

1 Title:

2 Protein interactions of *Magnaporthe oryzae* protein
3 kinase CK2 and secondary data analysis of a large
4 number of transcriptomes suggest chaperone-like activity
5 is integral to its function

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19 **ABSTRACT**

20 CK2, a serine/threonine (S/T) kinase present in eukaryotic cells is known to have a vast number of
21 substrates. We have recently shown that it localizes to nuclei and at pores between hyphal
22 compartments in *M. oryzae*. We performed a pulldown-proteomics of *M. oryzae* CK2 catalytic subunit
23 MoCKa to detect interacting proteins. The MoCKa pulldown was enriched for septa and nucleoli
24 proteins and intrinsically disordered proteins (IDPs) containing a CK2 phosphorylation motif proposed
25 to destabilize and unfold alpha helices. This points to a function for CK2 phosphorylation and
26 corresponding phosphatase dephosphorylation in the formation of functional protein-protein aggregates
27 and protein-RNA/DNA binding. To test this as widely as possible we used secondary data downloaded

28 from databases from a large range of *M. oryzae* experiments and also for a relatively closely related
29 plant pathogenic fungus, *Fusarium graminearum*. We found that CKa expression was strongly
30 positively correlated with S/T phosphatases as well as with disaggregase (HSP104, YDJ1, SSA1) and
31 an autophagy indicating protein (ATG8). The latter points to increased protein aggregate formation at
32 high levels of CKa expression. Our results suggest a general role for CK2 in aggregation and
33 disaggregation of IDPs and their binding to proteins, DNA and RNA interactions.

34

35

36 INTRODUCTION

37 CK2 is a serine/threonine (S/T) kinase constitutively active in eukaryotes (Meggio and Pinna, 2003).
38 The CK2 holoenzyme is a heterotetrameric structure consisting of two α -units and two regulatory β -
39 subunits (Ahmad *et al.*, 2008). In yeast these units can each be of two types and are named $\alpha 1$, $\alpha 2$, $\beta 1$
40 and $\beta 2$ (Padmanabha *et al.*, 1990) while several other fungi, including *M. oryzae*, only contain one CK α
41 (Zhang *et al.*, 2019). CK2 have been shown to phosphorylates a large number of proteins (Meggio and
42 Pinna, 2003; Ahmad *et al.*, 2008; Götz and Montenarh, 2016) and seems involved in many cellular
43 processes (Götz and Montenarh, 2016). Interestingly, intrinsically highly disordered SRP40 protein in
44 yeast (homologous to Nopp140; Insect, *Drosophila* and Ndc1; Mammal, Human) has been shown to
45 become phosphorylated to varying degrees by CK2 with effects on the SRP40 conformation, binding,
46 and aggregation properties with further effects on the diverse functions of the protein (Tantos *et al.*,
47 2013; Na *et al.*, 2018). The large number of substrates of CK2, could possibly be explained if CK2
48 phosphorylation destabilizes/unfolds alpha helices in IDPs (Zetina, 2001) and CK2 activity in
49 combination with phosphatases facilitates conformation changes and binding of IDPs.

50 We have previously argued that filamentous fungi are better models than yeast for investigating
51 eukaryotic cellular functions involved in cell-cell communication when we studied CK2 localization
52 and involvement in pathogenesis (Zhang *et al.*, 2019). We showed that the *M. oryzae* CK2 holoenzyme
53 (MoCK2) accumulates in the nucleolus, that it localizes in structures near septal pores, and assembles
54 to form a large ring structure perpendicular to the appressorium penetration pore (Zhang *et al.*, 2019).

55 To further investigate the general hypothesis that CK2 is especially involved in phosphorylating IDPs
56 and proteins known to be localized at septa and in the nucleolus we made a pulldown followed by
57 proteomics using the GFP-CK α (Zhang *et al.*, 2019) as our bait to find which proteins CK2 interacts
58 with. Since CK2 is known to interact with a large number of proteins (Meggio and Pinna, 2003) we
59 expected to find many proteins, and we did. To aid in interpreting the results we made a functional
60 classification of the interacting proteins. The pulldown was overrepresented for proteins known to be
61 present in nucleoli and at septa as expected and we found that CK2 interacts preferentially with
62 intrinsically disordered proteins (IDPs) carrying a phosphorylation motif that can destabilize alpha
63 helix folding (Zetina, 2001).

64 To test this destabilization/unfolding hypothesis in a general way we decided to make an analysis of
65 secondary data already published (Smith *et al.*, 2011; Zaritsky, 2018) and downloaded a large range of
66 transcriptomic data for *M. oryzae*. The following hypotheses resulting from the general hypothesis
67 were tested: Sub-hypothesis 1: If unfolding-refolding activity is important for IDP aggregation then a
68 high CKa expression should be correlated with expression of some S/T phosphatases present in the
69 pulldown that can act as a counterpart to the CK2 S/T kinase activity. Sub-hypothesis 2: Higher general
70 aggregation activity should lead to more aggregates of misfolded proteins, especially of IDPs.
71 Disaggregases and ATG8 are known to be needed to remove aggregates formed from misfolded
72 proteins (Glover and Lindquist, 1998; Wong and Cuervo, 2010; Watabe and Nakaki, 2011, 2012) and
73 the expression of these genes should be correlated with CKa expression. The correlations implied by
74 Sub-hypothesis 2 should also apply for other fungi. To test this hypothesis further we thus downloaded
75 a set of secondary data for another plant pathogenic fungus, *Fusarium graminearum*, and tested if
76 similar correlations between homologous genes apply. We found that CK2 expression correlates with
77 several CK2 phosphatase expression supporting the first sub-hypothesis. We also found that CK2
78 expression correlates with both disaggregase gene expression and ATG8 in both fungi tested giving
79 support for the second sub-hypothesis.

80 Our work provides evidence supporting the view that one of the main roles for the CK2 holoenzyme is
81 as a general inducer of binding, conformational changes and aggregate formation of intrinsically
82 disordered proteins.

83

84 **RESULTS**

85 **Identification of potential septal and nucleolar and other substrates for MoCK2 by GFP-CKa** 86 **pulldown and investigation if these proteins contain an CK2 phosphorylation unfolding motif.**

87 The CK2 localization pattern suggested that CK2 may have substrates associated with septa and
88 nucleolar function (Zhang *et al.*, 2019). To explore this, we performed co-immunoprecipitation to
89 identify proteins interacting with CK2 using GFP-CKa as a "bait", and in addition to the bait, identified
90 1505 proteins (**Data S1**). There is the risk of false positives in a pulldown. We estimated the number of
91 potentially false positives and removed 155 (~10%) of the lower abundance proteins to arrive at a list
92 of 1350 CKa interacting proteins (see Methods). Both CKb1 and CKb2 were found in the pulldown

93 with GFP-CKa (Data S1) although the amounts found in the pulldown were not supporting that 2CKa
94 are always interacting with 1 CKb1 and 1 CKb2 to form the CK2 holoenzyme (Zhang *et al.*, 2019). The
95 CK2 holoenzyme can be formed with only type of CKb subunit (Ahmad *et al.*, 2008) although our
96 previous results showed that both MoCKb subunits are needed for normal growth and pathogenicity of
97 *M. oryzae* (Zhang *et al.*, 2019).

