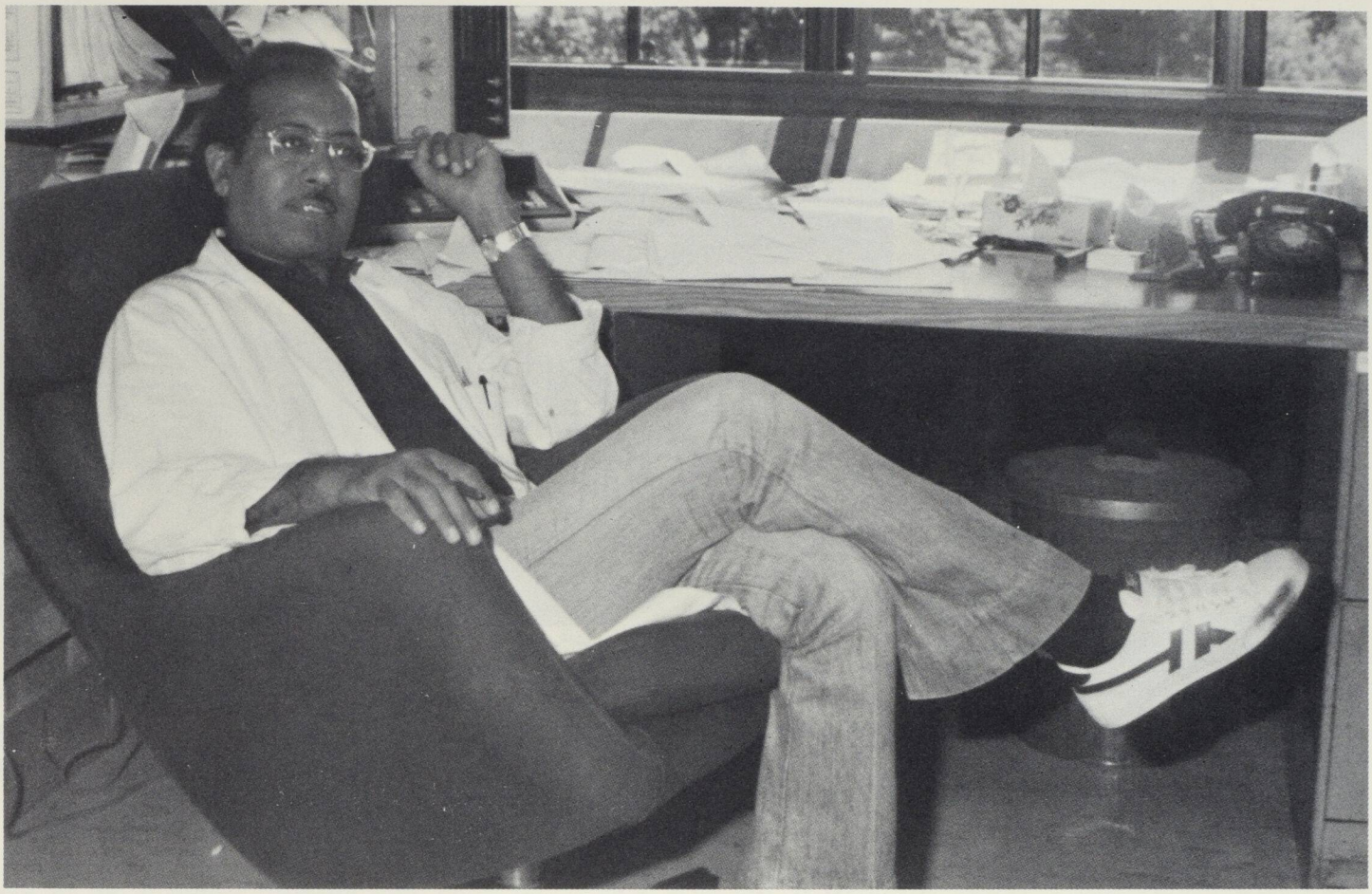

Saran Narang: The early dream was to make a really large gene that would function like the real thing.

gene like proinsulin, but they still didn't know if the bug would go to the next important step and translate the gene message into protein. "Even if it did," says Narang, "we suspected that the bacterium contained enzymes whose job it was to break down such 'outsider' proteins.

Nonetheless, they went ahead with their plan to build the proinsulin gene. Ottawa would synthesize it in segments and Cornell would string them together with ligase enzymes. Other laboratories, particularly Walter Gilbert's, and Howard Goodman's at the University of California, were also attempting to clone proinsulin, but they were isolating the natural material rather than building it, and there were problems. To get the proinsulin gene, they had to backtrack, using the messenger RNA for proinsulin retrieved from a living cell to build the DNA gene that coded for it. This retrieval and the enzyme surgery that went along with stitching the gene into a plasmid were very difficult procedures. And, for everyone in the field, there were problems that went beyond the merely technical (see "The double edge of DNA").

The proinsulin gene that Narang and Wu eventually built was 258 base letters of double-stranded or duplex DNA. To get enough material to synthesize such a long molecule, they took advantage of the plasmid techniques to clone the individual chains. These early experiments also allowed Wu's Cornell team to become familiar with manipulating plasmids and work with the 'transformed' bacteria that carried them. The field was so new that virtually everyone enjoyed an amateur status.

"We cloned the A chain first," says Narang. "It was the shortest of the three segments. When you clone a gene, you also need to add the DNA triplets ATG and TGA immediately before and after it on the run of the helix. These are the universal signals that tell the bug's translator system that it is to START building an amino acid chain, and finally, to STOP building it. Also, to each end of this assembly, we had to attach 'linker' molecules, the short DNA target regions of the plasmid splicing enzymes."

In this way, the two laboratories acquired enough of the three chains to allow Wu to patch them together to form the proinsulin gene code, B-C-A. With the START-STOP and linker regions, the finished molecule stretched to 287 base letters in length, one of the longest ever built. Wu's lab then spliced it into a plasmid, introduced the hybrid loop into *E. coli*, and waited to see what their three years of work would bring.

Narang recalls the tense days during which the Cornell team 'screened' the bacterial cultures for evidence that a transformed *E. coli* was, in fact, cloning their gene. "Finally, the screen turned up a bug that was doing it. From Gilbert's work with the natural gene, we knew that it was possible, but still, you're never certain until it actually happens. Equally important, we showed that the *E. coli* was also translating the gene into the protein, though at very low levels."