1   The memory of hyperosmotic stress response in yeast is modulated by gene-positioning

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15   Running title: Gene positioning modifies stress memory

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18   Abstract (174 words/175)

19   Cellular memory is a critical ability displayed by microorganisms in order to adapt to potentially detrimental

20   environmental fluctuations. In the unicellular eukaryote *S. cerevisiae* cellular memory can take the form of a

21   faster or a decreased response following repeated stresses in cell population. Using microfluidics and

22   fluorescence time-lapse microscopy, we studied how yeasts respond to short-pulsed hyperosmotic stresses at

23   the single-cell level by analyzing the dynamical behavior of the stress responsive STL1 promoter fused to a

24   fluorescent reporter. We established that pSTL1 shows variability in its successive activations following two

25   repeated short stresses. Despite this variability, most cells displayed a memory of past stresses through a

26   decreased activity of pSTL1 upon repeated stress. Notably, we showed that genomic location is important for

27   the memory effect since promoter displacement to a pericentromeric chromatin domain leads to a decreased

28   transcriptional strength of pSTL1 and to the loss of memory. This study provides a quantitative description of a

29   cellular memory that includes single-cell variability and points towards the contribution of the chromatin

30   structure in stress memory.

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34   Introduction

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36   Cellular memory can be defined as a cellular response to transient and repeated stimuli. These latter can

37   emanate from constantly fluctuating and potentially stressful environments, thus possibly exerting a selective

38   pressure on cell viability [1]. To ensure their survival, living organisms have developed various strategies to

39   cope with environmental changes. One possible way for cells to maintain their biological functions in a

40   challenged environment is to regulate gene transcription [2]. The active genetic response allowing cells to

41   survive a single stimulus is referred to as cellular adaptation. Factors such as histone post-translational

42   modifications, chromatin remodelers, specific proteins produced during the stress or even changes in

43   chromatin conformation have been determined to be causal factors for adaptation to environmental changes

44   [3, 4]. What happens when cells encounter consecutive stresses is less well understood, however it has been

45 observed in some cases that the adaptation to a first stress can serve as a learning process to a better

46 adaptation to a consecutive stress. This process is defined as memory. Biological mechanisms known to

47 underlie cellular memory involve chromatin remodeling (epigenetic memory) or proteins synthesized during

48 the first stress, which might leave a trace of the first event (cytoplasmic memory) [5-7].

49 In the budding yeast, studies on cellular memory showed that cells confronted to successive environmental

50 stresses can respond differently. For instance, the so called galactose memory is characterized by a faster

51 transcriptional reactivation of the GAL cluster, while an example of the memory of long hyperosmotic stresses

52 is characterized by a reduced activity of the osmo-responsive gene *GRE2* without any difference in the time of

53 reactivation of this gene [5, 7].

54 All eukaryotes have a highly organized nucleus. Yeast chromosomes follow a Rabl organization; centromeres

55 are tethered to the spindle pole body, while telomeres are anchored to the nuclear periphery [8, 9].

56 Interestingly, the galactose or inositol memory appears to rely on 3D gene positioning, since repositioning of

57 the *INO1* gene or *GAL* cluster towards the nuclear periphery, in an H2AZ and nucleoporin-dependent manner,

58 is important for memory [7, 10, 11]. The nuclear organization may also play a critical role in stress response as

59 most stress response genes are located in subtelomeres. Subtelomeres lack essential genes but are enriched in

60 fast-evolving non-essential gene families that are needed to adapt to environmental changes [12].

61 Subtelomeres are subjected to silencing by proteins of the Silent Information Regulator (SIR) complex, but

62 stress conditions can lift this repression [13, 14].

63 Most studies questioning memory effects are carried out on isogenic populations of cells, giving information

64 on the mean behavior of the population [15]. Nevertheless, cells populations are heterogeneous due to

65 extrinsic noise, such as age, size or position in the cell cycle (for reviews, [16, 17]). Moreover gene expression

66 is an inherently stochastic phenomenon, because of the low number and availability of transcription factors,

67 accessibility of the promoter or functional regulatory networks [18]. Overall, stochasticity causes genetically

68 identical cells to exhibit different behavior when encountering the same stimuli.

69 The response to osmotic changes in the budding yeast has proven to be a good tool to study the emergence of

70 adaptation and cellular memories in this organism [19, 20]. When yeast faces an osmolarity increase in its

71 environment (hyperosmotic stress), intracellular water flows out of the cell, leading to its shrinkage [21]. The

72 imbalance of osmotic pressure is detected by osmosensors that activate the High Osmolarity Glycerol (HOG)

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73   pathway that phosphorylates the cytoplasmic protein Hog1 [22, 23]. Phosphorylated Hog1 translocates into

74   the nucleus where it participates in the activation and regulation of an estimated 10% of the genome,

75   including the osmo-responsive gene STL1 [24]. Thanks to the HOG pathway, yeast can physiologically adapt to

76   a hyperosmotic stress in 15-30 min [25], notably by producing glycerol, which leads to homeostasis. The

77   dephosphorylation of Hog1 and its exit from the nucleus signals the end of the adaptation to the

78   hyperosmotic stress.

79   We here present a single-cell study of *S. cerevisi*ae cells submitted to short pulsed hyperosmotic stresses in a

80   well-controlled system based on time lapse fluorescence microscopy and microfluidics [26, 27]. Hundreds of

81   single cells receiving repeated osmotic stresses were tracked and analyzed. We found that in response to two

82   consecutive hyperosmotic stresses separated by 4h, individual cells display variability in the dynamical activity

83   of pSTL1 in response to the two stresses. Despite the existence of this pronounced dynamical variability, most

84   of the cells adopt the same behavior, consisting in decreased amplitude of response upon stress. We called

85   this specific behavior memory effect. Importantly, we found that the chromatin environment modulates cells

86   response to pulsed stresses. Relocation of the promoter of interest close to the centromere causes a reduced

87   activity of pSTL1 and loss of the memory effect. Overall, our study suggests that the specific location of pSTL1

88   at the subtelomere is required for an optimal level of transcription that can go beyond a simple stochastic

89   behavior and lead to the emergence of a memory in response to short osmotic stresses.

