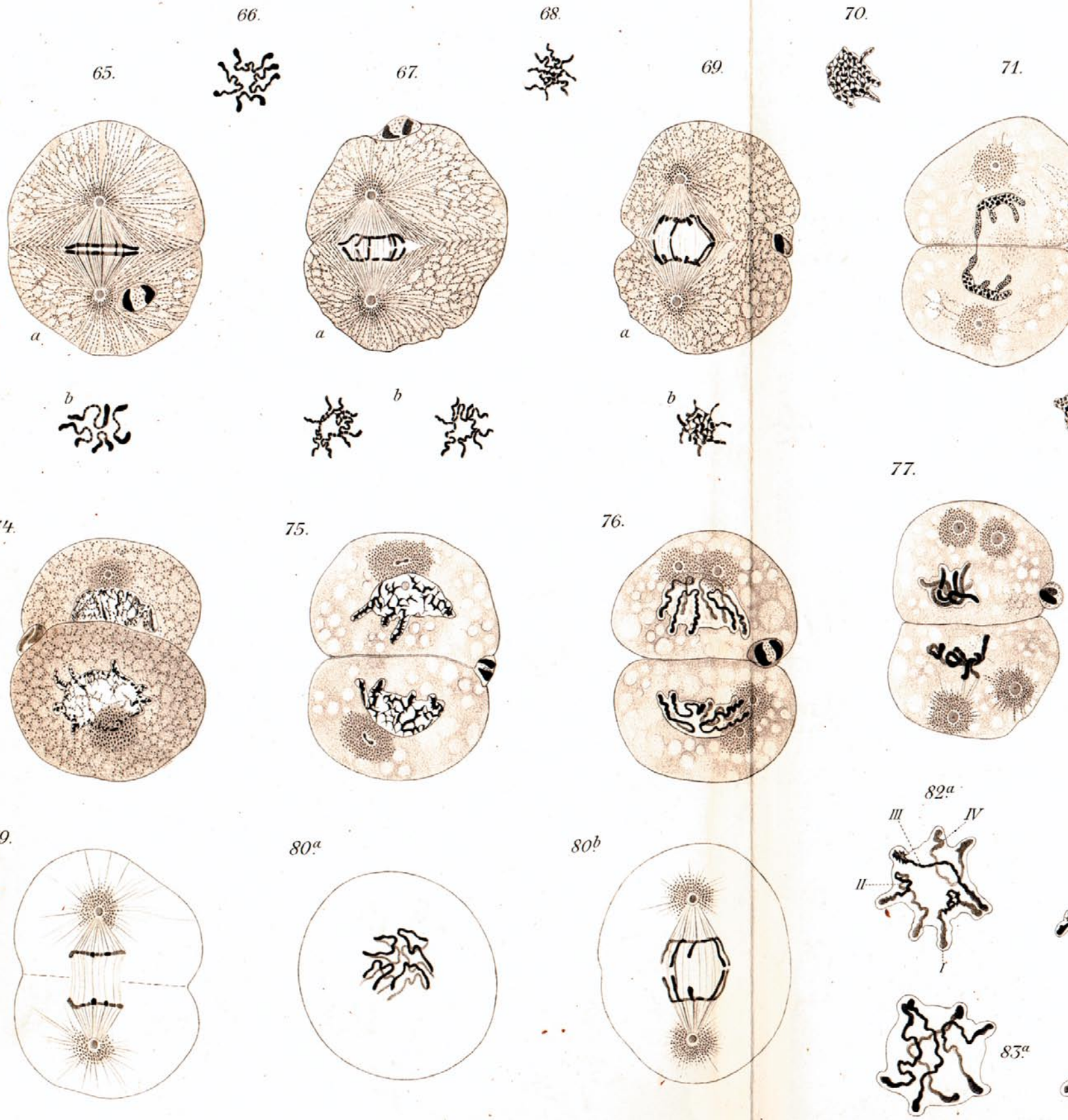
