bioRxiv preprint doi: https://doi.org/10.1101/357301; this version posted June 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

1 **Type I-E CRISPR-Cas system as an immune system in a eukaryote**

2

3 Devashish Rath[1,2*], Lina Amlinger[2], Gargi Bindal[1], Magnus Lundgren[2]#

4

5 [1]Molecular Biology Division, Bhabha Atomic Research Centre, Mumbai, India[a;]

6 [2]Department of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden[b]

7

8 #Address correspondence to Magnus Lundgren, magnus.lundgren@icm.uu.se

9 *Present address: Molecular Biology Division, Bhabha Atomic Research Centre,

10 Mumbai, India.

11

bioRxiv preprint doi: https://doi.org/10.1101/357301; this version posted June 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

12 **Abstract**

13 Defense against viruses and other mobile genetic elements (MGEs) is important in
14 many organisms. The CRISPR-Cas systems found in bacteria and archaea constitute
15 adaptive immune systems that acquire the ability to recognize MGEs by introducing
16 nucleic acid samples, spacers, in the CRISPR locus. The CRISPR is transcribed and
17 processed, and the produced CRISPR RNAs guide Cas proteins to degrade matching
18 nucleic acid sequences. No CRISPR-Cas system is found to occur naturally in
19 eukaryotic cells but here we demonstrate interference by type I-E CRISPR-Cas
20 system from *Escherichia coli* introduced in *Saccharomyces cerevisiae*. The designed
21 CRISPR arrays are properly expressed and processed in *S. cerevisiae*. Targeted
22 plasmids display reduced transformation efficiency, indicative of DNA cleavage.
23 Unlike *e.g.* Cas9-based systems, which can be used to inactivate MGEs in eukaryotes
24 by introducing specific mutations, type I-E systems processively degrade the target.
25 The type I-E system thus allows for defense without knowledge of MGE gene
26 function. The reconstituted CRISPR-Cas system in *S. cerevisiae* can also function as a
27 basic research platform for testing the role of various factors in the interference
28 process.

29

30 **Introduction**

31 Viruses and other mobile genetic elements (MGEs) are potential threats to most
32 studied cellular organisms, by acting as predators or by reducing fitness. In response,
33 organisms have evolved multiple defense strategies, largely grouped into innate and
34 adaptive systems. Innate systems are characterized by being activated by certain
35 preset features of infection. Adaptive systems, on the other hand, can learn to
36 recognize previously unrecognized pathogens. For a long time the vertebrate adaptive

bioRxiv preprint doi: https://doi.org/10.1101/357301; this version posted June 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

37 immune system was the only known example, but the CRISPR-Cas systems of
38 archaea and bacteria have been demonstrated to be a *bona fide* adaptive immune
39 systems (1). All studied CRISPR-Cas systems are based on short DNA or RNA
40 sequences (protospacers) from *e.g.* virus genomes being stored as DNA spacers in the
41 Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) locus. A long
42 precursor CRISPR transcript (pre-crRNA) is processed into CRISPR RNA (crRNA)
43 and used by CRISPR associated (Cas) protein effectors to locate and destroy matching
44 targets. The target can be DNA or RNA depending on the type of CRISPR-Cas
45 system (2, 3). CRISPR-Cas systems are grouped into class 1 and 2, depending on if
46 the effector is a protein complex or a single enzyme, respectively. Each class contains
47 several functionally different types of systems (4).
48
49 Programmable nucleases such as Zinc finger nucleases (ZFNs), transcription activator
50 like effector nucleases (TALENs), and Cas9 can function as anti-MGE systems in
51 eukaryotic cells by inducing crippling mutations. Particularly Cas9, which has
52 revolutionized gene editing in eukaryotes, has been demonstrated to effectively target
53 several human viruses (5). In basic Cas9 technology DNA cleavage is directed by a
54 single guide RNA (sgRNA). The break can be repaired by Homology Directed Repair
55 (HDR) or error-prone Non-Homologous End Joining (NHEJ) (2). In the case of
56 viruses, if a gene is repaired by the error-prone NHEJ it may be inactivated, resulting
57 in inability of the virus to proliferate, as demonstrated for *e.g.* HIV1 (6). However,
58 this approach requires thorough understanding of the virus' biology, as randomly
59 targeting a virus gene does not guarantee reduced proliferation.
60

bioRxiv preprint doi: https://doi.org/10.1101/357301; this version posted June 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

