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A cell line that is under control

Cell lines were nothing new in 1961, when a young medical student called George Todaro joined Howard Green's New York University laboratory to work for a single semester. Ten years earlier George Gey had isolated cervical carcinoma cells from Henrietta Lacks and turned them into the HeLa cell line. But this and subsequent cell lines made from noncancer cell types were transformed—they did not show contact inhibition in vitro and they caused tumors when injected in vivo. "These lines were not evolved according to any protocol that would ensure defined properties," says Green.

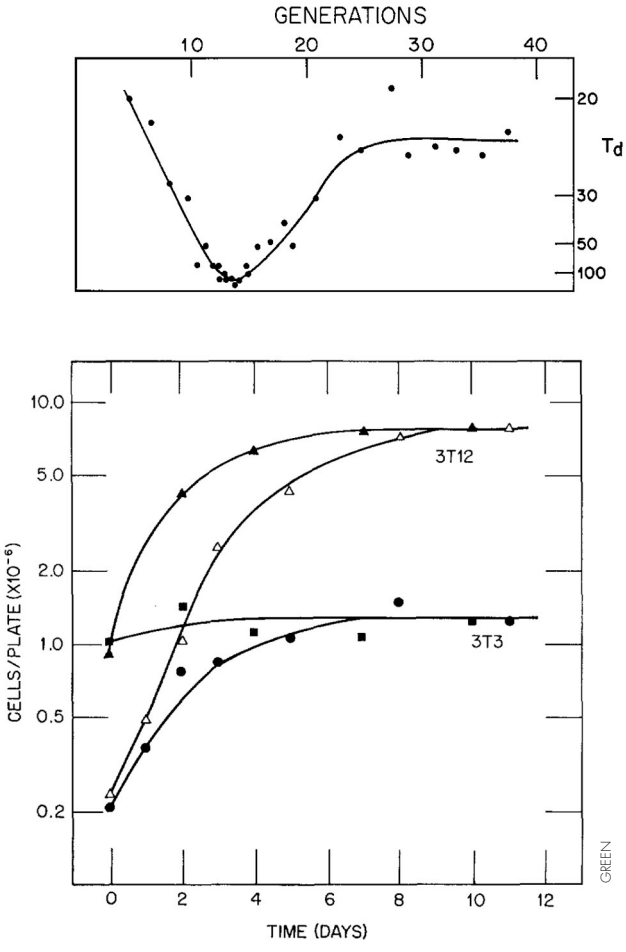
George Todaro and Howard Green establish the 3T3 cell line—the first well behaved, contact-inhibited cell line.

In this mess of uncontrolled growth it was difficult or impossible to pick out cells that had been transformed after infection with oncogenic viruses, thus slowing the study of this transformation process. Todaro and Green (1963) took a different approach. "I wanted to do an experiment that somebody else could reproduce," says Green. He and Todaro controlled both inoculation density and frequency of transfer so that cell density would be strictly regulated. This was unlike the culturing techniques used by most researchers. "They would just transfer whenever they liked—it was haphazard," says Green. "It's the way most people still do it."

Establishment of cell lines was thought to be a rare event, but with their strict protocol Todaro and Green succeeded in deriving stable cell lines in 9 of 11 attempts. Cells inoculated at higher density also grew optimally at higher density, but the most interesting line was one that was inoculated at a lower density of 3×10^5 cells per plate and transferred every three days. These 3T3 cells grew slowly at first, then recovered and grew stably to reach a reversible resting state. This state was reached at a much lower cell density than for other cell lines, and uniquely, these cells never grew over each other and did not show a reduction in size.

These unexpected and unique properties prompted a reassessment, still in progress today, of what steps are necessary for both the establishment and transformation processes. As Todaro and Green wrote in their paper, "the malignant properties of many established lines may be the result of the selective processes usually operating in cell culture and not related to the process of establishment per se."

As Todaro's single semester turned into a postdoc of several years he found that 3T3 cells were an excellent target for transforming viruses (Todaro et al., 1964), as the growing, transformed cells could easily be picked out from the background of resting cells. 3T3 cells were similarly useful when cellular oncogenes were discovered, and Green also developed them as a model for adipose differentiation (originally dismissed as lipid accumulation secondary to cell death). They became a widely used feeder cell for the culturing of other cell types; Rheinwald and Green (1975) used them in this role to derive the first human keratinocyte cultures, which led to treatments for burn patients. 3T3 cells, in their many guises, have now been cited in over 25,000 publications. JCB



3T3 cells were established after a temporary dip in reproductive ability (top); once established they entered a resting state at a low cell density (bottom).

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Text by William A. Wells
wellsw@rockefeller.edu

Defining junctional complexes

There are four major types of cell–cell junctions in the polarized epithelial cells of vertebrates. Farquhar and Palade (1963) defined three of them.

