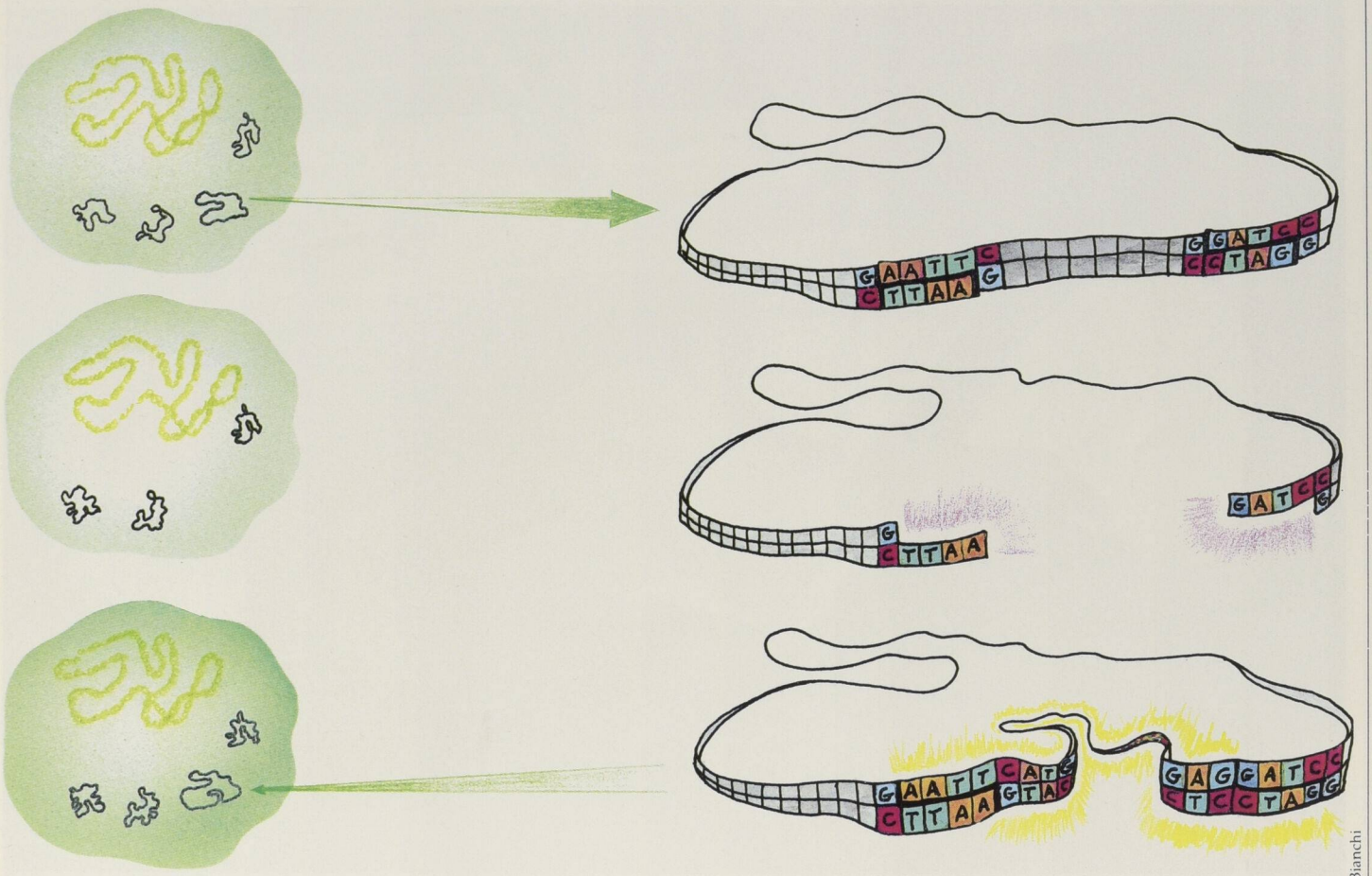

John Bianchi

reverse of that on the other." This gives the cut loop "sticky ends," or short stretches of single-stranded DNA dangling on the ends. All Narang and others had to do was build the same kind of single strands on the ends of their genes, but make the letter sequences "complementary" to the loop strands. That way, the gene ends would stick to their complements on the open plasmid, and the loop could be reformed or ligated with enzymes.

Saran Narang: "These enzymes and their easily synthesized target sequences, which we call 'linker' molecules because they are used to connect different DNA regions in plasmids, are the basic tools of the technology. It's really a form of gene surgery, and we routinely design plasmids or 'cloning vehicles,' with great precision."

He opens a drawer and removes yet another drawing. It contains a wheel-like figure, broken up into several segments by lines, the various regions identified by strange, abbreviated names. It looks like an engineering drawing of some strange, esoteric machine. "The plasmid that produced all that protein in the micrograph," he comments.

Getting up and moving to the blackboard, Narang chalks a large, but flattened Z-shape. On the top bar of the letter he writes B, on the oblique mid-bar, C, and on the bottom bar, an A. "When the insulin protein is translated from the messenger RNA molecule in our pancreas cells," he explains, "it is in this form, which we call 'proinsulin.' The molecule comes off the protein assembly line as a linear amino acid chain, BCA, but what we call 'steric' forces within the chain itself cause it to fold roughly into this kind of Z-shape. When this happens, enzymes form two 'disulfide' bonds between the A and B chains, and the C-chain is snipped out of the centre . . ." Narang rubs out the mid-bar and joins A and B with two red -s-s- bridges, ". . . to give the active hormone, insulin. The centre C-chain is only there to orient A and B so that the disulfide bonds can form."

Gene splicing, the heart of 'recombinant DNA' technology. The first step involves getting one of the bacterium's own small loops of DNA, called a plasmid, out of the cell, and cut open, by special enzymes, two in this case, each of which does its scissoring at very specific base letter sequences along the helix. Note that each of these sites, GAATTC and GGATCC, is a palindrome: it reads the same in either direction. Thus opened, the plasmid, also called a 'cloning vehicle,' loses a section of DNA, and is left with two special 'sticky' ends. When bioscientists want to clone a gene like insulin, they simply sculpt the gene ends so that they have the right base letter 'fits' for the plasmid's opened ends. These join sites are then annealed by 'ligase' enzymes and the recombined plasmid DNA is reinserted into the bacterium. During growth and reproduction, the bacterium also reproduces the inserted gene and in many instances, proinsulin being one of them, translates the gene into protein.

To get a hormone that worked, then, the scientists would have to produce the proinsulin protein, since it was known that the A and B chains could not be linked efficiently to form the active structure. Narang and Wu were aware by then that *E. coli* would probably clone a large