REPORT

Inactivation of fission yeast Erh1 de-represses *pho1* expression: evidence that Erh1 is a negative regulator of *prt* lncRNA termination

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ABSTRACT

Fission yeast Erh1 exists in a complex with RNA-binding protein Mmi1. Deletion of *erh1* up-regulates the phosphate homeostasis gene *pho1*, which is normally repressed by transcription in *cis* of a 5' flanking *prt* lncRNA. Here we present evidence that de-repression of *pho1* by *erh1*Δ is achieved through precocious 3'-processing/termination of *prt* lncRNA synthesis, to wit: (i) *erh1*Δ does not affect the activity of the *prt* or *pho1* promoters per se; (ii) de-repression by *erh1*Δ depends on CPF (cleavage and polyadenylation factor) subunits Ctf1, Dis2, Ssu72, Swd22, and Ppn1 and on termination factor Rhn1; (iii) de-repression requires synthesis by the Asp1 IPP kinase of inositol 1-pyrophosphates (1-IPPs); (iv) de-repression is effaced by mutating Thr4 of the RNA polymerase II CTD to alanine; and (v) *erh1*Δ exerts an additive effect on *pho1* de-repression in combination with mutating CTD Ser7 to alanine and with deletion of the IPP pyrophosphatase Aps1. These findings point to Erh1 as an antagonist of lncRNA termination in the *prt*–*pho1* axis. In contrast, in *mmi1*Δ cells there is a reduction in *pho1* mRNA and increase in the formation of a *prt*–*pho1* read-through transcript, consistent with Mmi1 being an agonist of *prt* termination. We envision that Erh1 acts as a brake on Mmi1's ability to promote CPF-dependent termination during *prt* lncRNA synthesis. Consistent with this idea, *erh1*Δ de-repression of *pho1* was eliminated by mutating the Mmi1-binding sites in the *prt* lncRNA.

Keywords: Erh1; Mmi1; *Schizosaccharomyces pombe*

INTRODUCTION

Fission yeast Erh1 is a 104-amino acid nuclear protein identified initially by virtue of its homology to the metazoan protein Enhancer of Rudimentary (Krzyzanowski et al. 2012). *Schizosaccharomyces pombe erh1*Δ cells are viable but display abnormal morphology in stationary phase and sensitivity to several stressful growth conditions (Krzyzanowski et al. 2012). Subsequent studies implicated Erh1 in preventing the deleterious expression of meiotic genes during vegetative growth, which it accomplishes via its physical association with Mmi1, a YTH-family RNA binding protein that recognizes DSR (determinant of selective removal) sequences in target RNAs and promotes their elimination by the nuclear exosome (Yamashita et al. 2013; Sugiyama et al. 2016; Shichino et al. 2018). Mmi1 and Erh1 colocalize in nuclear foci in vegetative cells and are recovered as a stoichiometric Mmi1–Erh1 complex after Erh1-affinity

purification from fission yeast cell extracts (Sugiyama et al. 2016). Mmi1 consists of: (i) a carboxy-terminal YTH-fold RNA binding domain with a distinctive mode of recognition of the DSR element UNAAAC and (ii) a low-complexity unstructured amino-terminal domain that is needed for formation of nuclear foci and mediates binding to Erh1 (Chatterjee et al. 2016; Wang et al. 2016; Shichino et al. 2018; Stowell et al. 2018; Xie et al. 2019). A crystal structure of Erh1 (expressed as a fusion in *cis* to a 28-aa segment of Mmi1 that suffices for binding to Erh1) showed that Erh1 is a homodimer and that each protomer has a Mmi1 peptide bound on its surface (Xie et al. 2019). In addition to its role in targeting RNA decay, Erh1 has imputed functions in the assembly of facultative heterochromatin (Sugiyama et al. 2016; Xie et al. 2019).

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Article is online at <http://www.majournal.org/cgi/doi/10.1261/rna.076463.120>.

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Our interest in *Erh1* was prompted by the results of transcription profiling of an *erh1Δ* mutant whereby it was found that the phosphate homeostasis gene *pho1* was up-regulated by sevenfold vis-à-vis a wild-type *erh1⁺* control (Sugiyama et al. 2016). Fission yeast phosphate homeostasis entails a transcriptional response to phosphate availability that affects three genes that comprise a *PHO* regulon encoding proteins involved in extracellular phosphate acquisition, these being a cell surface acid phosphatase *Pho1*, an inorganic phosphate transporter *Pho84*, and a glycerophosphate transporter *Tgp1* (Carter-O'Connell et al. 2012). Expression of the *pho1*, *pho84*, and *tgp1* genes is actively repressed during growth in phosphate-rich medium by the transcription in *cis* of a long noncoding (lnc) RNA from the respective 5' flanking genes *prt*, *prt2*, and *nc-tgp1* (Lee et al. 2013; Ard et al. 2014; Shah et al. 2014; Chatterjee et al. 2016; Garg et al. 2018a; Sanchez et al. 2018a).

Our studies have shown that the repressive action of the lncRNAs on the *PHO* genes is governed by the RNA polymerase II (Pol2) CTD code, the 3' cleavage/polyadenylation factor complex CPF, transcription termination factor *Rhn1*, and synthesis of inositol 1-pyrophosphates (1-IPPs) (Schwer et al. 2014, 2015; Chatterjee et al. 2016; Sanchez et al. 2018b, 2019). Current evidence supports a model for the repressive arm of phosphate homeostasis whereby transcription of the upstream lncRNA interferes with expression of the downstream mRNA genes by displacing the activating transcription factor *Pho7* from its binding site(s) in the mRNA promoters that overlap the lncRNA transcription units (Carter-O'Connell et al. 2012; Schwer et al. 2017; Garg et al. 2018b, 2019). Genetic manipulations of the aforementioned governors can elicit either hyper-repression or de-repression of the *PHO* genes, as depicted in Figure 1. For example, a *CTD-T4A* mutation

that precludes installation of the Pol2 Thr4-PO₄ mark hyper-represses the *PHO* genes under phosphate-rich conditions, as do deletions or loss-of-function mutations in the *Ctf1*, *Dis2*, *Ssu72*, *Swd22*, and *Ppn1* subunits of CPF, deletion of termination factor *Rhn1*, and ablation of the IPP kinase *Asp1* that synthesizes 1,5-IP₈ and 1-IP₇ from 5-IP₇ and IP₆, respectively. In contrast, a *CTD-S7A* mutation that eliminates the Pol2 Ser7-PO₄ mark de-represses the *PHO* genes in phosphate-replete cells, as do IPP pyrophosphatase-inactivating mutations that increase IP₈. RNA analyses, transcriptomics, and genetic epistasis analyses cohere around a model whereby: (i) hyper-repression is the result of reduced lncRNA termination and hence increased transcription across the mRNA promoter; and (ii) de-repression results from precocious termination of lncRNA transcription prior to the mRNA promoter and thus relief from transcription interference.