90

91   Results

92   **The response of a population to successive hyperosmotic stresses suggests cellular memory.**

93   A population of growing yeast cells in a microfluidic device was submitted to short and repeated hyperosmotic

94   stresses, using 1M Sorbitol (figure 1a). To measure the response to hyperosmotic stress, we used a reporter of

95   the activity of the HOG pathway in which pSTL1 promoter is tagged with the yECITRINE fluorescent protein

96   (yEFP) allowing for fluorescence quantification at the single cell level as a function of time [28] (figure 1b).

97   During the time course of an experiment, each cell is tracked allowing for quantification of the first and second

98   activation of pSTL1 due to the short and transient activation of the HOG pathway (figure 1c). The limited

99   duration of the stress (8 min) and the long delay between the two stresses (4 hours) guaranteed i)

100   investigation on the genetic response to hyperosmotic stress before adaptation is established, 15-30 min after

4

101 stress [29] and ii) full recovery of the cells from the first stress, thus allowing to compare the dynamics of

102 response to the first and second osmotic stress. Moreover daughter cells born between the two stresses were

103 not considered, since they did not receive the first stress and may blur the stress response. Rather, we focus

104 exclusively on the population that received both the first and the second stress (figure 1c). In such a

105 population, we calculated the mean pick of fluorescence reached during the first and second stress, taking the

106 basal fluorescence level prior to the corresponding stress as a reference. It reaches 77,14 ± 6,71 (a.u.) after the

107 first stress and 55,44 ± 4,26 (a.u.)after the second stress, indicating a decrease of fluorescence intensity by

108 20% in average after the second stress (figure 1d). No difference in the time required to reach the peak of

109 fluorescence was detected between the two picks (figure 1e). The decrease of fluorescence amplitude

110 correlated with decreased protein amount, as detected by Western Blotting (supplementary figure 1),

111 suggesting it is independent of photo bleaching.

112 Altogether, these observations suggest that at the level of stressed cells population, there is a memory of the

113 first stress event. Moreover, the decrease of fluorescence intensity at the second stress is seemingly due to a

114 reduction of protein production rate rather than a shortened duration of transcription events.

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116 **At the level of single cells, most cells, but not all, show a cellular memory.**

117 Yet, this memory effect was not shared equally among cells. Indeed, single-cell analysis reveals a dynamic

118 variability in the individual response to the repeated stresses. We classified single-cell fluorescent trajectories

119 according to typical behaviors based on the first and second responses to stress (figure 2a). The most frequent

120 behavior (55% ± 11%) is in line with the population memory effect in which the cells submitted to the second

121 stress showed lower fluorescence intensity (figure 2b). However, up to 18%±7% of cells display an opposite

122 behavior, with a stronger response at the second stress (figure 2b). Very few cells showed similar responses to

123 both stresses. Interestingly, we observed two subpopulations of cells that did not respond to one of the stress

124 (figure 2b), although we confirmed that these cells indeed perceived the stress by visualization of transient cell

125 shrinkage upon stress (Supplementary video & supplementary figure 2). Altogether our results show that the

126 population behavior hides a richer set of dynamic behaviors of single-cell responses, which are likely the traces

127 of the variability of the activation of pSTL1 by a hyperosmotic stress.

128

Cellular memory overcomes the stochasticity of gene expression on average.

To determine the importance of the intrinsic variability on setting the different dynamical behaviors shown in figure 2a, that is the stochastic nature of the pSTL1 activation, we performed stochastic simulations based on the Gillespie algorithm [30]. We simulated the transcription of pSTL1 and the translation of the fluorescent reporter of 1000 cells submitted to two 8min stresses separated by 4h (figure 2c). The rates of production and degradation of mRNA and proteins were set as established previously [31] (supplementary Table 1). Such a model implies that the computed cells will necessarily respond to both stresses. However, our experiments show that cells exposed to an 8min stress do not necessarily respond to a stress (figure 2d), conversely to cells submitted to a longer stress (figure 2e). More specifically, the absence of response disappears for longer stress duration since for a 1h stress 100% cells showed an activation of pSTL1, while for 8 min stress 80% of cells showed a response (figure 2f). This experimental observation suggests the existence of a critical time from which all cells will eventually respond to a stress. A stochastic time of activation of the STL1 promoter was therefore added to the model. As expected in such a memory-free system, cells were clustered equally in the two main categories obtained experimentally, and the population did not display any memory effect. Of note, transcriptional delay made possible clusters 4 and 5 appearance (figure 2g).

The differences in clusters observed in vivo and through simulation suggest that a biological mechanism other than noise in transcription and translation is at play in the memory effect.

Memory to pulsed hyperosmotic stress does not require de novo protein synthesis during the stress.

In order to investigate the biological origin of the memory effect, we first wanted to determine if the memory effect was linked to one or several long-lived proteins synthesized during the first episode of stress. To test this hypothesis, we inhibited transcription upon stress using thiolutin, a well-studied molecule that inhibits all three RNA polymerases in the yeast in a reversible manner [32] (figure 3a, b). As expected, treatment of cells with thiolutin led to the loss of pSTL1 activity: when cells were treated for 1h with thiolutin (50µg/µL), no cells showed a fluorescent signal when stimulated by an 8 min hyperosmotic stress, while 80% ± 20% cells showed a signal in response to a hyperosmotic stress in the absence of thiolutin (figure 3a, b). We next tested the cells ability to respond back to a hyper-osmotic stress after treatment with thiolutin (figure 3c, d). After 1h in the presence of thiolutin, the inhibitor was washed out and cells were submitted to an 8 min hyperosmotic stress,

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157 4h later. At the population level, a response similar to the response to the first stress without thiolutin

158 treatment was observed (figure 3c, d). In the presence of thioluthin, stress response is however slightly

159 decreased and the maximum of intensity after 8 min of stress is 88,55 +/- 7,7%, as compared to 100 +/- 4,74 %

160 in non-treated cells, suggesting that not all of the thioluthin effect is erased. We then performed a thiolutin

161 treatment during 1h, including during the first stress, then washed the inhibitor and submitted cells to a

162 second stress 4h later (figure 3e, f). In these conditions, cells showed a marked decrease of the YFP signal by

163 40%, comparable to the 47% decreased response to a second stress without thiolutin treatment. Consequently,

164 this experiment suggests that the memory effect is not primarily driven by de novo synthesis of proteins

165 during the first stress, which could help the cells to respond to the second stress. To explain the observed

166 memory effect, we can hypothesize that the first stress induces chromatin modifications independently of the

167 activity of any RNA polymerases, but with an effect on subsequent transcription events at the pSTL1 locus. A

168 possibility could be that chromatin marks would appear in most cells during the first stress and alter the

169 response dynamics of cells during the second stress.

