Correspondence

Ctf7p/Eco1p exhibits acetyltransferase activity — but does it matter?

Alex Brands and Robert V. Skibbens*

In eukaryotic cells, faithful chromosome segregation depends upon the physical pairing, or cohesion, between sister chromatids. Budding yeast *CTF7/ECO1* (herein termed *CTF7*) encodes an essential protein required to establish cohesion during S-phase and associates with DNA replication factors [1–10]. However, the molecular mechanism by which Ctf7p establishes cohesion remains unknown. *In vitro* characterization of Ctf7p as an acetyltransferase led to the model that this activity provides for Ctf7p's essential function [11]. However, *in vivo* Ctf7p substrates have yet to be documented, nor has an *in vivo* acetyltransferase activity been demonstrated even when Ctf7p is overexpressed [11] (A. Brands and R.V. Skibbens, unpublished data). In fact, the effects of acetylation-defective Ctf7p (*ctf7^{ack-}*) in yeast remain to be rigorously tested, leaving unanswered the critical questions of whether Ctf7p acetyltransferase activity is essential for cell viability and to what extent this activity is required for the establishment of cohesion. Here, we show that yeast strains harboring acetyltransferase-defective alleles [11] as the sole source of Ctf7p function exhibit robust growth and high fidelity chromosome transmission.

We first used a plasmid loss assay to test whether *ctf7^{ack-}* alleles could support cell viability. *CEN TRP1* plasmids containing wild-type *CTF7* or *ctf7^{ack-}* alleles exhibiting abrogated or greatly diminished acetyltransferase activity *in vitro* [11] were transformed into an *ade2;ade3* yeast assay strain in which the sole source of Ctf7p function was provided by a *CEN-ADE3-CTF7* plasmid. After growth on medium selective for both plasmids, transformants were placed on rich non-selecting medium to allow for random plasmid loss. Cells transformed with *CEN-TRP1-CTF7* exhibited *CEN-ADE3-CTF7* plasmid loss — an event easily detected by white sectors in an otherwise red colony [12]. Cells transformed with vector alone produced solid red colonies, confirming that the *CEN-CTF7-ADE3* plasmid was required in these cells. Importantly, three of the four *ctf7^{ack-}* alleles produced white-sectored colonies identical to cells transformed with wild-type *CTF7* (Figure S1, Supplemental data). A fourth allele produced thin and infrequent white sectored colonies, but was competent to perform the essential function of Ctf7p. To test whether the resulting *ctf7^{ack-}* strains might exhibit conditional growth phenotypes, serial dilutions were spotted onto non-selective rich medium plates and incubated at 23°C, 30°C or 37°C. Three of the *ctf7^{ack-}* mutant strains exhibited wild-type growth at all temperatures tested (Figure S2, Supplemental data). Plasmid rescue and DNA sequencing confirmed that the *ctf7^{ack-}* alleles (tested for R222G/K223G) provided the sole source of Ctf7p function. In summary, these findings reveal that *ctf7^{ack-}* alleles are competent to provide for the essential function of Ctf7p *in vivo*. Cells containing the G211D *ctf7* allele exhibited conditional growth defects, suggesting that this mutation may exhibit phenotypes beyond that associated with loss of acetyltransferase activity.

We next tested whether *ctf7^{ack-}* alleles, integrated at single copy, were competent to maintain both cell viability and high fidelity chromosome transmission. Using established gene replacement methods, the wild-type *CTF7* coding sequence was replaced with single copy integrated *ctf7^{ack-}* alleles controlled by the endogenous *CTF7* promoter [13]. The ability to obtain numerous healthy *ctf7^{ack-}* transformants further indicates that the acetyltransferase activity of Ctf7p may not be necessary for sister chromatid cohesion – a process that is essential for viability. For controls, we included wild-type *CTF7* and two alleles (*ctf7-108* and *ctf7-109*) obtained from the original chromosome transmission fidelity collection [14]. Gene replacement was performed in a sectoring yeast assay strain in which the phenotypic red colony color caused by an *ade2* mutation is suppressed by a non-essential *TRP1 SUP11* chromosome fragment [14]. Transformants were placed on rich medium to allow for loss of the *TRP1-SUP11* chromosome fragment, an event scored as red sectors in a white colony background. *CTF7* cells exhibited high fidelity transmission of the chromosome fragment, evidenced by solid white colonies. As expected, *ctf7-108* cells (and *ctf7-109*, data not shown) exhibited low fidelity transmission to produce highly red-sectored colonies. Importantly, all three *ctf7^{ack-}* strains tested produced solid white colonies indistinguishable from those produced by *CTF7* cells (Figure 1). These results reveal that *ctf7^{ack-}* mutant strains exhibit both robust cell viability and high fidelity chromosome transmission.