In the present study, we find that ablating *Erh1* de-represses *pho1* expression in phosphate-replete cells via a pathway that depends on CTD-Thr4, CPF subunits *Ctf1*, *Dis2*, *Ssu72*, *Swd22*, and *Ppn1*, termination factor *Rhn1*, and synthesis of 1-IPPs. Deletion of *erh1* exerts an additive effect on *pho1* de-repression in combination with *CTD-S7A* and deletion of the IPP pyrophosphatase *Aps1*. These genetic interactions, and the findings that *erh1Δ* has no impact on the activity per se of the *prt* lncRNA and *pho1* mRNA promoters, point to *Erh1* as a negative influence on lncRNA termination in the *prt-pho1* axis. In *mmi1Δ* cells there is a reduction in *pho1* mRNA and increased production of a *prt-pho1* read-through transcript, consistent with *Mmi1* being a positive effector of lncRNA termination. We suggest that *Erh1* acts as a damper on *Mmi1* such that when *Erh1* is absent *Mmi1* is unrestrained in eliciting precocious *prt* termination and thus de-repression of *pho1* mRNA synthesis.

FIGURE 1. Synthesis of *prt* lncRNA represses *pho1* mRNA transcription. Models for the *pho1* hyper-repressed (A) and de-repressed (B) states of the *prt-pho1* locus under phosphate-replete conditions. The genetic perturbations that elicit these states are indicated at right.

RESULTS AND DISCUSSION

Deletion of Erh1 de-represses Pho1 expression

We constructed a fission yeast *erh1* Δ strain and tested the effects of Erh1 loss on growth on YES agar medium at 20°C to 37°C (Fig. 2A) and on *pho1* expression during exponential growth at 30°C in liquid culture under phosphate-replete conditions (Fig. 2B). The *erh1* Δ strain grew as well as the wild-type control strain at 30°C to 37°C, as gauged by colony size, but displayed cold-sensitivity, manifest as small colony size at 25°C and failure to thrive at 20°C (Fig. 2A). Acid phosphatase activity (a gauge of Pho1 enzyme level that correlates with *pho1* mRNA levels) was quantified by incubating suspensions of serial dilutions of the phosphate-replete cells for 5 min with *p*-nitrophenylphosphate and assaying colorimetrically the formation of *p*-nitrophenol. The basal Pho1 activity of wild-type cells was de-repressed by sixfold in *erh1* Δ cells (Fig. 2B).

The loss of Erh1 might de-repress *pho1* expression either by (i) reducing the activity of the *prt* lncRNA promoter that drives transcription interference with the *pho1* promoter or (ii) increasing the intrinsic activity of the *pho1* mRNA promoter independent of lncRNA synthesis. To address these issues, we used a plasmid-borne *prt-pho1* reporter (Fig. 3A) that was introduced into fission yeast cells in which the chromosomal *pho1* gene was deleted. This reporter faithfully reflects known homeostatic controls on the

native *pho1* locus (Chatterjee et al. 2016; Sanchez et al. 2019). We found that the *prt-pho1* reporter was responsive to ablation of Erh1, whereby Pho1 levels under phosphate-replete conditions were de-repressed by eightfold in *erh1* Δ cells compared to the wild-type *erh1*⁺ control (Fig. 3D). We then tested a mutated version of the *prt-pho1* reporter construct in which the *prt* promoter is inactivated by nucleotide changes in the HomolD and TATA box elements that drive *prt* lncRNA synthesis (Fig. 3B; Chatterjee et al. 2016). This mutant reporter provides a readout of the intrinsic activity of the *pho1* promoter, absent interference by transcription of the flanking *prt* lncRNA. The Pho1 activity of the mutant plasmid in wild-type cells is high (i.e., de-repressed) and is not different from the Pho1 activity in *erh1* Δ cells (Fig. 3E), thereby indicating that the de-repressive effect of *erh1* Δ on *pho1* expression from the wild-type *prt-pho1* locus is not caused by up-regulation of the *pho1* promoter per se. The effect of *erh1* Δ on the *prt* promoter was assessed using a different plasmid reporter (Fig. 3C) in which the *prt* promoter directly drives expression of the *pho1* ORF. Pho1 expression from this plasmid was the same in wild-type and *erh1* Δ cells (Fig. 3F), signifying that the de-repression of native *pho1* by *erh1* Δ is not caused by decreased activity of the *prt* promoter. These results suggest that *erh1* Δ affects *pho1* expression by enhancing precocious termination of *prt* lncRNA synthesis, à la the Pol2 *rpb1*-CTD-S7A allele (Fig. 1).

FIGURE 2. De-repression of Pho1 in *erh1* Δ cells requires the synthesis of inositol 1-pyrophosphates by Asp1 kinase. (A) Growth of *S. pombe* strains with the indicated *erh1*, *asp1*, and *aps1* alleles. Cells were inoculated in YES broth and grown at 30°C. Exponentially growing cultures were adjusted to A_{600} of 0.1, and aliquots (3 μ L) of serial fivefold dilutions were spotted on YES agar and then incubated at the temperatures specified. (B,C) *S. pombe* strains with the indicated *erh1*, *asp1*, and *aps1* alleles were grown to A_{600} of 0.5 to 0.8 in liquid culture in YES medium at 30°C. Cells were then harvested, washed with water, and assayed for Pho1 acid phosphatase activity by conversion of *p*-nitrophenylphosphate to *p*-nitrophenol. Activity is expressed as the ratio of A_{410} (*p*-nitrophenol production) to A_{600} (input cells). Each datum in the bar graph is the average of assays using cells from at least three independent cultures $\pm$ SEM. (D) Structures of the 5-IP7, 1-IP7, and 1,5-IP8 are shown. Asp1 kinase converts 5-IP7 to IP8 (and also IP6 to 1-IP7) and the Asp1 pyrophosphatase reverses this process.