61 In this study we take advantage of the properties of the type I-E CRISPR-Cas system
62 to test its ability to function as an anti-MGE system in eukaryotes that do not require
63 understanding of target gene function. The key factors in target degradation by type I-
64 E systems are the Cascade protein-RNA complex and the Cas3 enzyme. Cascade
65 processes and retains crRNA, and uses the crRNA to identify DNA targets flanked by
66 a protospacer adjacent motif (PAM) (7). Once a target is identified, the Cas3 enzyme
67 is recruited to degrade the target. Unlike the DNA-targeting class 2 enzymes, which
68 perform blunt or staggered double-stranded cut, Cas3 destroys the target in a
69 processive manner (8). This property makes Cascade-Cas3 a poor choice for gene
70 editing but highly suitable for removing virus genomes as repair that restore virus
71 function is less likely to occur after processive degradation. As the effect should be
72 the same irrespective of target sequence, Cascade-Cas3 would be especially
73 advantageous for use against poorly characterized viruses. A type I-E system in a
74 eukaryote could also be beneficial for basic research. It allows testing the role and
75 effect of Cas and non-Cas proteins, as well as other factors, for their effect on
76 CRISPR-Cas immunity, independent of their native cellular background.
77
78 As a proof-of-concept we adapted the type I-E system from *E. coli* for use as a
79 programmable anti-MGE system in *S. cerevisiae*, chosen for its role as a eukaryotic
80 model system. As targets we use plasmids, which allow comparison with similar
81 experiments in bacterial systems (9-12).
82
83 **Results**
84 <u>Design and reconstitution of type I-E CRISPR-Cas system in *S. cerevisiae*</u>

bioRxiv preprint doi: https://doi.org/10.1101/357301; this version posted June 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

85 Our basic system for expressing Cascade, Cas3, and crRNA in *S. cerevisiae* was
86 different plasmids from the pRSGal series, where each produced one of the
87 components (fig. 1A), but we also designed several alternative systems (fig. 1B-D).
88 pCascade, expressing Cascade proteins, had three cassettes with different *cas* genes
89 under control of bidirectional Gal promoters. The cassettes were separated by *S.*
90 *cerevisiae* CYC1 terminator to prevent formation of antisense transcripts.
91 For production of targeting crRNA, pCRISPR was constructed by inserting a CRISPR
92 array containing four copies of the J3 spacer (4×J3) (13) under control of a Gal
93 promoter, with a CYC1 terminator preventing read-through.
94 For expression of the Cas3 nuclease we designed pCas3 by cloning *E. coli cas3* under
95 a Gal promoter. An alternative version of the plasmid, pCas3::Cse1, carried a
96 naturally occurring *cas3-cse1* fusion (8). Co-expression of this plasmid with pCascade
97 would result in incorporation of the Cas3-Cse1 fusion in a part of the Cascade
98 complexes. To test the effect of different crRNAs we combined a CRISPR with two
99 alternative spacers, J1 and J2, with Cas3 or Cas3-Cse1 fusion to generate
100 pCRISPR+Cas3 and pCRISPR+Cas3::Cse1, respectively.
101 Two different target plasmids were designed. pTargetHigh carried a phage Lambda J
102 gene fragment containing the PAMs and protospacers for J1, J2, and J3 (table S4) on
103 a high copy vector. pTargetLow, carried the same J fragment on a low copy
104 backbone. Empty vectors lacking the J fragment were used as non-targeted controls.
105

106 Processing of crRNA in *S. cerevisiae*

107 To test function of crRNA processing in yeast we analyzed the formation of crRNA in
108 *S. cerevisiae* W303 after expression from pCascade, pCas3, and pCRISPR was
109 induced by addition of galactose. The analysis was performed by northern blot with a

bioRxiv preprint doi: https://doi.org/10.1101/357301; this version posted June 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

110 radioactively labeled probe complementary to the J3 spacer. The assay demonstrates
111 formation of a 61 nt RNA species, equal in size to crRNA produced in *Escherichia*
112 *coli*. The amount of crRNA produced was assayed two, four, and five hours after
113 induction, and crRNA was detected at all time points (fig. 2A).
114
115 Interference by CRISPR-Cas in *S. cerevisiae*
116 Functionality of Cascade-Cas3 interference was assessed by comparing
117 transformation efficiency (TE) of a plasmid targeted by the CRISPR-Cas system with
118 that of the same plasmid lacking the target fragment. Transformations were routinely
119 done 240 min after induction of *cas* and CRISPR expression. Using pCas3, pCascade,
120 and pCRISPR in *S. cerevisiae* W303, a 47% decrease in TE of pTargetHigh was
121 observed (fig. 2B). Tests with 150 min induction resulted in similar results (fig. S1).
122 Alternative systems for expressing Cascade, Cas3, and crRNA were tested for
123 improved target interference. Cas3-Cse1 fusion construct, pCas3::Cse1, co-expressed
124 with pCascade and pCRISPR resulted in approximately 73.5% reduction in TE of
125 pTargetHigh in *S. cerevisiae* W303 (fig. 2C). Expression of pCRISPR+Cas3 or
126 pCRISPR+Cas3::Cse1 together with pCascade in *S. cerevisiae* BY418 resulted in
127 88.6 % and 88.7 % reduction in TE of pTargetLow, respectively (fig. 2D and 2E).
128
129 Effect of interference
130 Interference assays demonstrated that CRISPR-Cas targeting reduced TE but
131 transformants were still observed on selective plates. These transformants could be
132 carrying target plasmids that had not been cleaved by Cascade-Cas3, plasmids that
133 had been cleaved but subsequently been repaired or plasmids that had acquired escape
134 mutations in the region targeted by CRISPR. NHEJ repair or presence of different