170

171 **Chromosome positioning influences the dynamical activity of pSTL1.**

172 The STL1 locus is located on the right arm of the chromosome IV, in its subtelomeric region, prone to silencing

173 in non-stress conditions. To investigate the influence of the chromatin context on the dynamics of activation of

174 pSTL1, we moved a region containing the promoter of STL1 and the yECITRINE fluorescent reporter to a

175 distinct, centromeric chromatin domain (figure 4a). The displaced DNA region included 1kb upstream of the

176 STL1 locus, enough to have a fully functional STL1 promoter [33]. To compare pSTL1 activity between its

177 endogenous position and the centromeric one, we first submitted both strains to a 2h hyperosmotic stress and

178 used flow cytometry to quantify the fluorescence at several time points. The activity of centromeric pSTL1 was

179 significantly lower than in wild type cells in two independent clones (figure 4b and supplementary figure 3),

180 although the integrity of the promoter was preserved [33].

181 Patterns of consecutive responses to two 8min hyperosmotic stresses separated by 4h were then compared

182 between endogenous and displaced pSTL1. Cells expressing the STL1 promoter at this centromeric position

183 showed a more uniform distribution into the five defined clusters and there was a decrease in the amount of

184 cells displaying the memory effect (from 55%±11% to 28%±4%, figure 4c) compatible with a purely stochastic

7

185 process. Such a result indicated that the chromatin environment might be involved in the dynamical

186 transcriptional activity of pSTL1. Although a functional pSTL1 promoter has been displaced, potential

187 subtelomeric regulatory elements could have been lost during gene displacement. We have ruled out this

188 hypothesis using a Crispr/dCas9VPR system [34] to bypass regulatory elements and force the activation of

189 pSTL1 in non-stress conditions (supplementary figure 4). We designed guide RNAs to target Crispr/dCas9VPR

190 within the 1kb sequence of displaced pSTL1 and successfully induced the fluorescent reporter expression

191 independently of any stress at both the peri-centromeric and subtelomeric positions. We observed a decrease

192 in the activity of pSTL1 at the peri-centromeric position compared to the endogenous one, meaning that the

193 observed differences of expression of pSTL1 are not linked to the sequence of the promoter itself or the

194 presence of regulatory elements, but is rather related to the chromatin environment.

195 Taken together, our results show that the chromatin environment sets the variability of single-cell dynamical

196 response for short stresses and can consequently act on cellular memory.

197

198 **Discussion**

199 In the current study, we investigated how individual yeast cells dynamically behave in response to short pulsed

200 hyperosmotic stresses. Focusing our study on short stresses allowed to analyze the genetic response to

201 hyperosmotic stresses exclusively and probe the cell-cell variability that finds its origin in the onset of

202 transcriptional events.

203 The in-depth single-cell analysis reveals that yeasts display various behaviors in response to two repeated

204 stresses that we have clustered according to several typical profiles. Response to the second stress could be

205 similar, higher or lower than the response to the first stress. The latter case corresponds to the most frequent

206 response and was named memory effect. We have also considered two additional profiles where cells do not

207 respond to one of the two stresses exclusively. These last two profiles might depend on transcriptional delay

208 as verified by simulation and validated by the experimental observation that all cell respond to a long stress, as

209 observed previously [35]. Using stochastic simulations, we have established that the five profiles of response

210 could be explained by gene expression stochasticity. Yet, the single-cell quantification obtained with such a

211 model does not account for the prevalence of the memory effect, indicating it is not a reflection of gene

212 expression stochasticity alone.

8

213   Studies on cellular memories in budding yeast in response to repeated stresses typically describe a faster

214   dynamics of gene expression, or lower amplitude of response as two possible ways to respond [5, 7, 36].

215   Similarly to the response to a long hyperosmotic stress, we observe a decrease in amplitude of pSTL1 response

216   after pulsed stresses, without any difference in the time of reactivation [5]. We speculate that a diminished

217   response could be a strategy for the cells to lessen their burden compared to the case where transcriptional

218   response to two stresses would be similar.

219   What is the mechanism that could drive a diminished response to a hyperosmotic stress? It is possible that, as

220   observed in the response to hyperosmotic stress triggered by Nacl, a long-lived protein triggered by the stress,

221   could remain in the nucleus upon the first stress and hamper the promoter of interest for a similar activation

222   upon the second stress [5]. However, it is known that after stress, phosphorylated Hog1 translocates within 3

223   min. into the nucleus, leading to the up regulation of stress response genes, including STL1 and Hog1 rapidly

224   exits the nucleus, 15-30 min after the start of the stress, making this assumption less likely [25] . Alternatively,

225   transcriptional inhibition experiments show that the memory effect does not seem to require de novo protein

226   synthesis. Since transcriptional inhibitor thiolutin inhibits all three RNA Pol and de novo transcription, but does

227   not prevent potential transcription factors from binding to the promoter's sequence, specific histone marks

228   are possibly left after first stress. Those marks could serve as traces of previous activities of the promoter and

229   could explain a diminished response. Such an interpretation awaits single cell ChIP to be validated.

230   We further showed that the dynamic variability distribution between single cells was dependent on the

231   positioning of the pSTL1 locus on the chromosome. When displaced in a pericentromeric domain, pSTL1

232   shows a decreased activity. We could discard potential loss of regulatory sequences by showing that activation

233   of pSTL1 by a CrispR-dCAS9-VPR construct, which bypasses the need for stress factors for the response, still

234   depends on the genomic position. At this pericentromeric position, the stochasticity of gene expression

235   prevails and the memory effect is lost. Consequently, an active mechanism might occur in the subtelomeric

236   regions upon stress, allowing for the memory effect to become predominant among cell population.