FIGURE 3. Does *erh1Δ* affect the *prt* lncRNA or *pho1* mRNA promoters? (A) Schematic of the plasmid-borne *prt-pho1* reporter in which *pho1* expression is repressed by *prt* lncRNA transcription. (B) A reporter of *pho1* promoter activity in which *prt* lncRNA transcription is abolished by mutations (indicated by X) in the HomolD and TATA box elements in the *prt* promoter (Chatterjee et al. 2016). (C) A reporter of *prt* promoter activity in which the *prt* promoter directly drives transcription of the *pho1* gene. (D–F) The indicated reporter plasmids were transfected into *erh1*⁺ (WT) or *erh1Δ* strains in which the chromosomal *pho1* locus was deleted. Transformants were selected and single colonies of individual transformants were pooled (>20) and grown in plasmid-selective liquid medium to A₆₀₀ of 0.5–0.8. Aliquots were harvested for acid phosphatase activity measurements. Each datum in the bar graph is the average of assays using cells from three independent cultures ± SEM.

erh1Δ de-repression of Pho1 expression depends on 1-IPP synthesis

Previous studies showed that de-repression of Pho1 elicited by the *rpb1-CTD-S7A* allele depends on the activity of the IPP kinase Asp1 (Sanchez et al. 2019). Asp1 is a bifunctional enzyme composed of an amino-terminal IPP kinase domain that converts 5-IP7 to 1,5-IP8, and a carboxy-terminal IPP pyrophosphatase domain that converts 1,5-IP8 back to 5-IP7 (Fig. 2D; Pascual-Ortiz et al. 2018; Dollins et al. 2020). Asp1 can also phosphorylate IP6 to yield 1-IP7 and de-phosphorylate 1-IP7 back to IP6. The in vivo effect of an *asp1Δ* null allele or a kinase-dead *asp1-D333A* allele is to eliminate intracellular IP8 and 1-IP7 and to increase the level of 5-IP7; the in vivo effect of a pyrophosphatase-defective *asp1-H397A* allele is to increase the level of IP8 (Pascual-Ortiz et al. 2018). To see if IPP status affects Pho1 de-repression by *erh1Δ*, we crossed *erh1Δ* to an *asp1-D333A* strain. As noted previous-

ly (Sanchez et al. 2019), *asp1-D333A* cells formed slightly smaller colonies than the wild-type control strain (Fig. 2A). The lack of IP8 in *asp1-D333A* cells resulted in a fivefold hyper-repression of Pho1 acid phosphatase activity (Fig. 2B). We obtained viable *erh1Δ asp1-D333A* haploids after mating and sporulation; the double-mutant was slow-growing on YES agar and cold-sensitive (Fig. 2A). The instructive finding was that the de-repression of Pho1 activity by *erh1Δ* was erased in the *asp1-D333A* background (Fig. 2B), signifying a requirement for 1-IPP synthesis, presumably via 1-IPP's role as an agonist of 3'-processing/termination during *prt* transcription (Sanchez et al. 2019).

Pho1 is de-repressed in IP8 pyrophosphatase-defective *asp1-H397A* cells by virtue of precocious termination of *prt* lncRNA transcription when IP8 levels are elevated (Fig. 2C; Sanchez et al. 2019). Pho1 is also de-repressed in *aps1Δ* cells (Fig. 2C; Sanchez et al. 2019) that lack the Nudix-family IPP pyrophosphatase Aps1, which converts 1,5-IP8 to 1-IP7 (Fig. 2D; Safrany et al. 1999). Here we tested the genetic interactions of *erh1Δ* with *asp1-H397A* and *aps1Δ* by pairwise mating and recovery of viable *erh1Δ asp1-H397A* and *erh1Δ aps1Δ* haploids after sporulation (Fig. 2A). The 11-fold de-repres-

sion of Pho1 activity elicited by *asp1-H397A* vis-à-vis wild-type *asp1*⁺ was virtually unaffected by the *erh1Δ* deletion (Fig. 2C). In contrast, the fivefold de-repression of Pho1 in the *aps1Δ* strain was enhanced additively to 12-fold in the *erh1Δ aps1Δ* background (Fig. 2C).

De-repression of *pho1* expression by *erh1Δ* depends on CPF subunits and Rhn1

To test our hypothesis that loss of Erh1 leads to precocious termination during *prt* lncRNA synthesis, we introduced the *erh1Δ* mutation into knockout strains lacking the Dis2, Ctf1, Ppn1, or Swd22 subunits of the CPF complex, a strain with a catalytically dead (C13S) version of the Ssu72 protein phosphatase subunit of CPF, and a strain that lacks the transcription termination factor Rhn1. Viable double-mutant haploids were recovered after pairwise matings and sporulation of the diploids (Fig. 4A). As noted previously (Sanchez et al. 2018b), the CPF and

FIGURE 4. De-repression of *pho1* expression by *erh1Δ* depends on CPF subunits and Rhn1. (A) *S. pombe* strains with the genotypes indicated at left were inoculated in YES broth and grown at 30°C. Exponentially growing cultures were adjusted to A_{600} of 0.1, and aliquots (3 μ L) of serial fivefold dilutions were spotted on YES agar and then incubated at the temperatures specified. (B) *S. pombe* strains bearing the indicated *erh1* alleles (wild-type or *erh1Δ*) in combination with CPF subunit or Rhn1 mutations as specified were grown in liquid culture at 30°C and assayed for acid phosphatase activity.

Rhn1 mutations per se resulted in hyper-repression of Pho1 in phosphate-replete cells (Fig. 4B). The instructive findings were that the de-repression of Pho1 by *erh1Δ* was effaced in *rhn1Δ*, *ssu72-C13S*, *ctf1Δ*, *ppn1Δ*, and *swd22Δ* cells and was attenuated in *dis2Δ* cells (Fig. 4B). Thus, the increase in Pho1 expression in *erh1Δ* cells requires CPF subunits and Rhn1, consistent with the precocious termination model (Fig. 1B).

Genetic interactions of *erh1Δ* with Pol2 CTD phospho-site mutants

The de-repressive effect of *erh1Δ* on Pho1 expression and its genetic reliance on IP8 synthesis, CPF subunits, and Rhn1 reported above is similar to the IP8/CPF/Rhn1-dependent de-repression of *pho1* observed in *rpb1-CTD-S7A* cells and contrasts with the hyper-repression of Pho1 in *rpb1-CTD-T4A* cells (Chatterjee et al. 2016; Sanchez et al. 2018b, 2019). To query epistasis relationships, we mated *erh1Δ* with the CTD phospho-site mutants and recovered viable haploid *erh1Δ* CTD-S7A and *erh1Δ*

CTD-T4A progeny after sporulation (Fig. 5A). Assays of acid phosphatase activity showed that the S7A allele enhanced the de-repression of Pho1 by *erh1Δ*, whereas the T4A allele completely erased Pho1 de-repression (Fig. 5B). Thus, T4A “wins out” over *erh1Δ* with respect to Pho1 expression, just as it does over other mutations (*S7A*, *asp1-H397A*, or *aps1Δ*) that de-repress the *prt-pho1* locus.