98 Since CK2 localizes to septa we looked for known septal proteins in the pulldown. All previously
99 identified proteins by Dagas et al. (Dagdas *et al.*, 2012) that are involved in appressorium pore
100 development, were found in the pulldown as was a protein annotated as the main Woronin body protein,
101 Hex1 (MGG_02696). Since the Woronin body in Ascomycetes is tethered to the septal rim by Lah
102 protein (Ng *et al.*, 2009; Plamann, 2009; Han *et al.*, 2014) we searched for a homologue in *M. oryzae*
103 and found a putative MoLah (MGG_01625) with a similar structure as in *Aspergillus oryzae* (Han *et al.*,
104 2014) that is also present in the pulldown. In addition to the Lah, 18 other intrinsically disordered
105 septal pore associated proteins (Spa) were described for *Neurospora crassa* (Lai *et al.*, 2012). We
106 identified putative orthologs for 15 of the 18 Spa proteins in *M. oryzae* (See link from **Table 1**). Of these
107 putative MoSpa proteins, six were present in the CKa pulldown, MoSpa3 (MGG_02701), MoSpa5
108 (MGG_13498), MoSpa7 (MGG_15285), MoSpa11 (MGG_16445), MoSpa14 (MGG_03714) and
109 MoSpa15 (MGG_15226). MoSpa3, MoSpa5 and MoSpa15 also contain the CK2 phosphorylation
110 alpha helix unfolding motif (**Data S1**). The presence of the a MoLah and several MoSpa homologues
111 in the CKa pulldown is in agreement with the view that localization of the GFP-fusion protein (Zhang
112 *et al.*, 2019) gives a proper representation of CK2 localization. In addition, and further supporting this
113 conclusion, the six septal pore associated proteins (SPA) are homologues for intrinsically disordered
114 proteins that are expected to form temporary gels that are used to reversibly plug septal pores and
115 regulate traffic through septa (Lai *et al.*, 2012). CK2 could actively be involved in the gelling/un-
116 gelling of the regions near septa to create a membraneless organelle controlling the flow through septa.

117

118 Since many other of the proteins we found in the pulldown were IDPs we also searched the *M. oryzae*
119 proteome for proteins containing the CK2 phosphorylation helix unfolding motif identified by Zetina
120 (Zetina, 2001) using the FIMO tool at the MEME-suite website (<http://meme-suite.org/>) and found 1465
121 proteins with the motif out of a total of 12827 proteins annotated for *M. oryzae*. We found 275 of the
122 proteins contain at least one unfolding motif for alpha helixes (Zetina, 2001). Thus, there is an

123 overrepresentation of the motif among the pulldown proteins (P-value for the null hypothesis of same
124 frequency as in the whole proteome = 4×10^{-19} , Fisher's Exact test) lending support for the proposed role
125 for this motif in these proteins as a target for CK2 phosphorylation and protein unfolding. The finding
126 of overrepresentation of this signal in the set of CK2 interacting proteins corroborates the previous
127 suggestion that CK2 is involved in the destabilization/binding of intrinsically disordered proteins
128 (Zetina, 2001; Tantos *et al.*, 2013; Na *et al.*, 2018) and is consistent with the strong accumulation of
129 both CK2 and intrinsically disordered proteins in the nucleolus (Frege and Uversky, 2015) and also at
130 pores between cell compartments (Lai *et al.*, 2012; Zhang *et al.*, 2019).

131 To further explore the general hypothesis that CK2 could interact with and possibly phosphorylate
132 intrinsically unfolded proteins we used FungiFun (<https://elbe.hki-jena.de/fungifun/>) to make a
133 functional classification of the pulldown proteins with a special interest for those containing the alpha
134 helix unfolding motif (Zetina, 2001). All the protein categorization classes for the pulldown is
135 accessible at the FungiFun entry in **Table 1**. We found strong overrepresentation for proteins involved
136 in rRNA processing among the pulldown proteins containing the alpha helix unfolding motif (Funcat
137 category, 11.04.01 rRNA processing, *p*-value for same abundance as in whole genome = 1.53×10^{-26}) as
138 well as for proteins that, themselves, are known to interact with other proteins, DNA, and RNA (16.01
139 protein binding *p* 1.07×10^{-9} , 16.03.01 DNA binding *p* 1.45×10^{-6} , 16.03.03 RNA binding *p* 2.77×10^{-11} , *p*-
140 values for same abundance as in whole genome). These classes of proteins are enriched for intrinsically
141 disordered proteins. Such intrinsically disordered proteins can interact with each other to form ordered
142 subregions that have been described as membraneless organelles, such as nucleoli (Wright and Dyson,
143 2015). Since CK2 localizes to the nucleolus (Zhang *et al.*, 2019) we were especially interested in the
144 interaction of CK2 with nucleolar localized proteins. We identified homologues to the well described *S.*
145 *cerevisiae* nucleolar proteins and found a total of 192 proteins in *M. oryzae* homologous to yeast
146 nucleolar proteins. We found 120 (63%) of the nucleolar proteins in the pulldown and 60 of these (50%
147 of the ones found) had the alpha helix unfolding motif (**Data S1**) (also Table 1, linked from "*M. oryzae*
148 homologues to *S. cerevisiae* nucleolar proteins"). The nucleolar proteins were thus highly
149 overrepresented in the pulldown (P-value for the null hypothesis of same frequency as in the whole
150 proteome 9×10^{-43} Fisher's Exact test) compared to the whole proteome as was also nucleolar proteins
151 having the unfolding motif (P-value for the null hypothesis of same frequency as in the whole proteome
152 *p* 2×10^{-13} , Fisher's Exact test).

153 Ck2 is known to interact with the disordered nucleolar protein SRP40 that has a multitude of CK2
154 phosphorylation sites and in addition CK2 is known to change the protein binding activity towards
155 other proteins depending on the SRP40 phosphorylation status. When SRP40 becomes highly
156 phosphorylated it binds to and inhibits CK2 activity in a negative feedback loop ensuring that CK2
157 phosphorylation level will balance (Tantos *et al.*, 2013; Na *et al.*, 2018). We identified a putative
158 MoSRP40 (MGG_00613) and it is disordered in a similar way as other SRP40 proteins and it is well
159 conserved in filamentous fungi (**Method S1**). The MoSRP40 protein was highly represented in the
160 CKa pulldown indicating that it interacts with CKa (**Data S1**).

161 Interestingly, proteins that are imported into mitochondria and involved in oxidative phosphorylation
162 (Functat category, 2.11 “electron transport and membrane-associated energy conservation”) were
163 enriched in the pulldown (62 in the pulldown of 130 in the whole proteome, *p*-value for the null
164 hypothesis of same frequency as in the whole proteome 2.26E-14). In contrast to septal and nucleolar
165 interacting proteins, the mitochondrial proteins were not enriched for the known unfolding motif.

166 Although CK2 has been implicated to be located in mitochondria in earlier studies in other organisms,
167 no proteomic study of yeast mitochondria has detected the presence of CK2 (Rao *et al.*, 2011). Hence,
168 we do not expect MoCK2 to be present in mitochondria, and we saw no evidence for mitochondrial
169 localization of GFP-CKa (Zhang *et al.*, 2019). Of special interest was however the strong
170 overrepresentation of mitochondrial proteins among the CKa pulldown proteins without the alpha helix
171 phosphorylation unfolding motif. Since such proteins need to be imported into mitochondria in an
172 unfolded state, this may point to the existence of CKa phosphorylation and unfolding motifs other than
173 the one identified by Zetina (Zetina, 2001) that together with chaperone HSP70 (Yang *et al.*, 2018)
174 help keep these proteins unfolded until they reach their destination inside the mitochondria.

175 There was no enrichment for specific pathogenicity related proteins in the whole pulldown proteome
176 (Functat category: 32.05 “disease, virulence and defence”) but a significant depletion (*p*-value for the
177 null hypothesis of same frequency as in the whole proteome 3.31E-17) was found. This is generally
178 true within the main Functat category related to stress and defence (32 “CELL RESCUE, DEFENSE
179 AND VIRULENCE”) (*p*-value for the null hypothesis of same frequency as in the whole proteome
180 8.32E-05) with the exception of proteins involved in the unfolded protein response that was
181 overrepresented (32.01.07 “unfolded protein response”, e.g. ER quality control) among the pulldown
182 proteins without motif (*p*-value for the null hypothesis of same frequency as in the whole proteome

183 4.69E-04). This is notable since an involvement of CK2 in protein import into the ER has be
184 established (Wang and Johnsson, 2005).