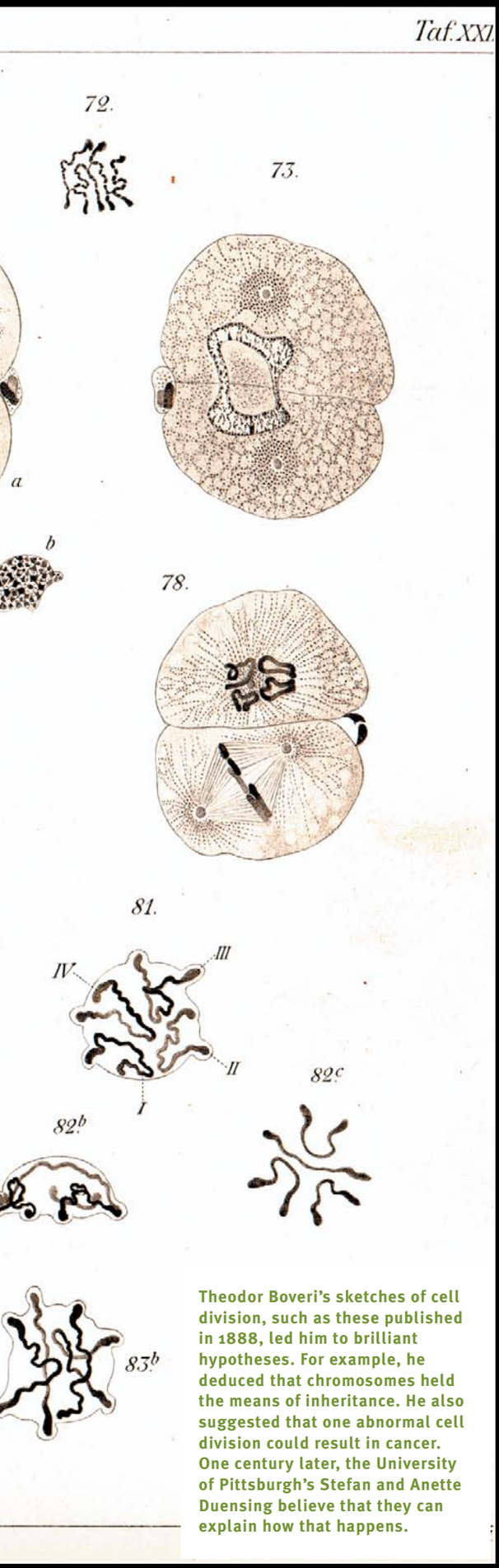


