MASSIVE MACHINES IN

BEAMS OF ELECTRONS ARE REVEALING THE INN

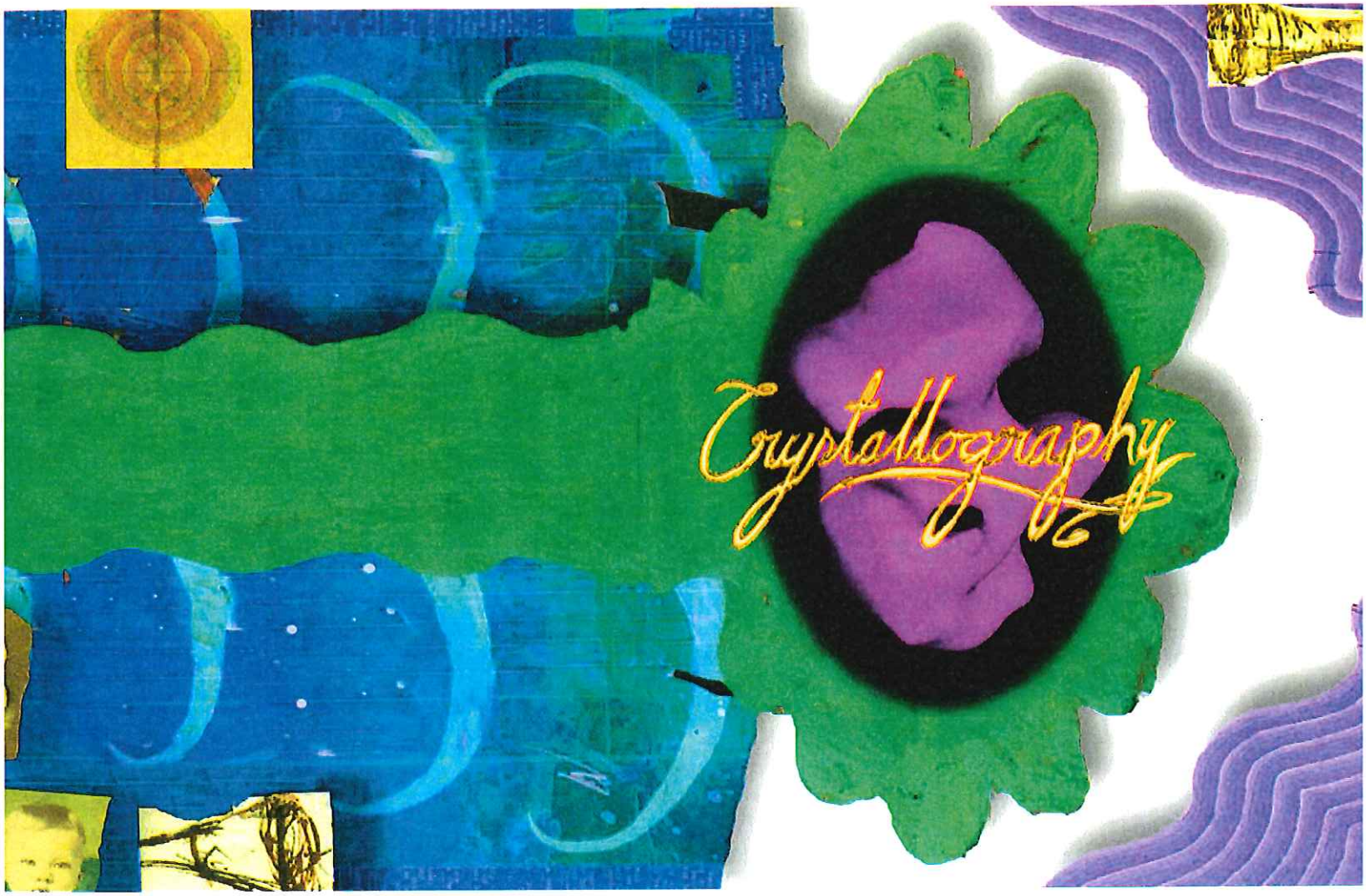
AS KERSTIN LEUTHER DRAGS HER CURSOR ACROSS THE COMPUTER SCREEN, A STRANGE AND WONDERFUL PURPLE OBJECT SPINS INTO VIEW, ROTATING TO SHOW OFF A BROAD INDENTATION ON ONE SIDE AND A HOLE CLEAN THROUGH ANOTHER. With its graceful curves and bizarre shape, the form looks like a scientist's attempt at abstract sculpture. But the object is a still life, an accurate representation of a human protein that patches together broken DNA to prevent cancerous changes. That indentation and hole may be the crucial sites where damaged DNA first threads into the protein machine before being stitched together.

