Acetylation regulates the oligomerization state and activity of RNase J, the major ribonuclease of *Helicobacter pylori*

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32 **Abstract**

33 In *Helicobacter pylori*, post-transcriptional regulation strongly relies on the
34 activity of an RNA degradosome, composed of the essential ribonuclease
35 RNase J and the DEAD-box RNA helicase RhpA. Here, we describe post-
36 translational modifications of this protein complex that affect its activity. Cell-
37 extracted RNase J is acetylated on multiple residues, one of which, K649,
38 strongly impacts RNase J oligomerization, which in turn influences ribonuclease
39 activity. Corroborating the link between oligomerization and activity, mutations
40 targeting K649 and other residues affect the dimerization and *in vitro* activity of
41 RNase J. Our crystal structure of RNase J reveals three loops that gate access
42 to the active site and rationalizes how oligomerization state influences activity.
43 The acetylated residues of RNase J are important for *H. pylori* morphology,
44 highlighting that the modifications affect the RNase J cellular function. We
45 propose acetylation as a regulatory level controlling the activity of RNase J and
46 the *H. pylori* RNA degradosome.

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51 **Key words:** post-transcriptional regulation, RNA degradosome, RNase J,
52 *Helicobacter pylori*, post-translational modifications, protein acetylation.

53

54 Introduction

55 Post-transcriptional regulation is one of the most important levels of control of
56 gene expression in every kingdom of life. Ribonucleases (RNases) are key
57 enzymes in this regulation, that are involved in RNA maturation and
58 degradation. In several bacteria, major RNases act in concert with DEAD-box
59 RNA helicases, that help to unfold RNA secondary structures, within protein
60 machines called RNA degradosomes¹.

61 We previously demonstrated the existence of a minimal RNA degradosome
62 in *Helicobacter pylori*². This Gram-negative bacterial pathogen chronically
63 colonizes the stomach of half of the human population worldwide. Infection
64 leads to chronic gastritis and, in some cases, to further gastric pathologies such
65 as peptic ulcers, MALT lymphoma, or gastric adenocarcinoma that causes
66 800,000 deaths each year worldwide³. *H. pylori* possesses a small genome (1.6
67 Mb) with few transcriptional regulators, and several lines of evidence indicate
68 that post-transcriptional regulation plays a major role in gene expression control
69 in this organism⁴⁻⁶. The *H. pylori* RNA degradosome is composed of RNase J, a
70 77.6 kDa enzyme that possesses both 5'-3' exoribonuclease and
71 endoribonuclease activities, and RhpA, a DEAD-box RNA helicase, with a
72 molecular weight of 55.8 kDa^{2,7}. RNase J is essential in *H. pylori* and plays a
73 major role in the degradation of both mRNAs and antisense RNAs⁸. RhpA is
74 the sole DEAD-box RNA helicase encoded by the *H. pylori* genome and is
75 essential for colonization in the mouse infection model⁷. Furthermore, we
76 recently demonstrated that, in *H. pylori*, the RNA degradosome is
77 compartmentalized into clusters at the inner membrane whose formation is
78 regulated and likely represents a major level of control of its activity⁹.

79 RNase J-encoding genes are present in 57 % of sequenced bacteria¹,
80 including the Gram-positive organisms *Bacillus subtilis*, *Staphylococcus aureus*
81 and *Deinococcus radiodurans*. *B. subtilis* and *S. aureus* encode two paralogues
82 of RNase J, called J1 and J2 while *H. pylori*, *Streptomyces pyogenes* and *D.*
83 *radiodurans* possess only one gene for this protein. Compared to the RNase J
84 proteins of these three organisms, the *H. pylori* enzyme possesses a unique N-
85 terminal extension of 132 residues that is predicted to be predominantly
86 disordered^{2,9}. In *B. subtilis*, while both RNase J paralogues (*Bsu*RNases J1 and
87 J2) have equivalent endoribonuclease activities, *Bsu*RNase J2 seems to lack
88 the 5'-3' exoribonuclease activity that predominates in *Bsu*RNase J1¹⁰.
89 Furthermore, these two proteins interact and form a heterotetramer *in vitro* that
90 has different cleavage specificity *in vitro* than each protein alone¹⁰. *In vitro*
91 analysis of the RNase J1 enzyme from *S. aureus* showed that it predominantly
92 assembles into dimers, but also forms tetramers, independently of divalent
93 cations¹¹. The single RNase J from *S. pyogenes* forms dimers and tetramers *in*
94 *vitro* without divalent cations being observed in the dimerization surface¹². In *D.*
95 *radiodurans*, RNase J forms a dimer in a Mn-dependent fashion *in vitro*, and the
96 interaction between the two monomers is mediated by the C-terminal domain of
97 *Dra*RNase J¹³. Several crystal structures of RNase J proteins have been
98 published¹²⁻¹⁴, where RNase J is generally found to be a tetramer (dimer of
99 dimers). However, it is unknown whether the different oligomeric states of
100 RNase J detected *in vitro* have differences in activity.

101 Almost nothing is known about post-translational modifications of these
102 protein complexes and whether this might influence their activity. Acetylation
103 has emerged as a major post-translational regulatory mechanism in

104 bacteria^{15,16}. Although the acetylation of lysine, cysteine, serine and threonine
 105 residues has been reported, lysine acetylation is the most prominent
 106 modification, particularly on its ϵ -amino group¹⁵. This modification has been
 107 shown to regulate multiple properties of the target proteins, including enzymatic
 108 activity, DNA-binding capability or stability, among others¹⁶. Interestingly, some
 109 proteins involved in RNA metabolism have been reported to be lysine
 110 acetylated, including (i) *Escherichia coli* RNase R, whose acetylation regulates
 111 its stability¹⁷; (ii) *E. coli* RNase II, regulated at the activity level¹⁸; and (iii) the
 112 CshA DEAD-box RNA helicase from *B. subtilis*, where acetylation has been
 113 proposed to influence its interaction with RNA polymerase independently from
 114 its association with RNase Y, the major ribonuclease of the *B. subtilis* RNA
 115 degradosome¹⁹.

116 The central importance of RNase J in post-transcriptional regulation in *H.*
 117 *pylori* and its participation in an RNase J-based degradosome prompted us to
 118 investigate the structure, oligomeric state and post-translational regulation of
 119 the *H. pylori* RNase J and to assess its stoichiometry in the RNA degradosome.
 120 Here, we report the crystal structure of *H. pylori* RNase J without its N-terminal
 121 extension. We also show that RNase J is in equilibrium between monomeric
 122 and dimeric states *in vitro* and that, together with its monomeric RNA helicase
 123 partner RhpA, assembles into a minimal degradosome complex with a 1:1
 124 stoichiometry. Our data suggest that the dimeric form constitutes the active form
 125 of RNase J *in vitro*. Furthermore, we found for the first time that RNase J is
 126 acetylated at several residues by multiple mechanisms. We concluded that the
 127 acetylation state regulates the oligomerization state and activity of RNase J *in*

128 *vitro* and its activity in *H. pylori*, and thereby constitutes an important level of
129 control of the RNA degradosome.
130

131 **Results**

132

133 **Structure of the *H. pylori* RNase J**

134 To better understand the structure-function relationship of the *H. pylori*
 135 RNase J protein, we first decided to determine its crystal structure. Several
 136 attempts to purify sufficient amounts of full-length RNase J from *H. pylori* to
 137 obtain crystals were unsuccessful. As we previously reported⁹, the N-terminal
 138 part of RNase J (first 120 amino acids) that is specific of the *Helicobacter*
 139 species is predicted to be an intrinsically disordered region (IDR). After
 140 truncating this region, well diffracting crystals were obtained from this RNase J
 141 form comprising residues 137-691 (from *H. pylori* strain 26695, uniprot P56185).
 142 The crystal structure of *H. pylori* RNase J was solved at 2.75 Å by molecular
 143 replacement using the *Streptomyces coelicolor* homologue as a search model
 144 (Table S1). One molecule occupies the asymmetric unit of the crystal, and
 145 through crystal symmetry a homotetramer can be generated that is organized
 146 as a dimer-of-dimers (Figure 1A). The core region of the enzyme, residues 137-
 147 586, encompasses the beta-CASP and beta-lactamase domains that define this
 148 family²⁰, but there are no detectable metal cofactors in the map at the catalytic
 149 site. An X-ray Zn-peak scan also confirmed that there are no detectable Zn ions
 150 in the crystal.