bioRxiv preprint doi: https://doi.org/10.1101/357301; this version posted June 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

135 escape mutations would result in target sequence heterogeneity in the population of
136 transformed plasmids. To determine sequence heterogeneity in the transformed
137 plasmids we performed the Surveyor assay, which cleave DNA with mismatched
138 bases formed by denaturing and reannealing DNA strands with sequence differences.
139 The target region of pTargetHigh was amplified by PCR and analyzed by Surveyor
140 assay. Sequence heterogeneity at J3 target site would result in two cleavage
141 fragments, both of about 160-170 bp. No such cleavage products could be detected in
142 strains expressing pCRISPR, pCascade and pCas3, similar to interference-negative
143 control strains (fig. S2).

144

145 **Discussion**

146 We demonstrate successful reconstitution of bacterial type I-E CRISPR-Cas
147 interference in the eukaryote *S. cerevisiae*. We detect accurate processing of crRNA
148 by northern blot and, importantly, a reduction of target plasmid transformation rate
149 compared to a non-targeted control plasmid. We could not detect sequence
150 heterogeneity in plasmids recovered from transformed cells, demonstrating that the
151 level of interference observed was due to the activity of Cascade-Cas3 and not by
152 varying levels of escape mutations in the target. The lack of sequence heterogeneity
153 also demonstrates that NHEJ repair could not be detected, though this type of repair is
154 rare in *S. cerevisiae*. Our results suggest that the programmable processive DNA
155 degradation by type I-E CRISPR-Cas system can be restored in eukaryotic cells.

156

157 In our initial design, Cascade, Cas3, and pre-crRNA is expressed from different
158 plasmids, and this resulted in 50% reduction in plasmid transformation rate. To
159 improve activity we developed improved systems. Co-expression of a Cas3-Cse1

bioRxiv preprint doi: https://doi.org/10.1101/357301; this version posted June 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

160 fusion results in a subpopulation of Cascade complexes permanently co-localized with
161 Cas3, which reduced transformation by 73.5 %, a level similar to when J3 crRNA is
162 used against phage Lambda in *E. coli* expressing the LeuO activator (13). We
163 hypothesize that this fusion improves the kinetics of target degradation, as the factors
164 do not need to find each other. Further, we altered the CRISPR to produce two
165 different crRNA targeting the plasmid to potentially increase activity and at the same
166 time expressed Cas3 and pre-crRNA from the same plasmid to reduce the load of
167 carrying multiple plasmids. These modifications resulted in further reduction of
168 transformation, and combination of all improvements result in about one-in-ten
169 plasmids escaping degradation.
170
171 Different crRNAs result in different levels of CRISPR-Cas system interference (7, 14,
172 15), so other spacer sequences may further increase efficiency of target degradation in
173 yeast. We also speculate that the Cascade and Cas3 in the absence of nuclear
174 localization signals (NLS) locate to the cytoplasm, thus allowing only a narrow
175 window of opportunity to destroy their target plasmids while they are en route to the
176 nucleus. This likely causes a reduction in target access and interference compared to
177 bacteria and archaea, which typically lack a membrane around their nucleoid. Other
178 factors that may further improve interference in *S. cerevisiae* are increased expression
179 of Cas protein and pre-crRNA, codon optimization, prevention of detrimental post-
180 translational modification of Cas proteins, and co-expression of *E. coli* host factors
181 that may assist interference.
182 The large type I-E system is more difficult to establish heterologously than the more
183 compact class 2 systems. However, complete target clearance by type I systems is a
184 distinct advantage over inactivation of MGEs by mutagenesis using class 2 systems.

bioRxiv preprint doi: https://doi.org/10.1101/357301; this version posted June 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