237   Interestingly, it has been already observed that changing the position of a gene in the genome can alter its

238   expression [37]. It was proposed that change in expression level could be due to changes in transcription noise,

239   or a in the noisy steps when cells transition between two expression levels [37]. The latter case has been

240   shown to enhance the cellular memory when cells were subjected to glucose limitation stress [20].

9

241 One key difference between the two genomic positions we have analyzed is the variation in the amplitude of

242 pSTL1 response. We thus propose that transcriptional marks or transactivators will induce a high level of

243 transcriptional activity during the first stress, required to overcome the stochasticity of gene expression and

244 lead to the emergence of a cellular memory. A parallel can be drawn with previous studies performed in *B.*

245 *subtilis*, where transcriptional events occurring above a certain threshold have been described to lead to the

246 emergence of a cellular memory [38]. Although this organism is a prokaryote, some similarities might exist

247 between those two microorganisms in regard to the biology of a memory.

248 It could be hypothesized that a high activity of the promoter of interest could mean an opened chromatin.

249 Although marks of acetylation are usually associated with a high level of gene expression, the activity of pSTL1

250 is reduced in absence of the histone deacetylase Rpd3 [39]. In the experimental context studied here, marks of

251 deacetylation are possibly involved for a high level of transcriptional activity. It would be interesting to

252 investigate the potential role of (de)acetylation by, for instance, forcing a high level of (de)acetylation during

253 the first stress only.

254 Stochastic gene expression gives a diversity of behaviors. From an evolutionary point of view, this diversity of

255 responses to repeated stresses allows the selection of the most adapted one. In our experimental conditions,

256 the preference for a memory effect suggests that the specific subtelomeric position of pSTL1 offers an optimal

257 regulation level to perform better adaptation.

258 Altogether our work shows how critical single-cell studies are for stress memory analyses. It also indicates that

259 establishment and transmission of memory does not require a long stress and can start after short-pulsed

260 stresses. Our work could serve as a basis to broader studies of the positioning of stress response genes in the

261 budding yeast in response to fluctuating environments.

262

263 **Materials and Methods**

264 Flow Cytometry: all flow cytometry experiments were performed with a flux cytometer Gallios (Beckman

265 Coulter) equipped with 10 colors, 4 lasers (488nm Blue, 561nm Yellow, 638nm Red, 405 nm Violet). We used

266 the excitation laser 488nm and the emission filter at 530nm +/- 30 nm.

267

268 Yeast strains and cell culture: our experiments were made using a pSTL1::yECITRINE-His5 (yPH53) strain

269 derived from S288C and gifted to us by Megan McClean. The yeast were grown overnight in SC+2% glucose at

270 30°C. Cells were diluted next morning to reach OD600=0.5 at the moment of the experiments. Genotypes of

271 all strains used in this study are indicated in table 2. To move pSTL1 reporter construct in the peri-centromeric

272 region of chromosome IV, pSTL1-yECITRINE-HIS5 construct was PCR amplified using primers

273 TATTGAGCACGTGAGTATACGTGATTAAGCACACAAAGGCAGCTTGGAGTCAATGATTCTGAAATACTCCTTTTACA and

274 TGCAGGCAAGTGCACAAACAATACTTAAATAAATACTACTCAGTAATAACATTATTGGTGCGGCAAGG with 50 bases

275 homologies to the TRP1 locus. [HIS+ TRP-] yeast transformants were verified by PCR and PCR fragment

276 subsequently sequenced, ensuring the absence of any mutations in the construct.

277

278 Crispr dCAS9 experiments: we used the plasmid pAG414GPD-dCas9-VPR from Addgene plasmid (# 63801) to

279 express the inactivated form of CAS9 fused to transcriptional activator VPR. The guides were cloned under

280 SNR52 promoter in plasmid pEF534 using the enzyme BsmBI. Digesting the resulting plasmid by NotI / XbaI

281 and cloning the Guide containing fragment into pRS425 similarly digested, performed marker exchange. We

282 designed two guides targeting pSTL1, respectively gRNA1, GAAAGTGCAGATCCCGGTAA and gRNA2,

283 GCGCCGAATACCCCGCGAAA.

284

285 Single-cell clustering: To categorize cells in different classes, we compared the maximum level of fluorescence

286 reached during the first and second stress, while taking the basal fluorescence level prior to the corresponding

287 stress as a reference. The ratio between the maximum amplitude of the first and the second stress was then

288 evaluated. Cells categorized according to profile 1 had a ratio superior to 1, cells categorized according to

289 profile 2 had a ratio inferior to 1 and cells displaying profile 3 had a ratio equal to 1. The cells with a maximum

290 of amplitude equal to 0 (no expression) during the second or the first stress were categorized separately

291 (figure 2b). All ratios were established with a 5% threshold.

292

293 Microfluidics: we used an H-shaped microfluidic device to confine the yeast in channels of 3.7μm high. This

294 microfluidic device was made using soft lithography techniques. The hyperosmotic stresses were triggered

295 using SC+2% glucose supplemented with 1M sorbitol. The media were flown in the microfluidic chip using a

296 peristaltic pump ISMATEC set at 120µL/min flow rate. Mask design is in sup figure 1.

297

298 Transcriptional inhibition: cells were exposed to SC with addition of thiolutin (Abcam ref ab143556) at

299 50µg/mL (diluted in DMSO) for 1h prior and during the stress. To wash out Thiolutin in the microfluidics

300 experiments, SC medium was delivered to the cells during 4h using a peristaltic pump set at a flow rate of

301 120µL/min.

302

303 Microscopy: we used an inverted microscope Olympus IX71. Yeasts were observed with an objective x100

304 UplanFLN 100x/ 1.3 Oil Ph3 Ul2. Images were recorded with a camera Cool Snap HQ2 Princeton Instruments.

305 All experiments were made at 30°C. Yeast were imaged every 5min with 20ms exposure in bright light and

306 200ms in fluorescence light. The microscope was controlled by the open source software MicroManager

307 interfaced with Matlab.

308

318

319 Author Contributions

320 ZBM, PH and EF designed the experiments and wrote the article. ZBM performed the experiments. YK,

321 performed Matlab analyses.

