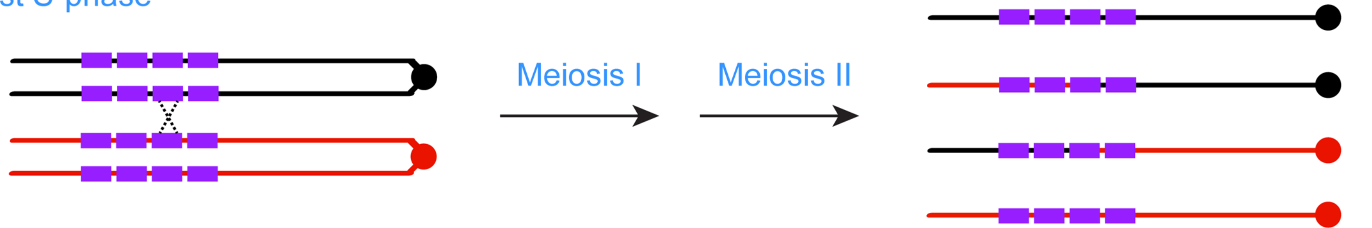
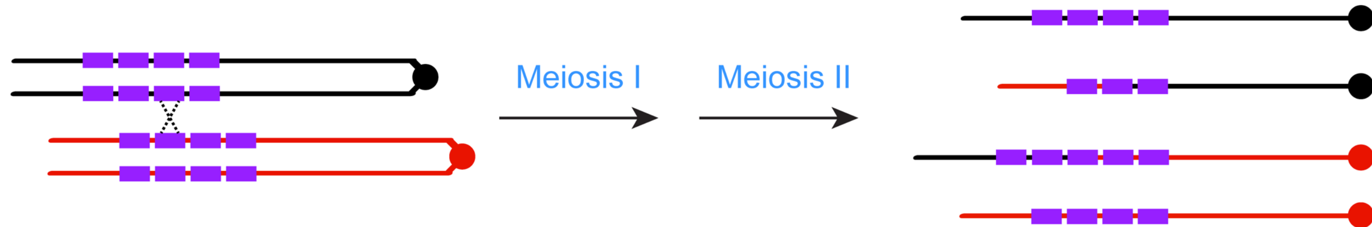
Gene Conversion and Recombination models

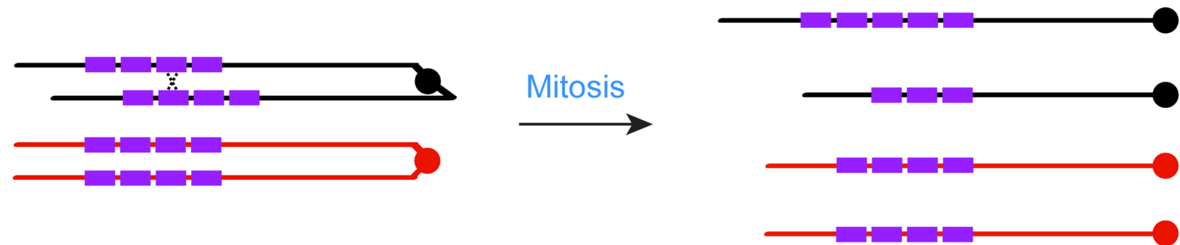
Unequal exchange:
Recombination between repetitive sequences