151 The structure predicted by the Robetta server, based on machine learning²¹,
 152 is in excellent agreement with the X-ray model for the core, with the notable
 153 exception of the three loop regions 174-183, 431-441 and 575-586 at the
 154 entrance to the active site, for which the predicted loop positions are closer to
 155 those seen in the crystal structure of the *S. coelicolor* RNase J (Figure 1B-C).

156 The electron density over these regions is well defined in the *H. pylori* map and
157 modelled with high confidence. Comparing the structures, it is apparent that the
158 position of the loops will enforce a different path for the RNA substrate in *H.*
159 *pylori* RNase J compared with the *S. coelicolor* enzyme (Figure 1C).

160 The C-terminal domain (CTD), from residues 587 to 691, is poorly ordered in
161 the crystal lattice and the density was not sufficiently well resolved to model the
162 polypeptide. The Robetta model for the C-terminal domain fits well and fitted
163 into the map, showing good agreement with the predicted helical segments.
164 The predicted model is also in agreement with a high confidence homology
165 model prepared by PHYRE2 using the *Thermus thermophilus* homolog as
166 template (PDB 3BK2). This domain makes a self-complementary interaction
167 through crystal lattice contact. The Robetta model predicted with low confidence
168 an extended helical region of the N-terminal residues 1-136 that were not
169 included in the crystallized protein, and this region seems unlikely to have a
170 compact fold. The final model to fit the X-ray data is a fusion of the fitted crystal
171 structure of the core and the Robetta model for the C-terminal domain with side
172 chains removed due to poor resolution of this part of the electron density map.

173 In the dimeric unit, the protomer interfaces are formed by self-complementary
174 interactions made mostly by the CTD. The surface area buried at the CTD-CTD
175 interface is estimated to be 1165 Å². The beta-CASP and beta-lactamase
176 domains of RNase J also make a self-complementary interface in the principal
177 dimer, with an estimated area of 672 Å² that is a little more than half of the
178 CTD-CTD contribution. Stronger interactions typically have a surface area of
179 more than 2000 Å² ²². The interface between the beta-CASP and beta-
180 lactamase domains is very similar to that seen in other dimers of RNase J, for

181 instance in the homologue of *S. coelicolor*¹². The interfaces that are formed
182 between the dimer units to make the tetramer are also made by the beta-CASP
183 and beta-lactamase interfaces, and these have a total buried area of 2070 Å².

184 An overlay of RNase J of *H. pylori* and of *S. coelicolor*, where an RNA
185 substrate is bound, suggests that the path of the RNA to the active site cannot
186 be the same for the two enzymes, and that the nucleic acid would need to be
187 rerouted in the *H. pylori* enzyme. The obstructions are caused by exposed
188 peptide loop regions that are positioned to accommodate the CTD. It is possible
189 that changes in the oligomerization state will impact on the loops and access to
190 the active site.

191 It is notable that the CTD has extensive charged side chains on the surface
192 of proteins that likely form complementary charge interactions. Such an
193 organization may stabilize the fold of the small domain^{23–25}. As the self-
194 complementary interactions of the C-terminal domain are a key component of
195 the assembly, any changes in the stability of the CTD may impact on the
196 oligomeric state. This prompted us to investigate the *in vitro* oligomeric state of
197 RNase J and its RhpA partner.

198

199 ***In vitro* analysis of the oligomeric state of the two partners of the *H. pylori*** 200 **RNA degradosome**

201 The crystal structure of *H. pylori* RNase J and of several other RNase J
202 proteins correspond to tetramers. However, for some of these proteins, other
203 oligomeric states (dimers and monomers) were found in solution and the activity
204 of the different forms was unclear. To evaluate the oligomeric state of *H. pylori*
205 RNase J and RhpA *in vitro*, we performed analytical ultracentrifugation (AUC)

with His-tagged versions of RNase J and RhpA purified from *E. coli* (Supp Figure S1). The sedimentation coefficient distribution of RNase J indicates that this protein forms two oligomeric species *in vitro* with concentration-independent sedimentation coefficients (S_0) of 5.8 and 8.8 (Fig. 2A). Based on the structural data of *H. pylori* RNase J presented above, the calculation of the sedimentation coefficients with the HYDROPRO software was 5.4 and 8.9 for a monomer and dimer, respectively. Moreover, the calculated sedimentation for the monomer suggests that the C-terminal end of the monomer is probably flexible and less extended in solution, explaining the small difference in sedimentation coefficient. On the other hand, the calculated sedimentation for the dimer is fully compatible with the extended arrangement of the C-terminal end, showing that dimerization locks the structure in an extended conformation. Therefore, we concluded that the two forms of RNase J observed by AUC are indeed a monomer and a dimer (Figure 2A) that have a structural arrangement in solution which is compatible with the observed crystal structure. The monomeric population predominates (~60%) over the dimer (~40%) independently of the protein concentration, showing that these species were not in equilibrium but were dependent on other modifications.

AUC of RhpA shows behavior as a monomer independent of concentration with an S_0 of 3.85 (Figure 2B).

RNase J and RhpA form a 1:1 minimal degradosome complex

Next, we wanted to evaluate the stoichiometry of the protein complex formed by RNase J and RhpA. We mixed both proteins at 5:1, 1:1 and 1:5 ratios and subjected them to AUC (Figure 2C). When using a 5:1 RNase J:RhpA ratio, we

231 identified three peaks corresponding to both proteins alone (RNase J monomer
232 and dimer and RhpA monomer). With a 1:1 RNase J:RhpA ratio, three peaks
233 could be detected. Based on the previous calculations, these peaks are
234 proposed to correspond to (i) RhpA monomer alone for the 3.85S peak, (ii) a
235 dimer of RNase J for the 8.8S peak, and (iii) a minor peak with a sedimentation
236 coefficient of 4.8S that would correspond to the RNase J-RhpA complex. This
237 latter peak was much more prominent when using a 1:5 ratio of RNase J:RhpA,
238 where only two peaks could be detected, corresponding to RhpA alone and
239 most probably the complex with a coefficient of 4.8S. From this peak and the
240 available frictional coefficients, we calculated a mass for the complex of
241 approximately 124 kDa that is only compatible with a complex with a 1:1
242 stoichiometry. Interestingly, neither the monomer nor the dimer of RNase J was
243 detected under this condition, and all RNase J was involved in complex
244 formation. We thus conclude that RNase J and RhpA form a 1:1 stoichiometry
245 complex *in vitro*.

246

247 **Bacterial two hybrid (BACTH) to identify residues important for the** 248 **oligomerization of RNase J**

249 As AUC results show that the formation of oligomer is not an equilibrium and
250 is independent of concentration, we then searched for the potential residues
251 involved in the self-interaction of RNase J using bacterial two hybrid (BACTH)
252 experiments that test the interaction of protein pairs in *E. coli*. First, a consistent
253 interaction of wild type RNase J with itself was detected (Figure 3A). Then,
254 different RNase J mutants were constructed and tested. As previously reported
255 for other RNase J proteins¹³, we found that the C-terminal region of RNase J is

256 essential for this interaction. Next, in order to pinpoint residues that are
257 important for RNase J multimerization, we constructed RNase J mutants in
258 which nine charged residues of this C-terminal domain were individually
259 replaced by alanine residues. As shown in Figure 3A, several mutants were
260 significantly affected in their capacity to homo-oligomerize, including RNase J
261 E618A, E654A, K660A, E663A and K649A. These data show that charged
262 residues at the C-terminal domain of RNase J are important for its self-
263 interaction.

264 These residues are not at the predicted dimerization interface of the C-
265 terminal domain, but they are likely to be involved in stabilizing the domain
266 through interactions with side chains of complementary charge.

267

268 **RNase J is acetylated at several lysine residues**

269 RNase R and RNase II from *E. coli* have been reported to be post-
270 translationally modified by acetylation but no such modification was reported on
271 RNase J. A mass spectrometry (MS) analysis was performed on an RNase J-
272 FLAG fusion protein expressed from the *H. pylori* chromosome (Supp Figure
273 S1) under the control of its native promoter. The RNase J-FLAG protein was
274 pulled down from *H. pylori* crude lysates using anti-FLAG antibodies and
275 extracted from a polyacrylamide gel. The experiment was performed on three
276 biological replicates from cultures in exponential phase. We found that nine
277 lysine residues of RNase J, spread across all protein domains, are consistently
278 acetylated: K134, K140, K323, K337, K397, K511, K547, K634 and K649 (Supp
279 Figure S2 and Supp Table S2).

