FEBS Letters 582 (2008) 497–502

The dosage of chromatin proteins affects transcriptional silencing and DNA repair in *Saccharomyces cerevisiae*

Sarah Z. Benbow, Michelle L. DuBois*

Seattle University, Department of Biology, 901 12th Avenue, Seattle, WA 98122, USA

Received 14 November 2007; revised 8 January 2008; accepted 12 January 2008

Available online 22 January 2008

Edited by Horst Feldmann

Abstract **Alterations in protein composition or dosage within chromatin may trigger changes in processes such as gene expression and DNA repair. Through transposon mutagenesis and targeted gene deletions in haploids and diploids of *Saccharomyces cerevisiae*, we identified mutations that affect telomeric silencing in genes encoding telomere-associated Sir4p and Yku80p and chromatin remodeling ATPases Ies2p and Rsc1p. We found that *sir4/SIR4* heterozygous diploids efficiently silence the mating type locus *HMR* but not telomeres, and diploids heterozygous for *yku80* and *ies2* mutations are inefficient at DNA repair. In contrast, strains heterozygous for most chromatin remodeling ATPase mutations retain wild-type silencing and DNA repair levels. Thus, in diploids, chromatin structures required for DNA repair and telomeric silencing are sensitive to dosage changes.**

Keywords: Telomere; Silencing; Chromatin remodeling; DNA repair; *Saccharomyces cerevisiae*

1. Introduction

A cell frequently modifies the composition and organization of its chromatin, and these changes may affect the accessibility to and expression of specific chromosomal regions. Many factors are involved in chromatin maintenance, including chromatin remodeling complexes and DNA repair factors [1,2]. Chromatin remodeling complexes interact directly with nucleosomes to shift their positions along the DNA and control gene accessibility. These complexes are typically composed of several proteins, including a catalytic subunit that possesses ATPase activity; particular non-catalytic subunits may be used in multiple remodeling complexes. These complexes must be reorganized after DNA is damaged to permit and assist the DNA repair machinery to recognize and repair the damage. Because types of DNA damage vary, DNA repair proceeds via several mechanisms, but chromatin changes are an inherent part of the repair process [3,4].

Additional variations in chromatin structure are found within heterochromatic regions of the nucleus. Heterochromatic regions, which exhibit little transcriptional activity, may cause the transcriptional silencing of genes in adjacent euchromatin, an effect termed position-effect variegation (PEV). The study of such regions in *Drosophila* has revealed that epigenetic inheritance of various chromatin components affects PEV. PEV also depends on a precise gene dosage of different chromatin factors; while two chromosomal copies of each chromatin factor results in wild-type PEV, one fewer or one more copy of some factors may suppress or enhance the effect [5,6]. This dosage dependence reflects the importance of the stoichiometry of the chromatin factors in gene expression.

Heterochromatic regions also include telomeres, the ends of linear chromosomes. Maintenance of telomeric chromatin structure requires a different complement of proteins than most genomic regions because chromosome ends must be differentiated from broken DNA ends and protected from nucleases and recombination events [7,8]. In *Saccharomyces cerevisiae*, telomeric structures are maintained by interactions among many factors, including Sir2p deacetylase, Sir3p and Sir4p, which bind DNA and interact with histones and other proteins near telomeres and the heterochromatic mating-type loci *HML* and *HMR*; Cdc13p and the Ku heterodimer, which bind the chromosome end; and Rap1p, which binds the telomeric DNA repeat [7–10]. The gene dosages of these proteins dramatically affect the heterochromatic structure that results in telomeric PEV [10,11]. For example, overexpression of *SIR4* also decreases telomeric silencing, but overexpression of *SIR2* or *SIR3* increases telomeric silencing. In contrast, deletion of *SIR2*, *SIR3* or *SIR4* reduces silencing of telomere-adjacent genes, as well as *HML* and *HMR*.

Because the chromatin structures that create PEV are sensitive to gene dosage, an investigation of its dosage dependence should reveal additional mechanisms required to maintain and modify these chromatin structures. Changing the dosages of chromatin proteins may affect their nuclear distributions, localizations, and availabilities of binding partners, thus altering the chromatin structure and transcriptional activity of nearby genes. In these experiments, we further explored effects of gene dosage on transcriptional silencing and DNA repair in *S. cerevisiae*.