Boveri del.

Verlag von Gustav Fischer, Jena.

DRAWINGS FROM THE COLLECTIONS OF THE ERNST MAYR LIBRARY, MUSEUM OF COMPARATIVE ZOOLOGY, HARVARD UNIVERSITY. BOVERI, T., ZELLEN-STUDIEN (VERLAG VON GUSTAV FISCHER, 1888).



Theodor Boveri's sketches of cell division, such as these published in 1888, led him to brilliant hypotheses. For example, he deduced that chromosomes held the means of inheritance. He also suggested that one abnormal cell division could result in cancer. One century later, the University of Pittsburgh's Stefan and Anette Duensing believe that they can explain how that happens.

DON'T SEND FLOWERS

DRAWINGS | THEODOR BOVERI

PHOTOGRAPHY | ANETTE & STEFAN DUENSING

TEXT | CHUCK STARESINIC

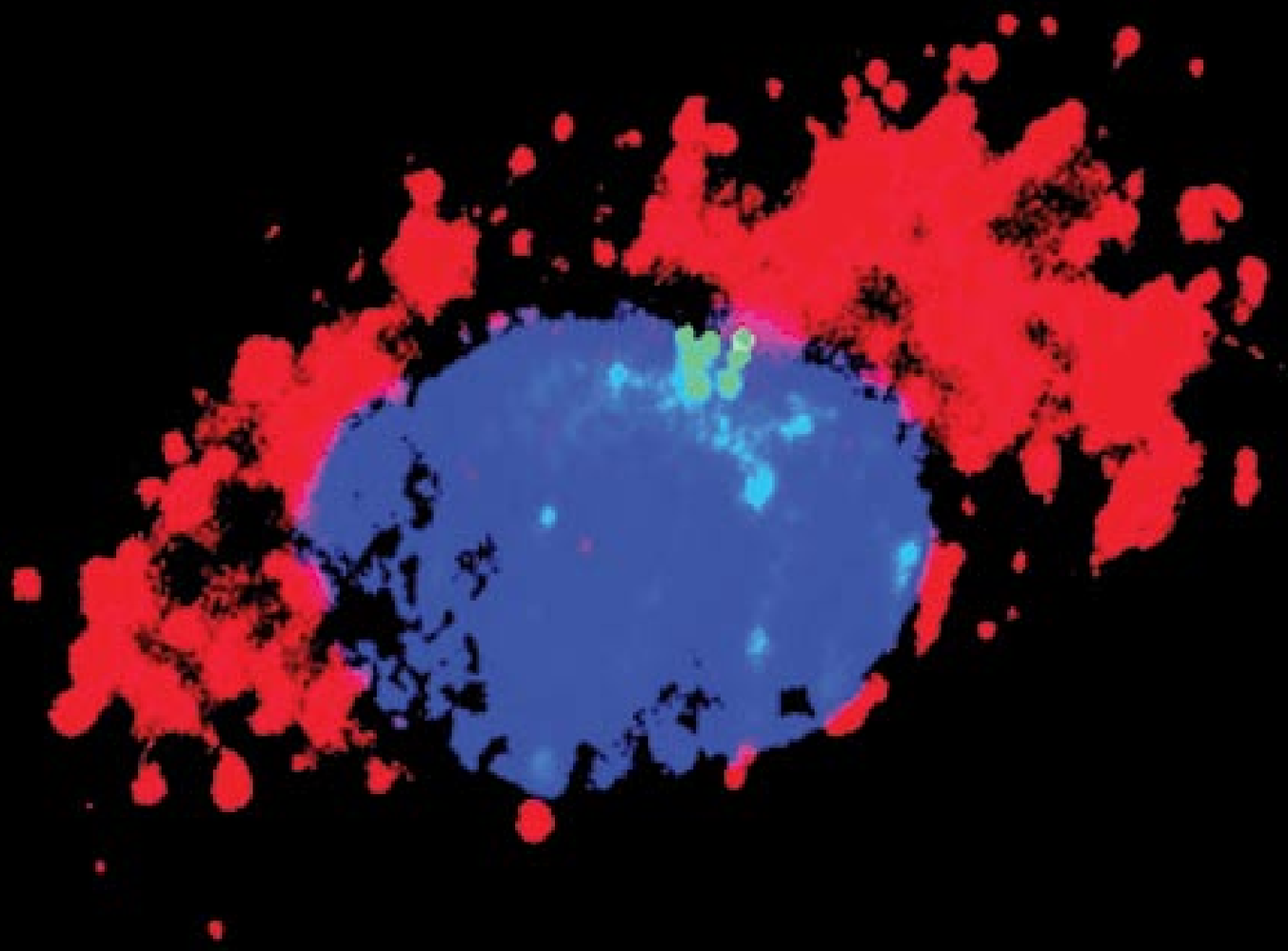
BIRTH OF A TUMOR

Beginning in the 1880s, a German scientist named Theodor Boveri probed some of the great mysteries of cell biology—the origin of cancer, for example—using just a microscope and a sketch pad.

Growing up in a distinguished family, Boveri excelled in painting and drawing. He was thought to be suited for a career in architecture or engineering. Instead, he became an academic physician. His colleagues at the University of Würzburg often photographed their microscope slides, but Boveri insisted that drawing led to more precise analysis. And drawing was a delight, he said. As he sketched, he theorized—rather brilliantly, as it turns out—about what he was observing.