280

281 **Lysine 649 is important for the oligomerization state of RNase J**

282 Lysine 649 (K649), localized at the RNase J C-terminal domain, was
 283 particularly interesting since this residue was both acetylated and required for
 284 RNase J homo-oligomerization in two hybrid experiments. Therefore, AUC
 285 analyses were performed with purified RNase J variants (Supp Figure S1) to
 286 test the importance of K649 in RNase J homo-oligomerization. As shown in
 287 Figure 3B, the purified RNase J K649A mutant assembles exclusively as a
 288 dimer *in vitro*, with a complete loss of the monomeric population seen with the
 289 WT protein. Circular dichroism was performed to verify that this effect was not
 290 due to a defect in the folding of RNase J K649A. The circular dichroism spectra
 291 of wild-type and K649A variants of RNase J did not show significant differences
 292 (Supp Figure S3) and the estimated proportions of the different secondary
 293 structure elements were maintained (Supp Table S3). This shows that mutation
 294 K649A does not introduce a major folding defect in RNase J secondary
 295 structure. Thus, K649 is important for RNase J dimerization both *in vitro* and in
 296 *E. coli*.

297

298 **Acetylation mimicry of Lysine 649 affects the oligomeric state of RNase J**

299 Given that K649 is acetylated, we next investigated whether RNase J
 300 acetylation at this residue could represent a control level of its oligomeric state.
 301 Therefore, we constructed RNase J mutants in which K649 was either replaced
 302 by an arginine residue (K649R), that mimics a non-acetylated state but
 303 maintains a positive charge at this position, or replaced by a glutamine residue
 304 (K649Q) which mimics an acetylated state while neutralizing the positive charge
 305 [a previously validated strategy²⁶]. As shown in Figure 3C, the AUC

306 sedimentation profile of RNase J K649R is similar to that of the wild type
307 enzyme. In contrast, RNase J K649Q shows a significantly increased proportion
308 of dimers over the monomers, similarly to the K649A mutant (Figure 3B-C).
309 These data show that the positive charge of K649 is important for the stability of
310 the RNase J monomeric state and that oligomerization is controlled by the
311 charge state of K649.

312

313 **Mutations in K649 alter the exoribonuclease activity of RNase J**

314 Since RNase J WT forms both monomers and dimers *in vitro*, we next
315 assessed whether the oligomeric state and the presence of a charge at position
316 649 were correlated with differences in the *in vitro* activity of this protein. The
317 exoribonuclease activity of RNase J was measured with a 24-nt long single
318 stranded RNA substrate from the 5'-untranslated region (UTR) of *rnj*, upon
319 which we previously found RNase J to be active⁸. This substrate was labeled
320 with 6-carboxyfluorescein (6FAM) at either the 3' or 5'-ends. As a control, a
321 mutant inactivated at the RNase J active site, D299A, was constructed. We
322 found, as expected, that the D299A inactive mutant did not degrade the
323 substrate while the wild type RNase J was active in its 5'-3' exoribonucleolytic
324 mode (Figure 4A-B and Table 1). This activity is evident from the rapid depletion
325 in the intact substrate bands labelled in 5' (Figure 4A) or 3' (Figure 4B) and,
326 from the additional accumulation of the 1nt product upon digestion of the 5'-
327 labeled substrate (Figure 4A).

328 The RNase J mutant with K649 replaced by R, that mimics a non-acetylated
329 state, and which behaves as monomers and dimers like the WT protein, was
330 strongly affected in its exoribonuclease activity (Figure 4 and Table 1). For the

331 K649R variant, the half-lives of the 3'- and 5'-labeled substrates increased by 5
332 and 10-fold, respectively (Table 1).

333 In contrast, the protein variants with K649 replaced either by A (that
334 abolishes both the positive charge at this position and the acetylation) or by Q
335 (acetylation-mimicking mutation) and that both mainly assembled into dimers,
336 presented an activity that was significantly higher than that of the RNase J
337 K649R variant. The half-lives of 3' and 5'-labeled substrates were 13 and 49-
338 fold lower for the K649A variant and 2.5 and 4-fold lower for the K649Q variant,
339 respectively. The half-lives of the substrates were calculated from three
340 independent experiments for the wild type and for every RNase J variant and
341 are shown in Table 1.

342 From these data, we conclude that, for these mutants, the dimeric form of
343 RNase J is most probably the active form *in vitro*. Altogether, we identified a
344 residue, K649, whose charge is important for RNase J activity and
345 oligomerization and that is acetylated in *H. pylori* cells. This indicates a
346 mechanism of control of RNase J activity that is based on the allosteric control
347 of its dimerization by acetylation.

348

349 **The activity of the RNase J-RhpA complex is not affected by K649** 350 **mutations**

351 It is not known whether the oligomerization state of RNase J can affect the
352 formation of the RNA degradosome complex. Therefore, we wanted to
353 determine whether mutations of K649 affect the activity of the RNase J-RhpA
354 complex. For this, we used a 45-nt long RNA substrate with hairpin structures at
355 both ends to prevent the exoribonuclease activity from the 5'-extremity and

356 labeled it with 6FAM at the 3' end [as in ¹³, Supp Figure S4A]. First, the activity
 357 of RNase J WT and mutant proteins alone was tested on this substrate. As
 358 compared to the linear 3'-labeled substrate, the degradation kinetics of this
 359 substrate by RNase J was significantly reduced but not abolished (half-life of
 360 the substrate is >4 fold lower, Table 1), suggesting that RNase J might attack
 361 this substrate by its endoribonuclease activity and then proceeds by
 362 exoribonuclease activity. As we previously reported, in a functional
 363 degradosome complex, RhpA will unwind the secondary structure elements and
 364 allow RNase J to act in its exoribonucleolytic mode. Most interestingly, the
 365 activity tests with a mixture of RNase J and RhpA in a 1:1 ratio resulted in a
 366 significant decrease in the half-life of the substrate to 6.3 ± 2.9 min as compared
 367 to the activity of RNase J alone (approx. 31.4 ± 17.6 min), compatible with a
 368 functional complex (Table 1). The half-life of the structured substrate incubated
 369 with the complex between RhpA and the RNase J K649A variant (that has
 370 increased exoribonuclease activity) was not affected (half-life 6.7 ± 0.5 min)
 371 (Supp Figure S4B and Table 1). We conclude that the activity of the RNase J-
 372 RhpA complex is not significantly affected by the K649A mutation.

373

374 **Residue D185 is important for oligomeric state and the activity of RNase J**

375 The RNase J structure suggests that residue D185 may indirectly support the
 376 stability of the monomer, as this residue is in an interaction network that
 377 supports a helical extension involving residues 584-594 that supports
 378 interactions with the C-terminal domain. In agreement with our predictions, AUC
 379 analysis of the RNase J D185A mutant revealed a much more predominant
 380 dimeric population, as compared to the wild type protein (Figure 5). Consistently

381 with what we found for mutant K649A, this was correlated with higher
382 exoribonucleolytic activity of RNase J D185A (Figure 4A-B and Table 1),
383 suggesting again that the structural destabilization of the monomer leads to an
384 increased amount of dimer with an enhanced enzymatic activity.

385

386 **Analysis of the consequences of RNase J mutations on *H. pylori*** 387 **morphology**

388 We next wanted to examine the consequences of the mutations affecting
389 RNase J oligomerization and/or acetylation state in live *H. pylori* cells. Since
390 RNase J is an essential protein, strains carrying mutations in the acetylated
391 residues identified above were constructed with the following strategy. *H. pylori*
392 strain B128 was transformed with plasmid pILL2157 derivatives either
393 expressing wild type or mutant variants of RNase J under the control of an
394 IPTG-inducible promoter. Then, the chromosomal wild type copy of RNase J
395 was deleted by homologous recombination and replacement with a kanamycin
396 resistance cassette.

397 In *B. subtilis*, mutants deficient in RNase J1 present an altered cell
398 morphology²⁷. Therefore, the morphology of our *H. pylori* RNase J mutant
399 strains was examined by phase contrast microscopy and quantified with the
400 MicrobeJ ImageJ plug-in²⁸. The wild type B128 strain presents cells that are
401 relatively heterogeneous in shape and with an average length of $2.15 \pm 0.67 \mu\text{m}$.
402 We observed that, in a strain expressing wild type RNase J from the system
403 described above, the *H. pylori* cells were longer in the absence of IPTG [lower
404 amount of RNase J as compared to the WT strain⁸], than upon induction with 1
405 mM IPTG (where the expression of RNase J is induced). The cell length was

406 2.44±0.61 μm without IPTG and 2.05±0.51 μm with IPTG (Figure 6). This
407 indicates that in *H. pylori*, RNase J is also regulating a factor required to
408 maintain wild type cell morphology. Interestingly, we noted that the observed
409 phenotype is not dependent on the activity of RNase J alone but rather on the
410 RNA degradosome complex, as IPTG induction of RNase J WT in a strain
411 lacking RhpA fails to complement this phenotype (average length of 3.41±0.83
412 μm) (Figure 6A).