185 A strong association of pathogenicity related proteins with CK2 was not expected although deletion of
186 any of CKb components severely affected pathogenicity (Zhang *et al.*, 2019). This either because of the
187 *in vitro* growth conditions to produce the biomass used for the pulldown or that CK2 is mainly
188 involved in the initial stages of infection to establish the biotrophic stage (Fernandez and Orth, 2018).

189 To have a dynamic function as an unfold of proteins by phosphorylation, CK2 should be partnered
190 with phosphatases as counterparts and their activity may track CK2 activity. Interestingly, five putative
191 S/T phosphatases (MGG_03154, MGG_10195, MGG_00149, MGG_03838, MGG_06099) were in the
192 pulldown set of proteins (**Data S1**). Conceivably these might de-phosphorylate CKa substrates as well
193 as substrates of other kinases to expand the reach of CK2 in regulating the phosphoproteome (Sub-
194 hypothesis 1). To examine the general relationship between the expression of CK2 and these
195 phosphatases as widely as possible, we downloaded expression data from a range of experiments with
196 *M. oryzae* and plotted the expression of the five phosphatases found in the pulldown, and an S/T
197 phosphatase not found in the pulldown, as a function of the CKa expression. We found that two of the
198 S/T phosphatases (MGG_00149 and MGG_03154) present in the pulldown were strongly correlated
199 with CKa expression and the others were less strongly correlated (**Fig. 1**) further supporting the view
200 that CKa-dependent phosphorylation/dephosphorylation plays a major role in shaping protein
201 interactions. Together with the high expression of CK2 in cells, this suggests an important function of
202 CK2 as a general temporary unfold of intrinsically disordered proteins, that comprise roughly 30% of
203 eukaryotic proteins (Vucetic *et al.*, 2003), in a similar way as it is known to interact with SRP40 and
204 influence its activity (Tantos *et al.*, 2013; Na *et al.*, 2018).

205 **CKa expression correlates with expression of genes associated with disaggregation and** 206 **autophagy**

207 Since CK2 activity has the potential to favour protein-protein binding between intrinsically disordered
208 proteins it consequently also has the potential to enhance protein aggregation and aggregation of
209 misfolded proteins. Misfolded proteins or protein aggregates may trigger the unfolded protein response
210 involved in disaggregation. Hsp104 is a disaggregase that cooperates with Yjdg1 and Ssa1 to refold
211 and reactivate previously denatured and aggregated proteins (Glover and Lindquist, 1998).

212 Alternatively, protein aggregates that cannot be dispersed by disaggregase may be degraded through

213 autophagy since these kinds of aggregates are too big for proteasome degradation (Wong and Cuervo,
214 2010). If this is the case, CK2 upregulation should be accompanied by higher autophagy flux or at least
215 there should not be low expression of key autophagy genes when CK2 expression is high (Wong and
216 Cuervo, 2010). ATG8 is a key autophagy protein for which its turnover rate can reflect autophagy flux
217 (Klionsky *et al.*, 2016). To test this second sub-hypothesis (Sub-hypothesis 2), we used the expression
218 data we downloaded for plant infection experiments with *M. oryzae* and also for another fungal plant
219 pathogen, *F. graminearum*, that has rich transcriptomic data available (see methods), to examine
220 expression of HSP104, YDJ1, SSA1, and ATG8 relative to CKa. For *M. oryzae*, we found an
221 approximately 60-fold increase in MoHSP104 expression associated with a doubling of MoCka
222 transcript levels. With increasing expression of MoCka the MoHSP104 levels did not increase further.
223 MoSSA1 expression had a similar pattern to MoHSP104 with a 16-fold increase across the initial 2-
224 fold increase in MoCka expression. For MoYDJ1, expression increased with MoCka expression, but
225 not as dramatically (**Fig. 2**). For *M. oryzae*, we find a log-log linear relationship between the MoCka
226 expression and MoAtg8 expression (**Fig. 2**). Since CKa expression disaggregase expression and ATG8
227 expression can be expected to all increase with growth rate these observed relationships could simply
228 be a reflection of increased growth rate if all activities are only growth rate correlated. If that is the case
229 the correlation should become very weak, disappear or become negative if compensated for growth rate.
230 The histones are tightly connected to growth rate since overexpression of histones are cytotoxic (Mei *et*
231 *al.*, 2017). The two rightmost plots (Fig. 2 and Fig. S2) shows that HSP104 and ATG8 well correlated
232 with CKa also when compensated for growth rate indicating that increased disaggregase and ATG8
233 activity is needed with higher CKa activity and that these relationships are not just growth rate
234 dependent. In the case of *F. graminearum*, we also found increased expression of all of the genes
235 correlated with FgCKa expression across a large range of experiments) (**Fig, S1A, B**). As for *M. oryzae*
236 these correlations were also strong when compensated for growth rate (Fig. S3 and S4). Within the *F.*
237 *graminearum* experiments a time course experiment was selected to examine expression of these genes
238 during the course of infection. Once again, the relationship could be observed (**Fig. S1C**). Overall,
239 these correlations support the hypothesis that protein disaggregation and autophagy are increasingly
240 needed to remove protein aggregates stimulated to form by increasing levels of CKa and its activity in
241 the cell.

242 In the absence of well-functioning autophagy removing incorrectly formed larger protein aggregates,
243 like those formed in brain cells of Alzheimer's patients (Zare-shahabadi *et al.*, 2015), CK2 activity also

244 facilitates protein aggregate formation and hastens the progression of Alzheimer's disease
245 (Rosenberger *et al.*, 2016) giving further support for a function of CK2 in facilitating the formation of
246 protein aggregates from intrinsically unfolded proteins that are then subjected to autophagy.
247 Interestingly a specific CK2 phosphorylation motif only present in primates has been shown to be
248 involved in aggressive formation and clearance by autophagy (Watabe and Nakaki, 2011, 2012)
249 pointing to a need for high aggregate clearance activity when CK2 levels are high. As autophagy is
250 important to appressorium development (Liu and Lin, 2008; Kershaw and Talbot, 2009), it will be of
251 interest to further examine the role of the CK2 ring structure during appressorial development and
252 infection (Zhang *et al.*, 2019).

253 However, as infection proceeds in into the necrotrophic phase for *F. graminearum* CKa is
254 progressively upregulated pointing to a role for it in the necrotrophic stage (**Fig. S1C**) but then mainly
255 in probably mainly in defence against plant defences stressing the fungus creating problems with
256 protein folding. The 32.01.07 unfolded protein response was the only sub-category within the main
257 category 32 "CELL RESCUE, DEFENSE AND VIRULENCE" that were overrepresented (*p*-value for
258 the null hypothesis of same frequency as in the whole proteome 1.81E-11).

259 As a counterpart to CK2 in gelling/un-gelling, disaggregase activity involving the MoHSP104 complex,
260 may be critical for control. Our observation of transcriptional co-regulation between CKa and HSP104
261 supports this notion. As MoCK2 is present both in the cytoplasm and nucleoplasm it would generally
262 assist intrinsically disordered proteins in protein interactions (Uversky, 2015). It also seems to be
263 essential for assembling ribosomes containing large numbers of intrinsically disordered proteins
264 (Uversky, 2015). All these functions also explains why CK2 is needed constitutively (Meggio and
265 Pinna, 2003) and we find it relatively highly expressed under all conditions but very high under
266 stressed conditions characterized by high levels of autophagy.

267 **Conclusion**

268 We conclude that CK2 likely has an important general role in the correct assembly/disassembly of
269 intrinsically disordered proteins in addition to its already suggested role in organelle biogenesis (Rao *et al.*
270 *et al.*, 2011). Our results further point to one of the main functions of the CK2 holoenzyme as a general
271 facilitator of protein-protein, protein-RNA and protein-DNA interactions important for a large range of
272 cellular processes. This includes a potential role for it influencing gel formation that creates

273 membraneless organelles at fungal septa, and maybe other pores between other eukaryotic cells,
274 through its interaction with (and modification of) intrinsically disordered proteins in or around the pore
275 opening. To cite Meggio and Pinna (Meggio and Pinna, 2003) “Using basket terminology, one would
276 say that CK2 looks like a “playmaker” not a “pivot”: hardly ever does it make scores; nevertheless, it is
277 essential to the team game”.