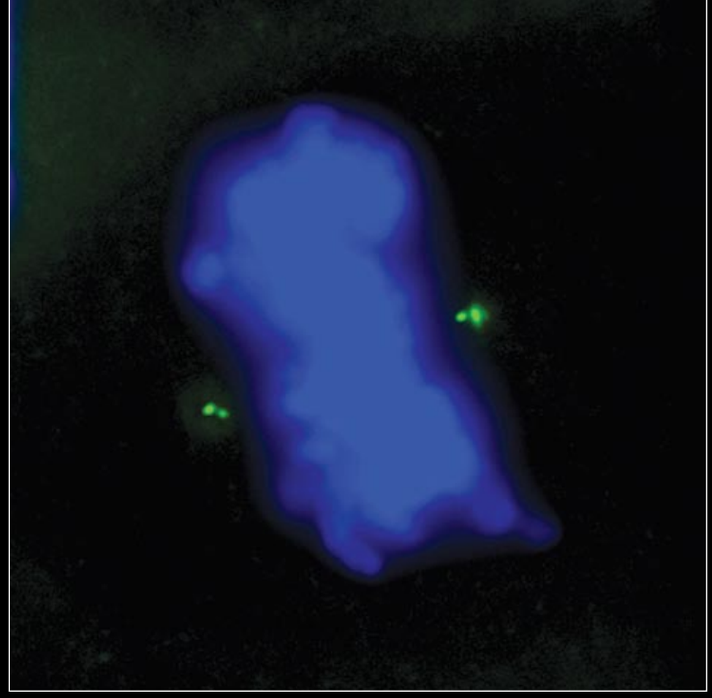
"Of course, the language is different," says the University of Pittsburgh's Stefan Duensing as he pages through a Boveri text from 1914. "But in this book, he predicts that there are oncogenes. He predicts that there are tumor suppressor genes."

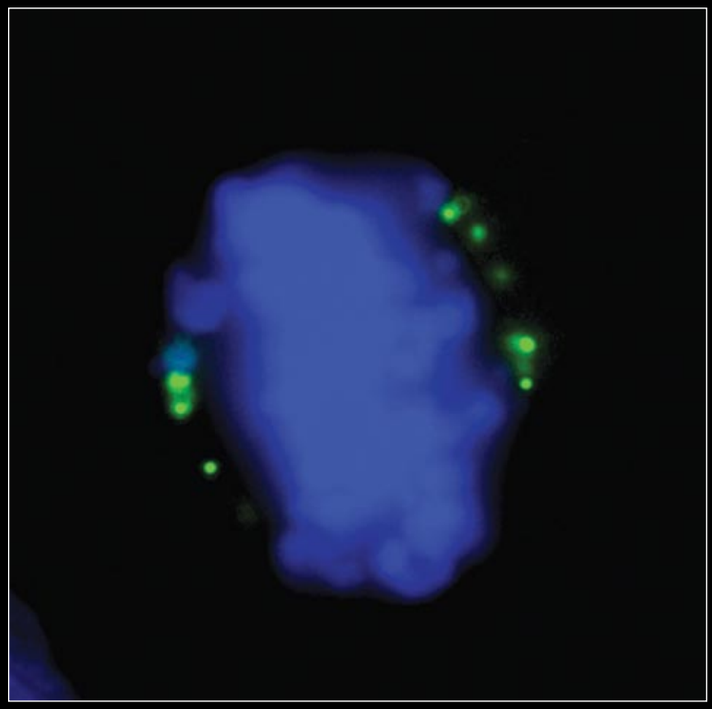
A century after his work, modern molecular biologists have verified much of what Boveri proposed. Duensing, an assistant professor of microbiology and molecular genetics, is resurrecting what was, until recently, thought to be one of the artist-MD's sketchier hypotheses.

