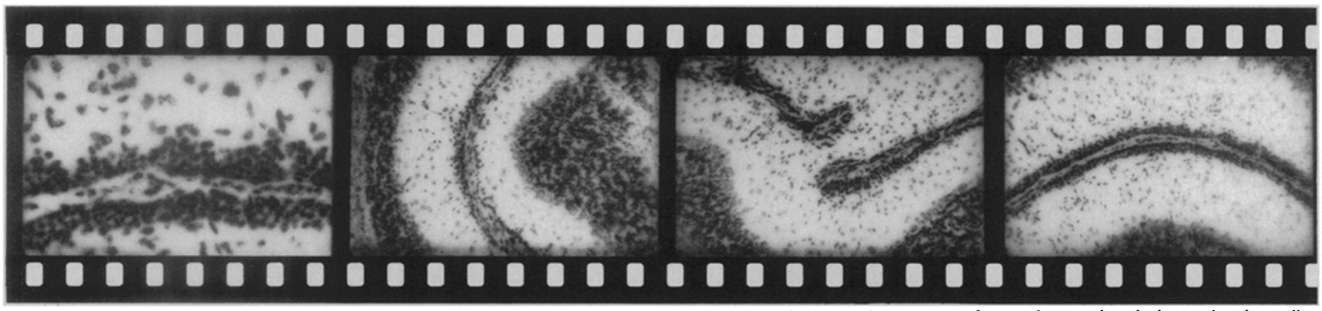
Picturing Protein



W.S.T. Griffin/UTHSCD

Black dots show early stages of protein synthesis in rat brain cells.

By JOANNE SILBERNER

In trying to pinpoint just when in a cell's life certain genes are "turned on," researchers have been limited to indirect approaches: They can measure the protein engineered by the gene, or they can find the gene itself.

But these methods are like trying to figure out what's going on in a car factory by looking for new cars in the lot or for the machinery needed to put the cars together. The presence of the protein in a cell sample doesn't mean it was made there, and the presence of the gene doesn't mean it is functioning. Without actually seeing the factory at work, there's no way to tell where the cars, or the protein, were manufactured.

Enter a process called *in situ* ("in place") hybridization, which now allows researchers to see the factory at work.

DNA is like a ladder, with two strands joined by "rungs." Each rung of the ladder lines up with a specific mate, so a piece of DNA that has been split down the middle will bind with, and only with, a specific matching half, whether artificial or natural. During protein manufacture, DNA splits down the middle, and messenger RNA (mRNA) lines itself up along one of the open strands. Messenger RNA then splits off and directs the synthesis of the new protein.

To do *in situ* hybridization, a radioactive DNA piece that is complementary to the messenger RNA for the gene in question is added to a slice of tissue. The resulting radioactive mRNA-DNA complex creates a black dot on photographic emulsion above the cell — a picture of a factory at work.

DNA probe techniques, used to identify particular pieces of DNA or RNA in a cell sample even if the genetic material is not active, are proving to be powerful tools for medical diagnosis (SN: 8/18/84, p. 104). *In situ* hybridization uses DNA probes to essentially take a snapshot of the gene in action in a slice of tissue.

The process, developed in the mid to late 1970s, is coming into its own as a tool for basic research. It was a big hit at the recent 7th International Congress of Endocrinology, where researchers spilled out into the hallways at sessions on the procedure.

By doing *in situ* hybridization on a tissue section (researchers in Australia are doing it on cross sections of entire mice), researchers can nail down protein manufacturing to specific cells. And in so doing, they are in the process of defining what goes on in particular cells in the brain and its underlying neighbor, the pituitary gland. The undertaking may keep them busy for a long time, since both organs are heterogeneous — adjacent cells can differ markedly in function.

In addition to exploring this heterogeneity, the technique is being used to study such mysteries as the how and when of brain development, and changes in the early stages of Alzheimer's disease.

Changes in the activity of individual cells can be masked by the activity of neighboring cells. Take, for instance, the case of brain cell production of mRNA. "If half the cells in a sample produce more mRNA in response to a signal, and the other half are decreasing their production, you'd get no net change," says Josiah Wilcox, who is working in Jim Roberts's laboratory at Columbia University in New York.

"If you want to look at gene expression in the brain, the problem is to dissect out a functionally related subgroup that responds uniformly," notes Wilcox. But with a heterogeneous group of cells like those in the brain, it gets beyond the ability to dissect.

Wilcox and others in Roberts's lab are looking at the interaction between estrogen and the production of a peptide known as one of the brain's "natural opiates," beta (β)-endorphin. When estrogen is administered to animals, β-endorphin production is reduced in the hypothalamus. Work from another laboratory showed that only a very few cells in that area of the brain have estrogen receptors and thus are sensitive to the hormone.

So somewhere in the hypothalamus is a handful of cells that produce less of the mRNA for β-endorphin when estrogen is administered. But how do you find them? You can't just take a hypothalamus and remove the cells that produce β-endorphin, because these cells are mixed in with other cells, explains Wilcox. *In situ* hybridization is "our next dissection down."