278

279 **Data availability**

280 Additional data that support the findings of this study are available from the corresponding authors
281 upon request.

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289

290 **Authors' contributions** Conceived and designed the study: L. Z., D. Z., Z. W., B. O. H. and S. O.
291 Performed the experiments: L. Z., D. Z., D. L., H. L., Conceived, developed and tested the new methods:
292 S. O. Prepared secondary data for analysis: B. O. H., Analysed the primary data: L. Z., and S. O.
293 Analysed the secondary data: B. O. H. and S.O. Wrote the paper: L. Z., D. Z., Z. W., B. O. H. and S. O.

294

295 MATERIALS AND METHODS

296 Material and methods details

297 Pulldown and identification of CKa interacting proteins

298 *M. oryzae*, strain GFP-MoCKa (GfpA) (Zhang *et al.*, 2019), was grown on liquid CM medium
299 consisting of 0.6% yeast extract (CAS No. 8013-01-2), 0.6% casein hydrolysate (CAS No. 65072-00-
300 6), 1% sucrose (CAS No. 57-50-1) in DD-water for 3 days at 25°C in the dark. The cultures were
301 harvested and total protein samples were extracted from vegetative mycelia and incubated with anti-
302 GFP beads (GFP-Trap®_A, Chromotek, Hauppauge, NY, USA) 90 minutes at 4°C with gentle shaking
303 following the manufacturer's instruction. After a series of washing steps, proteins bound to anti-GFP
304 beads were eluted. The eluted proteins were sent to BGI Tech (Shenzhen, Guangdong province, China)
305 and analysed by mass spectrometry for analysis of sequence hits against the *M. oryzae* proteome. The
306 transformant expressing GFP protein only was used as the negative control and the Ku80 background
307 strain (Zhang *et al.*, 2019) was used as Blank control. Data from three biological replicates were
308 analysed against the background of proteins that were bound non-specifically to the anti-GFP beads in
309 GFP transformant and in Ku80 to get the final gene list of genes that was pulldown with MoCKa (**Data**
310 **S1**).

311 Estimation of non-specific binding of proteins in the pulldown

312 We developed two methods to estimate the number of non-specific binding proteins found in the CKa
313 pulldown. The first approach is a chemistry-based reasoning and assumes that the degree of unspecific
314 association to the protein per protein surface area is the same for GFP specific hits and for the CK2
315 holoenzyme pulled down. Using this technique, we estimate that 44-132 proteins are false positive in
316 the CKa pulldown (all proteins pulled down by GFP-Beads or the Beads alone already removed from
317 the list) (**Data S1**). The Second approach is statistical where we assume that binding of the true
318 interacting proteins to CKa are log-normally distributed related to the abundance of each protein in the
319 pulldown, since the median is low and close to zero and negative amounts are impossible. Using the
320 deviation from the theoretical distribution, with higher than expected amounts of a specific protein, for
321 the less abundant proteins we estimate that 46-81 proteins found in the CKa pulldown (with controls
322 subtracted) were false positive. The higher number was used to set a conservative threshold for which
323 proteins should be included in the analysis (See **Data S1** for details of both methods).

324 **Finding *M. oryzae* proteins containing the helix unfolding motif**

325 The MEME motif LSDDDXE/SLEEEEXD (Zetina, 2001) was used to search through the proteome of
326 *M. oryzae* using the FIMO tool at the MEME suite website (<http://meme-suite.org/>). Results were then
327 downloaded and handled in MS Excel to produce a list of proteins with at least one motif hit (For data
328 see **Table 1**).

329

330 **Analysis of CKa expression in relation to disaggregase related protein, Atg8 and Ser/Thr**
331 **phosphatase expression**

332 For *M. oryzae*, transcriptome experiment data was downloaded as sra/fastq files from Gene Expression
333 Omnibus, <https://www.ncbi.nlm.nih.gov/geo/> and mapped onto the genome found at
334 http://fungi.ensembl.org/Magnaporthe_oryzae/Info/Index. The procedure was the following: Gene
335 Expression Omnibus (Barrett *et al.*, 2012) was queried for SRA files originating from *M. oryzae* and
336 the files downloaded. The conversion from SRA to Fastq was done using the SRA toolkit
337 (<http://ncbi.github.io/sra-tools>). The resulting samples were subjected to quality control using FASTQC
338 (Andrews, 2010). Quantification of RNA was performed using Kallisto with default settings (Bray *et al.*,
339 2016), the data was then normalized using the VST algorithm implemented in DESeq2 (Love *et al.*,
340 2014).

341 *F. graminearum* transcriptomic data (FusariumPLEX) was directly downloaded from the PlexDB
342 database selecting *in planta* experiments and treatments based on the information found in the database
343 (http://www.plexdb.org/modules/PD_general/download.php?species=Fusarium).

344 For each fungus an expression matrix was prepared with the different experiments as columns and gene
345 IDs as rows, using the MGG codes for *M. oryzae* and FGSG for *F. graminearum* according to BROAD,
346 respectively. From the resulting matrixes (For these data see Key resource **Table 1**) we used the data
347 needed to plot expression of selected proteins vs CKa expression for the two fungi.

348 *M. oryzae* data are expressed as log₂ of RPKM values. Similarly, for *F. graminearum*, these data were
349 already as log₂ of reported relative expression. Gene expression data used were from MoCKa,
350 MoHSP104, FgHSP109, MoYDJ1, FgYDJ1, MoSSA1 and FgSSA1 homologues identified in this
351 study (see results) as well as from, MoCKa (MGG_03696) (Zhang *et al.*, 2019), MoAtg8 (MGG_01062)

352 (Veneault-Fourrey *et al.*, 2006), FgCKa (FGSG_00677) (Wang *et al.*, 2011; Zhang *et al.*, 2019),
353 FgAtg8 (FGSG_10740) (Josefsen *et al.*, 2012), MoHistone2b (MGG_03578)(NCBI) FgHistone2b
354 (FGSG_11626). Data from the *M. oryzae* expression matrix was also used for plotting MoCKa
355 expression versus the expression of annotated serine/threonine phosphatases found in the CKa
356 pulldown.

357

358 DATA AVAILABILITY

359 Data and resources external to this paper and data from this study are available as listed in and linked
360 from **Table 1**.