Figure 1. The acetyltransferase activity of CTF7 is not required for viability.
Yeast cells harboring either wild-type *CTF7* (WT) or *ctf7^{ack-}* alleles (R222G/K223G, G225D or D232G) as the sole source of *CTF7* function retain the non-essential *TRP1 SUP11* chromosome fragment and produce non-sectored white colonies. Cells harboring *ctf7-108* (S108) or *ctf7-109* (not shown) alleles exhibit dramatic chromosome fragment loss and produce red-sectored colonies.

ctf7-108 and *ctf7-109* mutant strains both exhibit strong chromosome loss phenotypes [14]. We sequenced these alleles and found that *ctf7-108* and *ctf7-109* each contain single mutations in the putative zinc finger domain of Ctf7p [11] — outside of the acetyltransferase domain (Figure 2). Biochemical analyses of *ctf7-108* expressed in bacteria reveal that this allele exhibits acetyltransferase activity *in vitro* (data not shown). Thus, a mutation in the zinc finger domain of Ctf7p is sufficient to produce chromosome missegregation *in vivo*.

As the detection of Ctf7p acetyltransferase activity *in vivo* has failed so far, it formally remains possible that mutants defective for acetyltransferase activity *in vitro* may still retain residual activity *in vivo*. The importance of addressing this issue is underscored by the recent identifications of human (EFO1) and *Drosophila* (DECO) Ctf7p orthologs [15,16]. EFO1 exhibits acetyltransferase activity *in vivo*, revealing that this activity is conserved through evolution. In flies, *deco* mutants exhibit precocious sister chromatid separation. However, the acetyltransferase activity of these alleles has not been tested. Thus, the role in cell viability for the acetyltransferase activity of Ctf7p orthologs remains an open question.

Supplemental data

Supplemental data showing details of the viability assays are available at http://www.current-biology.com/cgi/content/full/15/2/R50/DC1/

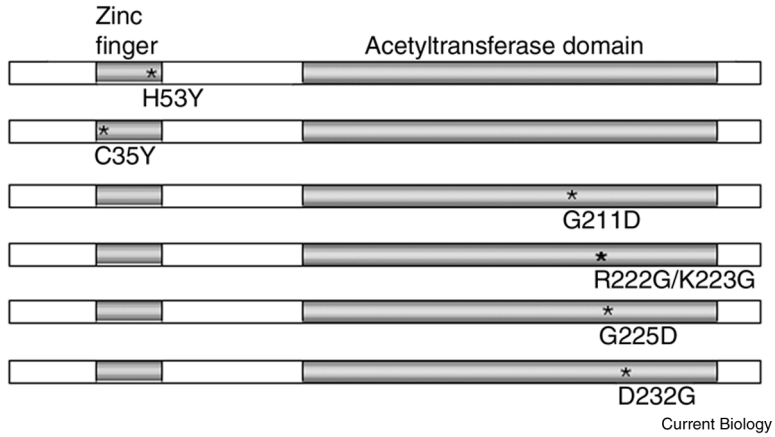

Figure 2. Sequence analyses comparing *ctf7* alleles.
Comparison of mutations of chromosome loss *ctf7* alleles to previously published sequence of *ctf7^{ack-}* alleles [10]. Single mutations in the zinc finger domain (H53Y = *ctf7-108*; C35Y = *ctf7-109*) are sufficient to produce massive chromosome missegregation.

