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MILESTONE 2

The dawn of recombinant DNA

The ability to make recombinant DNA molecules is the cornerstone of modern molecular biology. Yet 40 years ago, it was hardly a conceivable accomplishment.

In the 1960s, biologists had realized that DNA recombination happens in the cell — for example, when breaks caused by ultraviolet irradiation are repaired — and the search for an enzyme that could join DNA molecules was on. The breakthrough came at the beginning of 1967, when Martin Gellert at the National Institutes of Health showed that *Escherichia coli* extracts could convert λ phage DNA 'hydrogen-bonded circles' into a covalently circular form. Within 6 months, Gellert and three other groups independently purified the enzymatic activity, which formed phosphodiester bonds between DNA ends held by hydrogen-bond pairing in a double-stranded configuration.

DNA ligase, which was the first ingredient for making recombinant DNA, was then at hand, but other ingredients, like restriction enzymes, (see Milestone 4) remained to be discovered. Another key concept was the use of plasmids as vectors for shuttling DNA into bacteria. Stanley Cohen, who was studying the role of plasmids in bacterial resistance to antibiotics at Stanford University, first worked out a 'transformation' method to make bacteria take up purified plasmid DNA.

Then, in 1973, Cohen and his Stanford colleague Annie Chang, in collaboration with Herbert Boyer and Robert Helling at the University of California in San Francisco, reported the first *in vitro* construction of a bacterial plasmid. Using the restriction enzyme *EcoRI*, they generated

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fragments from two plasmids (each conferring resistance to one antibiotic), joined them using DNA ligase and applied the mixture to transform *E. coli*. As they had hoped, a fraction of the transformed bacteria became resistant to both antibiotics while carrying a single hybrid plasmid. Not only had they demonstrated that bacterial plasmids constructed *in vitro* were functional in bacteria, but they had also described the first plasmid vector.

Meanwhile, Paul Berg had devised a similar experiment to transfer foreign DNA into mammalian cells, using the tumour virus SV40 as a vector. In 1972, he made a hybrid molecule *in vitro* by inserting λ phage sequences into SV40. These reports immediately raised concerns, as *E. coli*, which is a natural habitant of the human gut, could now carry hybrid DNA molecules containing SV40 oncogenes or other potentially harmful sequences. These fears led the community to a self-imposed moratorium on recombinant DNA experiments. However, the foundation had been laid and progress soon resumed.

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