Cell's Gene-Copying Machinery Anchored

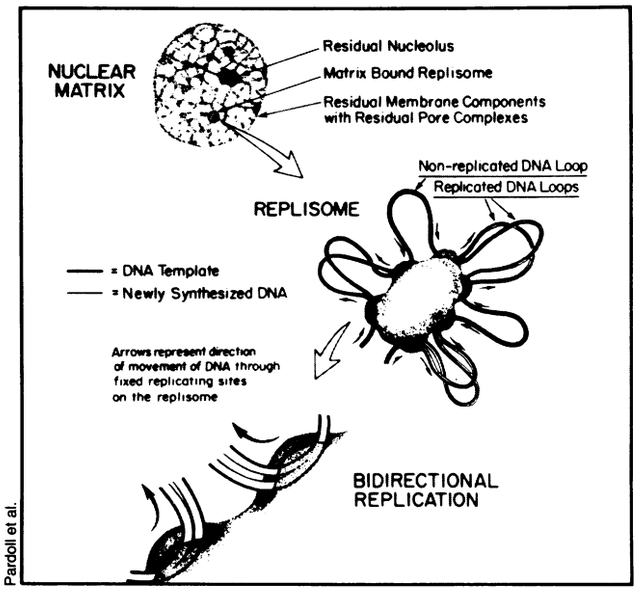
Keeping the thread of life untangled is one of the amazing accomplishments of reproducing cells. In one of the most impressive examples of delicate organization, 50 inches of DNA double helix is coiled into a cell nucleus only 0.0004 inches in diameter. Before a cell divides, all that genetic material must be unwound, copied and the two resulting strands held untangled, but coupled in a precise fashion for later distribution to the daughter cells.

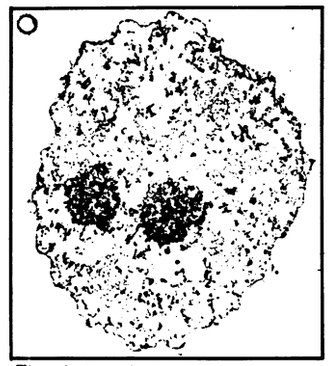
Scientists at Johns Hopkins University last week proposed a new model for that replication feat. They likened the complex of enzymes involved in DNA replication to the recording head of a tape recorder — the DNA reels through a fixed site. However, their model for replication is far more complex than operation of a single tape recorder. Drew M. Pardoll, Bert Vogelstein and Donald S. Coffey say that replication occurs simultaneously at many sites on a DNA molecule, and they suggest that between each two attachment sites DNA is cranked in from both sides, so that the newly produced strands form a loop. "Each nucleus contains many thousands of these loops and replicating sites, and this configuration allows the cell to duplicate its DNA in an orderly manner without entanglement," Coffey explains.

The nuclear matrix is the structure that seems to support all the replication complexes. It is made of protein and RNA and it extends from the surface of the cell nucleus to the center. In experiments using regenerating liver in rats, and also cells called fibroblasts growing under laboratory conditions, Coffey and colleagues employed detergents, salts and enzymes to strip cell nuclei of most of their components. All that was left were the matrix and tightly bound DNA. They biochemically nibbled at the loops of DNA until the only bits remaining were the segments where the loops attached to the matrix. Using radioactively labeled DNA components, they discovered that those DNA segments are the portions of the helix most recently synthesized. The researchers estimate that small segments of newly copied DNA arise simultaneously and are attached at as many as 15,000 sites on the nuclear matrix. Their data will be published in the February issue of CELL.

Earlier experiments in several laboratories demonstrated that in bacteria, which have no cell nuclei, the cell membrane plays a crucial role in organizing the many enzymes necessary for DNA replication. Scientists working on plant and animal cells, however, had been unable to find an unequivocal role for the nuclear membrane that surrounds plant and animal chromosomes. So DNA replication had been envisioned as the action of pairs of DNA-copying complexes moving in opposite directions along a double helix. Pardoll, Vogelstein and Coffey point out that if animal and plant cells use as many enzymes as bacteria do to replicate DNA it might be more economical energetically to anchor the replication complex and reel through the DNA, rather than the reverse situation.

Coffey and collaborators are now working to isolate replication sites from the nuclear matrix in order to analyze how the enzymes convert a double helix of DNA into daughter molecules. Further examination of the matrix could give clues to tumor growth, the scientists believe, especially because tumor cells have abnormally shaped nuclei. "In the sense that the nuclear matrix may be involved in determining the shape of the nucleus as well as in regulating DNA synthesis, it seems important to investigate further the structural nature of the matrix and how its alteration may be involved in the control of DNA synthesis," Coffey says. □





Fixed sites (called replisomes) in cell nucleus bind and copy DNA into loops, according to proposed model. Web-like structure that holds DNA coils is visible in electron micrograph.

EPA monitors wastes from cradle to grave

A roadmap to track hazardous wastes from cradle to grave is how Environmental Protection Agency Administrator Douglas M. Costle described three new regulations issued this week to stem illicit hazardous-waste disposal. They place on the nation's 750,000 generators of dangerous wastes the responsibility for reporting production of the wastes and for ensuring that such wastes are ultimately disposed of in an environmentally sound manner.

The rules set standards for generators and transporters of hazardous wastes and require thorough documentation of waste shipments. EPA leaves determination of whether a waste is hazardous up to the generator; in April EPA will issue a list of hazardous chemicals and criteria for measuring whether unlisted materials also come under the regulations. If hazardous, wastes must be so labeled and shipped to a licensed waste-disposal facility. A manifest accompanying the wastes must name the generator, all transporters and the waste-disposal facility in addition to describing the wastes. A signed copy of the manifest must be returned to the producer upon receipt of the wastes by the disposal firm. If the returned manifest doesn't arrive within 35 days, the generator must begin tracing the wastes. If it hasn't arrived by the 45th day, EPA must be notified and begin an investigation. Criminal and civil penalties associated with noncompliance can total $25,000 per day.

"The [manifest] system prevents illicit disposal and midnight dumping," Costle says, "because responsibility is fixed, and evasion requires extensive collusion." The economic incentive for illicit dumping is pointed out by the estimated cost of complying with EPA's new rules. The chemical industry, which generates an estimated 60 percent of all hazardous wastes, grossed about $146 billion in sales last year, Costle said. Proper disposal will cost that industry two to three percent of their total sales, Costle speculated, or an additional several billion dollars annually. □