185 To inactivate an MGE by mutation, thorough understanding of the MGEs function is
186 required to find a key component to target. Also, compared to Cas9-based systems,
187 type I systems can easily target several different sequences by adding more spacer-
188 repeat units to the CRISPR (14, 16), while current applications of Cas9 in eukaryotes
189 require insertion of multiple single guide RNA genes to achieve multiplexing (17).
190 Another benefit of our establishment of a type I-E system in *S. cerevisiae* is that it
191 functions as a platform for testing our current understanding of the system in a non-
192 native background. Such a platform would allow introduction and exclusion of *e.g.*
193 various proteins and other factors component to test their role in the immune system.
194 Finally, while we demonstrate interference in a eukaryote, further development could
195 reconstitute the entire adaptive capability of the system. This would require
196 expression of Cas1 and Cas2, and possibly also other factors such as IHF, indicated as
197 important for adaptation (18). As Richard Feynman stated, “what I cannot create I do
198 not understand”.
199

200 Materials and Methods

201 Strains and culture media

202 All cloning work was done in *E. coli* strain Top10. *E. coli* was cultured in Lysogeny
203 Broth (LB) with aeration at 37°C. When necessary, the media was supplemented with
204 kanamycin (50 μg/ml), ampicillin (100 μg/ml), streptomycin (50 μg/ml), tetracycline
205 (25 μg/ml), and chloramphenicol (15 μg/ml). For experiments in yeast *Saccharomyces*
206 *cerevisiae* W303 and BY418 strains were used. *S. cerevisiae* BY418 have full
207 deletion of chromosomal copies of genes used for selection, unlike W303 where they
208 are inactivated by point mutations. Yeast cells were cultured in SC medium (Yeast
209 nitrogen base 0.34 %, ammonium sulfate 0.5%, raffinose 2%, quadruple SC dropout

bioRxiv preprint doi: https://doi.org/10.1101/357301; this version posted June 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

210 mix (CSM, -His, -Leu, -Trp, -Ura; Formedium™, UK) at the rate of 1400 mg/L of
211 medium, pH 5.6). For certain induction experiments SC medium with 1% raffinose
212 was used. When necessary, the media was supplemented with adenine sulfate (80
213 µg/ml), uracil (20 µg/ml), tryptophan (40 µg/ml), histidine (20 µg/ml), and leucine (60
214 µg/ml). 1.5% agar was included in the medium to prepare solid medium. Yeast strains
215 were grown with aeration at 30°C. 2% galactose was added to the medium for
216 induction of Gal promoter as required. For a complete list of strains used see table S1.
217
218 Construction of vector expressing Cascade complex
219 A Gal1-10 promoter cassette from pRS425Gal was cloned between EcoRI/BamHI
220 sites of pBlueScript II SK (+) resulting in pUDM101. All *cas* genes were amplified by
221 colony PCR from *E. coli* MG1655, see table S2 for a complete list of primers. The
222 *cse1* and *cse2* genes were serially cloned into the pUDM101 XbaI and EcoRI sites,
223 respectively, to generate pUDM107. Similarly, *cas7* and *cas6e* were serially cloned in
224 pRS425Gal BamHI and SalI sites, respectively, to generate pUDM108. The four
225 genes were combined by subcloning a HindIII-NotI blunt fragment from pUDM107
226 into the blunted SacI site of pUDM108, thereby generating pUDM109. The *cas5e*
227 gene was cloned into pRS425Gal vector to generate pUDM110. CYC1 terminator
228 cassette was PCR amplified from pYES2 and subcloned into PstI site of pBlueScript
229 II SK (+) to generate pUDM102. A CYC1-*cas5e*-CYC1 cassette was constructed by
230 serially cloning EcoRV-SmaI fragment from pUDM102 into the SalI and SpeI sites
231 respectively of pUDM110 to generate pUDM315. Using XhoI and NotI this cassette
232 was excised from pUDM315, blunted and sub-cloned into the NotI site of pUDM109
233 to generate the final Cascade-expressing construct, pCascade. See table S3 for
234 complete list of plasmids used in this study.

bioRxiv preprint doi: https://doi.org/10.1101/357301; this version posted June 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

235

236 Construction of vectors expressing Cas3 or Cas3-Cse1 fusion

237 The *cas3* gene was PCR amplified from MG1655 to introduce flanking EcoRI sites
238 and cloned into the EcoRI site of pRS423Gal resulting in the Cas3-expressing plasmid
239 pCas3. A Cas3-Cse1 fusion fragment was amplified from pWUR657 and cloned
240 between the SpeI and NotI sites of pRS423Gal to generate pCas3::Cse1 expressing
241 the Cas3-Cse1 fusion.