322

323 Conflict of interest

324 Authors declare have no conflict of interest.

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414

415   Figure Legends

416   Figure 1

417   (A) Experimental setup. We use a multi-layer H-shaped microfluidics device composed of two large flow

418   channels of 50μm height and 40μm thin, observation chambers of 400μm x 400μm x 3.7μm. Cells are trapped

14

419 in the chambers and grow as a monolayer, facilitating cell segmentation and tracking. The medium flowing in

420 the channels diffuse in the chambers. The hyperosmotic stresses activating expression of pSTL1-yECRITRINE

421 are triggered using sorbitol 1M for 8min. SC, synthetic complete medium. (B) Example of cells in the

422 microfluid chamber, submitted to an 8min stress and left to recover for 4h (240min.). Cells are imaged every

423 5min in bright light (20ms exposure, upper raw) and fluorescence light (200ms exposure, lower raw). (C)

424 Fluorescent signal of Individual cells exposed to two successive 8 minutes stress separated by 4 hours.

425 Hyperosmotic stress is schematized by red bars. (D) Fluorescence response of a population of N= 97 cells. The

426 mean response of cells submitted to a first stress (dark blue), followed by another stress 4h later (light blue) is

427 represented with the standard error of the mean. Fluorescence levels are normalized by the value of the peak

428 of fluorescence during the first stress. The peak of fluorescence decreases upon stress. (E) Analyses of time

429 response to two consecutive stresses in the same population. Time between stress induction and fluorescence

430 peak reveals that the time of response is similar upon stress.

431

432 Figure 2

433   (A) Examples of five typical single-cell response profiles. Although the single-cell analysis reveals a dynamical

434 variability of the response, we have defined five typical profiles of response adopted by the cells (p1 to p5). (B)

435 Single-cell clustering. Using criteria on the values of the fluorescence peaks, dynamical variability of response

436 was clustered according to the five typical fluorescence response. Errors bars represent standard errors. 708

437 cells were analyzed from 3 independent experiments. (C) Modeling gene expression upon stress in a memory-

438 free system. Stochastic simulations with Gillespie algorithm were used to model the transcription of the

439 fluorescent reporter and the protein translation upon stress. (D) Sequence images of cells submitted to an

440 8min stress. The arrows on the last bright field image show the cells that do not respond to the stress. (E)

441 Sequence images of cells submitted to a continuous stress. All cells show a response to such a stress. (F)

442 Quantification of responsive cells. Upon receiving an 8min stress, 80% of cells show a response, whereas 100%

443 of cells respond to a 1h stress. (G) Single-cell quantification of computed cells according to the five typical

444 profiles. The model in this case includes a transcription delay randomly chosen between 0 and 10min for each

445 computed cells. This delay is also different during the two stresses. The simulation is run twice and the

446   fluorescence peaks values are used to cluster the computed cells responses according to the five typical

447   profiles of response.

448

449   Figure 3

450   (A) Principle of the transcription inhibition experiment with thioluthin. A 1h thiolutin treatment (pink

451   rectangle)) is performed prior and during the stress (red rectangle) in order to prevent the activity of pSTL1

452   upon stress. Non-stressed cells received no sorbitol (NC) and stressed cells received an 8 min hyperosmotic

453   stress (red rectangle, S). (B) Single-cell quantification of the fluorescence of cells after 70 min in non-stress

454   conditions (NC), hyperosmotic conditions (S) and hyperosmotic conditions in the presence of thiolutin (T + SC).

455   (C) Principle of the experiment controlling thiolutin. (Up) Cells are in the microfluidic device for 4h before

456   being exposed to an 8min hyperosmotic stress. (Down) Cells are treated with the transcriptional inhibitor for

457   1h in the microfluidic device, thiolutin is washed out and cells are allowed to grow for 4h before being exposed

458   to an 8min hyperosmotic stress. (D) pSTL1 fluorescence response of cells treated (N=101) or not (N=97) with

459   thiolutin after stress. A similar response is observed. Fluorescence levels are normalized by the value of the

460   peak of fluorescence in the non treated case. (E) Principle of memory effect quantification in the presence of

461   thioluthin. (Up) Cells are exposed to two 8min hyperosmotic stresses separated by four hours. (Down) As as in

462   A, cells are treated with thiolutin for 1h and exposed to a 8min hyperosmotic stress, thioluthin washed out

463   and after a 4h recovery, cells are exposed to second 8min stress. (F) Population quantification of stress

464   memory of cells submitted to a first stress in the absence (blue) or the presence of thioluthin (N=101, pink).

465   Fluorescence levels are normalized by the value of the peak of fluorescence in the non treated case presented

466   in (D). Decrease of fluorescence after second stress is seemingly similar in both cases.

467

468   Figure 4

469   (A) Displacement of pSTL1 towards the peri-centromere of chromosome IV. Sketch of the genomic position of

470   pSTL1 on chromosome IV. The promoter was moved at the TRP1 locus, on the same chromosome. Genomic

471   positions indicated in kb. (B) Decreased activity of the displaced pSTL1 upon stress. Fluorescence

472   quantification of promoter activity in response to a 2h hyperosmotic stress in the endogenous location (blue)

473   and when the promoter was moved (red). Standard deviation of triplicate experiment (C) Displacement of

16

474   pSTL1 leads to the loss of the memory effect. Single-cell quantification of cells containing displaced promoter

475   pSTL1 in response to two hyperosmotic stresses. Classification made according to the five typical profiles of

476   response as in figure 2.

477

478   Data availability

479   All data are available upon request.

480

481   Expanded View Figure legends

482   Supplemental Figure 1. Comparison of relative amounts of pSTL1-YFP by FACs analyses and Western Blotting.

483   (A) After 1h stress in 1M sorbitol, cells were sorted by FACS at different times after stress; 0min (t0), 50 min

484   (t1), 80min (t2), 110 min (t3), 140min (t) and 170 min (t5). Total fluorescence was normalized by fluorescence

485   levels before stress. Note that the minimum of response is reached after 240min. (B) Total protein extracts

486   were performed similarly. Actin was used to normalize the amount of YFP protein expressed. (C). ratios

487   between YFP protein amounts measured by western blot and fluorescence measured by FACS.