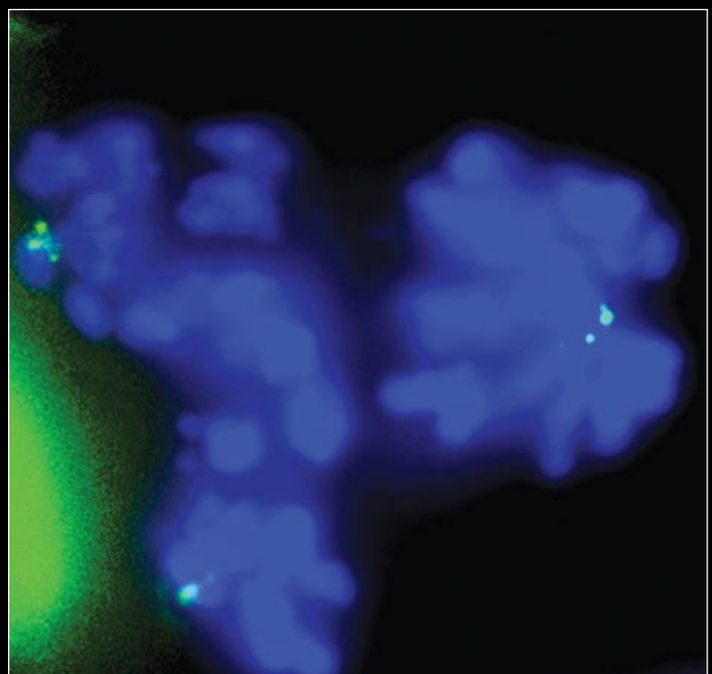


THIS PAGE: With this image, which appeared on the cover of the *Journal of Virology* in November 2003, Stefan Duensing showed that cells expressing a human papilloma virus (HPV) oncoprotein for just 48 hours produced an abnormal number of centrioles. The nucleus is stained blue, mitochondria are red, and centrioles are green.

OPPOSITE PAGE: A normal cell entering mitosis (top image) shows the centrioles (green) at opposite ends of the nucleus. In normal cell division, these centrioles will pull the duplicating chromosomes (blue) into two identical sets. Cells expressing just two viral oncoproteins from HPV-16 produce multiple abnormal centrioles (middle image). The resulting tug of war can divide the chromosomes unevenly (bottom image), perhaps creating extra and abnormal daughter cells. Boveri believed that a single such mishap could lead to cancer. The Duensings believe that is exactly what happens with cancers caused by HPV.







Nodding toward the Boveri text, Duensing notes, "He predicts that a tumor arises from a single abnormal cell, which we think is true because most tumors are clonal."

Duensing's molecular virology lab at the University of Pittsburgh Cancer Institute has uncovered mechanisms that can cause a single cell to divide abnormally and beget an abnormal daughter cell, which, if it keeps reproducing itself through cell division (mitosis), can become a tumor.

At Würzburg, Boveri sketched duplicating chromosomes. He deduced that they carried the means of inheritance, which we now know as DNA. He observed a structure he called the centrosome. In normal cell division, the centrosome duplicates in perfect synchrony with DNA. The two centrosomes then move to opposite ends of the nucleus, where they produce long spindly microtubules that pull the chromosomes into two identical sets in preparation for the cell to cleave.

In cancer, however, three centrosomes might engage in a tug of war over the chromosomes, which can lead to three daughter cells instead of two, says Duensing. And that's dangerous. The daughter cells end up with chromosomal imbalances of the sort that Boveri predicted. An abnormal cell may have an extra copy of a gene that promotes rampant growth, or it may completely lack a critical tumor suppressor gene. In either case, the cell is considered genomically unstable. Duensing believes just one single instance of centrosome overduplication can lead to cancer.

Duensing collaborates and shares a lab with his wife, Anette Duensing, a Pitt assistant professor of pathology. (Her primary interest is a rare gastrointestinal cancer.) Both received MDs in their native Germany and happened upon the work of their countryman Boveri during research fellowships at Harvard University.

To explore Boveri's single-cell hypothesis, the Duensings carefully observed the initiation of cancer, trying to see the moment when mitosis became abnormal. Because of its simplicity, they chose as their research model a cancer caused by HPV—human papilloma virus. A strain of virus called HPV-16 has two genes (oncogenes) that, once inserted into the host cell's genome, are enough to cause cancer. (Breast or lung cancers, by comparison, can have more than 200 such mutations.) The Duensings wanted to know whether these oncogenes could cause the abnormal number of centrosomes seen in HPV cancers. In fact, both did, and one caused it rapidly, within 24 to 48 hours. Those findings were published in 2000 in *Proceedings of the National Academy of Sciences.*

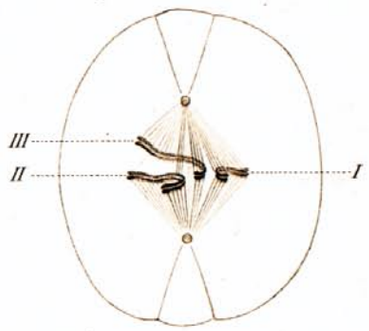
"This was the first report that an oncogene relevant to human cancer could stimulate centrosome overduplication," says Duensing.