361

362 REFERENCES

- 363 Ahmad, K.A., Wang, G., Unger, G., Slaton, J., and Ahmed, K. (2008) Protein kinase CK2 – A key suppressor of
364 apoptosis. *Adv Enzyme Regul* **48**: 179–187.
- 365 Andrews, S. (2010) FastQC: a quality control tool for high throughput sequence data. Available online at
366 <Http://www.bioinformatics.babraham.ac.uk/projects/fastqc>.
- 367 Barrett, T., Wilhite, S.E., Ledoux, P., Evangelista, C., Kim, I.F., Tomashevsky, M., et al. (2012) NCBI GEO: archive
368 for functional genomics data sets—update. *Nucleic Acids Res* **41**: D991–D995.
- 369 Bray, N.L., Pimentel, H., Melsted, P., and Pachter, L. (2016) Near-optimal probabilistic RNA-Seq quantification.
370 *Nat Biotechnol* **34**:
- 371 Dagdas, Y.F., Yoshino, K., Dagdas, G., Ryder, L.S., Bielska, E., Steinberg, G., and Talbot, N.J. (2012) Septin-
372 mediated plant cell invasion by the rice blast fungus, *Magnaporthe oryzae*. *Science* **336**: 1590–1594.
- 373 Fernandez, J. and Orth, K. (2018) Rise of a Cereal Killer: The Biology of *Magnaporthe oryzae* Biotrophic Growth.
374 *Trends Microbiol* **26**: 582–597.
- 375 Frege, T. and Uversky, V.N. (2015) Intrinsically disordered proteins in the nucleus of human cells. *Biochem*
376 *Biophys Rep* **1**: 33–51.
- 377 Glover, J.R. and Lindquist, S. (1998) Hsp104, Hsp70, and Hsp40: A Novel Chaperone System that Rescues
378 Previously Aggregated Proteins. *Cell* **94**: 73–82.
- 379 Götz, C. and Montenarh, M. (2016) Protein kinase CK2 in development and differentiation (Review). *Biomed*
380 *Rep* **6**: 127–133.
- 381 Han, P., Jin, F.J., Maruyama, J., and Kitamoto, K. (2014) A Large Nonconserved Region of the Tethering Protein
382 Leashin Is Involved in Regulating the Position, Movement, and Function of Woronin Bodies in *Aspergillus oryzae*.
383 *Eukaryot Cell* **13**: 866–877.
- 384 Josefsen, L., Droce, A., Sondergaard, T.E., Sørensen, J.L., Bormann, J., Schäfer, W., et al. (2012) Autophagy
385 provides nutrients for nonassimilating fungal structures and is necessary for plant colonization but not for
386 infection in the necrotrophic plant pathogen *Fusarium graminearum*. **8**: 13.
- 387 Kershaw, M.J. and Talbot, N.J. (2009) Genome-wide functional analysis reveals that infection-associated fungal
388 autophagy is necessary for rice blast disease. *Proc Natl Acad Sci* **106**: 15967–15972.
- 389 Klionsky, D.J., Abdelmohsen, K., Abe, A., Abedin, M.J., Abeliovich, H., Acevedo Arozena, A., et al. (2016)
390 Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). *Autophagy* **12**: 1–
391 222.

- 392 Lai, J., Koh, C.H., Tjota, M., Pieuchot, L., Raman, V., Chandrababu, K.B., et al. (2012) Intrinsically disordered
393 proteins aggregate at fungal cell-to-cell channels and regulate intercellular connectivity. *Proc Natl Acad Sci* **109**:
394 15781–15786.
- 395 Liu, X. and Lin, F. (2008) Investigation of the biological roles of autophagy in appressorium morphogenesis in
396 *Magnaporthe oryzae*. *J Zhejiang Univ Sci B* **9**: 793–796.
- 397 Love, M.I., Huber, W., and Anders, S. (2014) Moderated estimation of fold change and dispersion for RNA-seq
398 data with DESeq2. *Genome Biol* **15**:
- 399 Meggio, F. and Pinna, L.A. (2003) One thousand-and one substrates of protein kinase CK2. *FASEB J* **17**: 349–368.
- 400 Mei, Q., Huang, J., Chen, W., Tang, J., Xu, C., Yu, Q., et al. (2017) Regulation of DNA replication-coupled histone
401 gene expression. *Oncotarget* **8**:
- 402 Na, J.-H., Lee, W.-K., and Yu, Y. (2018) How Do We Study the Dynamic Structure of Unstructured Proteins: A
403 Case Study on Nopp140 as an Example of a Large, Intrinsically Disordered Protein. *Int J Mol Sci* **19**: 381.
- 404 Ng, S.K., Liu, F., Lai, J., Low, W., and Jedd, G. (2009) A Tether for Woronin Body Inheritance Is Associated with
405 Evolutionary Variation in Organelle Positioning. *PLoS Genet* **5**: e1000521.
- 406 Padmanabha, R., Chen-Wu, J.L.-P., Arnot, D.E., and Glover, C.V.C. (1990) Isolation, sequencing, and disruption
407 of the yeast CKA2 gene casein kinase II is essential for viability in *Saccharomyces cerevisiae*. *Mol Cell Biol* **10**:
408 4089–4099.
- 409 Plamann, M. (2009) Cytoplasmic Streaming in *Neurospora*: Disperse the Plug To Increase the Flow? *PLoS Genet*
410 **5**: e1000526.
- 411 Rao, S., Gerbeth, C., Harbauer, A., Mikropoulou, D., Meisinger, C., and Schmidt, O. (2011) Signaling at the gate:
412 Phosphorylation of the mitochondrial protein import machinery. *Cell Cycle* **10**: 2083–2090.
- 413 Rosenberger, A.F.N., Morrema, T.H.J., Gerritsen, W.H., van Haastert, E.S., Snkhchyan, H., Hilhorst, R., et al.
414 (2016) Increased occurrence of protein kinase CK2 in astrocytes in Alzheimer’s disease pathology. *J*
415 *Neuroinflammation* **13**: 1 – 14.
- 416 Smith, A.K., Ayanian, J.Z., Covinsky, K.E., Landon, B.E., McCarthy, E.P., Wee, C.C., and Steinman, M.A. (2011)
417 Conducting High-Value Secondary Dataset Analysis: An Introductory Guide and Resources. *J Gen Intern Med* **26**:
418 920–929.
- 419 Tantos, A., Sznka, K., Szabo, B., Bokor, M., Kamasa, P., Matus, P., et al. (2013) Structural disorder and local
420 order of hNopp140. *Biochim Biophys Acta BBA - Proteins Proteomics* **1834**: 342–350.
- 421 Uversky, V.N. (2015) The multifaceted roles of intrinsic disorder in protein complexes. *FEBS Lett* **589**: 2498–
422 2506.
- 423 Veneault-Fourrey, C., Barooha, M., Egan, M., Wakeley, G., and Talbot, N.J. (2006) Autophagic Fungal Cell Death
424 Is Necessary for Infection by the Rice Blast Fungus. *Science* **312**: 580–583.

- 425 Vucetic, S., Brown, C.J., Dunker, A.K., and Obradovic, Z. (2003) Flavors of protein disorder. *Proteins Struct Funct*
426 *Bioinforma* **52**: 573–584.
- 427 Wang, C., Zhang, S., Hou, R., Zhao, Z., Zheng, Q., Xu, Q., et al. (2011) Functional Analysis of the Kinome of the
428 Wheat Scab Fungus *Fusarium graminearum*. *PLoS Pathog* **7**: 1–21.
- 429 Wang, X. and Johnsson, N. (2005) Protein kinase CK2 phosphorylates Sec63p to stimulate the assembly of the
430 endoplasmic reticulum protein translocation apparatus. *J Cell Sci* **118**: 723–732.
- 431 Watabe, M. and Nakaki, T. (2012) CK2 as anti-stress factor. *Commun Integr Biol* **5**: 278–280.
- 432 Watabe, M. and Nakaki, T. (2011) Protein kinase CK2 regulates the formation and clearance of aggresomes in
433 response to stress. *J Cell Sci* **124**: 1519–1532.
- 434 Wong, E. and Cuervo, A.M. (2010) Integration of Clearance Mechanisms: The Proteasome and Autophagy. *Cold*
435 *Spring Harb Perspect Biol* **2**: 1–19.
- 436 Wright, P.E. and Dyson, H.J. (2015) Intrinsically disordered proteins in cellular signalling and regulation. *Nat Rev*
437 *Mol Cell Biol* **16**: 18–29.
- 438 Yang, J., Liu, M., Liu, X., Yin, Z., Sun, Y., Zhang, H., et al. (2018) Heat-Shock Proteins MoSsb1, MoSsz1, and
439 MoZuo1 Attenuate MoMkk1-Mediated Cell-Wall Integrity Signaling and Are Important for Growth and
440 Pathogenicity of *Magnaporthe oryzae*. *Mol Plant Microbe Interact* **31**: 1211–1221.
- 441 Zare-shahabadi, A., Masliah, E., Johnson, G.V.W., and Rezaei, N. (2015) Autophagy in Alzheimer’s disease. *Rev*
442 *Neurosci* **26**:
- 443 Zaritsky, A. (2018) Sharing and reusing cell image data. *Mol Biol Cell* **29**: 1274–1280.
- 444 Zetina, C.R. (2001) A Conserved Helix-Unfolding Motif in the Naturally Unfolded Proteins. *Proteins Struct Funct*
445 *Genet* **44**: 479–483.
- 446 Zhang, L., Zhang, D., Chen, Y., Ye, W., Lin, Q., Lu, G., et al. (2019) *Magnaporthe oryzae* CK2 Accumulates in
447 Nuclei, Nucleoli, at Septal Pores and Forms a Large Ring Structure in Appressoria, and Is Involved in Rice Blast
448 Pathogenesis. *Front Cell Infect Microbiol* **9**: 113.
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451 **Tables and figures.**