488   Supplemental Figure 2. Stress induces cell compression. (A) Example of cells before, during and after stress

489   viewed under nomarski and fluorescence illumination. A scheme of the cells is shown on the top, stress is

490   indicated as a red bar. (B) full video .

491

492   Supplementary Table 1: Gillespie parameters

493

Parameter	Definition	Unit	Reference Value	Source
k1	transcription rate	min-1	1.101	[31]
d1	mRNA decay	min-1	2.94.10-1	[31]
τ	time delay	min	Between 0 and 10 min	This study
k2	translation rate	min-1	9.47.10-1	[31]
d2	protein decay	min-1	4.10-3	[31]

494   Supplementary Table 2: strains genotype

yPH53 (YEF1095)	ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0 pSTL1::yECITRINE-HIS5MX
yPH142 (YEF1096)	ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0 Δ(pSTL1-STL1)::CaURA3
yPH212 (YEF1098)	ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0 Δ(pSTL1-STL1)::CaURA3 Δtrp1::pSTL1-yECITRINE- HIS5MX
yPH359	leu2Δ0 his3Δ1 lys2Δ0 pSTL1::yECITRINE-HIS5 Δtrp1::pURA3-URA3

495

496   Supplementary Table 3: list of primers

Deletion of pSTL1-STL1 forward	Gagtagaaaatttactaatgtggtctcgcgtgtgaatcaggtttagcttgcctcgtcccc
Deletion of pSTL1-STL1 reverse	taagtaaattacaaaatatgatttgtgagttgtgtgtgaaGTTTTCGACACTGGATGGCG
PCR of pSTL1-yECITRINE His selection with 50bp of TRP1 homology forward	TATTGAGCACGTGAGTATACGTGATTAAGCACACAAAGGCAGCTTGGAGTCAATGATTCTGAAATACTCCTTTTACA
PCR of pSTL1-yECITRINE His selection with 50 bp of TRP1 homology reverse	TGCAGGCAAGTGCACAAACAATACTTAAATAAATACTACTCAGTAATAACATTATTGGTGCGGCAAGG
Amplification of pSTL1-yECITRINE at TRP1 locus forward	CGCCAGATGGCAGTAGTGGAAG
Amplification of pSTL1-yECITRINE at TRP1 locus reverse	GCCTGCAGGCAAGTGCAC
Sequencing of pSTL1 at TRP1 locus forward 1	CCGATTAAGAATTCGGTCG
Sequencing of pSTL1 at TRP1 locus reverse 1	GGATCTGCACTTTCTCAG

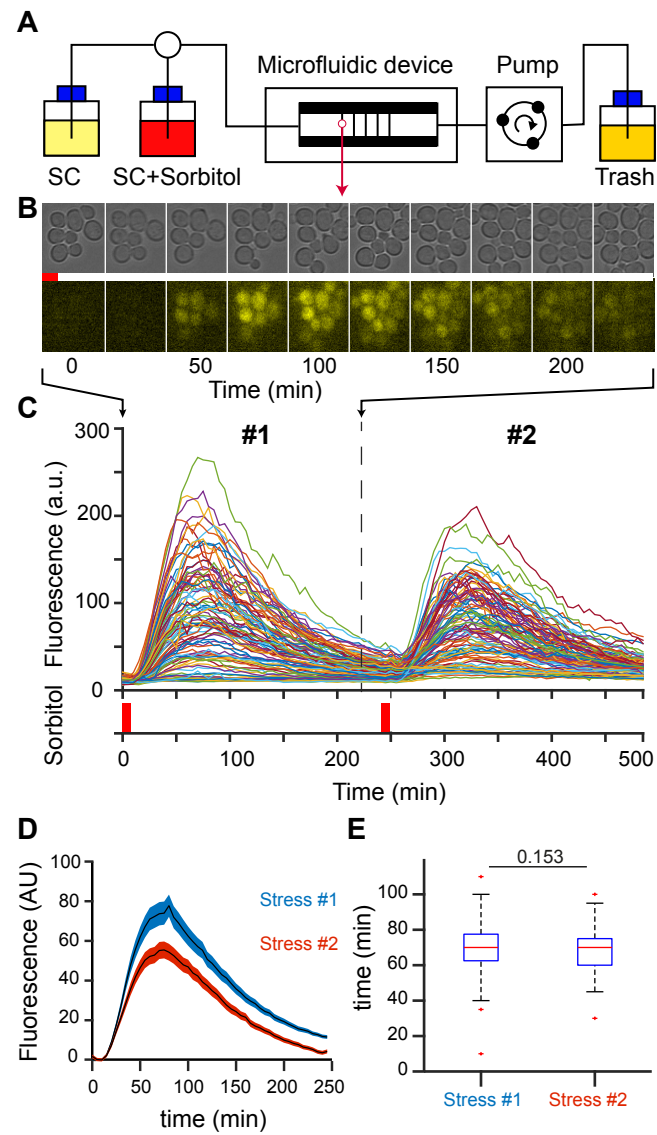|	
Sequencing of pSTL1 at TRP1 locus forward 2	CATTGCCAAGGCTAGGAG
Sequencing of pSTL1 at TRP1 locus reverse 2	catcaccttcaccttcacc
Primer gRNA 1 forward	gatcGAAAGTGCAGATCCCGGTAA
Primer gRNA 1 reverse	aaacTTACCGGGATCTGCACTTTC
Primer gRNA 2 forward	gatcGCGCCGAATACCCCGCGAAA
Primer gRNA 2 reverse	aaacTTTCGCGGGGTATTCGGCGC

497

Sequencing of pSTL1 at TRP1 locus forward 2	CATTGCCAAGGCTAGGAG
Sequencing of pSTL1 at TRP1 locus reverse 2	catcaccttcaccttcacc

19

A

SC   SC+Sorbitol   Microfluidic device   Pump   Trash

B

0   50   100   150   200
Time (min)

C

#1   #2

Fluorescence (a.u.)
300
200
100
0

Sorbitol

0   100   200   300   400   500
Time (min)