In a series of experiments, the Duensings showed that extra centrosomes appeared before the cell became genomically unstable. One problem with this story: Scientists who studied the centrosome said there was no way to produce that many centrosomes in a day or two. Textbooks reported that centrosomes doubled once per cell division cycle, which lasts about 24 hours. There was no way to get five, six, or seven centrosomes—like what the Duensings observed—that quickly.

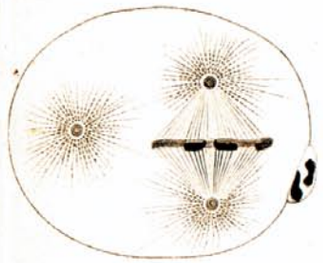
The obvious solution? Rewrite the textbooks. That's what's

OPPOSITE PAGE: COURTESY JOURNAL OF VIROLOGY, NOVEMBER 2003, VOL. 77, NO 22, © 2003, AMERICAN SOCIETY FOR MICROBIOLOGY. THIS PAGE AND P. 27: ADAPTED BY PERMISSION FROM MACMILLAN PUBLISHERS LTD: ONCOGENE, 26, 6280-6288 "CENTRIOLE OVERDUPLICATION THROUGH THE CONCURRENT FORMATION OF MULTIPLE DAUGHTER CENTRIOLES AT SINGLE MATERNAL TEMPLATES," © 2007.

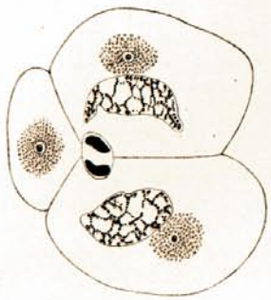
84b



85.



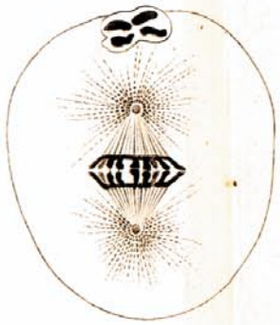
86.



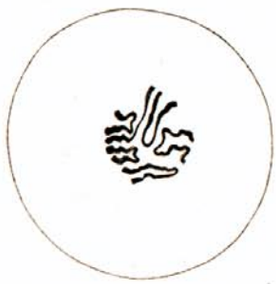
88.



89a



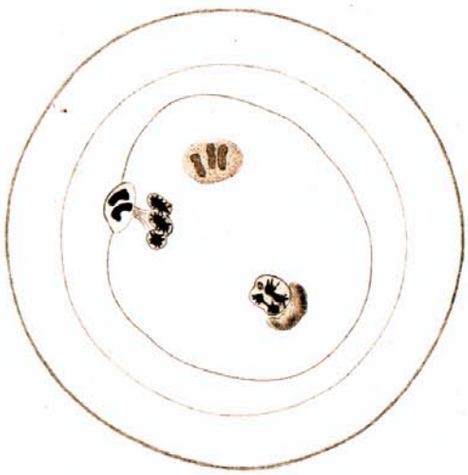
89b



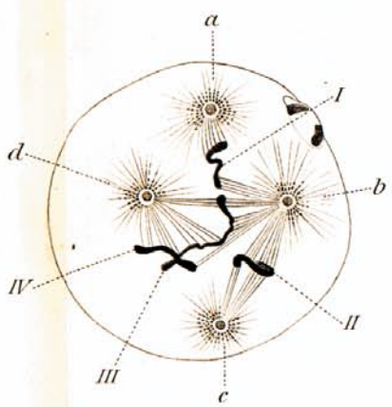
90.