413 When we performed these tests with strains that expressed different RNase
414 J variants, we identified two groups of mutations. In the first group, the RNase J
415 variants behaved similarly to the wild type enzyme, as their expression upon
416 IPTG addition resulted in a reduction of bacterial length. This was the case for
417 RNase J K323A, K547A and K649A and, to a lesser extent, RNase J K634A. In
418 the second group, that includes variants K134A, K140A, K337A, K397A and
419 K511A, the strains presented a strong growth defect and substantial cell lysis
420 and cellular debris were observed. In these cases, the cells were short with a
421 tendency towards a coccoid morphology that is typical of *H. pylori* cultures
422 under stress²⁹. This phenotype was particularly evident in the case of cells
423 expressing variant K397A. For these cultures, addition of IPTG favored bacterial
424 growth but failed to restore the typical helical form of healthy *H. pylori* cells,
425 suggesting that RNase J function is strongly impaired.

426 These results show that RNase J activity is important for the correct
427 regulation of growth and cell morphology in *H. pylori* and that several of the
428 residues that we found to be acetylated are important for RNase J function *in*
429 *vivo*.

430

431 RNase J is acetylated through a range of mechanisms in *H. pylori*

432 Several pathways contribute to acetylation in bacteria³⁰. We tried to elucidate
 433 which are the enzymes responsible for the different acetylation events that
 434 occur on the RNase J of *H. pylori*. Therefore, we used the *H. pylori* strain
 435 expressing an RNase J-FLAG fusion from the chromosome to introduce
 436 deletions in genes that we predicted as acetylase candidates. The RNase J-
 437 FLAG protein was enriched from these strains and analyzed by MS as
 438 explained above. The tests were performed from three biological replicates. The
 439 acetylase candidate genes were *hpb8_615* [henceforth referred to as *rimI*, as it
 440 presents homology to *rimI* from *E. coli*³¹] and *hpb8_1270* [that contains two
 441 Gcn5-acetyltransferase (GNAT) domains]. Non-enzymatic acetylation by acetyl-
 442 CoA and acetyl-phosphate also occurs in bacteria^{30,32}. The impact of this
 443 pathway was assessed in *H. pylori* by constructing a mutant in the *pta-ackA*
 444 operon that is in charge of acetyl-phosphate synthesis and metabolism. As
 445 shown in Figure 7, we found that several pathways influence the acetylation of
 446 RNase J lysine residues. RimI is partially responsible for the acetylation of
 447 RNase J residues K323, K397 and K649. The HPB8_1270 protein is partially
 448 responsible for the acetylation of residues K323, K397, K511 and K649. Acetyl
 449 phosphate-mediated non-enzymatic acetylation participated in the acetylation of
 450 K134, K323, K397 and K511 and K649. For other residues, namely K140,
 451 K337, K547 and K634, no change in acetylation levels was detected in the
 452 mutants, suggesting that other acetylation mechanisms are likely at play in *H.*
 453 *pylori*. These results suggest a high redundancy of the different acetylation
 454 mechanisms that act on RNase J, particularly on residues K323, K397 and
 455 K649.

456 Thus, multiple mechanisms participate in the acetylation of RNase J on
457 different residues.
458

459 Discussion

460 In this study, we defined the structure of the major *H. pylori* ribonuclease
461 RNase J and its *in vitro* oligomerization state alone and in the RNA
462 degradosome with its RhpA RNA helicase partner. We also identify *in vivo*
463 acetylation patterns of RNase J and demonstrate that these impact on
464 oligomerization and activity *in vitro* and *in vivo*.

465 We found, by AUC, that RNase J is in equilibrium between monomeric and
466 dimeric states. Earlier studies showed that *D. radiodurans* RNase J is also
467 distributed between monomers and dimers¹³. *Bsu*RNase J1 forms dimers and
468 tetramers *in vitro*¹⁴ and, when mixed with *Bsu*RNase J2, heterotetramers¹⁰.
469 Similarly, RNase J1 from *S. aureus* forms homodimers and homotetramers *in*
470 *vitro*³³. RNase J of *S. pyogenes* crystallizes as tetramers that are composed of
471 a dimer-of-dimers¹². In the present study, at the studied *H. pylori* RNase J
472 concentrations, no trace of tetramerization could be seen by AUC. It is likely
473 that the observed tetrameric structure in the X-ray structure is due to crystal
474 packaging and that it does not reflect the arrangement of RNase J in solution,
475 as the dimer seems to be the active form. In addition, RhpA alone behaves as a
476 monomer in solution by AUC. Together, RNase J and RhpA form a complex
477 that is compatible with a 1:1 stoichiometry.

478 Acetylation has emerged in recent years as a major post-translational
479 regulatory mechanism in prokaryotes¹⁶, and a few examples of its influence in
480 RNases and RNA-degrading enzymes have been described, including *E. coli*
481 RNase R¹⁷ and RNase II¹⁸ and *B. subtilis* CshA¹⁹. Here, we found that, in *H.*
482 *pylori*, as many as 9 lysine residues of RNase J were consistently acetylated,
483 although probably not at the same time, which constitutes the first evidence of

484 protein acetylation in *H. pylori*. One of these residues is K649, which we also
 485 found to be involved in the oligomerization state of RNase J by BACTH in *E.*
 486 *coli*. Interestingly, this residue is located at the C-terminal domain of RNase J
 487 (Supp Figure S2). This region was also shown to be important for the
 488 oligomerization and exoribonucleolytic activity of the RNase J of *D.*
 489 *radiodurans*¹³. Therefore, we examined the role of K649 in RNase J
 490 oligomerization (by AUC) and exoribonuclease activity. Three mutants were
 491 analyzed, RNase J K649A that abolishes the side chain of the lysine residue,
 492 K649R that mimics a non-acetylated state while maintaining the positive charge
 493 and K649Q that mimics an acetylated state²⁶. Whereas the K649R variant
 494 maintains a sedimentation profile similar to that of the wild type enzyme,
 495 variants lacking a positive charge in K649, either K649Q or K649A, had an
 496 increased proportion of dimer or displayed no monomeric form, respectively.
 497 Interestingly, we observed that the K649R variant is less active than the wild
 498 type RNase J whereas variants K649Q and K649A are more active than the
 499 K649R variant.

500 Our structural data indicated that residue K649 is not located at the
 501 interaction surface between the subunits of the dimer, but that it rather plays a
 502 structural role in the integrity of the overall structure. We conclude that
 503 elimination of the positive charge at residue 649 leads to a higher stabilization
 504 of the dimeric species. In agreement with this hypothesis, we observed that
 505 D185A, another mutation in a structurally important residue, had the same
 506 effect and favors the dimeric species. Interestingly, in *D. radiodurans*, the
 507 corresponding residue (D61) had already been identified as key for RNase J
 508 dimerization¹³. Electrostatic interactions of charged side chains on the surface

509 of proteins can strongly contribute to the fold stability²³⁻²⁵. We propose that the
 510 network of surface electrostatic interactions stabilizes the CTD of RNase J,
 511 which in turn supports the homodimer. The CTD is the primary self-
 512 complementary interface of the dimer, and the interface between the core
 513 (amino acids 137-586) and between the dimer-of-dimers formed by the core
 514 both have marginal stability in isolation. Thus, changes in the charge, through
 515 acetylation, can thereby impact on stability of the CTD fold and oligomerization
 516 state.

517 The question naturally arises of how the catalytic activity of RNase J is
 518 affected by its oligomerization state. A clue is suggested in the overlay of the
 519 structure of *H. pylori* RNase J with the *S. coelicolor* homologue, in which there
 520 is a bound RNA substrate engaged at the active site (Pei et al., 2015) (Figure
 521 1D). There are marked differences in the positions of loops at the entrance to
 522 the catalytic site that would obstruct the RNA from following the same route in
 523 the *H. pylori* enzyme, but the nucleic acid could approach the site from the
 524 opposite rim of the entrance. The exposed peptide loop regions are positioned
 525 to accommodate the CTD. It is possible that changes in the oligomerization
 526 state, through changes in CTD stability, will impact on the presentation of those
 527 loops and access to the active site in a gating mechanism.