D

Fluorescence (AU)
100
80
60
40
20
0

Stress #1
Stress #2

0   50   100   150   200   250
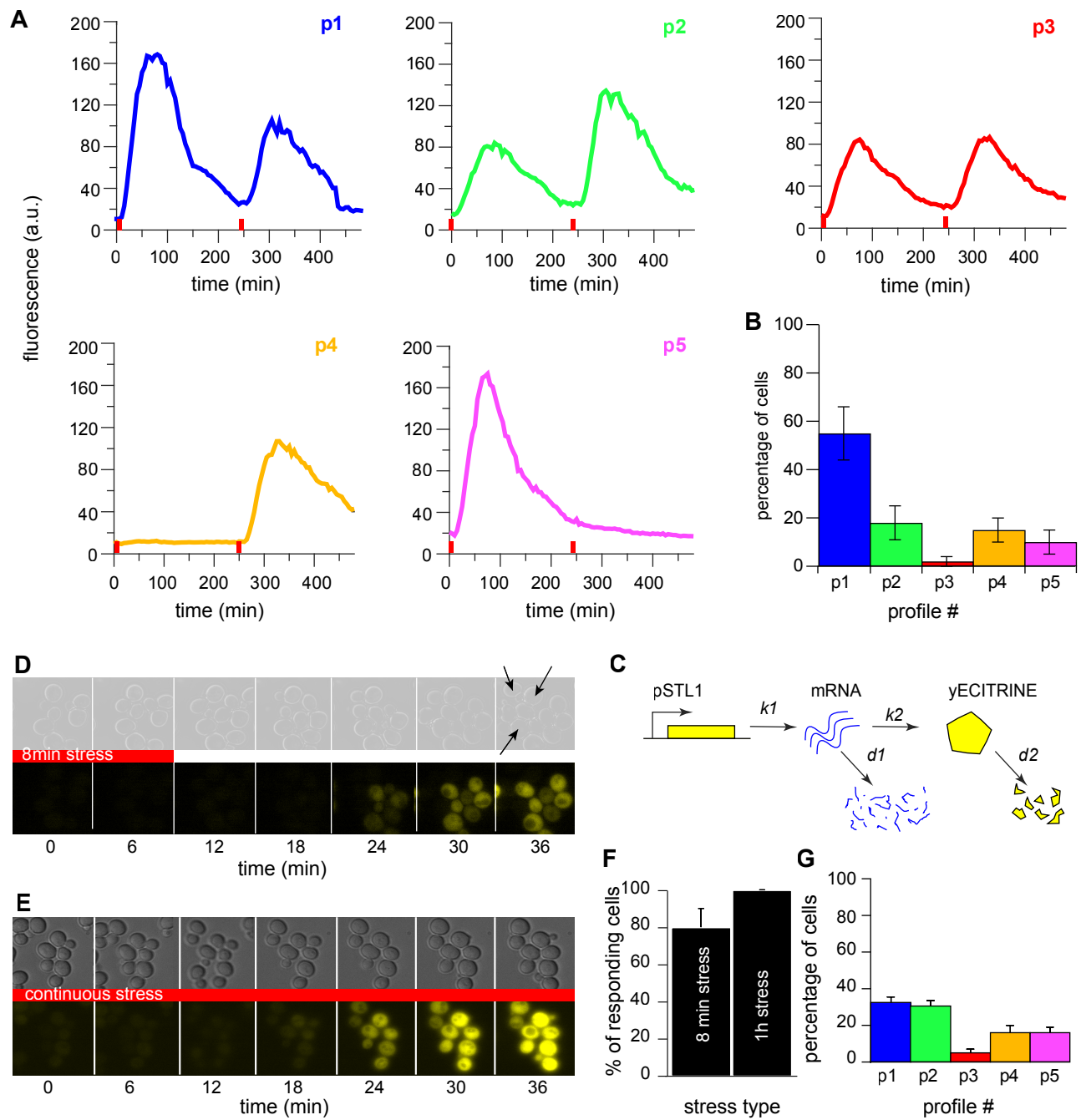time (min)

E

time (min)
100
80
60
40
20
0

0.153

Stress #1   Stress #2

Figure 1. Ben Meriem et al

Figure 2. Ben Meriem et al.,

A

NC — no change →

S — 8 min ▮ sorbitol →

T+S — 1h ▮▮ thiolutin + sorbitol →

B

Fluoresecne (a.u.)

NC   S   T+S

C

S — #1 ▮ 8 min sorbitol →

T+S — thiolutin 1h — 8 min sorbitol →

D

fluorescence (a.u)   #1

Time (min)

E

S — ▮ sorbitol 8 min →

T+S — #1 1h ▮ thiolutin   #2 ▮ sorbitol 8 min →

F

fluorescence (a.u)   #2

Time (min)