Leuther, PhD, a postdoctoral fellow in the laboratory of structural biology professor Roger Kornberg, PhD, first contemplated this protein while on a hike in the mountains of Colorado. The hike was a break during a conference, but it gave her a chance to quiz Gilbert Chu, MD, PhD, about the protein, called DNA-dependent protein kinase (DNA-PK). Chu, an associate professor in the departments of medicine and biochemistry, was fascinated by DNA-PK. He was intrigued by its ability to corral the broken ends of DNA together and repair the cut, and by its possible involvement in lymphomas and in a group of mysterious autoimmune diseases. If only he could see what a single DNA-PK molecule looks like — discern its structure — he might have a clue as to how it does its job. "Structure is just nooks and crannies and holes," says Chu, "but it suggests hypotheses for how the protein works. Then we can test those hypotheses using biochemistry."

A MICROSCOPIC WORLD
R WORKINGS OF THE CELL'S PROTEIN MACHINES.

BY WILLIAM WELLS

ILLUSTRATIONS BY HENRIK DRESCHER



There was just one problem with DNA-PK. In the words of Leuther: "It's huge."

Proteins, the cell's chemical workhorses and structural support, usually weigh in at a few tens of thousands of daltons, where one dalton is the weight of a single hydrogen atom. DNA-PK, at almost half a million daltons, is a protein behemoth.

Most structural biologists bounce X-rays off their favorite protein to figure out the position of every last atom. But more atoms in a single protein means more bouncing rays and more complicated calculations. Chu would eventually like to determine the structure of DNA-PK using X-rays. For now, however, he is realistic. After all, the 1997 Nobel prize in chemistry was awarded for determining the X-ray structure of a protein only two-thirds the size of DNA-PK.

Unfortunately, even a protein behemoth can't be seen under a light microscope. When a light microscope is used to observe an entire cell, the waves of light crash against the cell as though it were a cliff. A protein is more like a tiny pebble, and the light waves pass over it unperturbed.

The compromise solution is electron microscopy (EM). Electrons, like light waves, can be focused. This makes structure determination very much easier, so that something the size of DNA-PK is now a reasonable proposition.

But Chu's problems were not over yet. "The problem with the EM approach is that the electron beam is damaging to proteins,"

says Kornberg. "A typical protein will be destroyed by a beam strong enough to determine its position, let alone its structure."

Protein crystals make EM a viable approach. The researchers cannot use a strong beam to get lots of information from one protein molecule, so they use a weak beam to get less information from every one of the hundreds of thousands of protein molecules in the crystal. All molecules in the crystal are fixed in an identical orientation, like bricks in a wall. Electrons therefore bounce off every copy of the protein at the same angle, so the information from each copy of the protein can be added together.

Kornberg first came across electron crystallography as a postdoctoral fellow at Cambridge University, England. "This powerful method had been devised," he says, "but there was no way to systematically apply it to any protein, for lack of a way of making two-dimensional crystals." The crystals had to be two-dimensional — a single layer of perfectly aligned proteins — so that enough of the electron beam would pass through to form an image on the other side.

"To constrain a molecule in two dimensions you must bind it to a surface," says Kornberg, "But for it to crystallize it must be mobile," so that it can slot into position next to another molecule.

His solution had its basis in his graduate work at Stanford. In the late '60s, Kornberg worked out the motions of molecules in the lipid membrane — the outer lining of cells — in the laboratory of Stanford chemistry professor Harden McConnell, PhD. Kornberg and McConnell saw that the lipid membrane was a greasy molecular sandwich; individual lipid molecules could whiz about in either the upper or the lower half of the sandwich, but they almost never 'flip-flopped' to the other side.