2. Materials and methods

2.1. Yeast strains and growth conditions

S. cerevisiae media [10] and protocols for molecular genetic manipulations of yeast are described at the website, http://www.fhcrc.org/science/labs/gottschling/index.html. All yeast strains herein are derived from UCC3505 [12], UCC2225, YDW126 [13], and SUB593 (Table 1). Yeast strains were mutagenized by either transposon insertion (Tn) or PCR-mediated gene knockouts [14] of the entire open reading frame (Δ) with either the *kanMX* [14] or *hph* [15] gene; for detailed strain genotypes, refer to the Supplementary Material. For silencing

*Corresponding author. Fax: +1 206 296 5634.
E-mail address: mdubois@seattleu.edu (M.L. DuBois).

0014-5793/$32.00
doi:10.1016/j.febslet.2008.01.011

Table 1
Strain genotypes

Strain	Genotype	Source/parents
UCC3505	*MATa ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52 ppr1::HIS3 adh4::URA3-TEL-VIIL ADE2-TEL-VR*	Gottschling [12]
UCC2225	*MATa/MATα ade2-101/ade2-101 his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 lys2-801/lys2-801 trp1Δ63/TRP1+ ura3-52/ura3-52 ppr1::HIS3/ppr1::LYS2 adh4::URA3-TEL-VIIL/adh4::URA3-TEL-VIIL ADE2-TEL-VR/ADE2-TEL-VR*	UCC3505 × UCC3504 [12]
YDW126	*MATαade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 hmrΔA::TRP1*	Shore [13]
SUB593	*MATa/MATα ade2-101/ade2-1 his3-Δ200/his3-11 leu2-Δ1/ leu2-3,112 lys2-801/LYS2 trp1-Δ63/trp1-1 ura3-52/ura3-1 ppr1::HIS3/PPR1 adh4::URA3-TEL-VIIL/ADH4 ADE2-TEL-VR/VR*	UCC3505 × YDW126

assays, 10-fold serial dilutions of strains were spotted on synthetic complete plates (YC), YC plates lacking uracil or tryptophan, or YC plates containing 1 mg/ml 5′-fluoroorotic acid (FOA, US Biological), and grown at 30 °C for three days unless otherwise indicated. As a test of DNA repair, fivefold serial dilutions of strains were plated on YEPD and YEPD with 0.014% methyl methanesulfonate (MMS; Sigma–Aldrich) or 150 mM hydroxyurea (HU; Sigma–Aldrich); colonies grew at 30 °C on YEPD and YEPD + MMS for two days and on YEPD + HU for three days.

2.2. Genetic screen

UCC3505 and UCC2225 were mutagenized with transposon-containing genomic DNA plasmid libraries [16], a kind gift from M. Snyder. The libraries were mutagenized by the insertion of the mTn transposon that encodes the *lacZ*, *LEU2*, and β-lactamase genes [17]. NotI-digested library DNA was transformed into yeast, and transposon-containing transformants grew on YC lacking leucine plates. Approximately 23 000 transformants of UCC3505 and 88 000 transformants of UCC2225 were screened. Colonies were replica-plated onto YC medium lacking leucine and uracil, and white Ura$^+$ colonies were selected. Haploid isolates were mated and sporulated to observe segregation of *LEU2* and link the phenotype to the transposon, and diploid isolates were sporulated to examine haploid phenotypes. Transposon insertion locations were identified by directly sequencing inverse PCR products [18]; also see Supplementary Material.

3. Results and discussion

3.1. Screen for dosage effects

In an effort to identify genes whose dosages affect telomeric chromatin structure and organization, we performed a genetic screen with a diploid *S. cerevisiae* strain. If a diploid cell displays a mutant phenotype and the mutation affects only a single copy of the gene, the cell may be limiting for that gene product because the remaining wild-type copy cannot rescue the phenotype. Our screen was for mutations that resulted in the inability to transcriptionally silence two telomere-adjacent reporter genes. The isolation of such mutations would identify genes whose dosages are critical for telomeric silencing and perhaps other chromatin functions as well.

The telomere-adjacent reporter genes of *ADE2* (chromosome V, right end) and *URA3* (chromosome VII, left end) are commonly used for telomeric silencing assays [10]. In wild-type strains, these two telomeric loci are silenced, resulting in pinkish Ura$^-$ colonies. The pinkish appearance results from the combination of red Ade$^-$ cells (from a transcriptionally silenced *ADE2* gene) with small sectors of white Ade$^+$ cells that result from the epigenetic loss of silencing. The colony does not grow well on uracil-lacking medium because the telomeric *URA3* gene is transcriptionally silenced. In contrast, when a mutation inactivates transcriptional silencing of both the *ADE2-VR* and *URA3-VIIL* telomeric loci and renders both genes transcriptionally active, colonies are white and Ura$^+$.

