90
ABCB10	NP_036221.2		An04g07060	513	1,00E-145
2-OGC	CAA46905.1		An02g01730	216	7,00E-57
HCP1	Q96NT5.1		An15g02680	45,1	5,00E-05
FLVCR	AAH48312.1		An04g05210	124	1,00E-28
BCRP	AAC97367.1		An07g09170*	301	5,00E-82

Supplementary table 2.3 (continued)

silarity to:	MitoProt II: probability of export to mitochondria	MitoProt II predicted cleavage site	TargetP 1.1						predicted localization WoLFPSORT prediction
			mTP	SP	other	Loc	RC	Tplen	
strong similarity to molasses resistency protein Rtm1 - <i>Saccharomyces cerevisiae</i>	0.3398	37	0.027	0.844	0.125	S	2	36	plas: 17.0, mito: 5.0, E.R.: 4.0
similarity to calcium-related protein spray - <i>Neurospora crassa</i>	0.1328	12	0.019	0.979	0.038	S	1	22	plas: 17.0, E.R.: 4.0, extr: 3.0, golg: 2.0
strong similarity to hypothetical calcium-related spray protein 1A9.60 - <i>Neurospora crassa</i>	0.3344	not predictable	0.099	0.881	0.032	S	2	21	plas: 19.0, E.R.: 6.0
strong similarity to hypothetical calcium-related spray protein - <i>Neurospora crassa</i>	0.4218	29	0.102	0.863	0.028	S	2	22	plas: 20.0, extr: 3.0, E.R.: 3.0
similarity to calcium-related protein spray - <i>Neurospora crassa</i>	0.1328	12	0.019	0.979	0.038	S	1	22	plas: 17.0, E.R.: 4.0, extr: 3.0, golg: 2.0
strong similarity to hypothetical calcium-related spray protein 1A9.60 - <i>Neurospora crassa</i>	0.3344	not predictable	0.099	0.881	0.032	S	2	21	plas: 19.0, E.R.: 6.0
strong similarity to hypothetical calcium-related spray protein - <i>Neurospora crassa</i>	0.4218	29	0.102	0.863	0.028	S	2	22	plas: 20.0, extr: 3.0, E.R.: 3.0
similarity to calcium-related protein spray - <i>Neurospora crassa</i>	0.1328	12	0.019	0.979	0.038	S	1	22	plas: 17.0, E.R.: 4.0, extr: 3.0, golg: 2.0
strong similarity to hypothetical calcium-related spray protein 1A9.60 - <i>Neurospora crassa</i>	0.3344	not predictable	0.099	0.881	0.032	S	2	21	plas: 19.0, E.R.: 6.0
strong similarity to hypothetical calcium-related spray protein - <i>Neurospora crassa</i>	0.4218	29	0.102	0.863	0.028	S	2	22	plas: 20.0, extr: 3.0, E.R.: 3.0
strong similarity to ABC-type vacuolar membrane protein hmt1p - <i>Schizosaccharomyces pombe</i>	0.1262	65	0.054	0.741	0.147	S	3	38	plas: 20.0, E.R.: 4.0, extr: 2.0
strong similarity to peptide ABC transporter protein Mdl1 - <i>Saccharomyces cerevisiae</i>	0.8918	100	0.816	0.040	0.149	M	2	92	mito: 10.0, plas: 9.0, E.R.: 6.0
strong similarity to mitochondrial dicarboxylate transport protein Dtp - <i>Saccharomyces cerevisiae</i> ; localisation:mitochondrion	0.4450	103	0.369	0.075	0.583	O	4		mito: 22.0, nucl: 1.0, cyto: 1.0, plas: 1.0
strong similarity to tetracyclin resistance protein tetA - <i>Escherichia coli</i>	0.1541	11	0.444	0.020	0.644	O	5		plas: 20.0, E.R.: 5.0
strong similarity to feline leukemia virus subgroup C receptor FLVCR1 - <i>Felis catus</i>	0.0839	28	0.260	0.472	0.388	S	5	41	plas: 27.0
strong similarity to breast cancer resistance protein 1 BCRP1 - <i>Mus musculus</i>	0.2321	not predictable	0.164	0.883	0.016	S	2	23	plas: 16.0, extr: 8.0, E.R.: 2.0



Supplementary Fig 5.1: Schematic overview of the localization of the primers used for the complementation of A) $\Delta hemF$ and B) $\Delta hemH$. All possible primer combinations were applied during diagnostic PCR, generating fragments as described in Table 5.2.