After his postdoctoral work was concluded, Kornberg put the mobility of lipids to use in making two-dimensional crystals. He attached a small chemical to a lipid and then added a protein that normally attaches to that chemical. The protein latched onto the modified lipid, and so was fixed in two dimensions. Within those two dimensions, however, the protein was still free to roam. Thousands of wandering protein molecules coalesced and made a two-dimensional crystal.

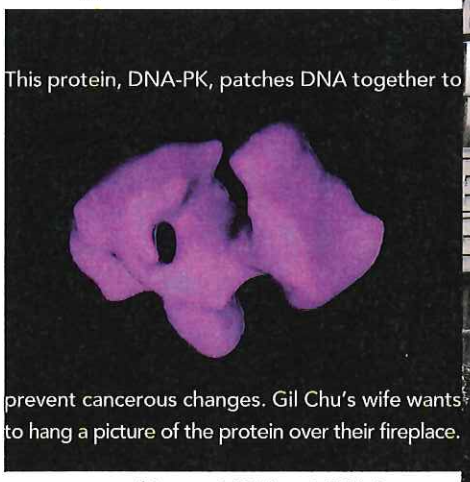
"The first results were, in retrospect, fairly dreadful because the crystals were very, very small. But at the time, the fact that they formed at all seemed absolutely remarkable," says Kornberg. "We declared victory."

ONE OF KORNBERG'S FIRST TARGETS FOR EM STRUCTURE ANALYSIS WAS THE BACTERIAL TOXIN THAT CAUSES HAVOC IN CHOLERA. The toxin's single A subunit and five B subunits attach to the outside of human cells, but only the A subunit gets into the cell. Once there, it interferes with the cell's communication network and convinces the cell to throw large numbers of salt ions out into the human gut. Water — and diarrhea — soon follow.

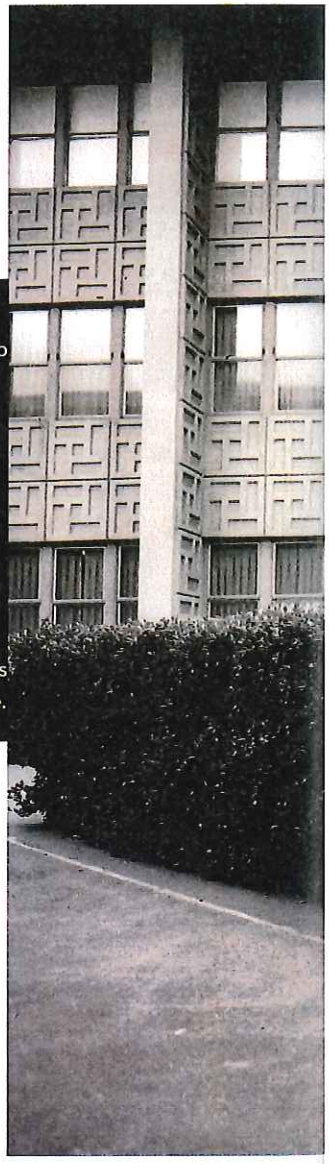
Researchers suggested that the five B subunits might form a tube through the cell membrane to allow the A subunit to get into the cell. The theory sounded reasonable but was hard to test without a direct look at the shape of the proteins.

In collaboration with Gary Schoolnik, MD, a professor of medicine and chief of Stanford's division of infectious diseases and geographic medicine, Kornberg made two-dimensional crystals of the five B subunits. The subunits formed a ring as predicted, but the ring sat on top of the membrane rather than forming a channel through it. When the A subunit was added, it lodged in the center of the ring and partially buried itself in the membrane underneath. Finally, Kornberg and Schoolnik mimicked events in the cell by cutting the A subunit. The majority of the A subunit completed its journey across the membrane, leaving only the smaller fragment associated with the ring of B subunits. The researchers calculated that the attachment of the B subunits to the underlying lipids held the lipids apart, giving the A subunit more room to burrow through the membrane into the cell. "It was the first time this method had been used to find out new biological information," says Kornberg.