In our genetic screen, we mutagenized two telomere-marked strains, the diploid UCC2225 and the haploid UCC3505 (for comparison), with transposon-containing genomic DNA libraries ([16], Table 1). Because the transposon insertion sites were random, insertions often disrupted genes and created truncation mutations. We screened for insertions that led to white Ura$^+$ colonies, which should contain mutations in genes that affect telomeric silencing, and mutations in the diploid should reveal genes that are limiting for telomeric silencing.

3.2. Dosage requirements for silencing

Some transposon insertion (Tn) mutations were isolated in both screens because they resulted in similar phenotypes in haploid and diploid strains. All mutations were characterized in both haploids and diploids. Because we were interested in dosage effects and transposon insertion can yield an allele other than a loss of function, we also compared the phenotype of each Tn mutation with the null in a haploid strain and the heterozygous null in a diploid strain.

In the screens, we isolated several mutations in *SIR4*, *SIR2*, and *YKU80*; representative mutations in *SIR4* and *YKU80* are shown (Fig. 1). The roles of these factors in telomeric silencing have been well described [8–11], and in haploids, the phenotypes of the Tn and the null mutations were essentially identical (Fig. 1A). Phenotypes were evaluated by comparing the growth of strains on media with and without uracil, and medium containing 5′-fluoroorotic acid (FOA, which is toxic to Ura$^+$ cells [10]). A wild-type colony that silences both its *ADE2* and *URA3* genes is pink-to-red, FOA$^+$, and Ura$^-$ (Fig. 1A). In contrast, both null and Tn mutations in *SIR4* and *YKU80* in haploids resulted in a loss of telomeric silencing at both loci, resulting in white, FOA$^-$, Ura$^+$ colonies. In heterozygous diploids (Fig. 1B), *yku80* mutations were recessive: one copy of *YKU80* restored the cells to wild-type levels of telomeric silencing. However, a single wild-type copy of *SIR4* was insufficient to restore silencing in the diploid; this dosage-dependent phenotype also was observed for null and