242

243 Construction of vectors expressing CRISPR RNA

244 For expression of crRNA, two CRISPR cassettes, one containing 4xJ3 spacer (13)
245 PCR amplified from pWUR630 and the other containing J1-J2 spacers (a synthetic
246 array ordered from ThermoFisher Scientific Inc.) were cloned into BamHI-NotI sites
247 of pRS424Gal_Cyc1 to place CYC1 terminator directly downstream of the CRISPR
248 array. All spacers targeted the J gene from bacteriophage Lambda. The J1-J2
249 CRISPR, along with the flanking CYC1 terminator, was excised as a BamHI-SacI
250 fragment and cloned in pCas3 cut with the same sites to generate pCRISPR+Cas3.
251 Additionally, the same BamHI-SacI fragment was blunted and cloned in the blunted
252 SalI site of pCas3::Cse1 to generate pCRISPR+Cas3::Cse1. See table S4 for details of
253 CRISPR and targets.

254 For expression crRNA in *E. coli*, a minimal CRISPR array with 54 nt of the leader,
255 the J3 spacer and two repeats was cloned into pZE12Luc (19) under the control of
256 PLlacO-1. The plasmid was amplified using primers PLlacO-C and pZE-Xba and the
257 minimal CRISPR array was amplified from pWUR564 (13) using primers LA009 and
258 LA013. The array was cloned by blunt-end ligation in the leader-end so that the first

bioRxiv preprint doi: https://doi.org/10.1101/357301; this version posted June 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

259 position of the partial leader corresponds to transcription start of the PLlacO-1 and in
260 the other end into the XbaI-site of pZE12Luc.
261
262 Construction of *S. cerevisiae* expressing type I-E CRISPR-Cas system
263 The vectors constructed as described above were transformed into the desired yeast
264 strains as required. Transformation of *S. cerevisiae* was performed using lithium
265 acetate method (20).
266
267 Construction of target vectors
268 To construct pTargetHigh, the 350 bp region of Lambda J from pWUR610 was
269 cloned into the BamHI and HindIII sites of pRS426Gal. The cloning replaced the Gal-
270 promoter fragment with the lambda DNA fragment. A low copy target vector,
271 pTargetLow, was constructed by PCR amplification of a region of Lambda J gene
272 using pTargetHigh as template with DR0015 and DR0016 primers and cloning the
273 fragment in the NheI site of pPS1739.
274
275 Analysis of crRNA processing
276 *Sample preparation and RNA purification: Yeast*
277 *S. cerevisiae* W303 carrying pCascade, pCRISPR (4xJ3 spacer), and pCas3 was
278 grown to stationary phase, diluted in fresh SC medium and grown with aeration at
279 32°C to an OD_{600} value of 0.3. Galactose was then added to induce the CRISPR-Cas
280 system. 10 ml culture was harvested by centrifugation 2, 4, and 5 hours after
281 induction. Total RNA was isolation using the hot phenol method (21).
282 *Sample preparation and RNA purification: Bacteria*

bioRxiv preprint doi: https://doi.org/10.1101/357301; this version posted June 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

283 Overnight culture of *E. coli* BL21AI harboring pWUR397 (Cas3), pWUR400
284 (Cascade) and pLA002 (minimal J3 CRISPR array), was diluted and grown to OD_{600}
285 ≈ 0.3. Cas-protein and crRNA expression was induced by addition of 0.2 % arabinose
286 and 1 mM IPTG. After 30 min, 5 ml sample was taken and mixed with 1 ml stop
287 solution (5 % phenol, 95 % ethanol), and pelleted at 4 °C. RNA was purified as
288 previously described (22).
289 *Gel and blotting*
290 Samples were run on a 10% denaturing polyacrylamide gel (10 % polyacrylamide, 7
291 M urea, 1X Tris-borate-EDTA (TBE)) in 1X TBE. Samples were mixed with loading
292 dye (95 % deionized formamide, 0.5 mM EDTA, 0.025 % bromphenol blue/xylene
293 cyanol, and 0.025 % SDS), and boiled 3 min prior to loading. The same was done
294 with the pre-labelled pUC8 size marker. 15 µg of total RNA was loaded for each
295 sample. As a positive control for processed crRNA, total RNA purified from the *E.*
296 *coli* BL21AI carrying pWUR 397, pWUR400 and pLA002, able to prevent phage
297 infection and plasmid transformation (data not shown), was also loaded on the gel.
298 Transfer to Hybond N+ membrane (Amersham) was done at 4°C overnight at 200
299 mA. The membrane was UV crosslinked and prehydridized in Church buffer (0.25M
300 sodium phosphate buffer pH 7.2, 1 mM EDTA, and 7% SDS) for 1 h at 42°C after
301 which 0.5 µM of radioactively labelled probe was added to the buffer. Hybridization
302 was done overnight. The membrane was washed two times 5 min with 2x SSC, 0.1%
303 SDS and exposed the PharosFX-system (BioRad).
304 The pUC8 size marker (Fermentas) was radioactively labeled using γ-^{32}P-ATP
305 (PerkinElmer) and PNK (Fermentas) in an exchange reaction according to the
306 manufacturer's instructions. The probe, LA014, was labelled the same way in a
307 forward reaction. Excess γ-^{32}P-ATP was removed using Illustra ProbeQuant G-50