The cholera toxin work was rewarding, says Kornberg, but it is talk of the next EM project that causes him to shift to the edge of his seat. He wanted to finally see RNA polymerase II, the subject of many years of his biochemical inquiries. Polymerase is a group of proteins that directs the decoding, or transcription, of DNA to make the messenger molecule mRNA. After genes are converted into mRNA, mRNA is converted into protein. Although the cell exerts some control over this latter process, protein production is largely controlled by turning transcription on and off. As organisms develop, age and encounter new environments, polymerase selects which genes should be decoded. Chemicals that poison polymerase kill everything from bacteria to humans, and polymerase action is needed before organisms can digest food, respond to hormones, or grow limbs. "Transcription," says Kornberg, "is arguably the most important and fundamental process in biology."



This protein, DNA-PK, patches DNA together to prevent cancerous changes. Gil Chu's wife wants to hang a picture of the protein over their fireplace.



Polymerase, says Kornberg, is a "large and complex beast" made of a dozen different proteins. Kornberg and a host of researchers around the world want to know how polymerase identifies special regions of DNA, called promoters, that mark the

THE CRYSTALS HAD TO BE TWO-DIMENSIONAL — A SINGLE LAYER OF
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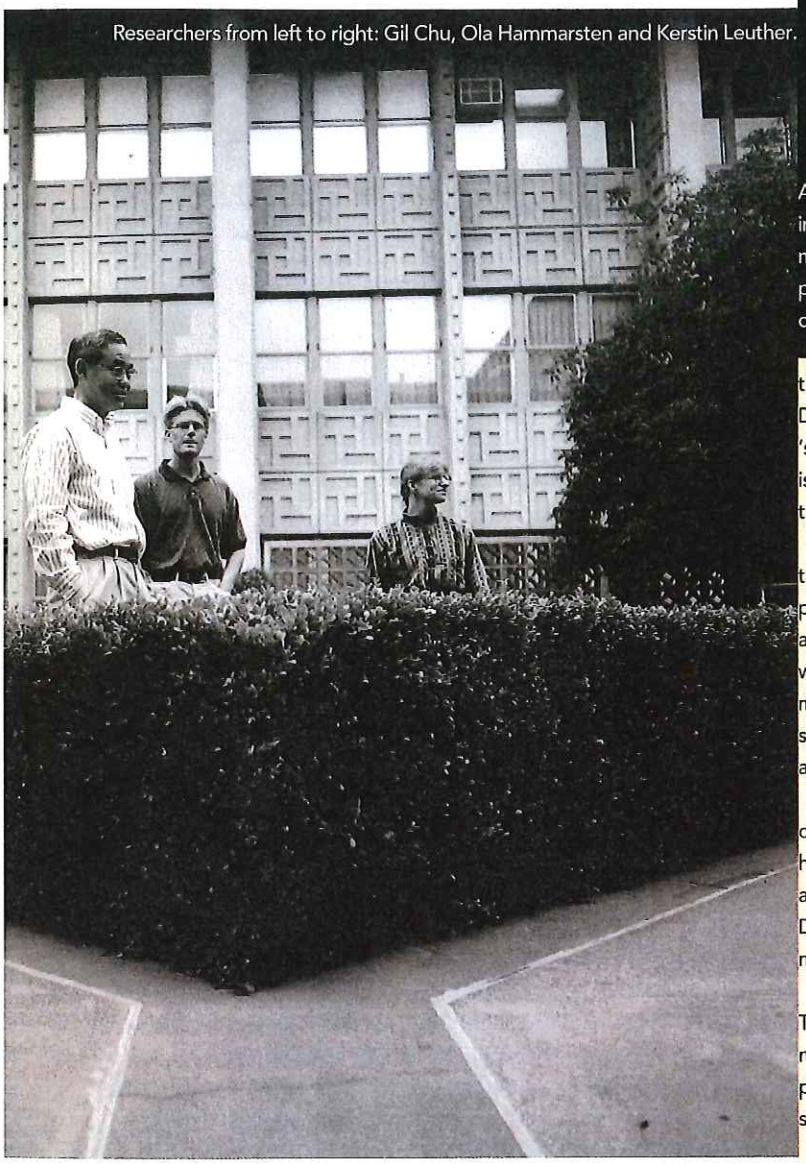
CATHERINE LEDNER

beginning of genes. How does polymerase choose, in any one cell, just a few thousand promoters from the several trillion DNA bases?