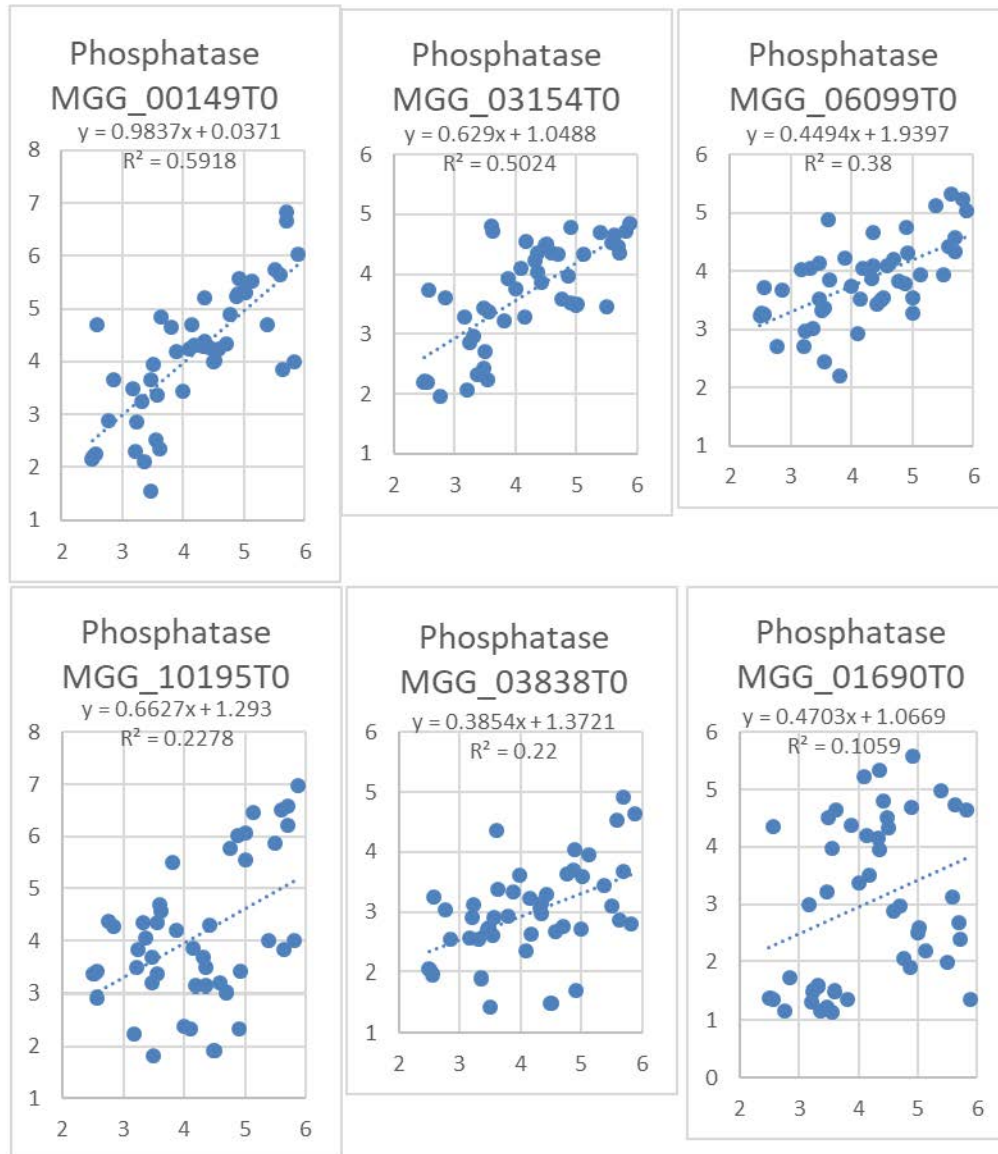
452 **Table 1. Key resources**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
anti-GFP beads	Chromotek, Hauppauge, Ny, USA	GFP-Trap®_A
Deposited Data		
Transcriptomic data sra/fastq files for <i>M. oryzae</i>	Gene Expression Omnibus	https://www.ncbi.nlm.nih.gov/geo
Genome for mapping transcriptomic data	EnsemblFungi	http://fungi.ensembl.org/Magnaporthe_oryzae/Info/Index
Transcriptomic data <i>F. graminearum</i>	PlexDB	http://www.plexdb.org/modules/PD_general/downloadphp?species=Fusarium
Protein ID codes and annotation (FGSG_codes for <i>Fusarium Graminearum</i> Ph1 and MGG_codes for <i>Magnaporthe oryzae</i> 70-15)	BROAD	ftp.broadinstitute.org/distribution/annotation/fungi/
List of proteins with unfolding motif according to (Zetina, 2001) found for <i>M. oryzae</i> found using the FIMO tool at the MEME-suite website.	Figshare, this paper	https://figshare.com/s/a25ef9d1b93240e72f96 DOI: 10.6084/m9.figshare.7066379
Nucleolar proteins in the MoCKa pulldown and annotation of <i>M. oryzae</i> with the help of <i>S. cerevisiae</i> annotation.	Figshare, this paper	https://figshare.com/s/752257b8b25dfe19a4e0 DOI: 10.6084/m9.figshare.7067897
<i>M. oryzae</i> transcriptomic data matrix covering a range of experiments	Figshare, this paper	https://figshare.com/s/64eec43151576417afbc DOI: 10.6084/m9.figshare.7068857
<i>F. graminearum</i> transcriptomic data matrix covering a range of experiments	Figshare, this paper	https://figshare.com/s/e28813e5cc7b9c394e08 DOI: 10.6084/m9.figshare.7068860
List of <i>M. oryzae</i> orthologs to <i>N. crassa</i> SPA proteins	Figshare, this paper	https://figshare.com/s/39c3962d1b8d7d969338 DOI: 10.6084/m9.figshare.7066382
FungiFun: Functat analysis of the <i>M. oryzae</i> CKA pulldown proteins	Figshare, this paper	https://figshare.com/s/231c764fad6e60855795 DOI:10.6084/m9.figshare.7066385
Experimental Models: Organisms/Strains		
<i>M. oryzae</i> Strain Ku80 transformed with the over-expressed GFP-MoCKa fusion protein (GfpA).	(Zhang <i>et al.</i> , 2019)	N/A
Software and Algorithms		
SRA toolkit	NCBI	http://ncbi.github.io/sra-tools

FIMO tool	The MEME suite	http://meme-suite.org
FASTQC (RNAseq quality control)	(Andrews, 2010)	N/A
Functional catalogue of fungal proteins	FungiFun	https://elbe.hki-jena.de/fungifun/
Other		
Proteomics services	BGI Tech, Schenzen, Guangdong province China	N/A