Another such case is the production of a hormone called ACTH (adrenocorticotrophic hormone), produced in the pituitary. ACTH stimulates production of substances called glucocorticoids in the adrenal glands. Removing the adrenal glands, which removes glucocorticoids, stimulates greater ACTH production by the pituitary in a vain attempt to restore glucocorticoid levels. There is a tripling in

Production

'Snapshots' of genes in action are showing how diverse brain cells can be

the amount of its mRNA.

But are all the cells—all the production lines in the factory—producing equally? Researchers in Roberts's lab found, using classical biochemistry, that the anterior portion of the pituitary increases ACTH production sixfold, while there is no change in the rest of the gland, netting the threefold increase. "The situation changes as you do smaller and smaller dissections," says Wilcox. But it, too, gets beyond the ability to dissect, so now they are doing *in situ* hybridization to see what cells of the anterior pituitary are involved.

The technique can take care of the what and the where, and some scientists are moving on to the when. At the University of Texas Health Science Center at Dallas, Sue Griffin and Marcelle Morrison are using it to follow the development of the cerebellum, a portion of the hindbrain that monitors movement. They are studying the production of tubulin, a structural protein involved in cell division, growth and transport of molecules within the cell.

"In the past," says Griffin, "you could grind up the tissue and look at gross levels, but you couldn't tell if cells doing a developmental task had an increase in message [messenger RNA].

"With the DNA probe, we can look at individual cells of the brain."

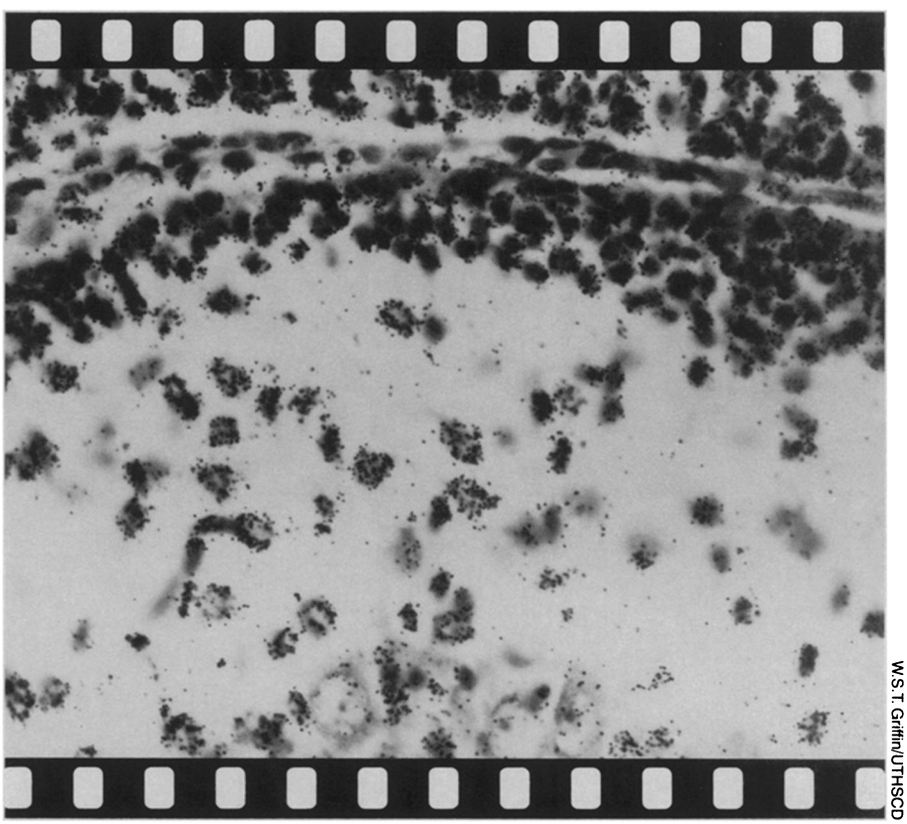
Griffin and Morrison looked at the cerebellum of the rat because the timing of its development—when the cells divide—is well known. "We found, just as expected, tubulin message increases in cells that are dividing. What this means is that the development of this specific protein is regulated at the level of the gene, rather than through degradation of the protein." The gene is turned off and on when needed, not constantly producing.

They are also using *in situ* hybridization to look at the development of Alzheimer's disease. While the affected area of the brain (SN: 9/15/84, p. 167) and a biochemical deficit (SN: 9/1/84, p. 132) have been defined, what starts the degenerative process is not known. Once one cell has a problem, it can cause a cascade of problems, killing off cells it is in contact with—the cells die without input.

Some neurotransmitters are found in reduced quantities in people with Alzheimer's disease; Griffin and Morrison are using *in situ* hybridization to follow the life histories of the enzymes involved in the production of these neurotransmitters in hopes of pinpointing early irregularities. "We need to get to the cell type that is the initiator of the cascade of events in Alzheimer's," says Griffin.

"It will be another problem, but at least if we can get to that cell we'll get beyond the innocent bystanders," she says. "We can start by looking at the genetic expression. That would give us a leg up on what causes Alzheimer's."

Clinical applications are a long way off, notes Griffin. "But they are now possibilities." □



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In situ hybridization of the cerebellum of a 14-day-old rat. Black dots indicate mRNA, which directs protein synthesis. High concentration of dots in the external granular layer at top of photo shows intense activity in these cells.