The initial EM structure solved by Kornberg and postdoctoral fellow Seth Darst (now at Rockefeller University, New York) gave only a few preliminary clues. A curve like a half-open hand looked to be just the right size for grabbing onto DNA. But the question of specificity remained, and the answer seemed to lie in proteins other than polymerase.

"The striking fact is that polymerase II alone, despite its size and complexity, is unable to recognize promoters or initiate the start of transcription," says Kornberg. "Many additional proteins are required."

Kornberg and other biochemists started by identifying each



Researchers from left to right: Gil Chu, Ola Hammarsten and Kerstin Leuther.

'ERFECTLY ALIGNED PROTEINS — SO
'ORM AN IMAGE ON THE OTHER SIDE.

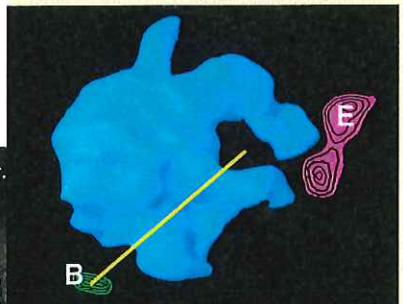
of these 16 additional proteins, or transcription factors. Kornberg uses a motor mechanic analogy to explain that this tactic, although a necessary first step, was unlikely to be enough. "If all the parts of an engine were laid out before you, you wouldn't have a clue as to how the engine worked," he explains. "But if a

THE ABCS OF TRANSCRIPTION

ELECTRON MICROSCOPY ALLOWS RESEARCHERS TO MAKE SENSE OUT OF ALPHABET SOUP.

BY WILLIAM WELLS

Transcription is the first step in turning genes into proteins, so the cell is very careful in controlling when and where it starts. The starting instructions are contained in the promoter, a piece of DNA at one end of each gene. The transcription engine is RNA polymerase, and the starting motor is the group of transcription factors — five tightly associated bundles of proteins called TFIIB, TFIID, TFIIE, TFIIF, and TFIIH.



A DNA molecule (yellow) lies in the groove in RNA polymerase (blue), and places its promotor near TFIIB (B). When the DNA and polymerase make contact, TFIIE (E) signals other proteins to allow transcription to start.

Transcription begins when TFIID finds a part of the promoter called the TATA box. TFIIB then attaches itself to TFIID, and polymerase attaches itself to TFIIB. So far so good, but the transcription factors are now far from the action. They are at the opposite end of the polymerase from the active site, the hand-like structure that holds onto and decodes the DNA. And they are attached to DNA that is 30 bases away from the 'start site,' the first DNA to be decoded. Leuther was puzzled. "TFIIB is incredibly important," she says, "but it's not even remotely close to the active site."

Everything fell into place when Kornberg and Leuther, using the electron microscopy-derived structures, oriented their computer-generated polymerase. The DNA between the TATA box and the start site lay neatly across a groove in the polymerase surface, and the polymerase active site was positioned at the start site, ready to go. "From this fairly simple-minded structure we have a geometric solution to start-site selection," says Leuther. "After two decades of biochemistry, all of a sudden we had a pretty simple picture."

To allow the DNA into its 'palm,' the active-site hand must initially be open. In the EM crystal with TFIIE, Leuther found not only that the hand had all but closed but that TFIIE spanned the remaining gap. Kornberg and Leuther think that TFIIE either helps clamp the polymerase to the DNA or senses closure and tells the rest of the proteins that the polymerase is ready to go.

The sequence of events is becoming clearer. TFIID finds TATA, then TFIIB attaches to TFIID, bringing polymerase with it. That orients polymerase so its active site wraps around the start site. TFIIE indicates that polymerase has made contact and brings in TFIIH, which parts the DNA strands to allow decoding.

The only other player, TFIIF, is always attached to polymerase. It is needed to stop polymerase from latching onto any old DNA. Now we know why TFIIF is essential: The groove along the polymerase surface from TFIIB to the active site is primed to attach to DNA. "TFIIF is a gatekeeper," says Kornberg, allowing polymerase near DNA only if it senses that TFIIB is already present on the DNA.