Diploid
Post S-phase

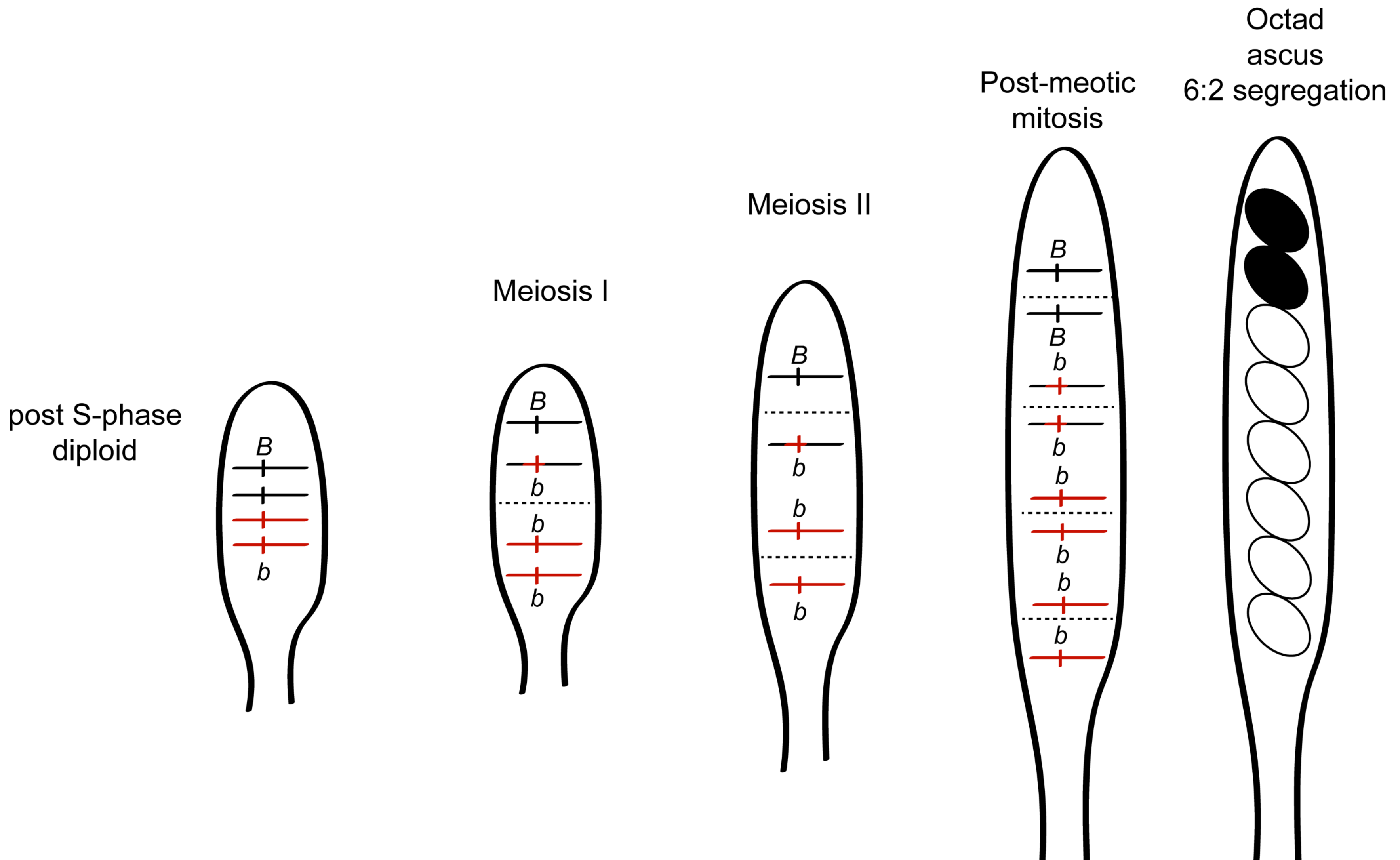




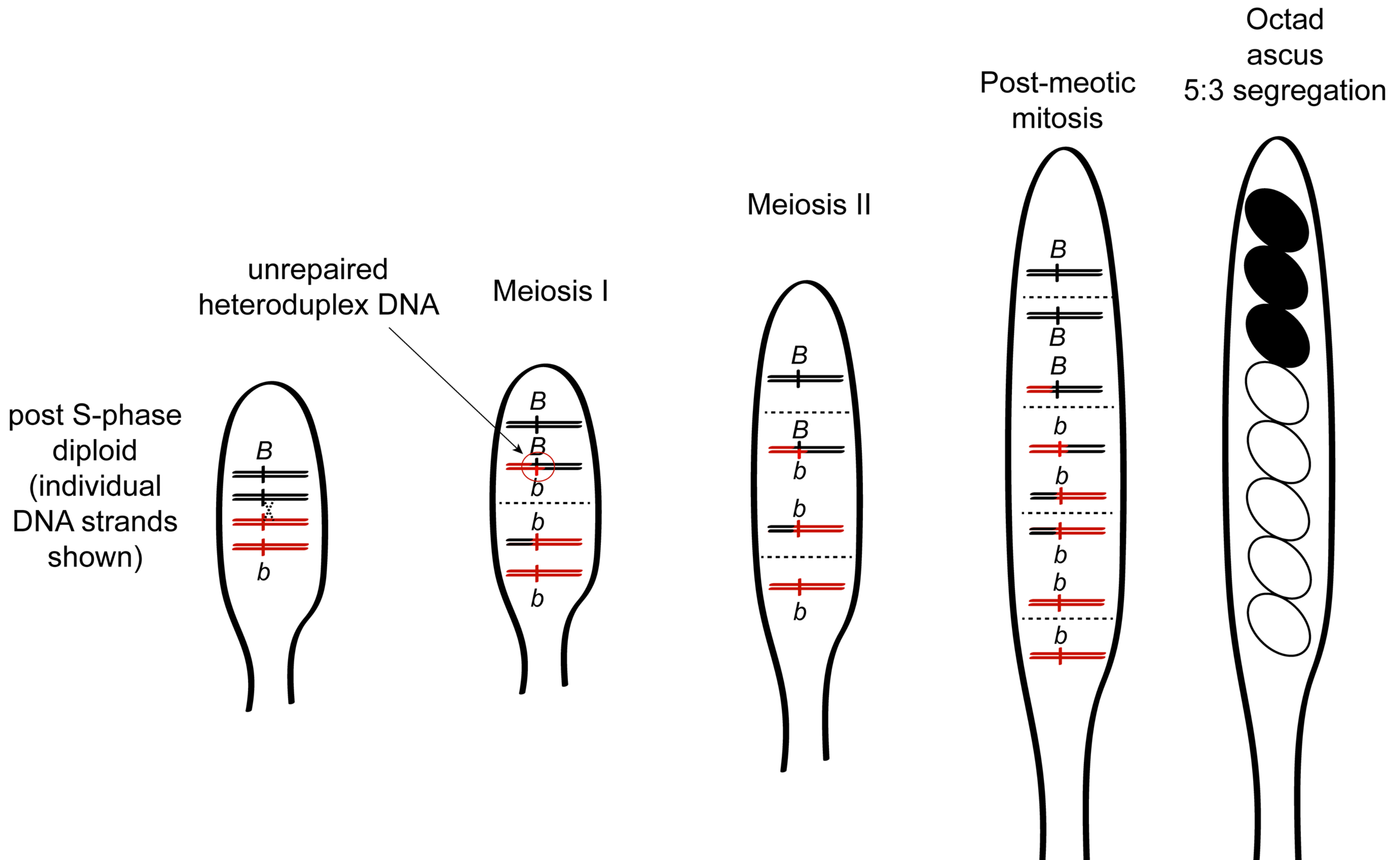
Inter-sister unequal exchange



Unequal Exchange: Gene conversion



Unequal Exchange: Heteroduplex DNA