528 Altogether, our results suggest that mutations that favor the dimeric RNase J
 529 conformation also increase the activity of RNase J of *H. pylori* *in vitro*. As the
 530 charge of residue 649 is important for dimerization, acetylation of K649 might tilt
 531 the balance of the RNase J species towards the dimeric form and potentially
 532 increase its activity. In this way, acetylation on K649 can modulate the activity of
 533 RNase J in *H. pylori* cells. Formation of the RNase J-RhpA complex is not

534 affected by mutations or modifications of residue K649 in the ribonuclease, and
535 thus such mutations do not significantly alter the activity of this complex. Earlier
536 studies indicate that RNase J and the RNase J-RhpA complex can act on
537 different transcripts^{2,8,34}. It can be envisaged that acetylation of K649 would
538 therefore have a differential impact on the subset of free RNase J that is not
539 part of a degradosome assembly.

540 Furthermore, we addressed the role of the acetylated residues that we
541 identified in *H. pylori* cells. It has been described that *B. subtilis* cells lacking
542 RNase J1 show morphology defects with a tendency to filament²⁷. We thus
543 evaluated the morphology of *H. pylori* cells expressing RNase J variants, in
544 which each acetylated lysine was replaced by an alanine residue. We observed
545 that cells with a reduced concentration of wild type RNase J are longer than
546 those in which RNase J expression is induced. This revealed that RNase J,
547 most probably indirectly, is involved in the control of *H. pylori* length.
548 Interestingly, this phenotype does not depend on the activity of RNase J alone,
549 but rather on the RNA degradosome complex, since the overexpression of
550 RNase J WT in a $\Delta rhpA$ strain does not reduce cell length. Given that we
551 previously showed that about 80% of *H. pylori* mRNAs are at least two times
552 more abundant in an RNase J-depleted mutant, it is difficult to define the target
553 mRNA that is causing the observed elongation phenotype⁸. One possibility
554 would be the overexpression of MreC (9.3-fold upregulated in an RNase J-
555 depleted strain), that has been shown to cause cell filamentation in *H. pylori*³⁵.

556 Alanine replacement of the different acetylated positions of RNase J leads to
557 different phenotypes, showing that acetylation is indeed important for RNase J
558 activity in *H. pylori*. Variant K649A behaves like the wild type enzyme in this

test, in agreement with the notion that the morphology phenotype depends on the activity of the whole RNA degradosome complex, which is not affected by whether there is a charge in this position or not. The phenotype of the K134A mutant suggests that it is affected in RNase J activity; interestingly this residue is located within the N-terminal extension of *Hp*RNase J (Supp Figure S2), that we previously found to be very important for efficient RNase J activity⁸. However, it is not known how these acetylated residues impact RNase J properties. They could affect its stability, activity, oligomerization, degradosome complex formation, compartmentalization, binding of other partners or RNA, or a combination of these.

We found that several mechanisms participate in the acetylation of the different residues of RNase J. Our results indicate that HPB8_1270 and the *H. pylori* RimI homologue indeed function as acetylases. In addition, we uncovered a role of non-enzymatic acetylation by acetyl phosphate that is produced and metabolized by the Pta and AckA enzymes. The *E. coli* RimI protein is a multifunctional acetyltransferase, that can perform ϵ -lysine acetylation and N α acetylation on the L31 and S18 ribosomal proteins, respectively^{31,36}, with high substrate specificity. In contrast, RimI from *Mycobacterium tuberculosis* is an N α acetylase with more relaxed substrate specificity *in vitro*³⁷. Here, we found that the *H. pylori* RimI targets K323, K397 and K649 of RNase J. HPB8_1270 is a protein belonging to the DUF2156 family, containing two GNAT domains. It presents homology with the BT_3689 protein from *Bacteroides thetaiotaomicron*, which contains an acetyl-CoA molecule in its active site (PDB 2HQY), suggesting its function as an acetyltransferase. Here, we found that HPB8_1270 is involved in the acetylation of K323, K397, K511 and K649,

584 showing that in *H. pylori* this protein is most likely an acetyltransferase. Lastly,
585 acetyl phosphate-mediated acetylation, which is responsible for most
586 acetylation events in *E. coli* and *B. subtilis*^{30,32}, was important for the acetylation
587 of K134, K323, K397, K511 and K649.

588 These data demonstrate that multiple mechanisms can target the same
589 lysine residue and that there is a high redundancy in the functions of these
590 acetylation mechanisms. Acetylation at residue K649 was strongly dependent
591 on three such mechanisms; this redundancy could testify of a critical importance
592 of acetylation at this position. In addition, other acetylation mechanisms could
593 occur in *H. pylori* that might account for some of these acetylation events. More
594 work is needed, in particular using multiple mutants to determine how many of
595 these mechanisms can target the different residues and the influence of the
596 different acetylation mechanisms on the activity and regulation of RNase J and
597 the RNA degradosome.

598 Using Clustal Omega 1.2.4³⁸, we performed an alignment of the RNase J
599 proteins of *H. pylori* and of the closely related organism *Campylobacter jejuni*,
600 but also of the more distant *B. subtilis*, *S. aureus*, *S. pyogenes* and *D.*
601 *radiodurans*. Interestingly, this revealed that most of the acetylated lysines are
602 conserved in *H. pylori* and *C. jejuni* RNase J proteins, and some of them are
603 also conserved in more distant organisms (Supp Figure S5). The case of K511
604 is particularly interesting, as it is conserved in *S. pyogenes*, *B. subtilis*, *H. pylori*
605 and *C. jejuni*, and in the case of *S. aureus* this position is a Q residue which
606 might behave as a “permanently-acetylated” lysine. In *H. pylori*, the mutant
607 expressing RNase J K511A variant presents a strong growth defect. The

608 relevance of lysine acetylation in the regulation of the activity of the RNase J
609 proteins from other organisms remains to be addressed.

610 In conclusion, we have established that RNase J is acetylated on multiple
611 lysine residues through different mechanisms, and that its acetylated residues
612 affect the activity of RNase J and phenotypes such as cell morphology.
613 Furthermore, our data suggests that acetylation on K649 can influence the
614 oligomeric state and activity of RNase J, and that the dimeric form could be the
615 most active one. Thus, acetylation is an important player in the modulation of
616 the activity of RNase J and of the RNA degradosome of *H. pylori*.

617

618 **Materials and methods**

619 *Bacterial strains and growth conditions*

620 The bacterial strains used in this study are summarized in Supp Table S4. *H.*
621 *pylori* strains were derivatives of strain B128^{39,40}. Plasmids (Supp Table S4)
622 were constructed and amplified using *E. coli* XL1-Blue (Agilent Technologies).
623 *H. pylori* strains were grown on Blood agar base 2 (Oxoid) plates supplemented
624 with 10% defibrinated horse blood and with an antibiotic-antifungal cocktail
625 composed of 2.5 µg/mL amphotericin B, 0.31 µg/mL polymyxin B, 6.25 µg/mL
626 trimethoprim and 12.5 µg/mL vancomycin. *H. pylori* mutants were selected
627 using 20 µg/mL kanamycin, 10 µg/mL apramycin or 6 µg/mL chloramphenicol.
628 For liquid cultures, we used Brucella broth supplemented with 10% fetal calf
629 serum (FCS) (Eurobio), the antibiotic-antifungal cocktail and, when necessary,
630 chloramphenicol to select for plasmid-containing bacteria. *H. pylori* cells were
631 grown at 37°C under a microaerophilic atmosphere (6% O₂, 10% CO₂, 84% N₂)
632 using an Anoxomat (Mart Microbiology) atmosphere generator.

633

634 *Plasmid preparation and transformations*

635 A NucleoBond Xtra midi kit (Macherey-Nagel) and a QIAamp DNA minikit
636 (Qiagen) were used for plasmid preparations for *H. pylori* transformation and
637 genomic DNA extractions, respectively. For plasmid preparations for *E. coli*
638 transformation and sequencing, a QIAprep Spin Miniprep kit (Qiagen) was
639 used. PCR was carried out with either DreamTaq DNA polymerase
640 (ThermoFisher) or with Q5 High-Fidelity DNA polymerase (NEB) when the
641 product required a high-fidelity polymerase. A list of the oligonucleotides that
642 were used can be found in Supp Table S5.