Distinctive effects of *erh1Δ* and *mmi1Δ* on *pho1* expression

Erh1 per se is a homodimer; it forms a 2:2 heterotetramer with Mmi1 (Sugiyama et al. 2016; Xie et al. 2019; Hazra et al. 2020). A pertinent question here is whether the *pho1* de-repressive effects of *erh1Δ* are recapitulated in the absence of Mmi1. To address this issue, we took advantage of the observation that Mmi1, which is essential for vegetative growth of fission yeast (by virtue of its ability to promote elimination of detrimental meiotic RNAs) can be deleted in a genetic background in which *mei4* is inactivated, for example, in the *mmi1Δ* strain SP1111 (Sugiyama and Sugioka-Sugiyama 2011) where we find there is a Cys125-Stop nonsense mutation in the *mei4* gene that truncates the 517-aa Mei4 protein. Figure 6A shows a northern blot analysis of total RNA from phosphate-replete wild-type, *erh1Δ*, and *mmi1Δ* cells probed with a radiolabeled DNA oligonucleotide complementary to the *pho1* ORF. Consistent with the Pho1 activity assays shown in the preceding sections, we find that the ~1.6 kb *pho1* mRNA is increased in *erh1Δ* cells vis-à-vis wild-type. In contrast, the *pho1* mRNA level is decreased slightly in

FIGURE 5. Genetic interactions of *erh1Δ* with Pol2 CTD phospho-site mutants T4A and S7A. (A) Serial dilutions of *S. pombe* strains with the *erh1* and *rpb1-CTD* alleles specified at left were spot-tested for growth at the indicated temperatures. (B) *S. pombe* strains with the indicated *erh1* and *rpb1-CTD* alleles were grown in liquid culture at 30°C and assayed for acid phosphatase activity.

FIGURE 6. Distinctive effects of *erh1Δ* and *mmi1Δ* on *pho1* expression. (A) Northern analysis. RNAs isolated from WT, *erh1Δ*, and *mmi1Δ* cells were resolved by formaldehyde-agarose gel electrophoresis and stained with ethidium bromide to visualize 28S and 18S ribosomal RNAs (bottom panel). The RNAs in the gel were transferred to membrane and hybridized to the *pho1* probe. Annealed probe was visualized by autoradiography. The positions and sizes (in kilobases) of RNA markers are indicated on the left. The *pho1* mRNA and *prt-pho1* readthrough transcript are indicated on the right. The three RNA samples were analyzed in parallel on the same gel, probed on the same membrane, and visualized in a single exposure; intervening lanes of the stained gel and blot were cropped out during figure preparation. (B) Primer extension analysis. Total RNA from WT and *mmi1Δ* cells was analyzed by reverse transcription primer extension using a mixture of radiolabeled primers complementary to the *pho1* and *act1* mRNAs. The reaction products were resolved by denaturing PAGE and visualized by autoradiography. The positions and sizes (nt) of DNA markers are indicated on the left. (C) *mmi1⁺* (WT) and *mmi1Δ* cells deleted for *pho1* and bearing the *prt-pho1* reporter plasmid (shown in Fig. 3A) were assayed for acid phosphatase activity as described in Figure 3D.

mmi1Δ cells and we detect the presence of a longer *prt-pho1* readthrough transcript (Fig. 6A) that extends from the *prt* lncRNA transcription start site to the *pho1* mRNA poly(A) site. We also performed primer extension analysis to simultaneously gauge RNAs initiated from the transcription start sites of the *pho1* and *act1* protein-coding genes (Fig. 6B), which affirmed the presence of properly initiated *pho1* mRNA in *mmi1Δ* cells at a slightly lower level than seen in wild-type cells. Our RNA analyses of *mmi1Δ* are

in accord with RNA-seq data reported previously, whereby: (i) *mmi1Δ* cells displayed increased read density over the *prt* lncRNA as well as persistent reads across the *pho1* coding region (Kilchert et al. 2015; Xie et al. 2019); and (ii) *erh1Δ* elicits a large increase in reads across the *pho1* ORF but no apparent increase in reads for the *prt* lncRNA (Xie et al. 2019). We conclude that Erh1 and Mmi1 exert distinctive effects on *pho1* expression at the RNA level. In addition, using the *prt-pho1* reporter plasmid to gauge Pho1 acid phosphatase expression, we found that Pho1 activity was lower in *mmi1Δ* cells than in wild-type cells (Fig. 6C), an effect counter to the de-repression of Pho1 activity from the *prt-pho1* reporter observed for *erh1Δ* cells (Fig. 3D).

The data presented thus far suggest a scenario in which Erh1 acts as a brake on Mmi1's ability to promote CPF-dependent termination during *prt* lncRNA synthesis. Previous studies have shown that the steady-state level of Erh1 is unchanged in *mmi1Δ* cells and the level of Mmi1 is similarly unaltered in *erh1Δ* cells (Sugiyama et al. 2016). Accordingly, we envision that when Erh1 is absent, Mmi1 is unrestrained in eliciting precocious *prt* termination and thus de-repression of *pho1* mRNA synthesis. No such de-repression is seen in *mmi1Δ* cells; rather there is a reduction in *pho1* mRNA and increase in the formation of a *prt-pho1* read-through transcript, reflective of decreased *prt* termination, augmented perhaps by decreased turnover of *prt* lncRNA in the absence of Mmi1. De-repression of *pho1* by *erh1Δ* depends on the CPF/Rhn1, CTD Thr4, and IP8/1-IP7 synthesis by Asp1. These are the same genetic dependencies that apply to *pho1* de-repression triggered by CTD-S7A and by increased IP8 levels (in cells defective for IPP pyrophosphatase activity) (Sanchez et al. 2018b, 2019), the unifying theme being that such de-repression results from precocious *prt* termination upstream of the *pho1* mRNA promoter (Fig. 1B). A nexus of Mmi1 in this interactome with respect to *pho1* control is fortified by several recent observations from the Grewal laboratory: (i) Mmi1 is present in CPF complex affinity-purified from fission yeast; (ii) ChIP-seq data show that CPF and Mmi1 are present at the *prt* locus, prominently over the 5' half of the lncRNA (wherein reside the sites to which Mmi1 binds); and (iii) the CPF ChIP signal over the *prt* locus is present but lower in *mmi1Δ* cells (Vo et al. 2019). They propose that Mmi1 recruits CPF to promote 3'-processing termination. Our model that Erh1 dampens Mmi1/CPF-dependent *prt* termination begets a prediction that the de-repression of *pho1* in *erh1Δ* cells should depend on the binding sites for Mmi1 in the *prt* lncRNA.