What is the molecular basis of recombination/GC? The Holliday model

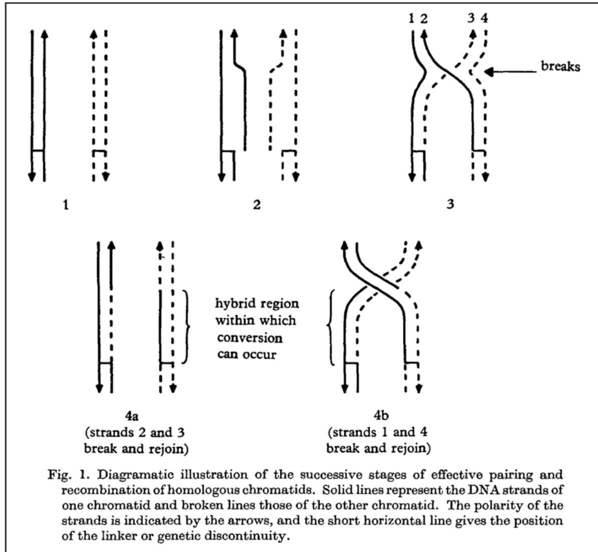
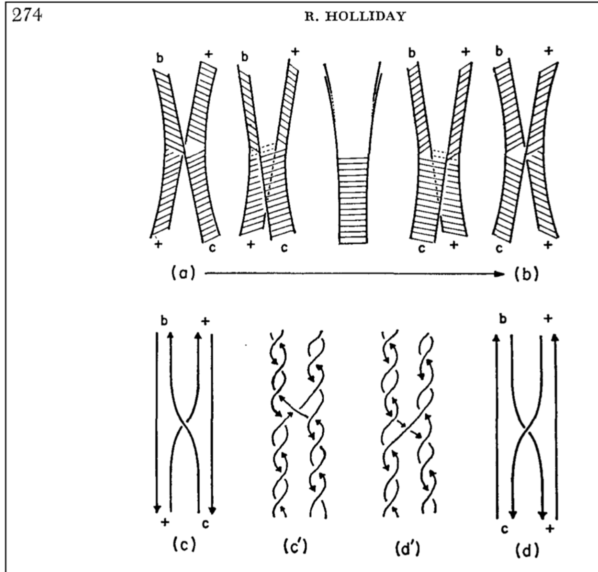
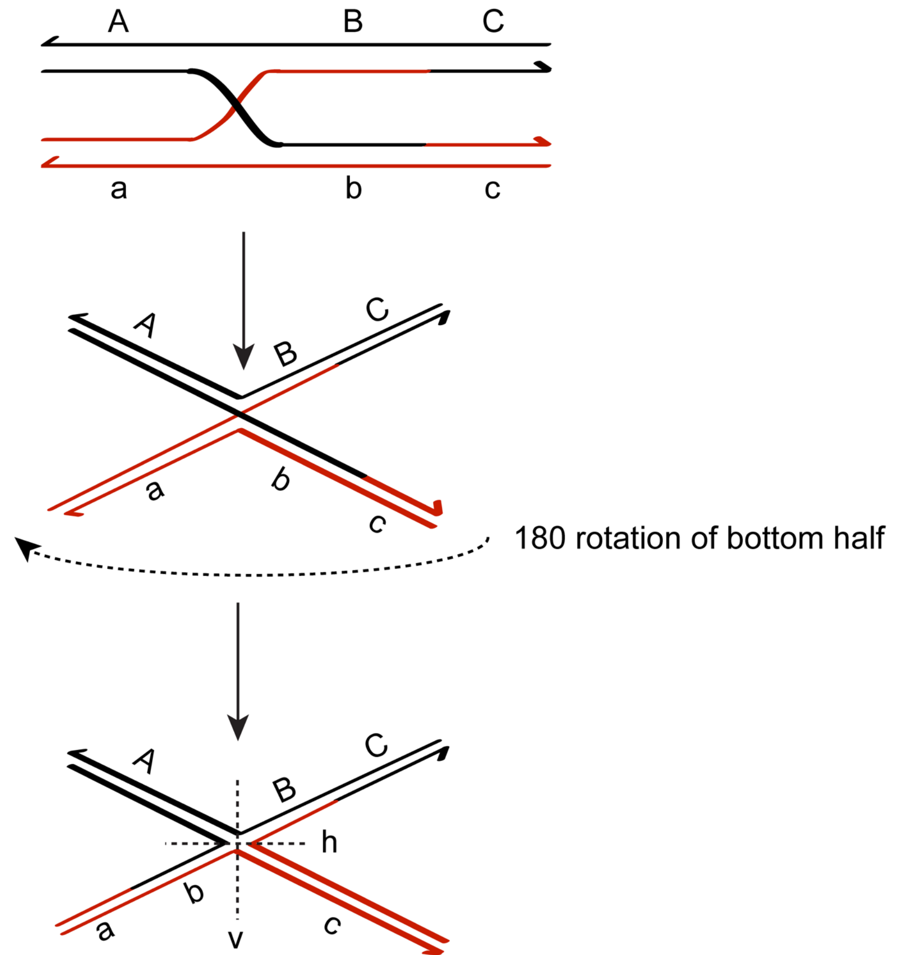