643

644 *Construction of H. pylori mutants and fusions*

645 The cassette for the construction of the *H. pylori* RNase J-FLAG strain was
646 constructed by the isothermal assembly technique⁴¹, by assembling in frame
647 the last 500 bp of the *rnj* gene followed by the FLAG-tag, the apramycin
648 resistance gene and the 500 bp immediately downstream from *rnj*. The resulting
649 fragment was PCR-amplified and 1 µg of the product was introduced into *H.*
650 *pylori* by natural transformation. Transformants were selected on plates with
651 apramycin. Strains carrying the pILL2157 plasmid with the wild type *rnj* gene²
652 and its derivatives with the different *rnj* mutants (constructed following standard
653 procedures with the primers compiled in Supp Table S5) were transformed in
654 the same manner with 1.5 µg of the plasmids and selected on plates with
655 chloramphenicol. The resulting strains were then used to transform by cassettes
656 constructed in a similar manner to delete the *rnj* gene and replace it by a
657 kanamycin resistance gene [as in ²]. The transformants were selected on plates
658 containing kanamycin, chloramphenicol and 1 mM IPTG to allow the expression
659 of the copy of *rnj* from the plasmid.

660 Plasmids for BACTH were constructed from plasmids pUT18 and pKNT25⁴²
661 by inserting wild type or mutant variants of the *rnj* gene in the *Pst*I and *Kpn*I
662 restriction sites following standard techniques using plasmids indicated in Supp
663 Table S4.

664 Deletion of the genes of interest, construction of fusions and/or insertion of
665 cassettes was verified by PCR and sequencing of the region of interest.

666

667 *Purification and crystallography of RNase J*

668 A vector co-expressing residues 21-465 of RhpA with a C-terminal 3C-
669 HALO-his6 tag and residues 139-691 of RNase J (C1hRSEj) (from *H. pylori*
670 strain 26695 gene HP1430, UniProt P56185) with a C-terminal hexa-histidine
671 tag was generated by the Oxford Protein Production Facility at Harwell. Cells
672 were grown in terrific broth supplemented with 0.5 M NaCl and 1 mM betain, 30
673 µg/mL kanamycin, 50 µg/mL carbenicillin and 20 µg/mL chloramphenicol. Cells
674 were grown at 37°C until optical density at 600 nm reached 0.3, then moved to
675 20°C and induced with 0.2 mM IPTG (isopropyl β-D-1-thiogalactopyranoside)
676 for 24 hours. Cells were harvested by centrifugation, then suspended in 50 mM
677 Tris pH 7.4, 50 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic
678 acid) pH 7.4, 500 mM NaCl, 1 mM TCEP (tris(2-carboxyethyl)phosphine),
679 0.05% v/v Triton X-100, 5% v/v glycerol and 10 mM imidazole and stored
680 frozen. The suspension was thawed, passed 5 times through a cell disruptor
681 (Avestin Emulsiflex C5 homogeniser, 1000 bars) and clarified by centrifugation
682 at 37,000 g for 30 minutes, 5°C. The supernatant was applied to a HiTrap Ni
683 chelating column, washed with lysis buffer containing 0.01% v/v Triton X-100,
684 and 15 mM imidazole, then eluted with a gradient in imidazole. The optimal
685 fractions were pooled and cleaved with 3CP-GST protease cleavage, using 300
686 µl of 0.85 mg/mL protease to cleave C1/RSE protein isolated from 1 L culture,
687 at 4°C overnight. This step removes HALO-Chis-tag on RhpA. The 3CP-cleaved
688 protein was loaded onto HALO-RESIN and the flow-through collected, which
689 contained the RNase J/helicase complex. The sample was concentrated with a
690 30kD MWCO centrifugal concentrator and applied to a S200 SEC column
691 equilibrated in 20 mM Tris-HCl pH 7.4, 20 mM HEPES pH 7.4, 150 mM NaCl,
692 5% glycerol, 0.1% v/v Triton X-100, 1 mM TCEP. The sample was concentrated

693 with a 30kD MWCO centrifugal concentrator to optical density at 280 nm of 3.38
694 and used in crystallization screens. Crystals were found in PEG I screen 0.1 M
695 HEPES pH 7.5, 25% wt/v polyethylene glycol 1000.

696 Diffraction data were collected at I24 at Diamond Light source at wavelength
697 0.9778 Å using a Pilatus6M detector. Crystals are in space group P4₁22 with
698 cell dimensions a=b=158 c=214.26 Å. The structure was solved by molecular
699 replacement using *Streptomyces* RNase J (PDB 3BK2) for with PHASER and
700 the CCP4 suite^{43,44}. There was no space in the lattice for the RhpA protein. The
701 model of RNase J 139-691 was built using COOT⁴⁵ and refined with PHENIX⁴⁶.
702 A homology model for the C-terminal domain, residues 587 to 691, was
703 prepared using PHYRE2⁴⁷, and the structure of the full-length protein was
704 predicted using the ROBETTA server²¹. The C-terminal domain from the
705 ROBETTA model was docked into the map and fitted as a rigid body and then
706 linked to the model of the well resolved core (139-586). As the density for the C-
707 terminal domain was poorly resolved due to disorder, the side chains were
708 removed. A final cycle of refinement using the core only (137-586) and not the
709 disordered C-terminal domain were used to provide the refinement parameters
710 summarized in the supplementary Table S1. A monomer of RNase J 139-691
711 occupies the asymmetric unit, and the tetramer is generated through
712 crystallographic symmetry. Estimates of buried surface area were made using
713 the PISA program in the CCP4 suite⁴⁸. The model and structure factors have
714 been deposited in the PDB with accession code 7PCR.

715

716

717 *Bacterial two-hybrid (BACTH)*

Bacterial two hybrid assays were carried out in *E. coli* strain BTH101 as previously described⁴². Briefly, strains carrying derivatives of the two vectors, pUT18 and pKNT25, were grown overnight in 1 mL LB with 40 µg/mL kanamycin, 100 µg/mL ampicillin and 0.1 mM IPTG. The OD at 600 nm of the resulting cultures was measured in a TECAN plate reader and the beta-galactosidase activity was calculated by mixing 500 µL of the cultures with 500 µL of buffer Z, 100 µL of chloramphenicol and 50 µL of 0.1% SDS, vortexing the cells and then adding 200 µL of 4 mg/mL ONPG. The reactions were incubated at 28°C until they turned yellow and the reactions were stopped by adding 500 µL of 1 M Na₂CO₃. Samples were centrifuged for 5 min at 14,000 rpm and the OD at 420 and 550 nm of the upper fraction was measured in a TECAN plate reader. The beta-galactosidase activity (Miller units) was calculated as described⁴⁹.

731

732 *Protein expression and purification*

Wild type and variant versions of RNase J and RhpA were cloned into a pET28a+ vector in *E. coli* XL1-Blue and then transformed in *E. coli* Bli5 for expression. The strains were grown in 800 mL cultures in LB with 40 µg/mL kanamycin and 25 µg/mL chloramphenicol and, once they reached an OD of 0.5-0.8, were induced with 1 mM IPTG. The cultures were incubated at 30°C and 160 rpm during 3 h. The cells were collected by centrifugation at 4000 rpm in an Avanti J-E centrifuge (Beckman Coulter) with a JA-14 rotor at 4°C during 30 min and pellets were frozen at -80°C until further processing.

Pellets were thawed on ice and resuspended on lysis buffer containing 25 mM Tris-HCl pH 7.8 for RNase J and pH 8 for RhpA, 500 mM NaCl, 10%

glycerol, 20 mM imidazole, 1 mM dithiothreitol (DTT), 50 U benzonase and protease inhibitor cocktail (c0mplete EDTA-free, Roche). Cells were lysed by sonication and centrifuged for 30 min at 4,000 rpm in a 5810R centrifuge (Eppendorf) at 4°C to eliminate cellular debris. The supernatant was then incubated overnight at 4°C and under agitation with 2 mL Ni-NTA resin (50% v/v, Thermofisher) pre-equilibrated with lysis buffer.