De-repression of *pho1* by *erh1Δ* depends on DSR elements in the *prt* lncRNA

The *prt* lncRNA contains two clusters of DSR elements—DSRx3-1 and DSRx3-2—each composed of three DSR

hexanucleotide elements (Fig. 7). The DSR clusters in the nascent lncRNA are binding sites for Mmi1 (Kilchert et al. 2015; Chatterjee et al. 2016). Previous studies highlighted the role of the DSR clusters in *prt*-promoted *pho1* repression, via tracking the effects of compound mutations (*mut1* and *mut2*) introduced into each of the hexanucleotide motifs comprising the *DSRx3-1* and *DSRx3-2* sequences of the *prt-pho1* reporter plasmid (Fig. 7; Chatterjee et al. 2016). As recapitulated here, *mut1* and *mut2* reduced acid phosphatase activity in phosphate-replete cells to 75% and 22% of the wild-type *prt* DSR control, respectively (Fig. 7). Combining the *mut1* and *mut2* DSR cluster mutations had an additive effect, reducing Pho1 activity to 11% of the wild-type *prt* level. The hyper-repressive effect of the *prt* DSR *mut1+mut2* changes on Pho1 expression were shown previously to “win out” over the de-repressive effects of *CTD-S7A* and *asp1-H397A* (i.e., increased IP8), suggesting that DSRs are key to establish the precocious termination of *prt* lncRNA synthesis that underlies Pho1 de-repression (Chatterjee et al. 2016; Sanchez et al. 2019). Here we queried the effect of DSR mutations on

de-repression of the *prt-pho1* reporter in *erh1Δ pho1Δ* cells. The *mut1*, *mut2*, and *mut1+mut2* changes reduced Pho1 expression in *erh1Δ* cells to 52%, 11%, and 2%, respectively, of the wild-type DSR reporter control, such that the level of Pho1 expression from the *mut1+mut2* reporter was virtually identical in the wild-type and *erh1Δ* strains (Fig. 7). We conclude that *erh1Δ* de-repression of *pho1* depends on the DSR elements in the *prt* lncRNA.

Synthetic genetic interactions of *mmi1Δ* with CPF and Rhn1 mutants

There is significant functional redundancy built into the fission yeast 3' processing/termination machinery, whereby the effects of ablating one inessential component are buffered by other inessential components, such that inactivation of both elicits synthetic lethality or severe synthetic sickness (Sanchez et al. 2018b, 2020). Because termination is one of several processes in which Mmi1 is implicated, we performed a synthetic genetic array analysis entailing pairwise mating of the *mmi1Δ* strain with *ctf1Δ*, *ppn1Δ*, *swd22Δ*, *dis2Δ*, *ssu72-C13S*, and *rhn1Δ* cells and screening large populations of random spores for the drug-resistance markers linked to the respective mutant alleles. We thereby found that *mmi1Δ* was synthetically lethal with *dis2Δ* (Supplemental Fig. S1). The viable *mmi1Δ* CPF/*rhn1* strains were grown in YES liquid medium and then spot-tested for growth on YES agar in parallel with the *mmi1Δ* single mutant (which grew well at 25°C to 37°C) (Supplemental Fig. S1). We found that: (i) *mmi1Δ* was synthetically sick at all temperatures with *ssu72-C13S*, *swd22Δ*, and *ppn1Δ*; and (ii) *mmi1Δ* exacerbated the temperature sensitivity of *rhn1Δ* such that *mmi1Δ rhn1Δ* cells failed to grow at 30°C (compare Supplemental Fig. S1 and Fig. 4A).

Probing the structural requirements for Mmi1's essential function in vegetative cells

The 488-aa Mmi1 protein (Fig. 8A) consists of carboxy-terminal YTH-fold RNA binding domain that suffices for DSR recognition (Chatterjee et al. 2016; Wang et al. 2016) and an unstructured amino-terminal domain that mediates protein-protein interactions. The Mmi1 segment from aa 95–122 (underlined in Fig. 8A) suffices for interaction with Erh1 and forms an extensive hydrophobic and hydrogen-bonding interface with Erh1 in the cocrystal structure (Xie et al. 2019). The Mmi1•Erh1 complex interacts physically in vivo with the multisubunit Ccr4-Not deadenylase complex (Sugiyama et al. 2016), and Mmi1 (in the absence of Erh1) is reconstituted as a stoichiometric component of a highly purified recombinant Mmi1•Ccr4-Not complex produced by coexpression using a baculovirus vector (Stowell et al. 2016). Deletion of the amino-terminal 56-aa of Mmi1 abolished interaction with Ccr4-Not (Stowell et al. 2016). In order to interrogate the contributions of the amino-

FIGURE 7. De-repression of *pho1* by *erh1Δ* depends on DSR elements in the *prt* lncRNA. The top panel shows a schematic of the *prt-pho1* locus in the reporter plasmid that highlights the two clusters of triplet DSR elements recognized by Mmi1. The nucleotide sequence of the wild-type DSR clusters and the base mutations introduced into the *mut1* and *mut2* variants are shown below the graph. The reporter plasmids with wild-type or mutated DSRs were transfected into *erh1⁺* (WT) or *erh1Δ* strains in which the chromosomal *pho1* locus was deleted. Acid phosphatase activity was determined as described in Figure 3D.

FIGURE 8. Probing the structural requirements for Mmi1's essential function in vegetative cells. (A) The amino acid sequence of *S. pombe* Mmi1 (Spo) is aligned to that of the homologous protein from *S. japonicum* (Sja). Positions of side chain identity/similarity are denoted by dots above the sequence. A conserved motif in the amino-terminal domain (aa 95–122) that suffices for Mmi1 interaction with Erh1 is underlined. The boundaries of the series of amino-terminal deletion alleles are indicated by *rightward-pointing bent arrows*. Amino acids in the carboxy-terminal YTH domain that were mutated to alanine are indicated by triangles above the sequence. (B) Viable *mmi1-ΔN* and *mmi1-Ala* strains were spot-tested for growth on YES agar at the indicated temperatures. Lethal *mmi1-ΔN* and *mmi1-Ala* alleles are indicated at *bottom*.