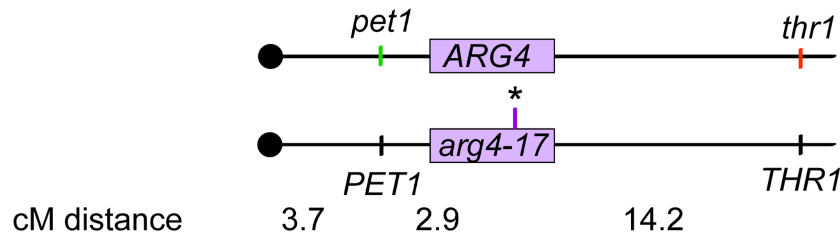
Fig. 1. Diagramatic illustration of the successive stages of effective pairing and recombination of homologous chromatids. Solid lines represent the DNA strands of one chromatid and broken lines those of the other chromatid. The polarity of the strands is indicated by the arrows, and the short horizontal line gives the position of the linker or genetic discontinuity.





Holliday 1964, 1974

Gene conversion is associated with crossing over



313 tetrads scored
22 conversions (3:1 + 1:3)
10 were associated with *PET1-THR1* CO

TABLE 2. *Frequency of conversion-associated recombination at arg4 relative to length of outside-marker interval*

Interval	Interval length (cM)	Number of conversions	Number of conversions with crossover	Fractions of conversions with crossover	X^2*
c-thr1	20.5	81	37	0.46	0.6
pet1-thr1	17.1	148	74	0.50	0.0
arg4-thr1	14.2	256	116	0.45	2.3
c-arg4	6.3	9	4	0.4	—
pet1-arg4	2.9	33	23	0.69	5.1†
arg4-a-arg4-b	<1	22	14	0.64	1.6
Total		549	268	0.49	0.31

* Chi-square test assumes that 50% of the conversions are associated with crossing-over.
† Significant at the 5% level ($P < 0.05$).

