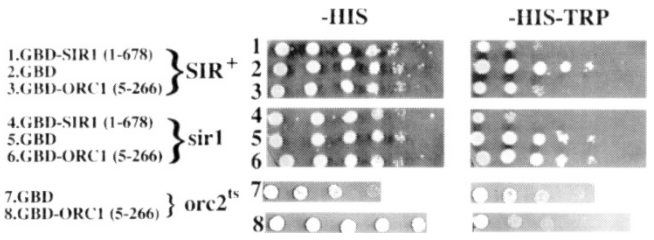

FIG. 3 Targeted silencing by GBD–ORC1 and GBD–SIR1. Rows 1–3 show results in YSB2, a *SIR*$^+$ strain (ref. 11), and rows 4–6 show results in RS1172, a *sir1* derivative of YSB2. The plasmid present in each strain is indicated (GBD, GAL4 DNA binding domain). Cell cultures were serially diluted (1/10) 6 times, and 3 μl of each dilution spotted from left (undiluted) to right (diluted to 1/10^6) on media lacking histidine (-HIS) to measure the total number of cells plated, and on media lacking histidine and tryptophan (-HIS-TRP) to measure the amount of silencing. The lack of growth seen on -HIS-TRP medium in rows 1, 3 and 4 is due to targeted silencing. Rows 7 and 8 show results in YJS10-5B, an *orc2-1*^{ts} derivative of YSB2, grown at 23 °C. Here the spots (5 μl) correspond to undiluted, 1/5, 1/10, 1/50 and 1/100 dilutions. The lack of growth on -HIS-TRP medium in row 8 signifies silencing by GBD–ORC1. Silencing by GBD–SIR1 could not be tested in the *orc2-1* mutant because SIR1 overexpression by this and other SIR1 plasmids was very toxic in the mutant. This is in marked contrast with the lack of any inhibitory effect of SIR1 overexpression in *ORC*$^+$ strains.

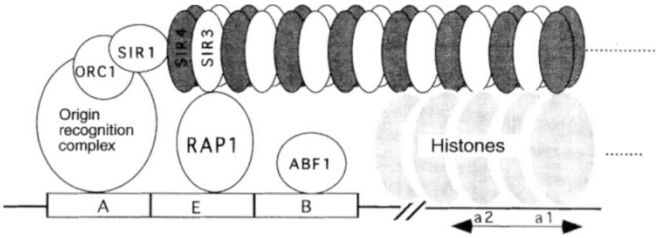

FIG. 4 A model for the establishment of silencing by SIR1 at the *HMR-E* silencer. The observed binding of SIR1 to ORC1 and SIR4 is indicated. Also shown is the binding of SIR3 to RAP1 (ref. 15), as well as the interactions of SIR3 and SIR4 with each other[13,15] and with H3 and H4 (ref. 16). The model proposes that the seeding of the SIR3–SIR4 array begins at the silencer and spreads to silence nearby genes. For simplicity the drawing shows the spreading to be unidirectional; actually it is likely to be bidirectional[18]. The roles of ABF1 and SIR2 in silencing are not known and are therefore ignored in the model.

functional ORC complex, we used an *orc2-1*^{ts} mutant. This mutant is temperature sensitive for growth and completely defective at silencing at the permissive temperature, as long as the *HMR-E* silencer is weakened by a deletion of its RAP1 binding site[3]. Surprisingly, ORC1 targeted silencing was totally unaffected by the *orc2-1* mutation (Fig. 3). Thus a small N-terminal domain of ORC1, when targeted to *HMR*, can establish silencing in the absence of a fully functional ORC. The silencing defects of *orc2* and *orc5* mutants indicate that ORC is important for HM silencing[3,6,7]. However, the ORC2 independence of ORC1 targeted silencing suggests that the presence of an intact ORC is not an absolute requirement; rather, the localization of SIR1 to the silencers by means of ORC1 is the crucial ORC-dependent event.

Previous data have suggested that efficient establishment of the silent state at the *HM* loci requires both SIR1 (refs 9–11) and passage through S phase[8]. Our data suggest a simple explanation, that the SIR1–ORC interaction is regulated by an S-phase-dependent modification or stabilization of one of these proteins.

We present a model for the initiation of silencing at the *HM* loci (Fig. 4). During S phase, SIR1 is recruited to the silencer by its binding to ORC1. We propose that this binding acts as a trigger to initiate the formation of silent chromatin. Whether SIR1 binds specifically to ORC at silencers or also to ORC bound at other chromosomal loci is not clear. The binding of SIR4 to SIR1 allows recruitment of that silencing protein. This brings SIR4 in close proximity to SIR3, which is bound by the neighbouring RAP1 (ref. 15; Fig. 4). As shown previously, SIR3 and SIR4 both homodimerize and heterodimerize with each other[13,15]. The recruitment of both SIR3 and SIR4 to the silencer acts to seed an array of SIR3/SIR4 complexes that spreads from the silencer. The interactions of SIR3 and SIR4 with the N-terminus tails of histones H3 and H4 (ref. 16) are likely to cause the compaction of nucleosomes into a heterochromatic state. □

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ADDENDUM

Structural evidence for a two-step process in the depinning of the superconducting flux-line lattice

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In this Letter, we provided structural evidence for a two-step depinning process of the flux lattice in 2H-$NbSe_2$ but failed properly to reference the previous work of S. Bhattacharya, M. J. Higgins and A. C. Marley[1–3]. In these papers, the occurrence of a regime intermediate between a pinned flux line lattice and a coherently moving one was inferred from transport and noise measurements on $NbSe_2$. We also regret that we inadvertently failed to acknowledge discussions with S. Bhattacharya. □

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