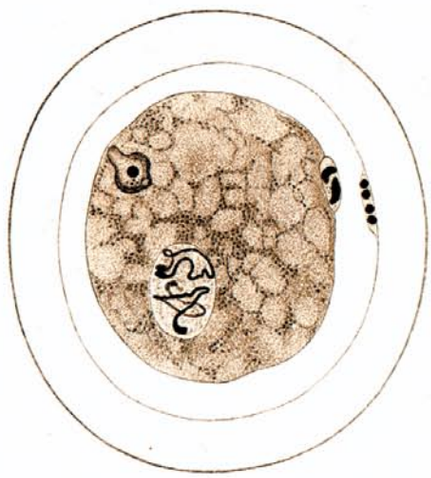
92.



93.

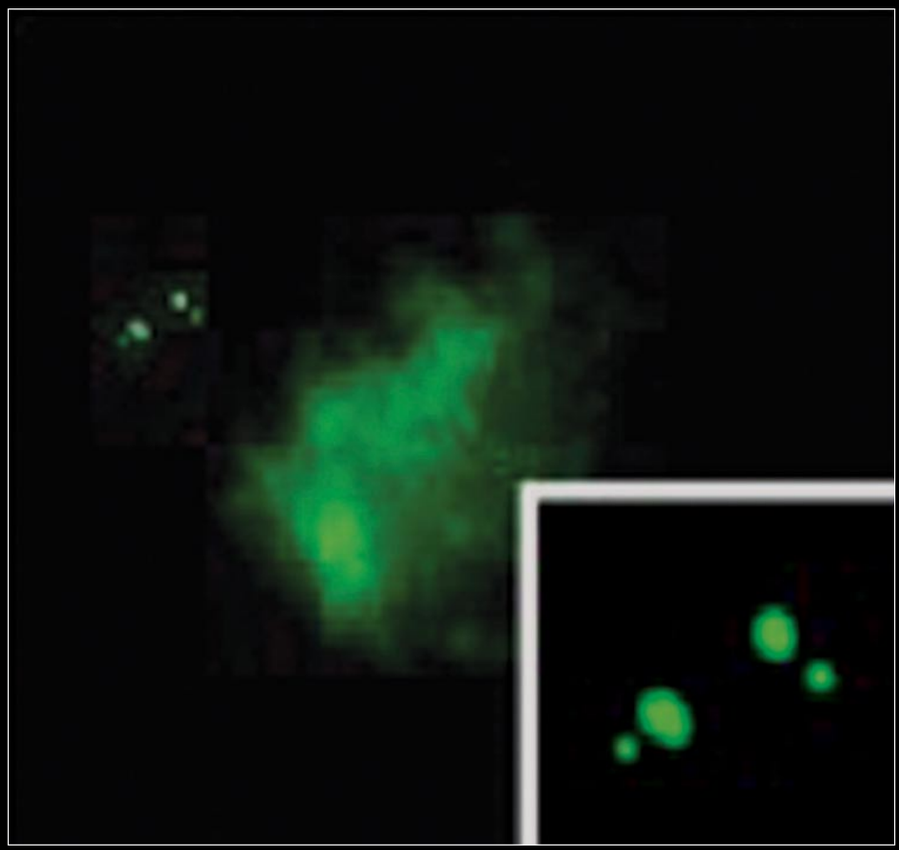


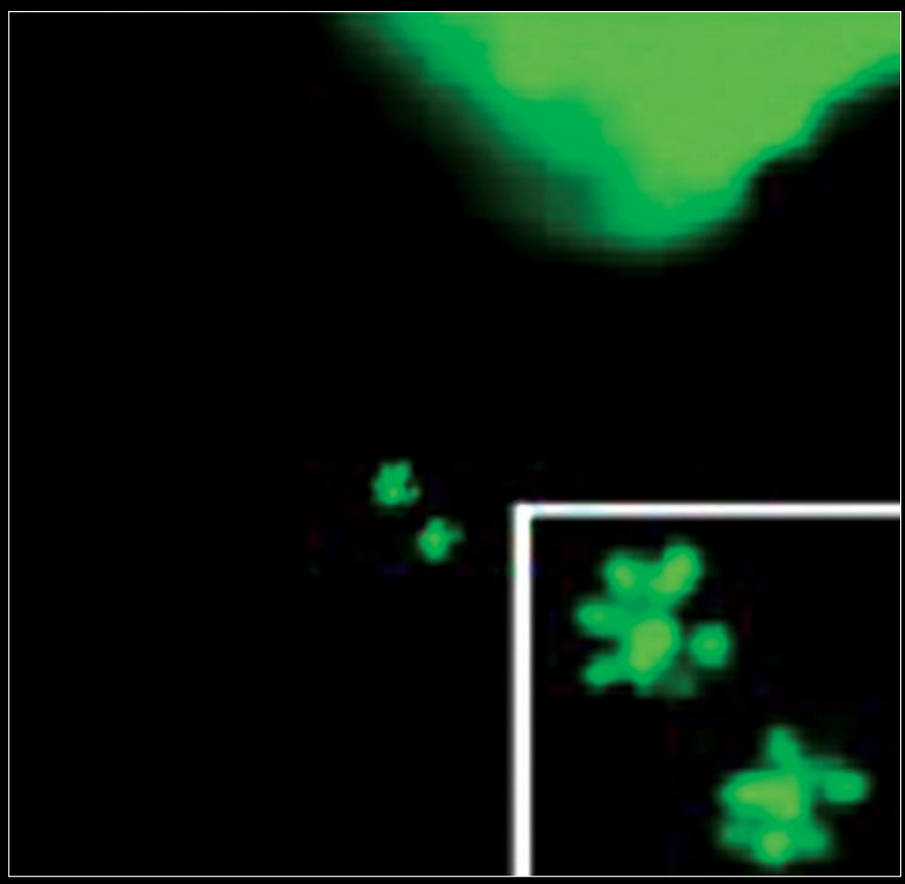
94.



Verlag von Gustav Fischer, Jena.

Lith. Anst. v. Werner & Winter, Frankfurt a/M.





been happening since Stefan Duensing made the biggest discovery of his young career in the summer of 2005. He describes it as a typical scientific experiment—one that required him to go nearly cross-eyed looking at hundreds of slides.

While examining extraordinarily thin slices from cells that had been treated with a drug so they would divide abnormally, he hoped to stumble upon a centrosome perfectly sliced to reveal a tiny structure within called a centriole. (A normal centrosome has two centrioles; they move apart and duplicate at the start of centrosome duplication.)

"It was one of these long, hot, and humid August Pittsburgh Saturdays," says Duensing. "I was about to go home, and I said, 'One more slide. Let's do it.' I looked under the electron microscope, and there it was—this flower under the electron microscope."

The "flower" was a centriole that was doing more than just doubling as the textbook said it should. Multiple, smaller daughter centrioles surrounded it like petals on a daisy.

