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TUNEL vision spots apoptotic cells

In 1992, Gavrieli et al. described a new assay to detect cell death in vivo.

By the early 1990s, scientists knew that programmed cell death, or apoptosis, was important for tissue development and homeostasis as well as for cancer progression. Because apoptotic cells activate nucleases to cleave their chromatin, cell death could be followed in vitro by extracting DNA from a population of tissue culture cells and running it out on an agarose gel to look for a characteristic "ladder" of nucleosome-sized fragments. But detecting individual apoptotic cells, especially within tissues, was much more challenging (1), at least until Gavrieli et al. described the TUNEL assay (2), a development that senior author Muli Ben-Sasson from The Hebrew University of Jerusalem describes as a "fortunate accident."

"I wanted to study the patterns of DNA methylation at different stages of cell maturation in situ," Ben-Sasson explains. His approach was to treat paraffin-embedded tissue sections with restriction enzymes showing different sensitivities to methylated DNA. The nicks generated by these enzymes could be detected using the enzyme terminal deoxynucleotidyl transferase (TdT) to incorporate biotinylated deoxyuridine at the cleavage sites, which could then be visualized by staining with the biotin-binding protein avidin conjugated to horseradish peroxidase.

"Luckily, I used the small intestine as a model tissue," says Ben-Sasson, referring to the self-renewing tissue's well-defined architecture, in which new cells generated at the base of intestinal crypts move up toward the villi tips, where they die and slough off into the intestinal lumen. "Of course, I did a control experiment with no restriction enzymes, and, to my surprise, I saw that the cells at the tip of the villi were beautifully stained."

Fortunately, Ben-Sasson didn't miss the significance of this result. "I was aware of the concept of apoptosis and knew that it involved DNA fragmentation, so it immediately struck me that I had a method to identify apoptotic cells in situ. There was a great need for this because nobody was able to track apoptosis in a physiological or pathological context. The lesson is that, even when you're doing research in your own narrow field, it helps to have a broader biological perspective."

Knowing how important this method could be for understanding apoptosis—the initial staining already indicated that older intestinal cells die as part of their internal differentiation program instead of being pushed to their death by younger cells moving up toward the villi tips—Ben-Sasson assigned graduate student Yael Gavrieli the task of improving the assay and demonstrating its applicability to a variety of tissues, even those in which apoptosis is a rare event.

However, when the researchers started to write up their findings, they discovered, buried deep within the existing literature, two papers from the 1970s describing a similar technique that detected DNA fragmentation in the lens (3, 4). Although they could have easily disregarded these forgotten studies, boosting their chances of obtaining a patent on the assay, Ben-Sasson says that they had no hesitation in citing the older papers: "For me it was a moral issue."

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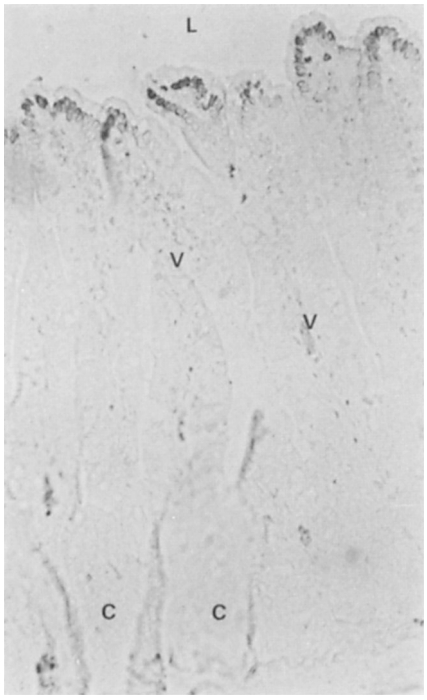
Nevertheless, to ensure they retained long-lasting recognition for their new and improved technique, even after some of those using it would stop crediting the original paper, Ben-Sasson took care to brand the assay with a catchy name: the TUNEL assay, for TdT-mediated dUTP-biotin nick end labeling.

The response was immediate. "Within a very short time of publication, I received hundreds of reprint requests and also many nice letters that complemented us for developing the method," Ben-Sasson recalls. "It was a straightforward assay that worked in people's hands the first time, so it was quickly adopted by the field."



The TUNEL assay labels apoptotic cells at the tips of villi in the small intestine.

The TUNEL assay has remained in use ever since, and Gavrieli et al.'s original study is the most cited *JCB* paper of all time. "The TUNEL assay translated DNA fragmentation, a hallmark of apoptosis, into a format that could be used on fixed cells and was compatible with immunostaining," says Junying Yuan, a senior editor at *JCB* and an expert in apoptosis research. "It therefore became one of the most popular assays in the apoptosis field."

1. Kerr, J.F.R., et al. 1987. Apoptosis. *In* Perspectives on Mammalian Cell Death. 93–128.
2. Gavrieli, Y., et al. 1992. *J. Cell Biol.* 119:493–501.
3. Modak, S.P., and F.J. Bollum. 1972. *Exp. Cell Res.* 75:307–313.
4. Appleby, D.W., and S.P. Modak. 1977. *Proc. Natl. Acad. Sci. USA*. 74:5579–5583.