terminal region to Mmi1's essential function in vegetative cells, we introduced cDNAs for full-length *mmi1* and a series of *mmi1* amino-terminal truncation alleles, each marked by a 3' flanking *hygMX* drug-resistance cassette, in lieu of one chromosomal *mmi1*⁺ locus of a diploid fission yeast strain. The diploids were sporulated and large populations of random haploid progeny were screened for hygromycin resistance linked to the alleles of interest. We recovered viable haploid truncation mutants *mmi1*-(31-488), *mmi1*-(63-488), and *mmi1*-(94-488) at ~50% frequency in the random spore analysis. Spot testing for growth on YES agar showed that these three truncation strains grew as well as the marked wild-type *mmi1*-*hygMX* control strain (Fig. 8B). The inessential 93-aa amino-terminal segment includes the module required for Mmi1 binding to Ccr4-Not, suggesting that this protein-protein interaction is dispensable for Mmi1 function during

vegetative growth. In contrast, we were unable to recover any viable hygromycin-resistant haploids bearing the *mmi1*-(129-488), *mmi1*-(166-488), *mmi1*-(222-488), or *mmi1*-(301-488) alleles (Fig. 8). The loss of viability coincided with deletion of Mmi1 aa 94–128, which embraces the Erh1 interaction motif. Yet the lethality of *mmi1*-(129-488) is unlikely to reflect necessity for Erh1•Mmi1 interaction, insofar as (i) Erh1 per se is not essential and (ii) a single Mmi1 missense mutation *W112A* within the Erh1 binding motif that eliminates binding to Erh1 in vitro is able to support vegetative growth (Xie et al. 2019). It is conceivable that the Mmi1-(94–128) segment has a salutary effect on Mmi1 activity separate from its interaction with Erh1.

We also queried the role of RNA binding in Mmi1 vegetative function by alanine scanning of selected amino acids at the interface of the YTH domain with the DSR element 5'-U¹U²A³A⁴A⁵C⁶ (Wang et al. 2016). We targeted RNA-binding residues Tyr352 (which makes hydrogen bonds to the A³ nucleobase), Lys436 (which makes hydrogen bonds to the U¹pU² and U²pA³ phosphates), and Tyr466 (which makes a hydrogen bond to the A⁴ nucleobase). As a control, we mutated Trp372, a residue conserved among YTH proteins and a key component of the aromatic cage that recognizes the modified m6A nucleobase.

base, but which is not involved in the binding of Mmi1 to DSR elements (Chatterjee et al. 2016; Wang et al. 2016). The salient findings were that alanine substitutions for DSR-binding residues Tyr352 and Lys436 were lethal and that a Y466A mutant was viable but extremely sick (Fig. 8B). In contrast, the aromatic cage mutant W372A grew as well as wild-type at 20°C–34°C and formed larger colonies than the WT control at 37°C (Fig. 8B). Thus, DSR recognition is essential for Mmi1 to sustain vegetative growth.

MATERIALS AND METHODS

Deletion of *erh1*

Using PCR amplification and standard cloning methods, we constructed a plasmid in which the *erh1* gene from nucleotides +1 to

+696 (relative to the translational start codon +1) was replaced by a *kanMX* antibiotic resistance cassette. The disruption cassette was excised from the plasmid and transfected into diploid *S. pombe* cells. G418-resistant transformants were selected and analyzed by Southern blotting to confirm correct integration at one of the *erh1* loci (thereby effectively deleting amino acids 1–99 of the 104-aa Erh1 protein). G418-resistant *erh1Δ* haploids were isolated upon sporulation of the heterozygous diploids. A nourseothricin-resistant *erh1Δ* strain was generated by marker switching (Hentges et al. 2005).

Allelic exchange at the *mmi1* locus

Strains harboring marked wild-type and mutated *mmi1* alleles were constructed as follows. We first built an integration cassette for wild-type *mmi1* that consisted of five elements in series from 5' to 3': (i) a 843-bp segment of genomic DNA 5' of the *mmi1*⁺ start codon; (ii) a 1467-bp cDNA fragment encoding wild-type Mmi1, flanked by BamHI and HindIII restriction sites immediately adjacent to the translational start and stop codon, respectively; (iii) a 270-bp segment including poly(A)/termination signals from the *nmt1*⁺ gene; (iv) a *hygMX* gene conferring resistance to hygromycin; and (v) a 815-bp segment of genomic DNA 3' of the *mmi1*⁺ stop codon. Amino-terminal truncation variants were generated by PCR using forward primers that introduced a BamHI site followed by a Met residue in lieu of Pro30, Asn62, Lys93, Ser128, Leu165, Ala221, or Ala300. Missense mutations in the full-length *mmi1* ORF were introduced by two-stage PCR overlap extension with mutagenic primers. The mutated DNA restriction fragments were inserted into the integration cassette in lieu of the wild-type *mmi1* ORF. All inserts were sequenced to exclude the presence of unwanted mutations. The integration cassettes were transfected into diploid *S. pombe* cells. Hygromycin-resistant transformants were selected and correct integrations at the target locus were confirmed by Southern blotting. The heterozygous diploids were then sporulated and hygromycin-resistant haploids were isolated. A segment of the *mmi1-hygMX* locus was amplified by PCR and sequenced to verify that the desired mutations were present and to ascertain that the crossovers had occurred upstream of the translational start codon (i.e., that none of the introns were retained).

Acid phosphatase activity

Cells were grown at 30°C in YES liquid medium. Aliquots of exponentially growing cultures were harvested, washed with water, and resuspended in water. To quantify acid phosphatase activity, reaction mixtures (200 μ L) containing 100 mM sodium acetate (pH 4.2), 10 mM *p*-nitrophenyl phosphate, and cells (ranging from 0.01 to 0.1 A_{600} units) were incubated for 5 min at 30°C. The reactions were quenched by addition of 1 mL of 1 M sodium carbonate, the cells were removed by centrifugation, and the absorbance of the supernatant at 410 nm was measured. Acid phosphatase activity is expressed as the ratio of A_{410} (*p*-nitrophenol production) to A_{600} (cells). The data shown in graphs are averages ($\pm$ SEM) of at least three assays using cells from three independent cultures.

prt-pho1 reporter plasmids and assays

The *prt-pho1* reporter plasmids, marked with a kanamycin-resistance gene (*kanMX*), were transfected into *pho1Δ* cells (Chatterjee et al. 2016) or *pho1Δ erh1Δ* cells and transformants were selected on YES agar medium containing 150 μ g/mL G418. Single colonies of individual transformants were pooled (≥ 20) and grown in liquid YES + G418 medium to A_{600} of 0.5–0.8. Aliquots were harvested by centrifugation for acid phosphatase activity measurements as described above.