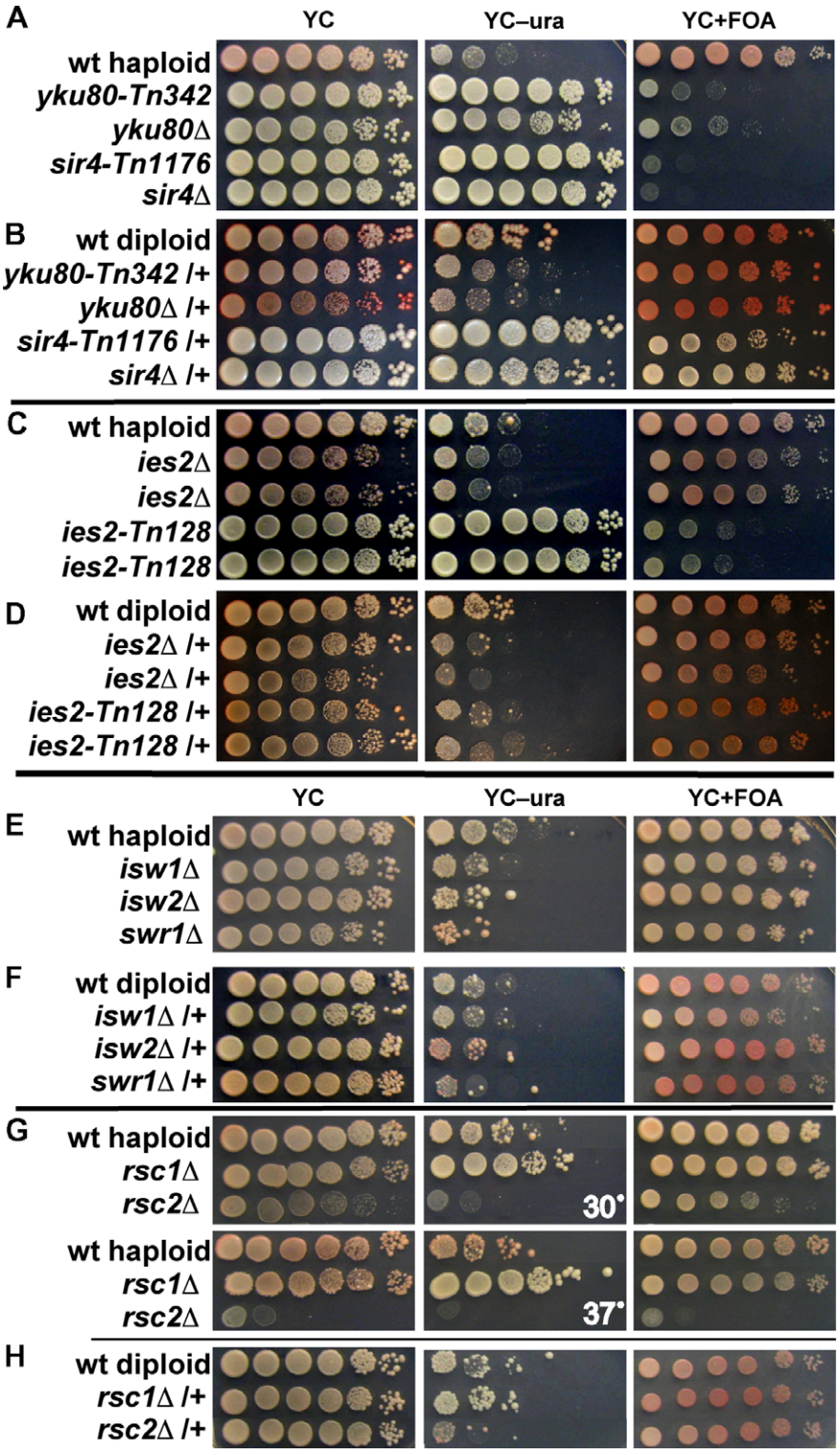

Fig. 1. Effects of transposon insertion mutations and gene dosage on telomeric silencing. Haploid and heterozygous diploid strains, derived from UCC3505 and UCC2225, respectively, were plated in 10-fold serial dilutions and grown at 30 °C (or 37 °C as shown) on indicated media. The wild-type (wt) haploid is UCC3505, and the wt diploid is UCC2225. All diploids are heterozygous for the mutations. Mutations are either null mutations (Δ) or transposon insertion mutations (Tn), and the site of transposon insertion is indicated in the mutation name (i.e. Tn342 at aa 342 of the open reading frame). The wild-type lengths of these proteins are: Yku80p = 629aa, Sir4p = 1358aa, Ies2p = 320aa. Two independent isolates with each *ies2* mutation are shown.

Tn mutations of *SIR2* (data not shown). Growth on both FOA-containing medium and medium lacking uracil indicates that the single copy dosage of Sir4p is sufficient to create silent chromatin in the diploid, but the decreased Sir4p (or Sir2p)

dosage may destabilize the structure, which increases accessibility to transcriptional machinery.

Another mutation isolated in the screen was *ies2-Tn128*, a mutation in the Ies2p subunit of the INO80 chromatin remodeling complex [2,19]. Although the Tn allele resulted in a dramatic decrease in telomeric silencing in the haploid, the null allele maintained wild-type silencing (Fig. 1C), and both mutations exhibited wild-type levels of silencing in diploids (Fig. 1D), indicating that the mutations are recessive. The difference between the null and Tn alleles is intriguing and may result from an effect of the truncation, which could titrate or interfere with an essential component for silencing, and the direct (or indirect) nature of these interactions is unknown.

The effects of these mutations on the silencing of the mating-type loci also were examined. We tested the ability of each mutant strain to silence a compromised *HMR* locus [13], which is a more sensitive assay than direct mating. These strains contain the locus *hmrΔA::TRP1*, in which the E silencer of *HMR* is deleted and the *a1* gene is replaced by the *TRP1* gene. Strong silencing was observed in the haploids for wild-type strains and mutations in *YKU80* and *IES2*, while the mutation in a *SIR4* haploid eliminated silencing (Fig. 2A), consistent with the telomere-specific silencing roles of Ku and Ies2p. In diploids, single copies of *YKU80*, *IES2*, and *SIR4* provided a sufficient dosage to restore silencing, suggesting that less Sir4p is required to maintain silencing of the mating-type loci than telomeres and telomeric heterochromatin is more sensitive than *HMR* to the dosage of Sir4p.