bioRxiv preprint doi: https://doi.org/10.1101/357301; this version posted June 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

308 Micro column (GE Healthcare) purification according to the manufacturer's
309 instructions.
310
311 CRISPR-Cas activity assays
312 CRISPR-Cas activity was assessed by plasmid interference assays. Yeast cultures
313 grown to stationary phase and diluted in fresh SC medium containing either 1% or 2%
314 raffinose as carbon source and grown with aeration at 32°C to an OD_{600} value of 0.2,
315 followed by addition of galactose inducer. 150-240 min after induction the cells were
316 harvested by centrifugation. The cells were then transformed with target vectors or
317 non-target control vectors using lithium acetate method (20), but excluding carrier
318 DNA. Plasmid interference by the CRISPR-Cas system was measured by plating
319 cultures transformed with target vector or non-target vector on selective media and
320 comparing TE.
321
322 Surveyor assay
323 *S. cerevisiae* BY418 cells harboring pCascade, pCRISPR, and pCas3::Cse1, or
324 negative controls lacking pCRISPR, pCascade or both were grown in SC medium
325 with or without the galactose inducer and transformed with pTargetHigh. The
326 transformed cells were grown for 24 hours in SC medium, pelleted by centrifugation
327 and grown again in equal volume of fresh medium for 24 hours.1.5 ml of the culture
328 was pelleted by centrifugation and resuspended in 500 µl of distilled water. The cells
329 were lysed by heating at 98°C for 10 minutes and 0.5 µl of the lysate was used as
330 template for PCR with primers DR003 and DR004 to amplify the target region. The
331 PCR products were analyzed by electrophoresis on 2 % agarose gel and used for
332 mutation detection with Surveyor mutation detection kit (IDT) as per manufacturer's

bioRxiv preprint doi: https://doi.org/10.1101/357301; this version posted June 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

333 instructions. The result of Surveyor assay was analyzed by Qiagen QIAxcel advanced
334 gel electrophoresis system.
335
336 **Acknowledgements**
337 We are deeply grateful to Erik Johansson (Umeå University), Hans Ronne (Swedish
338 University of Agricultural Sciences), Gerhart Wagner (Uppsala University), Pernilla
339 Bjerling (Uppsala University) and their respective research groups for providing
340 assistance, strains, and plasmids for this project. This project was funded by the
341 Wenner-Gren Foundations, the Swedish Research Council and the Royal Swedish
342 Academy of Sciences.
343
344 **Author Contributions**
345 ML conceived the concept. DR, GB, and ML performed experimental design and DR,
346 LA, and GB performed the experiments. DR and ML analyzed the data and wrote the
347 article.
348
349 **Conflict of Interest**
350 The authors declare no conflict of interest.

bioRxiv preprint doi: https://doi.org/10.1101/357301; this version posted June 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

351

352 **References**

353 1. Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S,
354 Romero DA, Horvath P. 2007. CRISPR provides acquired resistance
355 against viruses in prokaryotes. Science 315:1709-12.
356 2. Rath D, Amlinger L, Rath A, Lundgren M. 2015. The CRISPR-Cas immune
357 system: Biology, mechanisms and applications. Biochimie
358 doi:10.1016/j.biochi.2015.03.025.
359 3. Koonin EV, Makarova KS, Zhang F. 2017. Diversity, classification and
360 evolution of CRISPR-Cas systems. Curr Opin Microbiol 37:67-78.
361 4. Makarova KS, Wolf YI, Alkhnbashi OS, Costa F, Shah SA, Saunders SJ,
362 Barrangou R, Brouns SJ, Charpentier E, Haft DH, Horvath P, Moineau S,
363 Mojica FJ, Terns RM, Terns MP, White MF, Yakunin AF, Garrett RA, van der
364 Oost J, Backofen R, Koonin EV. 2015. An updated evolutionary
365 classification of CRISPR-Cas systems. Nat Rev Microbiol 13:722-36.
366 5. White MK, Hu W, Khalili K. 2015. The CRISPR/Cas9 genome editing
367 methodology as a weapon against human viruses. Discov Med 19:255-62.
368 6. Ebina H, Misawa N, Kanemura Y, Koyanagi Y. 2013. Harnessing the
369 CRISPR/Cas9 system to disrupt latent HIV-1 provirus. Sci Rep 3:2510.
370 7. Brouns SJ, Jore MM, Lundgren M, Westra ER, Slijkhuis RJ, Snijders AP,
371 Dickman MJ, Makarova KS, Koonin EV, van der Oost J. 2008. Small CRISPR
372 RNAs guide antiviral defense in prokaryotes. Science 321:960-4.
373 8. Westra ER, van Erp PB, Kunne T, Wong SP, Staals RH, Seegers CL, Bollen S,
374 Jore MM, Semenova E, Severinov K, de Vos WM, Dame RT, de Vries R,
375 Brouns SJ, van der Oost J. 2012. CRISPR immunity relies on the

