III (Fig. 2) (ref. 3). Oligonucleotides designed to form triple helix complexes that overlap half of the *Eco*RI recognition site were synthesized with CT, ^{Me}CT or ^{Me}C^{Br}U nucleotides[18]. The genetic map of yeast chromosome III (refs 22, 23) and affinity cleaving data[3] indicate that cleavage at the target site should produce two fragments 110±10 and 230±10 kilobases (kb) in size (Fig. 2).

Resolution of total yeast chromosomal DNA by pulsed-field gel electrophoresis[24,25] revealed that chromosome III was cut exclusively at the target site when a ^{Me}CT oligonucleotide was used for triple helix formation at pH 7.6 (Fig. 3, lane 1). No cleavage was detected on any other chromosomes under these conditions nor was cleavage observed in the absence of oligonucleotide (lane 2) or in a yeast strain lacking the target sequence (lane 3). The expected 110-kb product was visualized with ethidium bromide staining and confirmed by Southern blotting with a *HIS4* (ref. 26) marker (Fig. 3*c*). The 230-kb product comigrated with chromosome I, but was detected by Southern blotting with a *LEU2* (ref. 24) marker (Fig. 3*b*). The cleavage efficiency was 94±2%. Similar efficiencies were seen with a CT oligonucleotide up to pH 7.4 and ^{Me}CT and ^{Me}C^{Br}U oligonucleotides past pH 7.8 (Fig. 4). The cleavage efficiency with all oligonucleotides was gradually reduced with longer methylation times, suggesting that the oligonucleotide dissociation rate might be the limiting factor for efficiency in this system[27].

The specificity of triple helix formation has been shown to be pH-dependent[1-3]. By lowering the pH, sequences of near but imperfect similarity can be bound and cleaved[1-3]. In agreement with this observation, secondary cleavage sites were revealed as a function of oligonucleotide composition and pH (Fig. 4). Cleavage at the secondary site was preferentially reduced by longer methylation times, suggesting differential oligonucleotide dissociation rates between the primary and secondary target sites. Cleavage at all secondary sites could be eliminated at a threshold pH for each oligonucleotide.

Whereas affinity cleaving using oligonucleotide-EDTA·Fe identifies all sites of oligonucleotide binding[1-3], triple-helix-mediated endonuclease cleavage exposes only those sequences that also partially overlap a restriction site[4,5]. The sequence requirement of a methylation-restriction site increases the cleavage specificity but reduces the number of available sites. To partially overcome this limitation, other commercially available methylation and restriction enzyme pairs with homopurine half sites were tested on plasmid DNA. In addition to *Taq*I (ref. 4) and *Eco*RI (ref. 5), single-site protection of plasmid DNA was possible with *Msp*I, *Hpa*II and *Alu*I methylases. *Bam*HI, *Hae*III and dam methylases could potentially be used, but remain untested.

The generalizability of triple helix-mediated enzymatic cleavage affords high specificity that can, in principle, be customized to unique genetic markers without artificial insertion of a target sequence. The use of degenerate oligonucleotides in this technique to rapidly screen genetic markers for overlapping triple-helix methylation-restriction sites could make it possible to cut chromosomal DNA uniquely and efficiently at endogenous sites with minimal sequence information (S.A.S. and P.B.D., unpublished observations). □

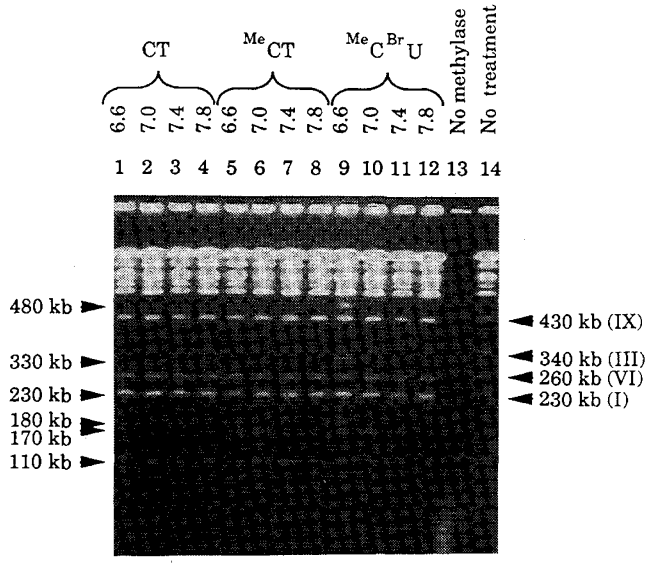

FIG. 4 Triple-helix-mediated enzymatic cleavage of the yeast genome as a function of oligonucleotide composition and pH. Lanes 1–12, reactions on a yeast strain containing the triple helix target site with oligonucleotides (CT, ^{Me}CT and ^{Me}C^{Br}U) and pH values (6.6, 7.0, 7.4 and 7.8) as indicated above figure. Methylation time was 4.5 h. 170-kb and 650-kb (unresolved) secondary cleavage products were observed with both ^{Me}C substituted oligonucleotides at or below pH 7.4. The cleavage site can be assigned to chromosome II (820 kb) by two-dimensional pulsed-field gel electrophoresis in which the DNA was triple helix-protected and methylated before the first dimension and restriction enzyme-digested before the second dimension (data not shown). Additional secondary cleavage sites were observed with the ^{Me}C^{Br}U oligonucleotide at pH 6.6 (lane 9). The 180- and 480-kb products were assigned to chromosome XI (660 kb), and the 330 and 780 kb (unresolved) products were assigned to the VII/XV doublet (1,100 kb) by the same method (data not shown).

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ERRATUM

Meteoritic silicon carbide: pristine material from carbon stars

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