Here was the evidence the Duensings had hoped for. Now the couple had convincingly shown that an abnormal number of centrioles could occur in just one cell division cycle. The centriole flower—a term the Duensings have introduced into the scientific vernacular—would help explain how cell division gone awry initiates cancer.

Boveri presumably came to his hypothesis about abnormal mitosis leading to cancer as he gazed through a microscope in Würzburg and meticulously refined sketches of centrosomes pulling chromosomes into abnormal bunches. One century later in Pittsburgh, Duensing snapped a picture.

"It was just the best day I ever had," he says. "I took a ton of pictures. I went home, showed it to Anette, and said, 'Here we go. The paper is done.'" ■

OPPOSITE PAGE: Boveri drew pictures of cells undergoing abnormal mitosis, including some that had too many centrosomes. In the image marked with a numeral 93, four centrosomes tug at the chromosomes, potentially leading to abnormal cells and even cancer.
LEFT AND BELOW: On a muggy Saturday in Pittsburgh, Duensing looked through an electron microscope and snapped a grainy black and white photo of what he termed "a centriole flower." The center of the "flower" is a structure within the centrosome called a centriole (also shown in green), which produces one daughter in normal mitosis. Duensing had found something unheard of: a centriole producing multiple daughter centrioles that surround it like daisy petals. This could be the first step in the overduplication of centrosomes.