The history of cell–cell contacts goes all the way back to the 1830s. For much of the 19th century an intercellular cement was thought to surround and bind cells over their entire surface area. The opposing idea of specific sites of adhesion was, however, hinted at even at the birth of the cell theory. Theodor Schwann (who with Matthias Schleiden enunciated cell theory in 1839) suggested that animal cells might fuse at specific points via cytoplasmic bridges. Several investigators singled out the spines of certain epidermal cells as contact points and potential intercellular channels. Bizzozero confirmed that these densely staining regions were contact points but found that the cytoplasm was not continuous between the cells. The structures were first named the nodes of Bizzozero; they were subsequently generalized to other tissues and in 1920 renamed desmosomes (bonding bodies).

Electron microscopy supplied the means to look at adhesion structures, but by the early 1960s the field had settled only on desmosomes (Wood, 1959) and something called a terminal bar, which may or may not have been a collection of desmosomes. Other investigators came across structures that they named quintuple-layered cell interconnections, external compound membranes, and nexuses. But there was no study that brought together and organized these various observations.

Farquhar and Palade remedied this situation by defining, based on their very different characteristic appearances and relative locations, three structures. The zonula occludens (closing belt; now known as the tight junction) was nearest the apical surface of the columnar epithelium. It featured closely apposed membranes, and was shown to act as a diffusion barrier. Next was the zonula adherens (adhering belt; now known as the adherens junction) with an intercellular space separating strictly parallel membranes, all framed by dense cytoplasmic matrices. Most basal were the desmosomes (here named the macula adhaerens [adhering spot], a name that did not last). Whereas the first two junctions were continuous and belt-like, the desmosomes were dispersed buttons of adhesion.

Farquhar and Palade (when they were both at Rockefeller University, New York, NY) had originally set out to study how the kidney glomerulus filters the blood to form urine. They induced glomerular damage and saw that filtration of tracers by the basement membrane was compromised. But in response the epithelium formed intercellular connections that were "tightened and increased in depth" (Farquhar and Palade, 1961). That morphological change associated with increased permeability made Marilyn Farquhar take a closer look. To see the tripartite structure of the junction properly, she says, "you need just the right section. It takes a lot of looking. You have to have a reason for looking, and people hadn't had that functional connection before."

The new work surveyed junctions in 13 different epithelial tissues in exhaustive detail. Farquhar says that she and George Palade were in no hurry to publish a less complete account. "It was not like now where there are multiple groups breathing down each others' necks," she says. "Nobody was doing work on this topic at this level of detail." As a result, she says, the 1963 paper "was pretty much accepted from the beginning as the definitive work in the area, because nobody had done anything comparable."

Farquhar acknowledges that in the 1960s "it was much easier to find new things with the electron microscope. But it was just as demanding as it is now to put that structure into a functional context." Many people had excellent microscopes and high quality preparations, but "once it got past the looking they were stuck." Adding the functional data, such as the impermeability of tight junctions to protein tracers, "was really a hallmark of the Rockefeller school at that time," she says.

The initial study reported on the orderly organization of a columnar epithelium, but a similar hierarchy was found in the less rigidly organized stratified cells of the epidermis (specifically in the basal layer; Farquhar and Palade, 1965). The depth (number of occluding strands) in a tight junction was later found to correlate with the leakiness or tightness of a tight junction (Claude and Goodenough, 1973). Eventually, the molecular components of the junctional complexes were determined: the occludin (Furuse et al., 1993) and claudins (Furuse et al., 1998) of tight junctions; actin-linked cadherins of adherens junctions; and intermediate filament-linked cadherins of desmosomes. JCB

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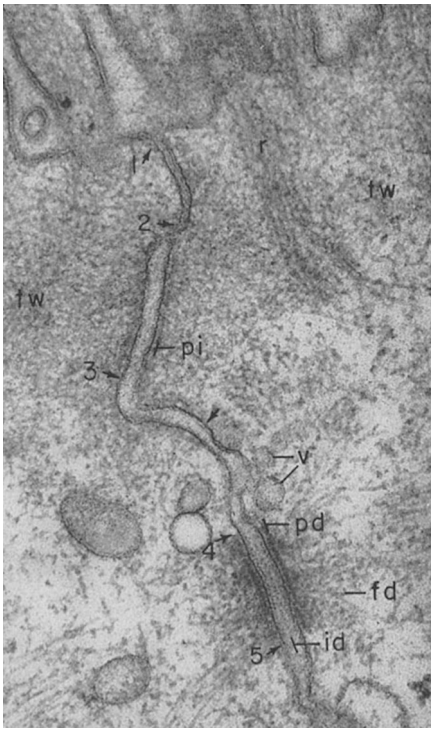

FARQUHAR

Three in one: The EM of an intestinal epithelium reveals a tight junction (arrows 1 to 2), adherens junction (arrows 2 to 3), and desmosomes (arrows 4 to 5).