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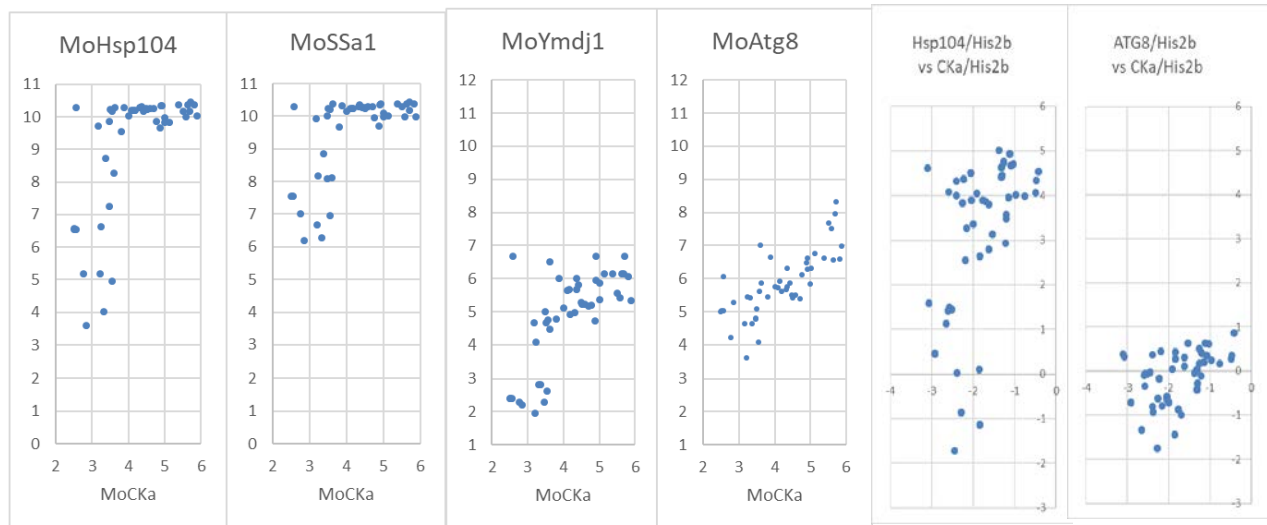
454



455

456 **Fig. 1** Plots of 6 putative serine/threonine protein phosphatase expression (y-axis) vs MoCKa expression (x-axis) in a
457 range of transcriptome datasets from different experiments. (Note: Log₂ scale on axes and grids are represented with
458 fixed “aspect ratio” to highlight the different slopes of the correlations). MGG_01690 is not in the pulldown while the
459 other five are and used to illustrate that not all S/T phosphatases are well correlated with CKa. The P values for the
460 Null hypothesis of no correlation with CKa are: MGG_00149 P=2.7E-10, MGG_03154 P=2.5E-8, MGG_06099
461 P=4.0E-6, MGG_10195 P=6.9E-4, MGG_03838 P=8.8E-4, MGG_01690 P=2.6E-2. Transcriptomic data was
462 downloaded from public websites so as to be able to test the relationship under many different growth conditions in
463 many experiments.

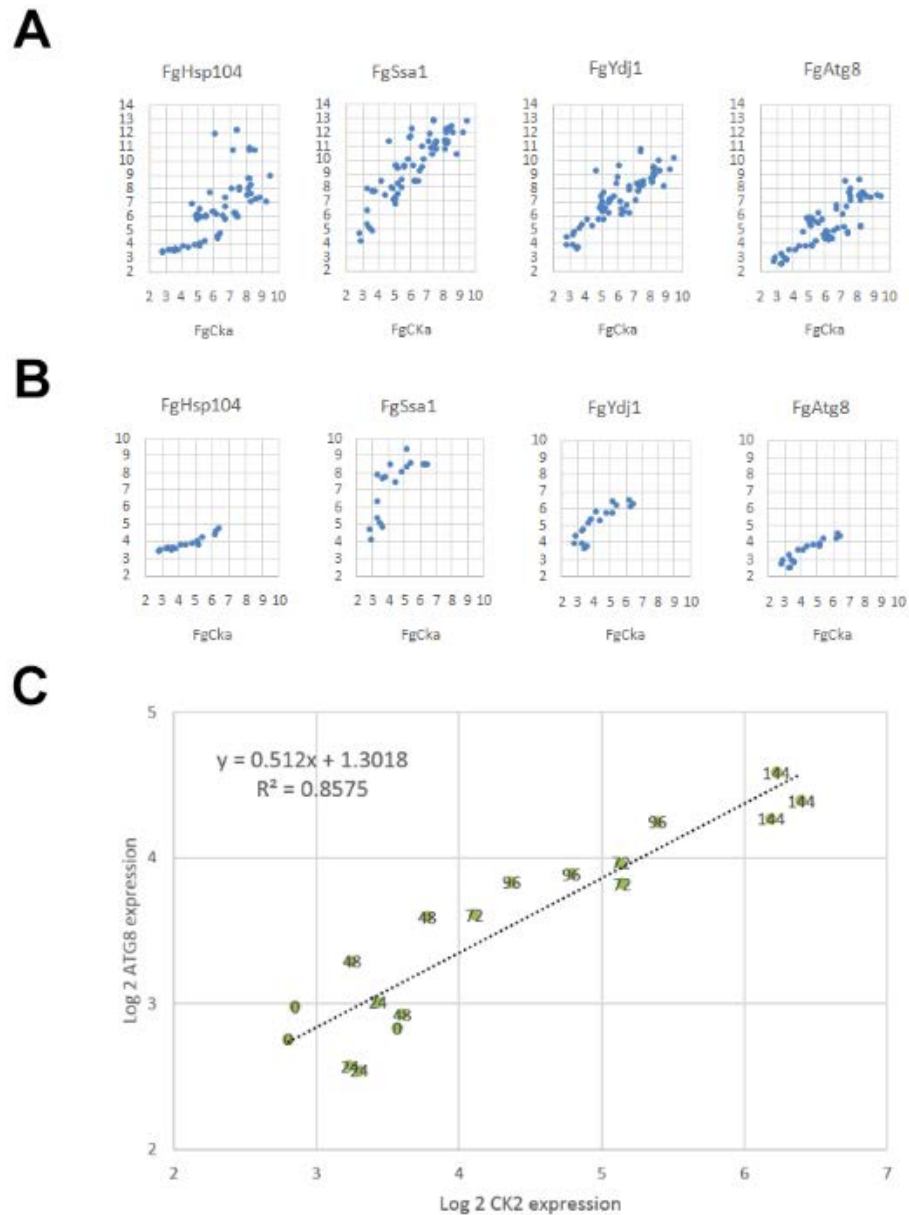
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465

466 **Fig. 2.** Plot of expression involved in protein quality control vs MoCKa expression in a range of transcriptomes from
467 different experiments (Note: Log2 scale on axes and grids are represented with fixed “aspect ratio” to highlight the
468 slope of the correlation). The two rightmost figures show growth compensated plots using ratio of expression in
469 relation MoHis2b expression that tightly correlates with new DNA synthesis. Hsp104, SSa1 and Ymdj1 has in yeast
470 been shown to cooperatively help aggregate proteins to be able to refold. The key protein with its main function in this
471 process appear to be Hsp104 (Glover and Lindquist, 1998).