TABLE 3. *Conversion-associated recombination in chromosome V*

c *thr3 his1* *arg6*

Zygote	Number of asci	Allele(s) converted	Interval	Interval length (cM)	Number of conversions	Number of conversions with crossover	Fraction of conversions with crossover
Z3735	975	*thr3-2*	*thr3-1-his1-315*	2.6	7	3	0.4
		his1-315	*thr3-2-his1-1*	2.6	21	7	0.3
		his1-1	*his1-315-arg6*	7.4	1	0	0.0
		his1-315 and *his1-1*	*thr3-2-arg6*	10.0	13	5	0.4
Z3910	485	*his1-16*	*thr3-arg6*	8.8	15	7	0.5
Total	1460				57	22	0.4

Hurst, Mortimer, and Fogel 1972

Plasmid Integration

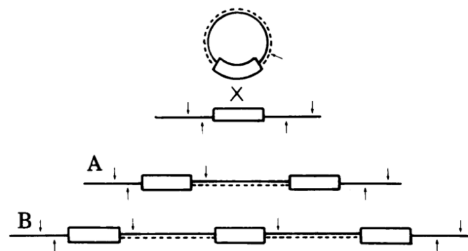


FIG. 1. Integration of a plasmid by recombination with a yeast chromosome. Two types of events occur at approximately equal frequencies: (*A*) integration of a single plasmid molecule by reciprocal recombination within the region of homology or (*B*) integration of two or more plasmid molecules. These events can be distinguished by restriction enzyme mapping. ——, chromosomal DNA; ----, vector sequences; ▭, homologous sequences on the plasmid and the chromosome; ↓, restriction cleavage site present only in the vector sequence; ↑, restriction enzyme site in flanking chromosomal DNA.

Orr-Weaver et al., 1981

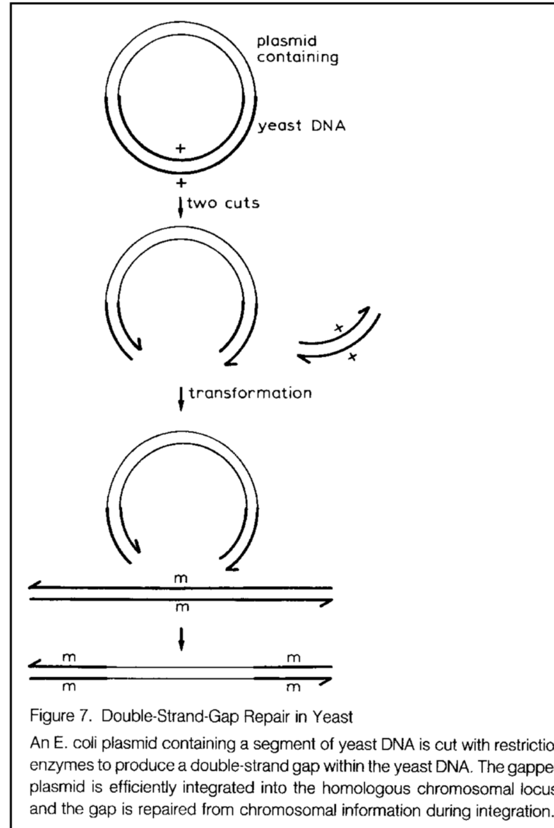


Figure 7. Double-Strand-Gap Repair in Yeast

An E. coli plasmid containing a segment of yeast DNA is cut with restriction enzymes to produce a double-strand gap within the yeast DNA. The gapped plasmid is efficiently integrated into the homologous chromosomal locus, and the gap is repaired from chromosomal information during integration.