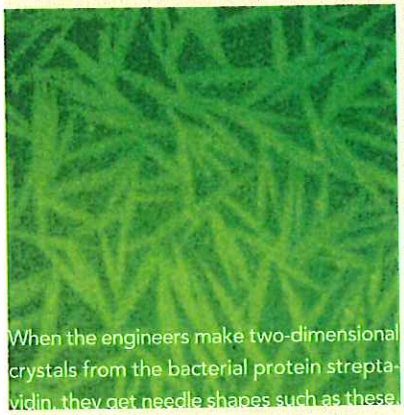
"This really is a plausible hypothesis and the first real understanding of the reason for complexity in transcription," says Kornberg. "This is a great triumph of the EM approach. You can see each component of what was previously an alphabet soup playing a simple and essential role."

CRYSTALS MADE TO ORDER

ENGINEERS HOPE TO PUT PROTEIN CRYSTALS TO WORK.

BY WILLIAM WELLS

Engineers and biologists use two-dimensional protein crystals in very different ways. ■ Alice Gast, PhD, and Channing Robertson, PhD, both professors in Stanford University's chemical engineering department, are using the crystals to help figure out how proteins arrange themselves on a surface, with an eye to making biosensors or biocompatible materials.



When the engineers make two-dimensional crystals from the bacterial protein streptavidin, they get needle shapes such as these.

Since proteins are specialists at taking one or more inputs — such as the amount of a toxic chemical or an infectious bacterium — and giving a precise, modulated output, proteins would be perfect for any number of diagnostic or medical devices. "At some point in time there will be an interaction between biological materials and inorganic materials [to make these devices]," says Gast. "But, first, an engineer wants control over organization of the surface."

In collaboration with Kornberg, Gast and Robertson have made crystals while varying the acidity of their solutions or the amount of a protein contaminant. EM structures show the researchers the different ways that the same protein packs against itself in the various crystals. They can then reason how the strength and extent of these contacts lead to differing rates of crystal growth in different directions, and so to the different shapes of the crystals. The pure protein crystallizes in simple rectangular or needle shapes, but the addition of contaminants causes complicated branching, yielding feathery snowflake shapes.

Streptavidin proteins form feathery shapes like these if crystallized with a contaminant.

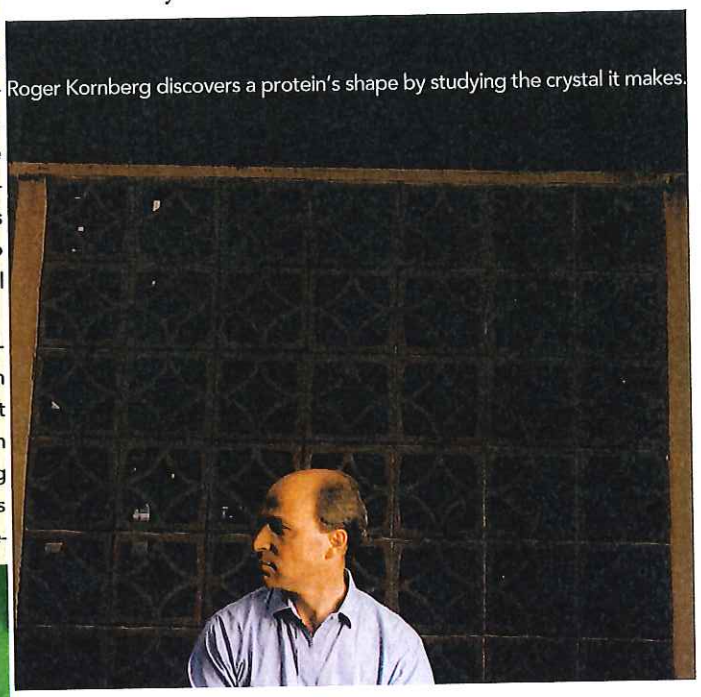
The full explanation from molecular structure to macroscopic shape is incredibly satisfying, says Gast, but working devices have not been created yet. "That is the light at the end of the tunnel," she says, "but it's some way off."

few components were organized into their proper arrangement — a piston in a cylinder for example — you would be able to intuit the general idea of how the engine works."

Nature has done some preliminary organizing. The transcription factors are found in five complexes — tightly associated bundles of several proteins — called TFIIB, TFIID, TFIIE, TFIIF, and TFIIH (pronounced "TFtwoB," etc.). What really matters is how these protein complexes interact with polymerase. Solving this problem by EM was Leuther's first task. She added TFIIB and TFIIE to the flat, accessible layer of a two-dimensional polymerase crystal. The added proteins appear as somewhat amorphous blobs on a much larger amorphous blob.