Figure 3. Ben Meriem et al.,

A

Tel IVL
0kb

Cen IV
448kb

Tel IVR
1525kb

TRP1
462kb

pSTL1
1508kb

● centromere
● subtelomere

B

C
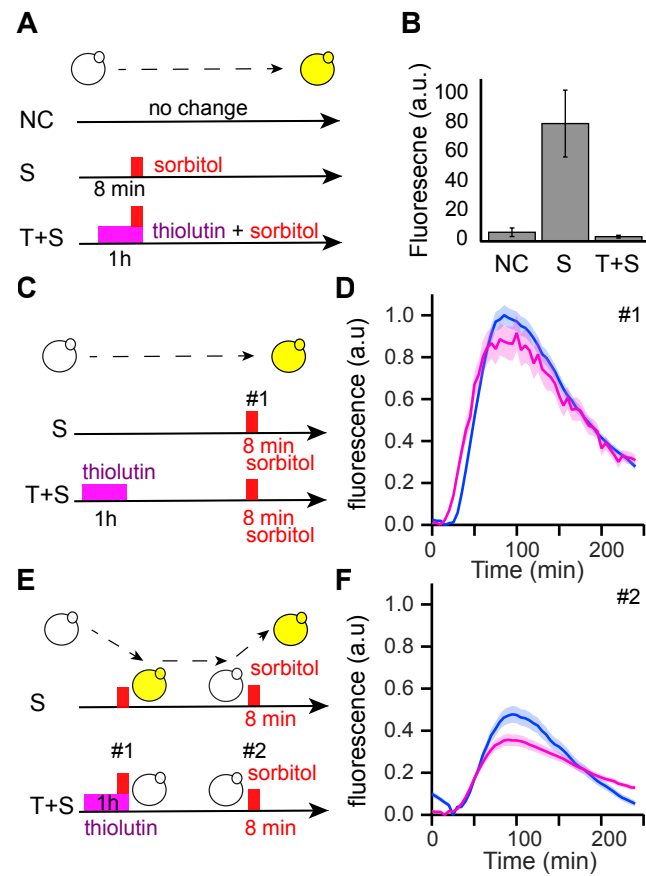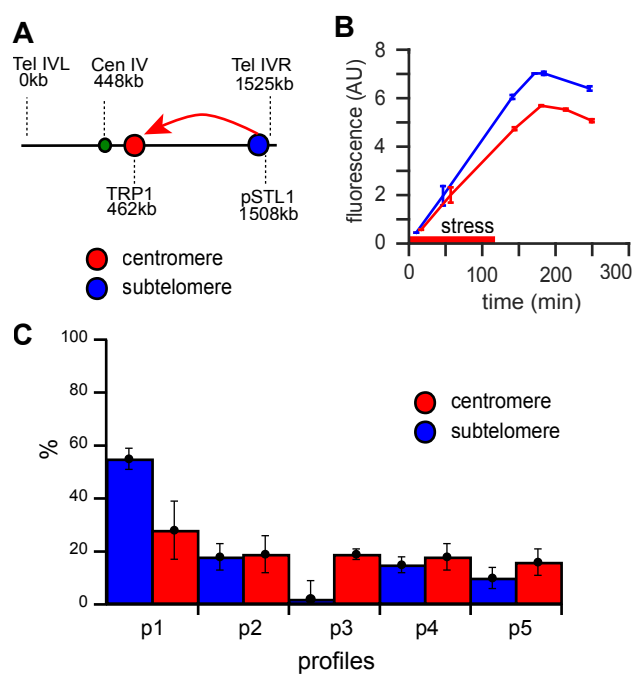
Figure 4. Ben Meriem et al.,

A
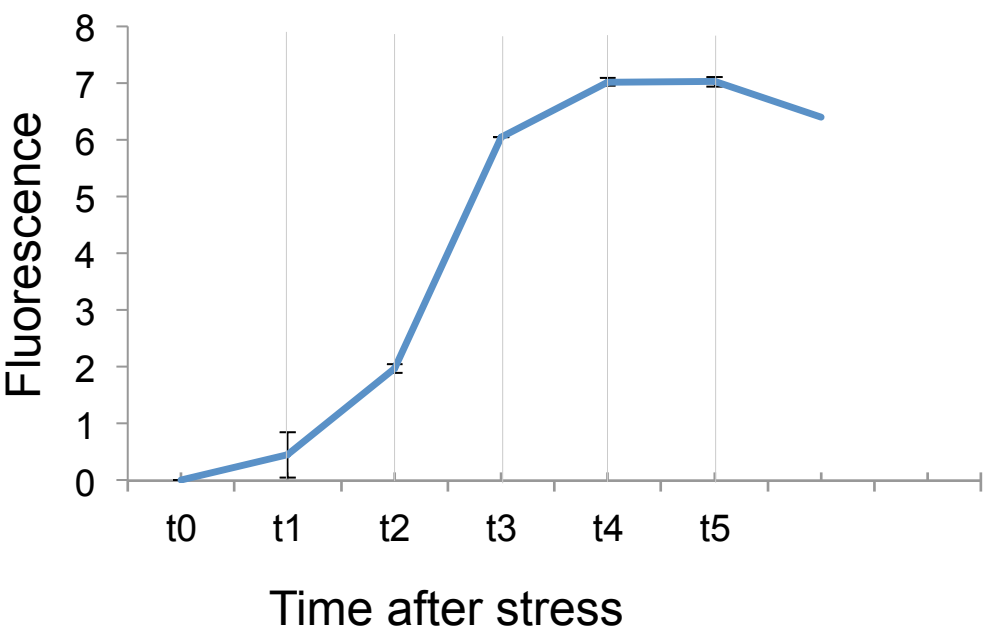
B
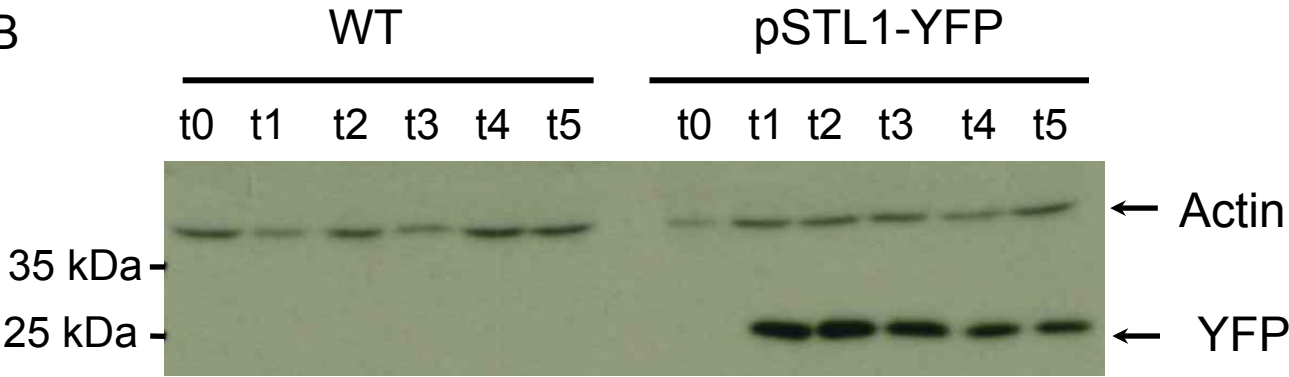
C

Relative YFP

	t1/t2	t2/t3	t3/t4	t4/t5
WB	0,91	1,12	1,3	1,06
FACS	0,91	1,11	1,2	1,05

Supp. Figure 1. Ben Meriem et al.,

0   6   24

Supp. Figure 2A. Ben Meriem et al.,