Szostak et al., 1983

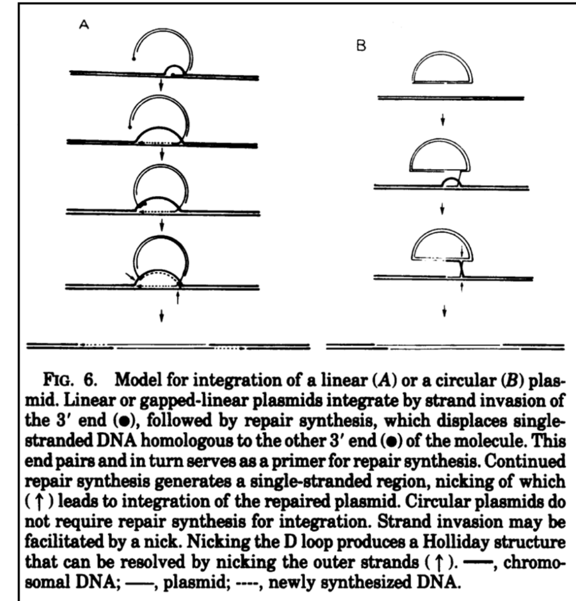
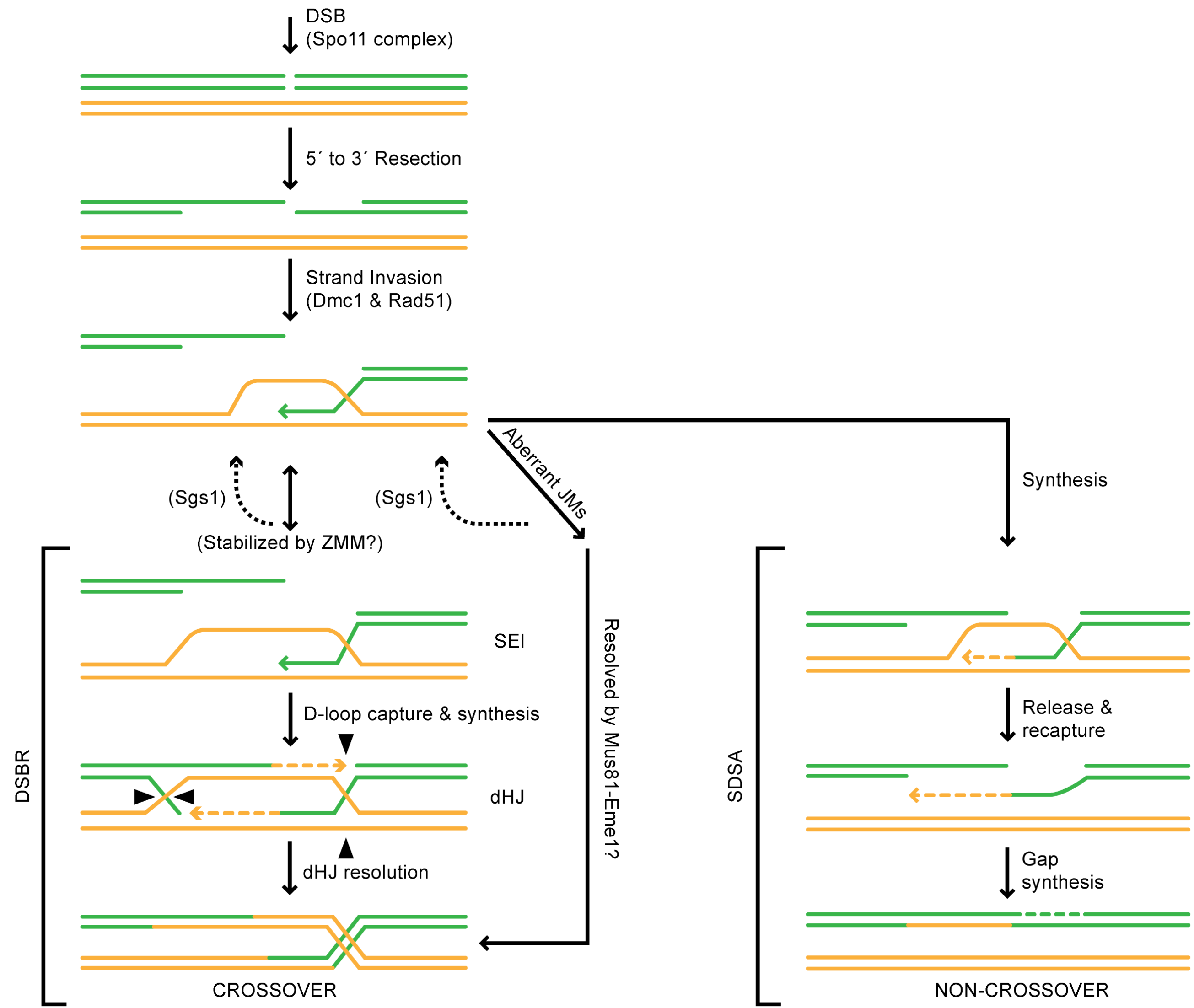


FIG. 6. Model for integration of a linear (*A*) or a circular (*B*) plasmid. Linear or gapped-linear plasmids integrate by strand invasion of the 3′ end (●), followed by repair synthesis, which displaces single-stranded DNA homologous to the other 3′ end (●) of the molecule. This end pairs and in turn serves as a primer for repair synthesis. Continued repair synthesis generates a single-stranded region, nicking of which (↑) leads to integration of the repaired plasmid. Circular plasmids do not require repair synthesis for integration. Strand invasion may be facilitated by a nick. Nicking the D loop produces a Holliday structure that can be resolved by nicking the outer strands (↑). ——, chromosomal DNA; ——, plasmid; ----, newly synthesized DNA.