RNA analyses

Total RNA was extracted via the hot phenol method (Herrick et al. 1990) from 20 A_{600} units of yeast cells that had been grown exponentially to A_{600} of 0.6 to 0.8 at 30°C. Aliquots (15 μ g) of total RNA were used as templates for M-MuLV reverse transcriptase-catalyzed extension of 5' ³²P-labeled oligodeoxynucleotide primers complementary to the *pho1* or *act1* mRNAs. The primer extension reactions were performed as described previously (Schwer et al. 1998) and the products were analyzed by electrophoresis of the reaction mixtures through a 22-cm 8% polyacrylamide gel containing 7 M urea in 80 mM Tris-borate, 1.2 mM EDTA. The ³²P-labeled primer extension products were visualized by autoradiography of the dried gel. The primer sequences were as follows: *act1* 5'-GATTCTTCTTCCATGGTCTTGTC and *pho1* 5'-GTTGGCACAAACGACGGCC. For northern blotting, aliquots (10 μ g) of total RNA were resolved by electrophoresis through a 1.2% agarose/formaldehyde gel. After photography under UV light to visualize ethidium bromide-stained rRNAs and tRNAs, the gel contents were transferred to a Hybond-XL membrane (GE Healthcare) and hybridization was performed with a randomly primed ³²P-labeled DNA fragment spanning nucleotides 590–1293 of the *pho1* ORF.

Mutational effects on fission yeast growth

Cultures of *S. pombe* strains were grown in YES liquid medium until A_{600} reached 0.6–0.8. The cultures were adjusted to A_{600} of 0.1 and 3 μ L aliquots of serial fivefold dilutions were spotted on YES agar. The plates were photographed after incubation for 2 d at 34°C, 2.5 d at 30°C and 37°C, 4 d at 25°C, 6 d at 20°C.

Tests of mutational synergies

Standard genetic methods were used to generate haploid strains harboring mutations/deletions in two differently marked genes. In brief, pairs of haploids with missense or null mutations were mixed on malt agar to allow mating and sporulation and then the mixture was subjected to random spore analysis. Spores (~ 1000) were plated on YES agar and on media selective for marked mutant alleles; the plates were incubated at 30°C for up to 5 d to allow slow growing progeny to germinate and form colonies. At least 500 viable progenies were screened by replica-plating for the presence of the second marker gene, or by sequentially replica-plating from YES to selective media. A finding that no haploids with both marker genes were recovered after 6 to 8 d of incubation at 30°C was taken to indicate synthetic lethality. By sequentially replica-plating and gauging the numbers

of colonies at each step, we ensured that wild-type (unmarked) and the differentially marked single mutant alleles were recovered at the expected frequencies. Growth phenotypes of viable double-mutants were assessed in parallel with the individual mutants and wild-type cells at different temperatures (20°C–37°C) as described above.

SUPPLEMENTAL MATERIAL

Supplemental material is available for this article.

ACKNOWLEDGMENTS

We thank Dr. Tomoyasu Sugiyama for generously providing the *mmi1Δ* strain SP1111. This work was supported by National Institutes of Health, National Institute of General Medical Sciences (NIH-NIGMS) grants R01-GM52470, R01-GM134021, and R35-GM126945.

Received May 16, 2020; accepted June 1, 2020.

REFERENCES

- Ard R, Tong P, Allshire RC. 2014. Long non-coding RNA-mediate transcriptional interference of a permease gene confers drug tolerance in fission yeast. *Nature Commun* **5**: 5576. doi:10.1038/ncomms6576
- Carter-O'Connell I, Peel MT, Wykoff DD, O'Shea EK. 2012. Genome-wide characterization of the phosphate starvation response in *Schizosaccharomyces pombe*. *BMC Genomics* **13**: 697. doi:10.1186/1471-2164-13-697
- Chatterjee D, Sanchez AM, Goldgur Y, Shuman S, Schwer B. 2016. Transcription of lncRNA *prt*, clustered *prt* RNA sites for Mmi1 binding, and RNA polymerase II CTD phospho-sites govern the repression of *pho1* gene expression under phosphate-replete conditions in fission yeast. *RNA* **22**: 1011–1025. doi:10.1261/ma.056515.116
- Dollins DE, Bai W, Fridy PC, Otto JC, Neubauer JL, Gattis SG, Mehta KP, York JD. 2020. Vip1 is a kinase and pyrophosphatase switch that regulates inositol diphosphate signaling. *Proc Natl Acad Sci* **117**: 9356–9364. doi:10.1073/pnas.1908875117
- Garg A, Sanchez AM, Shuman S, Schwer B. 2018a. A long noncoding (lnc) RNA governs expression of the phosphate transporter *Pho84* in fission yeast and has cascading effects on the flanking *prt* lncRNA and *pho1* genes. *J Biol Chem* **293**: 4456–4467. doi:10.1074/jbc.RA117.001352
- Garg A, Goldgur Y, Schwer B, Shuman S. 2018b. Distinctive structural basis for DNA recognition by the fission yeast Zn₂Cys₆ transcription factor *Pho7* and its role in phosphate homeostasis. *Nucleic Acids Res* **46**: 11262–11273. doi:10.1093/nar/gkx957
- Garg A, Goldgur Y, Sanchez AM, Schwer B, Shuman S. 2019. Structure of fission yeast transcription factor *Pho7* bound to *pho1* promoter DNA and effect of *Pho7* mutations on DNA binding and phosphate homeostasis. *Mol Cell Biol* **39**: e00132-19. doi:10.1128/MCB.00132-19
- Hazra D, Andric V, Palancade B, Rougemaille M, Graille M. 2020. Formation of *S. pombe* Erh1 homodimer mediates gametogenic gene silencing and meiosis progression. *Sci Rep* **10**: 1034. doi:10.1038/s41598-020-57872-4
- Hentges P, Van Driessche B, Tafforeau L, Vandenhaute J, Carr AM. 2005. Three novel antibiotic marker cassettes for gene disruption and marker switching in *Schizosaccharomyces pombe*. *Yeast* **22**: 1013–1019. doi:10.1002/yea.1291
- Herrick D, Parker R, Jacobson A. 1990. Identification and comparison of stable and unstable RNAs in *Saccharomyces cerevisiae*. *Mol Cell Biol* **10**: 2269–2284. doi:10.1128/MCB.10.5.2269
- Kilchert C, Wittmann S, Passoni M, Shah S, Granneman S, Visiljeva L. 2015. Regulation of mRNA levels by decay-promoting intron that recruit the exosome specificity factor Mmi1. *Cell Rep* **13**: 2504–2515. doi:10.1016/j.celrep.2015.11.026
- Krzyzanowski MK, Kozłowska E, Kozłowski P. 2012. Identification and functional analysis of the *erh1⁺* gene encoding enhancer of rudimentary homolog from the fission yeast *Schizosaccharomyces pombe*. *PLoS One* **7**: e49059. doi:10.1371/journal.pone.0049059
- Lee NN, Chalamcharia VR, Reyes-Turce F, Mehta S, Zofall M, Balachandran V, Dhakshnamoorthy J, Taneja N, Yamanaka S, Zhou M, et al. 2013. Mtr4-like protein coordinates nuclear RNA processing for heterochromatin assembly and for telomere maintenance. *Cell* **155**: 1061–1074. doi:10.1016/j.cell.2013.10.027
- Pascual-Ortiz M, Saiardi A, Walla E, Jakopc V, Künzel NA, Span I, Vangala A, Fleig U. 2018. Asp1 bifunctional activity modulates spindle function via controlling cellular inositol pyrophosphate levels in *Schizosaccharomyces pombe*. *Mol Cell Biol* **38**: e00047-18. doi:10.1128/MCB.00047-18
- Safra ST, Ingram SW, Cartwright JL, Falck JR, McLennan AG, Barnes LD, Shears SB. 1999. The diadenosine hexaphosphate hydrolase from *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* are homologues of the human disphosphoinositol polyphosphate phosphohydrolase: overlapping substrate specificities in a MutT-type protein. *J Biol Chem* **274**: 21735–21740. doi:10.1074/jbc.274.31.21735
- Sanchez AM, Shuman S, Schwer B. 2018a. Poly(A) site choice and Pol2 CTD Serine-5 status govern lncRNA control of phosphate-responsive *tgpl* gene expression in fission yeast. *RNA* **24**: 237–250. doi:10.1261/ma.063966.117
- Sanchez AM, Shuman S, Schwer B. 2018b. RNA polymerase II CTD interactome with 3' processing and termination factors in fission yeast and its impact on phosphate homeostasis. *Proc Natl Acad Sci* **115**: E10652–E10661. doi:10.1073/pnas.1810711115
- Sanchez AM, Garg A, Shuman S, Schwer B. 2019. Inositol pyrophosphates impact phosphate homeostasis via modulation of RNA 3' processing and transcription termination. *Nucleic Acids Res* **47**: 8452–8469. doi:10.1093/nar/gkz567
- Sanchez AM, Garg A, Shuman S, Schwer B. 2020. Genetic interactions and transcriptomics implicate fission yeast CTD prolyl isomerase Pin1 as an agent of RNA 3' processing and transcription termination that functions via its effects on CTD phosphatase *Ssu72*. *Nucleic Acids Res* **48**: 4811–4826. doi:10.1093/nar/gkaa212
- Schwer B, Mao X, Shuman S. 1998. Accelerated mRNA decay in conditional mutants of yeast mRNA capping enzyme. *Nucleic Acids Res* **26**: 2050–2057. doi:10.1093/nar/26.9.2050
- Schwer B, Bitton DA, Sanchez AM, Bähler J, Shuman S. 2014. Individual letters of the RNA polymerase II CTD code govern distinct gene expression programs in fission yeast. *Proc Natl Acad Sci* **111**: 4185–4190. doi:10.1073/pnas.1321842111
- Schwer B, Sanchez AM, Shuman S. 2015. RNA polymerase II CTD phospho-sites Ser5 and Ser7 govern phosphate homeostasis in fission yeast. *RNA* **21**: 1770–1780. doi:10.1261/ma.052555.115
- Schwer B, Sanchez AM, Garg A, Chatterjee D, Shuman S. 2017. Defining the DNA binding site recognized by the fission yeast Zn₂Cys₆ transcription factor *Pho7* and its role in phosphate homeostasis. *mBio* **8**: e01218-17. doi:10.1128/mBio.01218-17
- Shah S, Wittmann S, Kilchert C, Vasiljeva L. 2014. lncRNA recruits RNAi and the exosome to dynamically regulate *pho1* expression in response to phosphate levels in fission yeast. *Genes Dev* **28**: 231–244. doi:10.1101/gad.230177.113
- Shichino Y, Otsubo Y, Kimori Y, Yamamoto M, Yamashita A. 2018. YTH-RNA-binding protein prevents deleterious expression of