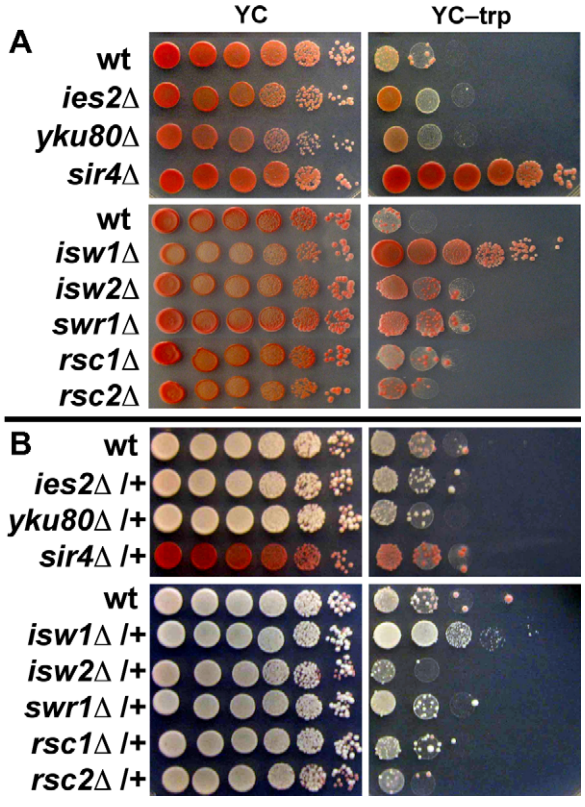

Fig. 2. The silencing of *hmr*ΔA in haploids and diploids. Tenfold serial dilutions of the indicated strains were grown at 30 °C on indicated media. (A) Haploid yeast strains with the indicated mutations were derived from YDW126 (wt haploid). (B) Diploid yeast strains were created from the mating of the haploid strains in Fig. 1 with YDW126; the wt diploid is SUB593.

3.3. Chromatin remodeling factor roles in silencing

Because the *IES2* mutation identified in our screen suggested a link between chromatin remodeling factors and telomeric silencing, we also investigated the dosage requirements for other chromatin remodeling complexes on silencing. Although telomeric DNA does not have standard nucleosomal patterning that is affected by chromatin remodeling complexes [7], the remodeling may affect subtelomeric regions. The INO80 chromatin remodeling complex has been implicated in the response to DNA damage [1] and the regulation of telomere structure [20], consistent with a telomeric role for Ies2p. Among other chromatin remodeling ATPases, Isw1p does not affect telomeric silencing but does have a role at the rDNA and *HMR* [21,22], Isw2p plays a role in epigenetic effects at the telomere [23], Rsc1p plays a role at *HMR* [24], and mutations in *RSC2* result in short telomeres, temperature sensitivity, and haploinsufficiency for sporulation [25–27].

We created null mutations of five ATP-dependent remodeling ATPases: *ISW1*, *ISW2*, *SWR1*, *RSC1*, and *RSC2* (Deletions of the *CHD1*, *INO80*, and *SNF2* genes were unstable in our genetic background.). Null mutations in *ISW1*, *ISW2*, *SWR1*, and *RSC2* exhibited wild-type levels of silencing in haploids (Fig. 1E and G) and in heterozygous diploids (Fig. 1F and H). The instability and temperature sensitivity of the *rsc2*Δ haploid strain also was observed (Fig. 1G), though the effect is recessive (Fig. 1H). In contrast, we observed a decrease in telomeric silencing in *rsc1*Δ haploid strains (Fig. 1G) that is recessive (Fig. 1H). The growth on both FOA-containing and uracil-lacking media in *rsc1*Δ strains is similar to the phenotype in the *sir4* heterozygotes. Rsc1p may normally remodel subtelomeric regions, and a reduced Rsc1p dosage may destabilize or improperly position nucleosomal and silencing structures, resulting in chromatin that is more accessible to transcriptional machinery.

At the *HMR* locus, a mutation in *ISW1* reduces silencing (Fig. 2A) as previously described [21], and Isw1p may be limiting, as silencing is not completely restored in the heterozygous diploid (Fig. 2B). Also, a minor decrease in silencing was observed for mutations in *ISW2* and *SWR1*, but these mutations do not affect silencing of *HMR* in a heterozygous diploid (Fig. 2A and B). Deletions of the *RSC1* and *RSC2* genes had no effect on silencing of *HMR* (Fig. 2A and B).