BUT TO LEUTHER THEIR POSITIONS WERE TELLING. ONCE SHE ADDED IN THE DNA, IT WAS APPARENT THAT TFIIB PUTS THE polymerase in exactly the right place, ready to start transcription at the promoter "start site." And TFIIE helps clamp the mouth of the polymerase around the DNA. Adding this information to knowledge of the other transcription factors made a coherent story of the way that transcription gets going (see *The ABCs of Transcription* sidebar). "We now understand the basis for the complexity of the system, and we get a simple picture of the whole process," says Kornberg. "To understand the process at this level is a real triumph."

In some ways the number of proteins involved in transcription is a blessing. Kornberg can add or remove proteins from his mix and see how many steps polymerase makes toward starting transcription. But DNA-PK does not allow for this strategy. DNA-PK is one huge DNA-mending machine: a single protein almost the size of the twelve proteins of polymerase combined. There is nothing to add or take away. So on that hike in Colorado, Chu jumped at Leuther's offer to try making DNA-PK crystals.



Roger Kornberg discovers a protein's shape by studying the crystal it makes.

Chu came to DNA-PK through another protein, Ku, that researchers had identified as the target of attacks by the immune system in autoimmune diseases such as scleroderma, lupus, Grave's disease and polymyositis. "People went to a lot of trouble to study this antigen because they thought they would gain some insight into these diseases," says Chu. "They haven't." Why the body attacks this protein, and how that is involved in the specific symptoms of each disease, remains a mystery.

Chu found that Ku is needed to repair DNA when both strands have been sheared. Though the cuts can come from X-rays or toxic chemicals, most often they come from free radicals generated by our own metabolism. Proteins in the immune system also purposely cut DNA to increase the diversity of antibody genes, which are used to fight infections.

Once a cut occurs, Ku seeks it out. "Ku is the placeholder," says Leuther. DNA-PK then identifies the DNA breaks initially recognized by Ku.

If the repair effort fails, wayward DNA fragments can reattach almost anywhere. If the attachment is near a promoter, it can cause havoc; the promoter can instruct polymerase to make excess mRNA and protein from the interloper DNA, and the extra protein can lead to uncontrolled cell growth and can-

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cer. Chu plans to see whether immune cells lose Ku or DNA-PK as the cells progress to a cancerous state.

For the finer details of DNA-PK functioning, however, he is looking to the structure for inspiration. Leuther's data did not disappoint.

"Often you just end up with an oblong blob," she says. "The DNA-PK structure is very nice because it has a lot of structural features that look interesting."

The indentation, for example, looks a lot like the cupped hand of polymerase that clamps onto DNA. ("If you have a hammer," says Leuther, "all your problems look like nails.")

And then there is that hole.

"The hole is pretty obvious, so we had better find a use for it," says Leuther. Chu and Leuther's current hypothesis starts with a protein "machine" propelled by DNA-PK chewing its way along one of the two strands of the first DNA fragment. Eventually it finds a match: some DNA bases that can partner with bases from the other DNA fragment so the repair job can proceed. But this search for a match should be a short one. The strand that is not being chewed up, which is left waving in the breeze, could be the signal to stop chewing. "Maybe as a signal, the single-stranded end goes into the tunnel and says, 'Stop! Try aligning the DNA,' " says Leuther. This, Chu and Leuther believe, may be DNA-PK's way of making sure that the machine doesn't chew through too much DNA before doing the repair. Consistent with this idea, no more than 15 to 20 DNA bases are ever chopped out when a break is repaired in cells.

"A lot of this is measured speculation," says Chu. "But the structure is guiding our experiments." Chu and postdoctoral fellow Ola Hammarsten, MD, PhD, have found, for example, that single DNA strands longer than 10 bases turn down the activity of DNA-PK.

And the structure serves another purpose. Even those who are not devoted to uncovering the secrets of DNA-PK, those with little interest in biochemical arcana, can now see the three-dimensional version of the protein and appreciate its complexity. "It's so beautiful," says Chu, "that my wife wants it above our fireplace." SM