References

1. Skibbens, R.V., Corson, L.B., Koshland, D., and Hieter, P. (1999). Ctf7p is essential for sister chromatid cohesion and links mitotic chromosome structure to the DNA replication machinery. Genes Dev. *13*, 307–319.
2. Toth, A., Ciosk, R., Uhlmann, F., Galova, M., Schleiffer, A., and Nasmyth, K. (1999). Yeast cohesin complex requires a conserved protein, Eco1p(Ctf7), to establish cohesion between sister chromatids during DNA replication. Genes Dev. *13*, 320–333.
3. Mayer, M.L., Gygi, S.P., Aebersold, R., and Hieter, P. (2001). Identification of RFC (Ctf18p, Ctf8p, Dcc1p): An alternate RFC complex required for sister chromatid cohesion in S. cerevisiae. Mol. Cell *7*, 959–970.
4. Hanna, J.S., Kroll, E.S., Lundblad, V., and Spencer, F.A. (2001). Saccharomyces cerevisiae CTF18 and CTF4 are required for sister chromatid cohesion. Mol. Cell. Biol. *21*, 3144–3158.
5. Wang, Z., Castano, I.B., De Las Penas, A., Adams, C., and Christman, M.F. (2000). Pol kappa: A DNA polymerase required for sister chromatid cohesion. Science *289*, 774–779.
6. Krause, S.A., Loupart, M.L., Vass, S., Schoenfelder, S., Harrison, S., and Heck, M.M. (2001). Loss of cell cycle checkpoint control in Drosophila Rfc4 mutants. Mol. Cell. Biol. *21*, 5156–5168.
7. Kenna, M.A., and Skibbens, R.V. (2003). Mechanical link between cohesion establishment and DNA replication: Ctf7p/Eco1p, a cohesion establishment factor, associates with three different Replication Factor C Complexes. Mol. Cell. Biol. *23*, 2999–3007.
8. Edwards, S., Li, C.M., Levy, D.L., Brown, J., Snow, P.M., and Campbell, J. (2003). Saccharomyces cerevisiae DNA polymerase varepsilon and polymerase sigma interact physically and functionally, suggesting a role for polymerase varepsilon in sister chromatid cohesion. Mol. Cell. Biol. *23*, 2733–2748.
9. Skibbens, R.V. (2004). Chl1p, a DNA helicase-like protein in budding yeast, functions in sister chromatid cohesion. Genetics *166*, 32–42.
10. Petronczki, M., Chwalla, B., Siomos, M., Yokobayashi, S., Helmhart. W., Deutschbauer, A., *et al.* (2004) Sister-chromatid cohesion mediated by the alternative RF-CCtf18/Dcc1/Ctf8, the helicase Chl1 and the polymerase-alpha-associated protein Ctf4 is essential for chromatid disjunction during meiosis II. J. Cell Sci. *117*, 3547–3559.
11. Ivanov, D., Schleiffer, A., Eisenhaber, F., Mechtler, K., Haering, C.H., and Nasmyth, K. (2002). Eco1 is a novel acetyltransferase that can acetylate proteins involved in cohesion. Curr. Biol. *12*, 323–328.
12. Koshland, D., Kent, J.C., and Hartwell, L.H. (1985). Genetic analysis of the mitotic transmission of minichromosomes. Cell *40*, 393–403.
13. 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 establishment of sister chromatid cohesion. Mol. Cell. Biol. *24*, 1232–1244.
14. Spencer, F., Gerring, S.L., Connelly, C., and Hieter, P. (1990). Mitotic chromosome transmission fidelity mutants in Saccharomyces cerevisiae. Genetics *124*, 237–249.
15. Bellows, A.M., Kenna, M.A., Cassimeris, L., and Skibbens, R.V. (2003). Human EFO1p exhibits acetyltransferase activity and is a unique combination of linker histone and Ctf7p/Eco1p chromatid cohesion establishment domains. Nucl. Acids Res. *31*, 6334–6343
16. Williams, B.C., Garrett-Engele, C.M., Li, Z., Williams, E.V., Rosenman, E.D., and Goldberg, M.L. (2003). Two putative acetyltransferases, san and deco, are required for establishing sister chromatid cohesion in Drosophila. Curr. Biol. *13*, 2025–2036.

Department of Biological Sciences, 111 Research Dr., Lehigh University, Bethlehem, Pennsylvania 18015, USA.
*E-mail: rvs3@lehigh.edu