3.4. Dosage requirements for DNA repair

A phenotype common among mutant chromatin proteins is sensitivity to DNA-damaging agents, suggesting a role for these proteins in DNA repair. Haploid strains with mutations in *IES2*, *ISW1*, *RSC1*, *RSC2*, *SWR1*, and *YKU80* have been observed to display decreased growth in the presence of either methyl methonate sulfate (MMS), hydroxyurea (HU), or other DNA-damaging agents [19,28–31]. The dosage dependence of the sensitivity was tested with the same set of haploid and diploid strains (Fig. 3). As expected from its key role in DNA repair, both haploid and heterozygous diploid strains with mutations in *YKU80* show decreased viability in the presence of MMS and HU (Fig. 3A and B). The haploid *sir4*Δ strain also was sensitive to the agents, but the heterozygous diploid grew efficiently. The *sir4-Tn1116* haploid grew well and thus

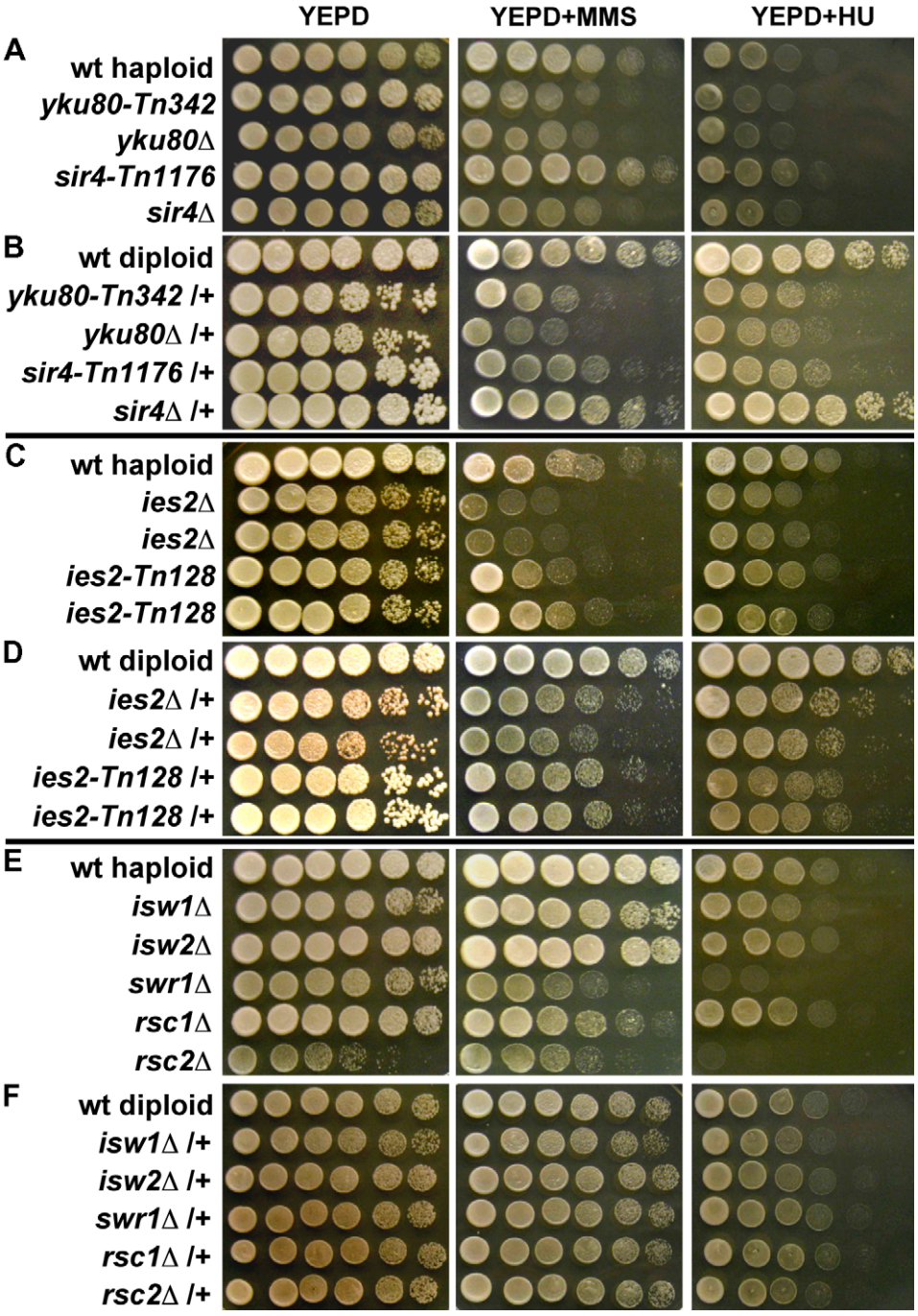

Fig. 3. Effects of gene dosage on MMS and HU sensitivity. Fivefold serial dilutions of the strains used in Fig. 1 were grown at 30 °C on indicated media.

