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MILESTONE 4

Making the cut

I remember clearly the first time I made a supervised trip, as an undergraduate student, to the departmental freezer to obtain a precious aliquot of restriction enzyme. Its real value, however, only dawned on me when I created the first of countless recombinant DNA constructs.

It is unlikely that Stuart Linn and Werner Arber were aware of the far-reaching consequences of their discovery when they stumbled across restriction enzymes in the late 1960s. While studying a phenomenon called host-controlled restriction of bacteriophage growth, they showed that restriction enzymes of the host cells cleave unmethylated phage DNA in numerous places, thereby limiting their growth. A couple of years later, Hamilton Smith and Kent Wilcox reported the isolation and characterization of the first restriction enzyme — endonuclease R (later renamed *Hin*dII) — from extracts of *Haemophilus influenzae* strain Rd. Importantly, the enzyme degraded foreign DNA, such as that of phage T7, but did not affect native *H. influenzae* DNA.

Smith and Wilcox demonstrated that endonuclease R produces double-stranded 3′-hydroxyl, 5′-phosphoryl cleavage products. They proposed that the enzyme recognizes a specific sequence on the foreign DNA, and estimated from the number of breaks that the site would have to be five or six bases in length. Smith, together with Thomas Kelly, determined the recognition sequence using end-labelling techniques. This was an exceptional technical feat, as there was no method at the time for the analysis of terminal sequences beyond the dinucleotide level. They postulated that the internal symmetry of the recognition sequence, which was cleaved in the middle, was not surprising given that the enzyme carries out a symmetrical reaction on opposite strands.

Before long, Kathleen Danna and Daniel Nathans pioneered the application of restriction enzymes. They used endonuclease R to characterize the small oncogenic DNA virus SV40: the resulting 11 fragments were resolved by polyacrylamide gel electrophoresis, and their molecular weights were determined. Their prediction that restriction-enzyme analysis would be useful to map a genome region and to localize specific genes by testing for biological activity turned out to be visionary.

The 'recombination' potential of restriction enzymes was first demonstrated by Janet Mertz and Ronald Davis. They showed that the R1 restriction endonuclease produces 'staggered' breaks, generating 'cohesive' ends that are identical and complementary. Their findings suggested that any R1-generated ends can be joined by incubation with DNA ligase to generate hybrid DNA molecules. Thus, the era of recombinant DNA technology was born.

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Nature Reviews Molecular Cell Biology

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