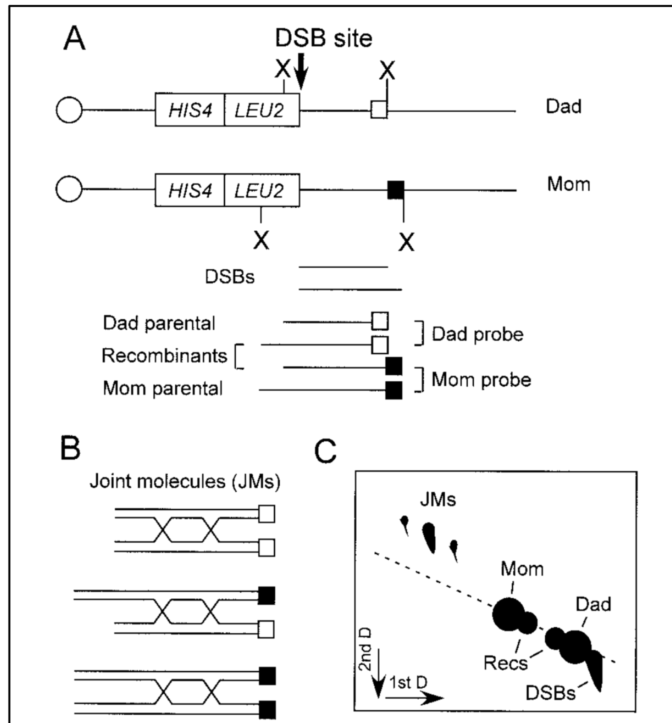
Orr-Weaver et al., 1981

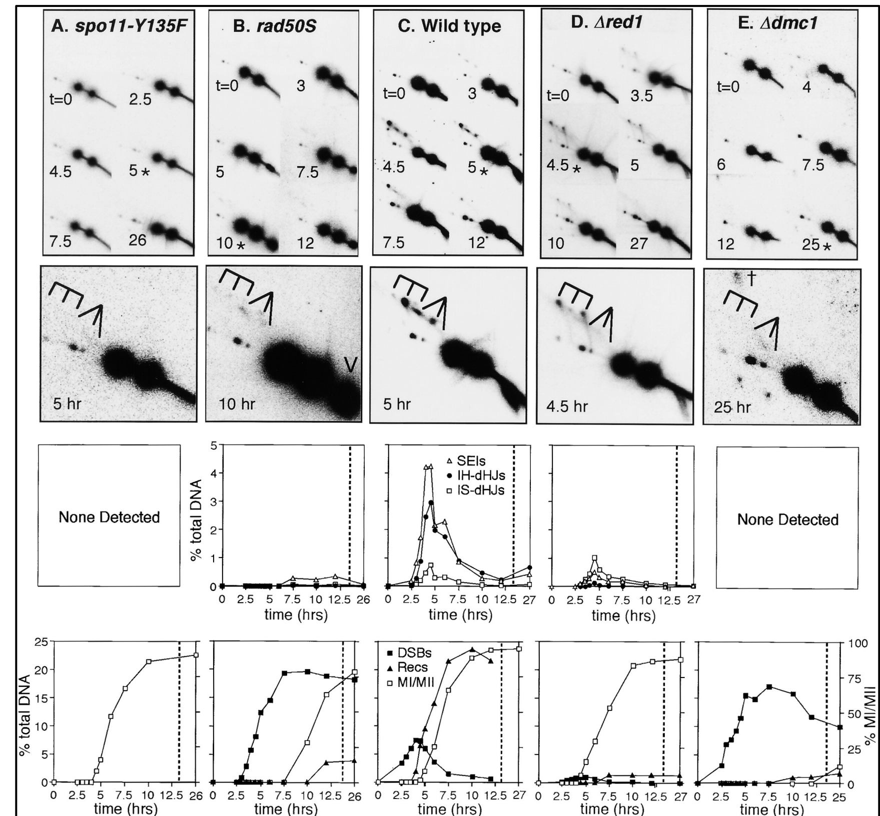
Double Strand Break Repair model of recombination