Resins were packed onto Poly-Prep Chromatography columns (Bio-Rad) and washed with 10 volumes of 25 mM Tris-HCl at the appropriate pH, 300 mM NaCl, 1 mM DTT and 20 mM imidazole, 10 volumes of the same buffer with 50 mM imidazole and 10 volumes of the same buffer with 100 mM imidazole. The elution was carried out with an elution buffer containing 25 mM Tris-HCl at the appropriate pH, 300 mM NaCl, 1 mM DTT and 500 mM imidazole, in 1 mL fractions. Aliquots of the resulting fractions were run on a 4-20% Mini-Protean TGX stain-free precast protein gel (Bio-Rad) at 200V for 30 min and stained by Imperial protein stain (ThermoFisher). After destaining, the corresponding purest fractions were chosen, mixed and concentrated using Vivaspinn 20 tubes (MWCO 30,000 Da, GE Healthcare) down to 1 mL volumes. The buffer was replaced using G-25 columns (PD MiniTrap G-25, GE Healthcare) with 25 mM Tris-HCl, 300 mM NaCl, 1 mM DTT and 10% glycerol. Samples were aliquoted and frozen at -80°C.

Analytical ultracentrifugation

Samples of purified proteins were thawed on ice and the buffer was replaced using G-25 columns (PD MiniTrap G-25, GE Healthcare) with 25 mM Tris-HCl pH 8, 300 mM NaCl and 0.5 mM Tris(2-carboxyethyl)phosphine (TCEP, Sigma).

768 Appropriate amounts of the different proteins were mixed and diluted in the
769 same buffer with a final volume of 300 μ L that was charged into 2 sector
770 analytical ultracentrifugation cells. The concentration of the proteins was 3 mM
771 when there was only one protein, 3 mM:3 mM when two proteins were in
772 equimolar amounts and 1 mM:5 mM when the proteins were mixed at a 1:5
773 ratio. The centrifugation was performed for 16 h on an Optima analytical
774 ultracentrifuge (Beckman-Coulter) with an 8-hole AnTi50 rotor, at 42,000 rpm
775 and 20°C, measuring the optical density at 230 nm in order to determine the
776 sedimentation profile of the protein or complex. The data was analyzed with the
777 Sedfit software (NIH) using a diffusion deconvoluted continuous sedimentation
778 coefficient distribution $c(s)$ with one discrete component model⁵⁰. The model
779 was chosen in order to take in consideration the signal due to TCEP absorption.
780 The presented graphs correspond to a resolution of 300 points with a
781 confidence interval of 0.9. For the individual proteins, the sedimentation
782 coefficient independent of the concentration (S_0) was calculated.

783 The partial specific volume ($\bar{v}$) of the proteins was estimated by the software
784 Sednterp⁵¹ based on the amino acid sequence. This software was also used for
785 the calculation of the density and viscosity of the buffer used throughout the
786 experiments. Calculation of sedimentation coefficients for protein structures was
787 performed with the program Hydropro⁵².

788

789 *Circular dichroism*

790 The near-UV absorption spectra (195-260 nm) were measured, in the same
791 buffer as for the AUC experiments, by circular dichroism with a Circular
792 Dichroism Spectrometer Model 215 (Aviv instruments) using 0.2 mm optical

793 path cells with 5 mM of RNase J WT and K649A mutant. The proportions of the
794 different secondary structure elements were determined with the BeStSel
795 software^{53,54} based on the amino acid sequence.

796

797 *Ribonuclease activity tests*

798 The endoribonuclease and exoribonuclease activities were measured using
799 6-carboxyfluorescein (6FAM)-labeled RNA substrates either in 5' or in 3' as
800 listed in Supp Table S5. 500 nM RNA substrate were incubated with 500 nM
801 protein in a total volume of 10 µL with a buffer containing 50 mM Tris-HCl pH
802 7.8, 100 mM NaCl, 8 mM MgCl₂, 0.1 mM and 0.1 mg/mL bovine serum albumin
803 (BSA). The reactions were carried out at 37°C for 0-30 min for the
804 exoribonuclease activity tests and 0-75 min for endoribonuclease activity tests.
805 Reactions were stopped by adding 98% formamide and 10 mM ethylene
806 diamine tetraacetic acid (EDTA) and by incubating for 15 min at 95 °C.

807 The degradation products were run on a Mini-Protean TBE-Urea gels (Bio-
808 Rad) that was pre-run for 60 min at 200 V. The samples were run for 5 min at
809 50 V and then for 30 min at 200 V. Gels were visualized on a Chemidoc MP
810 Imaging System (Bio-Rad) with 0.2 s exposure at 488 nm. The gels were then
811 stained with ethidium bromide to reveal the molecular weight marker (ssRNA
812 molecular weight marker, Takara). Experiments were performed in triplicate and
813 images were quantified ImageLab (Bio-Rad, version 6.1). Decay coefficients, λ ,
814 were calculated with the formula $N(t) = N_0 e^{-\lambda t}$, where $N(t)$ is the amount of
815 substrate at time t and N_0 is the initial amount. The half-lives, $t_{1/2}$ were then
816 calculated with the formula $t_{1/2} = \frac{\ln(2)}{\lambda}$.

817

818 *Phase contrast microscopy*

819 Phase contrast microscopy was performed with an Axio Observer
820 microscope (Zeiss) equipped with an Axiocam camera with X100 magnification.
821 Image acquisition was performed with the axiovision software. Images were
822 cropped and adjusted using FiJi software. The length of wild type cells and that
823 of cells expressing wild type or mutant versions of RNase J from a plasmid, with
824 or without 1 mM IPTG, was measured by using the MicrobeJ plugin of ImageJ
825 ²⁸ with the following parameters: cell area above 0.7 μm^2 , minimal length of 1
826 μm and cell width between 0.4-1 μm .

827

828 *Pulldown and mass spectrometry*

829 *H. pylori* cultures of strains expressing FLAG-tagged RNase J were grown in
830 15 mL of Brucella medium until an OD_{600 nm} of 0.6-1.2 for exponential phase
831 cultures. Cells were pelleted by centrifugation for 10 min at 4 °C and 4,000 rpm
832 and resuspended in PBS to an OD_{600 nm} of 10. Cells were lysed by sonication
833 and cell debris were eliminated by centrifugation for 10 min at 13,000 rpm and 4
834 °C. The supernatant constitutes the total extract fraction.

835 Pulldowns were performed using 2 μL of monoclonal anti-FLAG M2
836 antibodies produced in mice (F3165, Sigma) per reaction and 50 μL of magnetic
837 protein G-coated Dynabeads according to the supplier's instructions
838 (Dynabeads Protein G for Immunoprecipitation, 10003D, ThermoFisher).
839 Proteins were eluted in the denaturing mode and run in a 4-20% Mini-Protean
840 TGX stain-free precast protein gel (Bio-Rad) at 200V for 30 min and stained by
841 Imperial protein stain (ThermoFisher) and destained.

Each band corresponding to RNase J-FLAG was cut and washed several times in 50 mM ammonium bicarbonate (ABC)-acetonitrile (ACN) (1:1) for 15 min at 37 °C. Disulfide bonds were reduced with 10 mM DTT and cysteines alkylated with 55 mM chloroacetamide. Trypsin (V5111, Promega) digestion was performed overnight at 37 °C in 50 mM ABC. Peptides were extracted from the gel by two incubations in 50 mM ABC-ACN-formic acid (FA) (50:50:0.5) for 15 min at 37 °C. After ACN evaporation in a Speed-Vac, resulting peptides were desalted with stage-tip⁵⁵ using C18 Empore disc and eluted with 80% ACN, 0.1% FA. Peptides were resuspended in 2% ACN, 0.1% FA prior to liquid chromatography coupled to mass spectrometry (LC-MS/MS) analysis.

For LC-MS/MS, a nanochromatographic system (Proxeon EASY-nLC 1200 - ThermoFisher Scientific) was coupled on-line to a Q ExactiveTM Plus Mass Spectrometer (ThermoFisher Scientific) using an integrated column oven (PRSO-V1 - Sonation GmbH, Biberach, Germany). For each sample, peptides were loaded on an in-house packed 26 cm nano-HPLC column (75 µm inner diameter) with C18 resin (1.9 µm particles, 100 Å pore size, Reprosil-Pur Basic C18-HD resin, Dr. Maisch GmbH, Ammerbuch-Entringen, Germany) after an equilibration step in 100 % solvent A (H₂O, 0.1% FA).

Peptides were eluted with a multi-step gradient using 2 to 7 % solvent B (80% ACN, 0.1% FA) during 5 min, 7 to 23% during 70 min, 23 to 45% during 30 min and 45 to 95% during 5 min at a flow rate of 300 nL/min over 132 min. Column temperature was set to 60°C.