bioRxiv preprint doi: https://doi.org/10.1101/357301; this version posted June 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

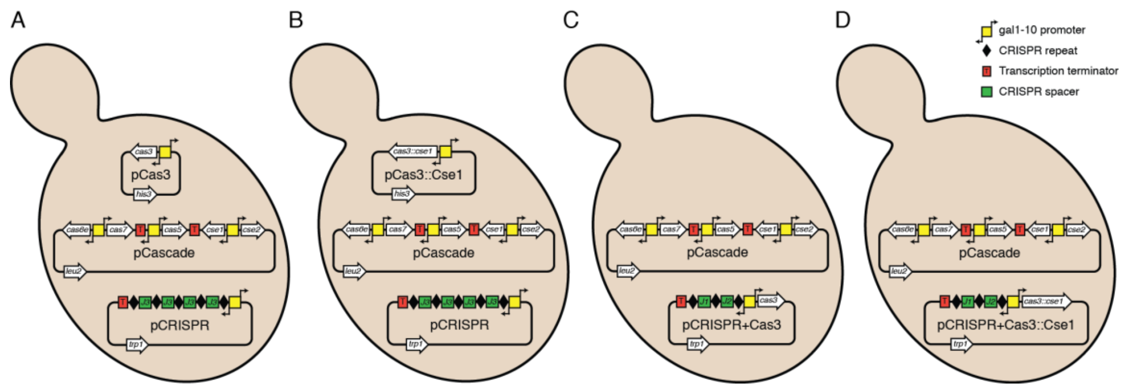
376 consecutive binding and degradation of negatively supercoiled invader
377 DNA by Cascade and Cas3. Mol Cell 46:595-605.
378 9. Sapranauskas R, Gasiunas G, Fremaux C, Barrangou R, Horvath P, Siksnys
379 V. 2011. The Streptococcus thermophilus CRISPR/Cas system provides
380 immunity in Escherichia coli. Nucleic Acids Res 39:9275-82.
381 10. Swarts DC, Mosterd C, van Passel MW, Brouns SJ. 2012. CRISPR
382 interference directs strand specific spacer acquisition. PLoS One
383 7:e35888.
384 11. Marraffini LA, Sontheimer EJ. 2008. CRISPR interference limits horizontal
385 gene transfer in staphylococci by targeting DNA. Science 322:1843-5.
386 12. Garneau JE, Dupuis ME, Villion M, Romero DA, Barrangou R, Boyaval P,
387 Fremaux C, Horvath P, Magadan AH, Moineau S. 2010. The CRISPR/Cas
388 bacterial immune system cleaves bacteriophage and plasmid DNA. Nature
389 468:67-71.
390 13. Westra ER, Pul U, Heidrich N, Jore MM, Lundgren M, Stratmann T, Wurm
391 R, Raine A, Mescher M, Van Heereveld L, Mastop M, Wagner EG, Schnetz K,
392 Van Der Oost J, Wagner R, Brouns SJ. 2010. H-NS-mediated repression of
393 CRISPR-based immunity in Escherichia coli K12 can be relieved by the
394 transcription activator LeuO. Mol Microbiol 77:1380-93.
395 14. Rath D, Amlinger L, Hoekzema M, Devulapally PR, Lundgren M. 2015.
396 Efficient programmable gene silencing by Cascade. Nucleic Acids Res
397 43:237-46.
398 15. Xue C, Seetharam AS, Musharova O, Severinov K, Brouns SJ, Severin AJ,
399 Sashital DG. 2015. CRISPR interference and priming varies with individual
400 spacer sequences. Nucleic Acids Res 43:10831-47.

bioRxiv preprint doi: https://doi.org/10.1101/357301; this version posted June 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