appeared to repair like wild-type, but the heterozygous diploid had decreased growth; these effects may be due to a dominant negative effect of the truncated *sir4-Tn1116* protein in the diploid. The haploid chromatin remodeling ATPase mutant strains that were most sensitive were *rsc1*Δ and *swr1*Δ (Fig. 3E), and diploid strains with these mutations grew like wild-type (Fig. 3F), suggesting the diploids can repair DNA efficiently and the mutations are recessive. However, all strains with a mutation in *IES2* exhibited decreased viability on both MMS and HU, and though diploid strains were healthier than haploids, diploids did not grow as well as wild-type (Fig. 3C and D). Thus, although a single-copy dosage of each ATPase was sufficient for repair in diploids, the Ies2p subunit seems to be required for optimal DNA repair in response to damage. Whether the Ies2p subunit or the entire INO80 complex is limiting is not known, but these data provide additional evidence for the importance of the INO80 complex in DNA damage response [1].

We have investigated the dosage requirements for some chromatin factors that play roles in silencing and chromatin remodeling. The regulation of gene dosage plays an important role in many cellular processes, from rDNA expression to

X-chromosome inactivation [32], and in many cases, the processes require the activities of multisubunit complexes and structures. To maintain wild-type activity, the stoichiometry of the subunits also must be maintained. Within the chromatin structures of *S. cerevisiae*, most of these factors are not limiting for silencing of telomeric genes nor *HMR* nor DNA repair in diploids, which may indicate that cells require only a low level of these proteins to function in chromatin, or that other proteins can compensate adequately for the decrease (but not absence) of the particular protein, or that expression of the single gene copy is upregulated. In contrast, diploids heterozygous for mutations in *YKU80*, *SIR4*, and *IES2* exhibited mutant phenotypes, suggesting that these factors are limiting in some silencing or DNA repair roles. These data indicate that a sensitive balance among chromatin factors is required to maintain the optimal structures involved in silent chromatin and DNA repair. An understanding of the dosage requirements of individual factors in chromatin will provide mechanistic insight regarding chromatin regulation and dynamics.

Acknowledgements: We thank Michael Snyder for the transposon libraries, Dan Gottschling and David Shore for yeast strains, and Glenn Yasuda, John Doedens, Brenda Bourns, and Toshi Tsukiyama for helpful discussions and comments. This work was supported by grants to M.L.D. from the M.J. Murdock College Research Program for Life Sciences.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2008.01.011.