- meiotic proteins by tethering their mRNAs to nuclear foci. *Elife* **7**: e32155. doi:10.7554/eLife.32155
- Stowell JAW, Webster MW, Kögel A, Wolf J, Shelley KL, Passmore LA. 2016. Reconstitution of targeted deadenylation by the Ccr4-Not complex and the YTH domain protein Mmi1. *Cell Rep* **17**: 1978–1989. doi:10.1016/j.celrep.2016.10.066
- Stowell JAW, Wagstaff JL, Hill CH, Yu M, McLaughlin SH, Freund SMV, Passmore LA. 2018. A low-complexity region in the YTH domain protein Mmi1 enhances RNA binding. *J Biol Chem* **293**: 9210–9222. doi:10.1074/jbc.RA118.002291
- Sugiyama T, Sugioka-Sugiyama R. 2011. Red1 promotes the elimination of meiosis-specific mRNAs in vegetatively growing fission yeast. *EMBO J* **30**: 1027–1039. doi:10.1038/emboj.2011.32
- Sugiyama T, Thillainadesan G, Chalamcharla VR, Meng Z, Balachandran V, Dhakshnamoorthy J, Zhou M, Grewal SI. 2016. *Enhancer of Rudimentary* cooperates with conserved RNA-processing factors to promote meiotic mRNA decay and facultative heterochromatin assembly. *Mol Cell* **61**: 747–759. doi:10.1016/j.molcel.2016.01.029
- Vo TV, Dhakshnamoorthy J, Larkin M, Zofall M, Thillainadesan G, Balachandran V, Holla S, Wheeler D, Grewal SI. 2019. CPF recruitment to non-canonical transcription termination sites triggers heterochromatin assembly and gene silencing. *Cell Rep* **28**: 267–281. doi:10.1016/j.celrep.2019.05.107
- Wang C, Zhu Y, Bao H, Jiang Y, Xu C, Wu J, Shi Y. 2016. A novel RNA-binding mode of the YTH domain reveals the mechanism for recognition of determinant of selective removal by Mmi1. *Nucleic Acids Res* **44**: 969–982. doi:10.1093/nar/gkv1382
- Xie G, Vo TV, Thillainadesan G, Holla S, Zhang B, Jiang Y, Lv M, Xu Z, Wang C, Balachandran V, et al. 2019. A conserved dimer interface connects ERH and YTH family proteins to promote gene silencing. *Nature Commun* **10**: 251. doi:10.1038/s41467-018-08273-9
- Yamashita A, Takayama T, Iwata R, Yamamoto M. 2013. A novel factor Iss10 regulates Mmi1-mediated selective elimination of meiotic transcripts. *Nucleic Acids Res* **41**: 9680–9687. doi:10.1093/nar/gkt763

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RNA 2020 26: 1334-1344 originally published online June 16, 2020
Access the most recent version at doi:[10.1261/rna.076463.120](https://doi.org/10.1261/rna.076463.120)

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