401 16. Zetsche B, Heidenreich M, Mohanraju P, Fedorova I, Kneppers J,
402 DeGennaro EM, Winblad N, Choudhury SR, Abudayyeh OO, Gootenberg JS,
403 Wu WY, Scott DA, Severinov K, van der Oost J, Zhang F. 2017. Multiplex
404 gene editing by CRISPR-Cpf1 using a single crRNA array. Nat Biotechnol
405 35:31-34.
406 17. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. 2012.
407 A programmable dual-RNA-guided DNA endonuclease in adaptive
408 bacterial immunity. Science 337:816-21.
409 18. Nunez JK, Bai L, Harrington LB, Hinder TL, Doudna JA. 2016. CRISPR
410 Immunological Memory Requires a Host Factor for Specificity. Mol Cell
411 62:824-33.
412 19. Lutz R, Bujard H. 1997. Independent and tight regulation of
413 transcriptional units in Escherichia coli via the LacR/O, the TetR/O and
414 AraC/I1-I2 regulatory elements. Nucleic Acids Res 25:1203-10.
415 20. Agatep RK, R.D.; Parchaliuk, D. L.; Woods, R. A.; Gietz, R. D. 1998.
416 Transformation of Saccharomyces cerevisiae by the lithium
417 acetate/single-stranded carrier DNA/polyethylene glycol protocol.
418 Technical Tips Online 3:133-137.
419 21. Collart MA, Oliviero S. 2001. Preparation of yeast RNA. Curr Protoc Mol
420 Biol Chapter 13:Unit13 12.
421 22. Blomberg P, Wagner EG, Nordstrom K. 1990. Control of replication of
422 plasmid R1: the duplex between the antisense RNA, CopA, and its target,
423 CopT, is processed specifically in vivo and in vitro by RNase III. EMBO J
424 9:2331-40.
425

bioRxiv preprint doi: https://doi.org/10.1101/357301; this version posted June 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

426 **Figures**



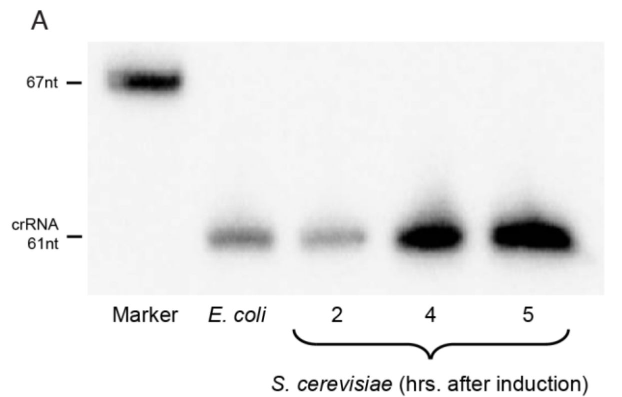
427

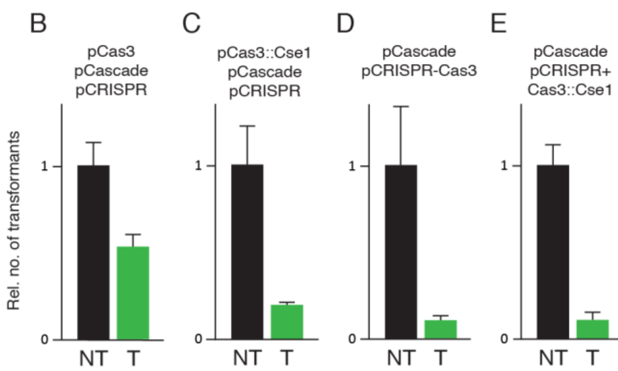
428 Figure 1. A-D: The different variants of the type I-E CRISPR-Cas system used for

429 expression in *S. cerevisiae*.

430

431





432

433 Figure 2. A: Analysis of crRNA by northern blot. *S. cerevisiae* samples were taken at

434 indicated time points after induction of *cas* genes and 4xJ3 CRISPR array expression.

435 RNA from an *E. coli* strain active for interference was used as positive control. B-E:

bioRxiv preprint doi: https://doi.org/10.1101/357301; this version posted June 28, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

436 Analysis of CRISPR-Cas interference on plasmids in different *S. cerevisiae* strains
437 (A-B: W303, C-D: BY418) with indicated CRISPR-Cas expression system.
438 Transformation efficiency was measured for plasmids containing a target sequence
439 (T) and non-targeted parent plasmids (NT). Data in B-E is an average of three
440 independent biological replicates normalized so that relative level of transformation
441 by the non-target plasmid is equal in the different panels. Error bars indicate one
442 standard deviation.