MS data were acquired using Xcalibur software using a data-dependent Top 10 method with a survey scans (300-1700 m/z) at a resolution of 70,000 and a MS/MS scans (fixed first mass 100 m/z) at a resolution of 17,500. The AGC

867 target and maximum injection time for the survey scans and the MS/MS scans
868 were set to 3E6, 20 ms and 1E6, 60 ms respectively. The isolation window was
869 set to 1.6 m/z and normalized collision energy fixed to 28 for HCD
870 fragmentation. We used a minimum AGC target of 1E4 for an intensity threshold
871 of 1.7E5. Unassigned precursor ion charge states as well as 1, 7, 8 and >8
872 charged states were rejected and peptide match was disable. Exclude isotopes
873 was enabled and selected ions were dynamically excluded for 30 seconds.

874 Raw data were analyzed using MaxQuant software version 1.6.6.0⁵⁶ using
875 the Andromeda search engine⁵⁷. The MS/MS spectra were searched against a
876 UniProt *Helicobacter pylori* [strain B8^{39,40}] database (download in 24/06/2019,
877 1,719 entries) and tagged RNase J. Usual known mass spectrometry
878 contaminants and reversed sequences of all entries were included.

879 Andromeda searches were performed choosing trypsin as specific enzyme
880 with a maximum number of five missed cleavages. Possible modifications
881 included carbamidomethylation (Cys, fixed), oxidation (Met, variable), Nter
882 acetylation (variable) and K acetylation (Lys, variable) for in-gel peptides. The
883 mass tolerance in MS was set to 20 ppm for the first search then 4.5 ppm for
884 the main search and 20 ppm for the MS/MS. Maximum peptide charge was set
885 to seven and five amino acids were required as minimum peptide length.

886 A false discovery rate (FDR) cutoff of 1 % was applied at the peptide and
887 protein levels.

888 Raw files and MaxQuant files (msms.txt) were processed in Skyline-daily
889 20.1.9.268⁵⁸ to generate MS1-XIC and perform peak integration for each K-
890 acetylated peptide. Normalization across samples was performed by using the
891 mean of the ratios from non-modified peptides (Supp Table S2).

892 The mass spectrometry data have been deposited to the
893 ProteomeXchange Consortium via de PRIDE partner repository⁵⁹ with the
894 dataset identifier PXD027670.

895

896 *Statistical analyses*

897 Statistical analysis was performed using Prism 9. We applied one-way
898 ANOVA with Dunnett's multiple comparisons test in Figure 2A, one-way ANOVA
899 with Sidak's multiple comparisons test in Figure 6B and two-way ANOVA with
900 Dunnett's multiple comparisons test in Figure 7.

901

902

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923

924 **Declaration of interests**

925 The authors declare no competing interests.

926 **Figure legends**

927

928 **Figure 1. Structure of RNase J from *H. pylori*.** Crystals were prepared from
 929 *H. pylori* RNase J residues 139-691, encompassing the beta-CASP, metallo-
 930 beta-lactamase and C-terminal domains. (A) View of the tetramer, which is a
 931 dimer-of-dimers. A monomer occupies the asymmetric unit, and the tetramer is
 932 generated through crystallographic symmetry. (B) Overlay of the predicted
 933 structure from Robetta server (beige) and the refined crystal structure (blue).
 934 The individual beta-CASP and metallo-beta-lactamase domains overlay well,
 935 but there are noted differences in the orientation of the three loop regions 174-
 936 183, 431-441 and 575-586 at the entrance to the active site (shifts indicated by
 937 orange arrows). The predicted structure is closer to the orientation seen in
 938 *Streptomyces coelicolor* RNase J, shown in overlay with *H. pylori* enzyme in the
 939 left panel of (C). The *S. coelicolor* enzyme structure (beige) is in complex with
 940 RNA, and the path of the RNA substrate along a channel to the active site is
 941 shown in the right panel. The *H. pylori* RNase J (blue) cannot bind RNA with the
 942 same orientation seen for the *S. coelicolor* enzyme due to steric clashes with
 943 the loops (left panel). The two zinc ions at the active site of the *S. coelicolor*
 944 enzyme are indicated in the right panel.

945

946 **Figure 2. The solution oligomerization state of RNase J, RhpA and the**
 947 **degradosome assembly.** The profiles are from analytical ultracentrifugation
 948 sedimentation of (A) RNase J, (B) RhpA and (C) the degradosome complex at
 949 different ratios of RNase J and RhpA. The profiles were recorded at 230 nm
 950 wavelength.

951

952 **Figure 3. Point mutations modify the oligomeric state of RNase J. (A)**

953 BACTH two hybrid experiments to assess homo-oligomerization of WT and
954 mutant RNase J proteins. Differences in the beta-galactosidase activity units
955 are indicated with ns (non-significant) ** p-value<0.005, *** p-value<0.0005, ****
956 p-value<0.0001. (B) Analytical ultracentrifugation sedimentation profiles of
957 RNase J WT and variant K649A at 230 nm wavelength. (C) Analytical
958 ultracentrifugation sedimentation profiles of RNase J WT and variants K649R
959 and K649Q at 230 nm wavelength.

960

961 **Figure 4. Exoribonuclease activity of RNase J wild type and mutants. (A)**

962 Exoribonuclease activity of RNase J WT and different variants using a 24-nt
963 RNA substrate labeled with 6-carboxyfluorescein (6-FAM) at its 5'-end over the
964 course of 30 min. (B) Exoribonuclease activity of RNase J WT and different
965 variants using a 24-nt RNA substrate labeled with 6-FAM at its 3'-end over the
966 course of 30 min.

967

968 **Figure 5. Impact of D185A mutation on RNase J oligomeric state. Analytical**

969 ultracentrifugation sedimentation profiles of RNase J WT and variant D185A,
970 recorded at 230 nm wavelength.

971

972 **Figure 6. *Helicobacter pylori* cell morphology is affected by RNase J**
973 **abundance and point mutations at lysine residues that are acetylation**

974 **targets. (A)** Phase contrast microscopy images of *H. pylori* Δrnj B128 strains
975 expressing WT or mutant versions of RNase J from plasmid pILL2157 without

976 (top panels) or with IPTG induction (bottom panels). The phenotype of a *H.*
 977 *pylori* Δrnj - $\Delta rhpA$ strains expressing WT RNase J from a plasmid is also shown.
 978 The scale bar represents 2 μ m. (B) Dot plots showing the distribution of the cell
 979 lengths of wild type and the different mutants without or with IPTG. Horizontal
 980 lines indicate the mean length and the error bars are the standard deviation. ns
 981 (non-significant), ***p-value<0.0005, ****p-value<0.0001.

982

983 **Figure 7. RNase J peptides containing acetylated residues extracted from**
 984 **different *H. pylori* strains.** The normalized intensities were obtained by mass
 985 spectrometry from strains expressing FLAG-tagged RNase J in a wild-type
 986 background or in a mutant strain lacking different acetylation mechanism. ns
 987 (non-significant) p-value>0.05, *p-value<0.05, **p-value<0.005, ***p-
 988 value<0.0005, ****p-value<0.0001. Error bars represent the standard deviation.

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995 **Table**

996 **Table 1. Half-lives of fluorescent RNA substrates in the presence of**
 997 **different variants of RNase J alone or in complex with RhpA.** The half-life
 998 values in brackets are estimated values extrapolated from the decay slope
 999 when the half-lives exceeded the time of the assay, over 30 min for the two first
 1000 and the last columns and over 75 min for the third column.

1001

	Half-life $\pm$ SD (min)			
RNase J variant	5'-labeled substrate (exo)	3'-labeled substrate (exo)	3'-labeled substrate with hairpins (endo)	3'-labeled substrate with hairpins + RhpA (complex)
WT	18.77 $\pm$ 3.16	7.37 $\pm$ 3.36	31.36 $\pm$ 17.57	6.29 $\pm$ 2.92
D299A	>30 [165.04 $\pm$ 93.36]	>>>30	>75 [231.05 $\pm$ 163.38]	n.d.
K649A	4.52 $\pm$ 0.52	2.75 $\pm$ 0.82	10.3 $\pm$ 0.56	6.95 $\pm$ 0.49
K649R	>30 [222.80 $\pm$ 175.05]	>30 [36.61 $\pm$ 12.04]	>75 [81.83 $\pm$ 6.81]	27.70 $\pm$ 5.4
K649Q	>30 [56.80 $\pm$ 13.02]	14.82 $\pm$ 9.11	>75 [92.52 $\pm$ 26.85]	>30 [37.33 $\pm$ 28.89]
D185A	9.14 $\pm$ 4.83	4.56 $\pm$ 1.15	28.6 $\pm$ 9.67	n.d.

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