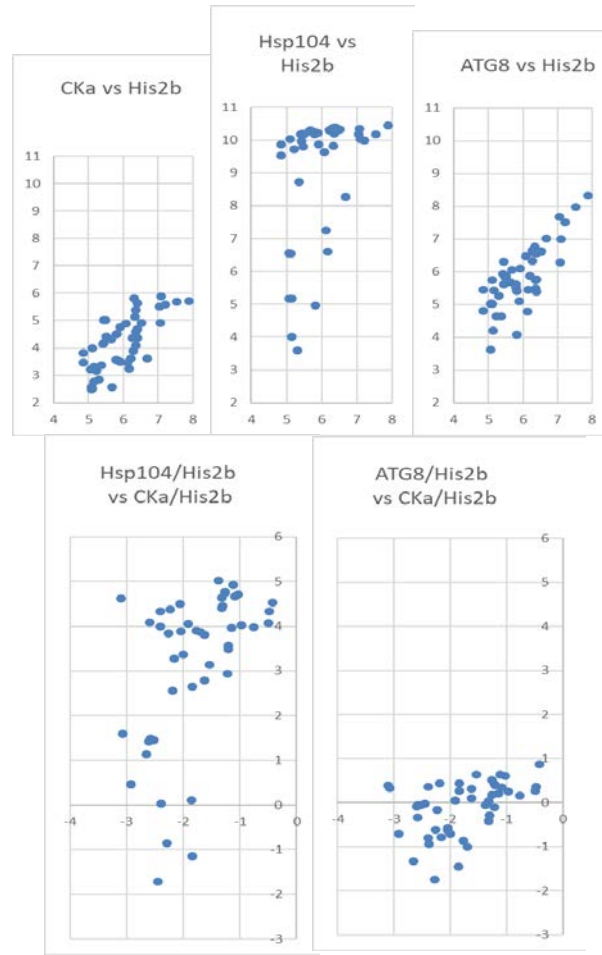
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473

474 **Fig. S1. Plot of expression involved in protein quality control vsFgCKa expression in a range of transcriptomes**
 475 **from different experiments** (Note: Log₂ scale on axes and grids are represented with fixed “aspect ratio” to highlight
 476 the slope of the correlation). (A). data from all experiments. (B) data from a time course infection experiment. Hsp104,
 477 Ssa1 and Ymdj1 have in yeast been shown to cooperatively help aggregate proteins to be able to refold. The key
 478 protein with its main function in this process appear to be Hsp104 (Glover and Lindquist, 1998).

479 (C) Plot of FgAtg8 (autophagy) expression vs FgCKa expression in a times series infection experiment with 3 replicates
480 where numbers in the plot indicate hours post infection (hpi). (Note: Log2 scale on axes and grids are represented with fixed
481 “aspect ratio” to highlight the slope of the correlation). P value for the Null hypothesis that there is no correlation = 3.6E-08.
482

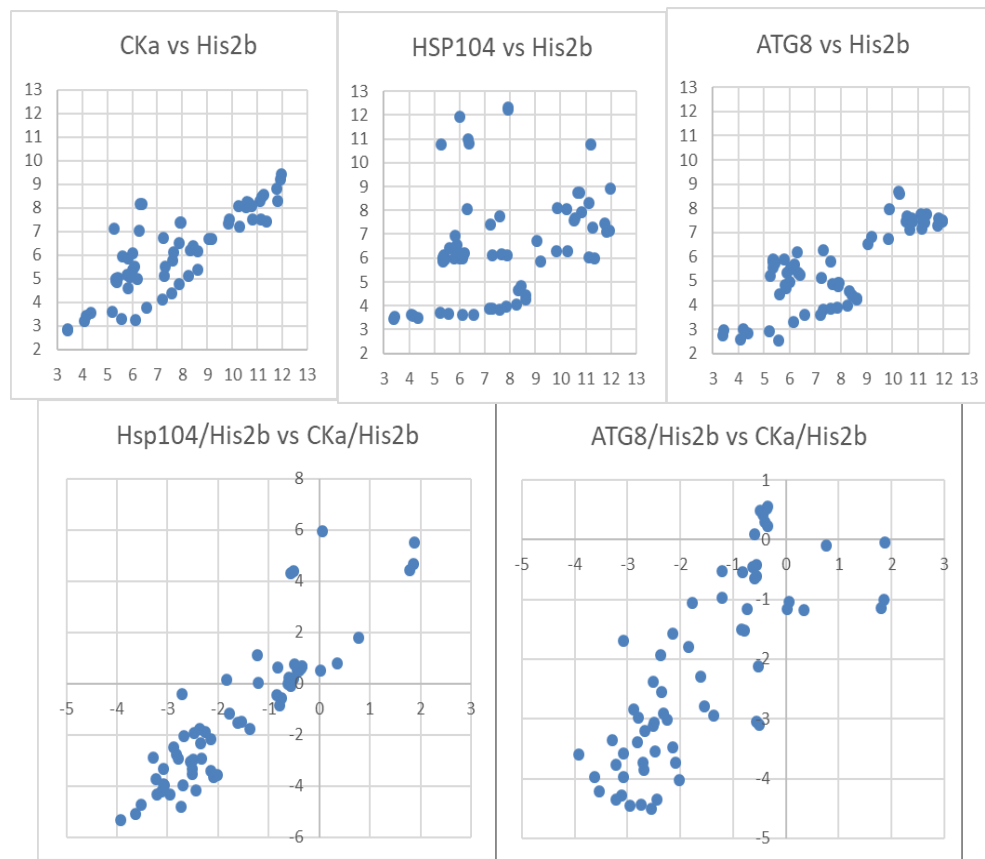


483

484 **Fig. S2 Expression of MoCKa, disaggregase MoHsp104 and MoATG8 in relation to growth as MoHis2b expression.**

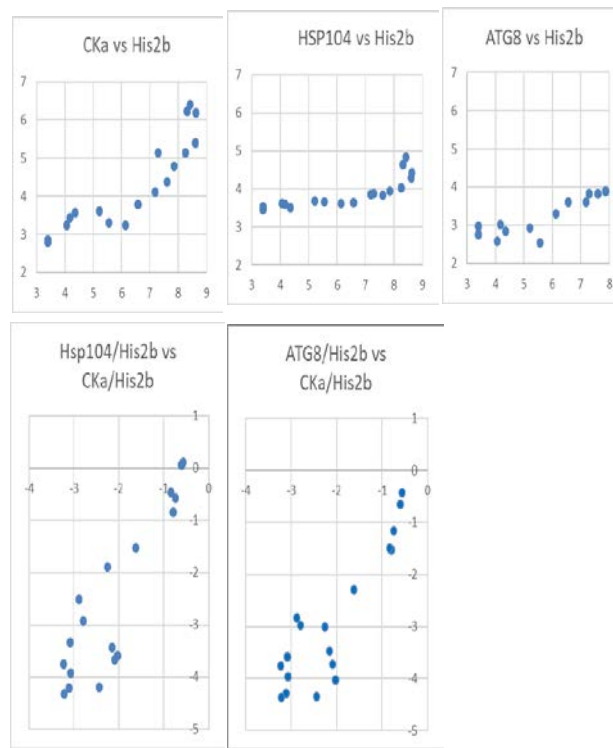
485 Top 3 diagrams show CKa, the disaggregase Hsp104 and ATG8 expression plotted against growth reflected by His2b
486 expression. The 2 bottom diagrams show growth adjusted plots where the expression of the disaggregase and the ATG8
487 expression per His2b expression are plotted against CKa/His2b expression as in a qPCR using His2b as reference gene to
488 detect changes in relation to growth. Scales are Log2.

489



490

491 **Fig. S3. Expression of *Fusarium graminearum* FgCKa, disaggregase FgHsp104 and FgATG8 in relation to growth as**
492 **FgHis2b expression.** Top 3 diagrams show CKa, the disaggregase Hsp104 and ATG8 expression plotted against growth
493 reflected by His2b expression. The 2 bottom diagrams show growth adjusted plots where the expression of the disaggregase
494 and the ATG8 expression per His2b expression are plotted against CKa/His2b expression as in a qPCR using His2b as
495 reference gene to detect changes in relation to growth. Scales are Log2.



496

497 **Fig. S4. Expression of *Fusarium graminearum* FgCka, disaggregase FgHsp104 and FgATG8 in relation to growth as**
498 **FgHis2b expression. Data from one experiment were Cka expression follows hours post infection (HPI) (Fig S1C).**
499 Top 3 diagrams shows Cka, the disaggregase Hsp104 and ATG8 expression plotted against growth reflected by His2b
500 expression. The 2 bottom diagrams shows growth adjusted plots where the expression of the disaggregase and the ATG8
501 expression per His2b expression are plotted against Cka/His2b expression as in a qPCR using His2b as reference gene to
502 detect changes in relation to growth. Top left show that both growth and Cka expression increases with HPI since we
503 previously showed that Cka increases with HPI. The two bottom diagrams shows that the growth compensated relative
504 expression of disaggregase and ATG8 increases with Cka relative expression.

505