References

[1] Osley, M.A., Tsukuda, T. and Nickoloff, J.A. (2007) ATP-dependent chromatin remodeling factors and DNA damage repair. Mutat. Res. 618, 65–80.
[2] Saha, A., Wittmeyer, J. and Cairns, B.R. (2006) Chromatin remodelling: the industrial revolution of DNA around histones. Nat. Rev. Mol. Cell Biol. 7, 437–447.
[3] Costelloe, T., FitzGerald, J., Murphy, N.J., Flaus, A. and Lowndes, N.F. (2006) Chromatin modulation and the DNA damage response. Exp. Cell Res. 312, 2677–2686.
[4] Su, T.T. (2006) Cellular responses to DNA damage: one signal, multiple choices. Ann. Rev. Genet. 40, 187–208.
[5] Ebert, A., Lein, S., Schotta, G. and Reuter, G. (2006) Histone modification and the control of heterochromatic gene silencing in *Drosophila*. Chromosome Res. 14, 377–392.
[6] Schotta, G., Ebert, A., Dorn, R. and Reuter, G. (2003) Position-effect variegation and the genetic dissection of chromatin regulation in *Drosophila*. Semin. Cell Develop. Biol. 14, 67–75.
[7] Blackburn, E.H. and Greider, C.W. (1995) Telomeres, Cold Spring Harbor Laboratory Press, Plainview, NY.
[8] Gilson, E. and Geli, V. (2007) How telomeres are replicated. Nat. Rev. Mol. Cell Biol. 8, 825–838.
[9] Teixeira, M.T. and Gilson, E. (2005) Telomere maintenance, function and evolution: the yeast paradigm. Chromosome Res. 13, 535–548.
[10] van Leeuwen, F. and Gottschling, D.E. (2002) Assays for gene silencing in yeast. Methods Enzymol. 350, 165–186.
[11] Gasser, S.M. and Cockell, M. (2001) The molecular biology of SIR proteins. Gene 279, 1–16.
[12] Singer, M.S. and Gottschling, D.E. (1994) TLC1: template RNA component of *Saccharomyces cerevisiae* telomerase. Science 266, 404–409.
[13] Wotton, D. and Shore, D. (1997) A novel Rap1p-interacting factor, Rif2p, cooperates with Rif1p to regulate telomere length in *Saccharomyces cerevisiae*. Genes Dev. 11, 748–760.
[14] Brachmann, C.B., Davies, A., Cost, G.J., Caputo, E., Li, J., Hieter, P. and Boeke, J.D. (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast 14, 115–132.
[15] Goldstein, A.L. and McCusker, J.H. (1999) Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. Yeast 15, 1541–1553.
[16] Burns, N., Grimwade, B., Ross-Macdonald, P.B., Choi, E.Y., Finberg, K., Roeder, G.S. and Snyder, M. (1994) Large-scale analysis of gene expression, protein localization, and gene disruption in *Saccharomyces cerevisiae*. Genes Dev. 8, 1087–1105.
[17] Siefert, H., Chen, E., So, M. and Heffron, F. (1986) Shuttle mutagenesis: a method of transposon mutagenesis for *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 83, 735–739.
[18] Ochman, H., Gerber, A. and Hartl, D.L. (1988) Genetic applications of an inverse polymerase chain reaction. Genetics 120, 621–623.
[19] Mizuguchi, G., Shen, X., Landry, J., Wu, W.H., Sen, S. and Wu, C. (2004) ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. Science 303, 343–348.
[20] Yu, E.Y., Steinberg-Neifach, O., Dandjinou, A.T., Kang, F., Morrison, A.J., Shen, X. and Lue, N.F. (2007) Regulation of telomere structure and functions by subunits of the INO80 chromatin remodeling complex. Mol. Cell Biol. 27, 5639–5649.
[21] Cuperus, G. and Shore, D. (2002) Restoration of silencing in *Saccharomyces cerevisiae* by tethering of a novel Sir2-interacting protein, Esc8. Genetics 162, 633–645.
[22] Mueller, J.E., Li, C. and Bryk, M. (2007) Isw2 regulates gene silencing at the ribosomal DNA locus in *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. 361, 1017–1021.
[23] Iida, T. and Araki, H. (2004) Noncompetitive counteractions of DNA polymerase epsilon and ISW2/yCHRAC for epigenetic inheritance of telomere position effect in *Saccharomyces cerevisiae*. Mol. Cell Biol. 24, 217–227.
[24] Jambunathan, N., Martinez, A.W., Robert, E.C., Agochukwu, N.B., Ibos, M.E., Dugas, S.L. and Donze, D. (2005) Multiple bromodomain genes are involved in restricting the spread of heterochromatic silencing at the *Saccharomyces cerevisiae HMR*-tRNA boundary. Genetics 171, 913–922.
[25] Askree, S.H. et al. (2004) A genome-wide screen for *Saccharomyces cerevisiae* deletion mutants that affect telomere length. Proc. Natl. Acad. Sci. USA 101, 8658–8663.
[26] Baetz, K.K., Krogan, N.J., Emili, A., Greenblatt, J. and Hieter, P. (2004) The ctf13-30/CTF13 genomic haploinsufficiency modifier screen identifies the yeast chromatin remodeling complex RSC, which is required for the establishment of sister chromatid cohesion. Mol. Cell Biol. 24, 1232–1244.
[27] Bungard, D., Reed, M. and Winter, E. (2004) RSC1 and RSC2 are required for expression of mid-late sporulation-specific genes in *Saccharomyces cerevisiae*. Eukaryot. Cell 3, 910–918.
[28] Begley, T.J., Rosenbach, A.S., Ideker, T. and Samson, L.D. (2002) Damage recovery pathways in *Saccharomyces cerevisiae* revealed by genomic phenotyping and interactome mapping. Mol. Cancer Res. 1, 103–112.
[29] Bennett, C.B., Lewis, L.K., Karthikeyan, G., Lobachev, K.S., Jin, Y.H., Sterling, J.F., Snipe, J.R. and Resnick, M.A. (2001) Genes required for ionizing radiation resistance in yeast. Nat. Genet. 29, 426–434.
[30] Rieger, K.J., El-Alama, M., Stein, G., Bradshaw, C., Slonimski, P.P. and Maundrell, K. (1999) Chemotyping of yeast mutants using robotics. Yeast 15, 973–986.
[31] Boulton, S.J. and Jackson, S.P. (1996) Identification of a *Saccharomyces cerevisiae* Ku80 homologue: roles in DNA double strand break rejoining and in telomeric maintenance. Nucleic Acids Res. 24, 4639–4648.
[32] Birchler, J.A., Yao, H. and Chudalayandi, S. (2007) Biological consequences of dosage dependent gene regulatory systems. Biochim. Biophys. Acta 1769, 422–428.