Adapted from numerous sources including Szostak et al., 1983

Testing the models: Contrasting genetic and biochemical approaches

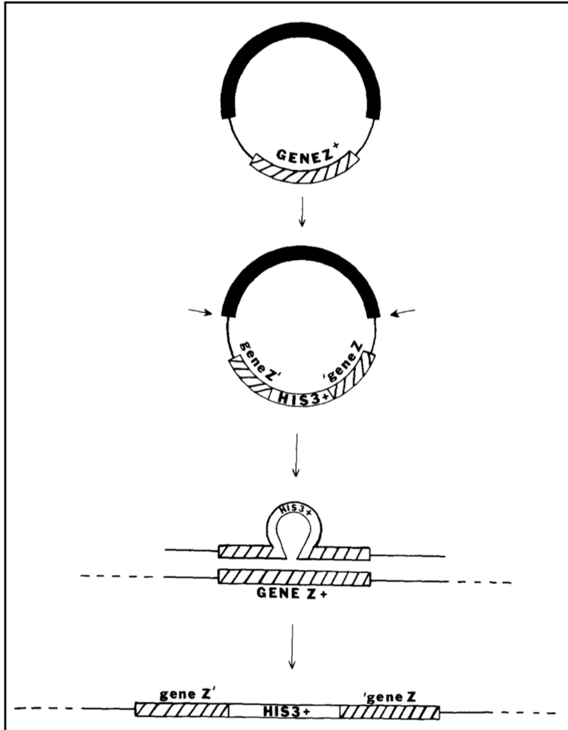




Symington 2001 review

Hunter & Kleckner 2001

Targeted gene disruption



Rothstein, 1983

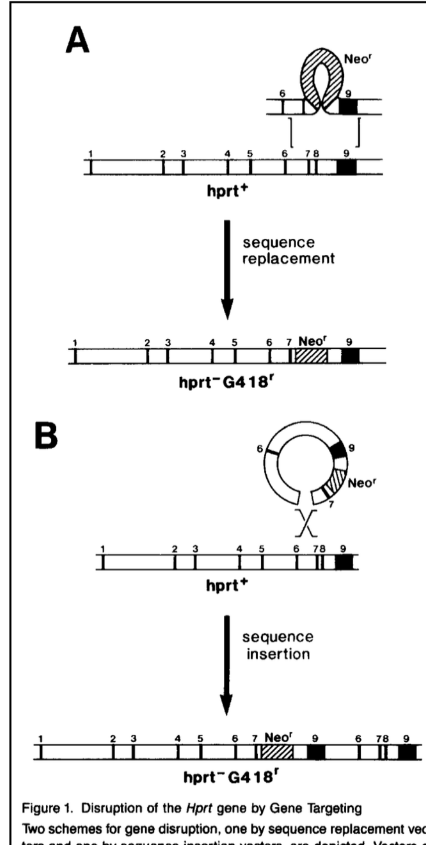


Figure 1. Disruption of the *Hprt* gene by Gene Targeting

Two schemes for gene disruption, one by sequence replacement vectors and one by sequence insertion vectors, are depicted. Vectors of both classes contain *Hprt* sequences interrupted in the eighth exon with the *neo^r* gene.

(A) Sequence replacement. Sequence replacement vectors are designed such that upon linearization, the vector *Hprt* sequences remain colinear with the endogenous sequences. Following homologous pairing between vector and genomic sequences, a recombination event replaces the genomic sequences with the vector sequences containing the *neo^r* gene.

(B) Sequence insertion. Sequence insertion vectors are designed such that the ends of the linearized vector lie adjacent to one another on the *Hprt* map. Pairing of these vectors with their genomic homolog, followed by recombination at the double strand break, results in the entire vector being inserted into the endogenous gene. This produces a duplication of a portion of the *Hprt* gene. Open boxes indicate introns; closed boxes indicate exons, numbered according to the map of Melton et al. (1984); the crosshatched box indicates the *neo^r* gene.

Thomas and Capecchi 1987

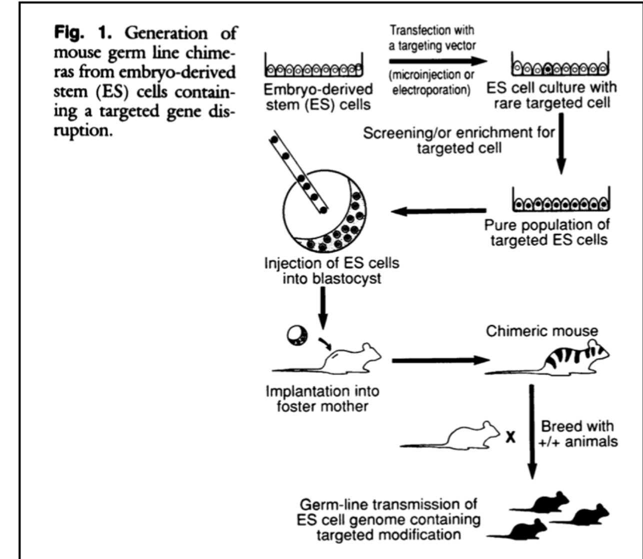


Fig. 1. Generation of mouse germ line chimeras from embryo-derived stem (ES) cells containing a targeted gene disruption.

Capecchi 1989