

From the Archive

Heterochromatin is late

In 1953—the year that Watson and Crick published those findings “of considerable biological interest”—Howard and Pelc (1953) showed that DNA synthesis occurred in a discrete phase of the cell cycle. They had labeled DNA with radioactive phosphate. But better spatial localization of replication required a lower energy and thus more highly localized radioactive probe. This was tritiated thymidine, which Taylor et al. (1957) used to show that DNA replication was restricted to one sister chromatid and thus semi-conservative. The biochemical proof of the same principle came only in the following year, from Meselson and Stahl (1958).

Into this flurry of activity came Lima-de-Faria (1959), who showed that heterochromatin replicated later than euchromatin. Heterochromatin was first identified as a darkly staining, condensed material whose significance was unclear. Lima-de-Faria injected tritium-labeled thymidine into grasshopper abdomens and then looked at developing spermatocytes. As the spermatocytes developed in a clear geographical sequence, replication events occurring at different times could be ordered.

“The evidence,” wrote Lima-de-Faria, “[was] clear. The tritium was incorporated into heterochromatin later than into euchromatin.” Heterochromatin in rye leaves also replicated at a different time, and late replication of heterochromatin was confirmed in detail by Taylor (1960).

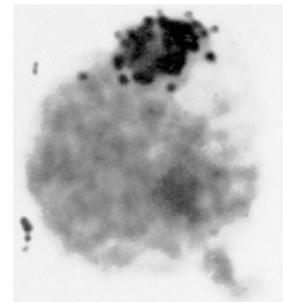
The biological importance of this finding “is an important issue,” says Danesh Moazed (Harvard Medical School, Boston,

MA). “But over the years nothing has come of it.” The late replication may help set up the heterochromatic state, but equally “it may be a side effect of the DNA being less accessible,” says Moazed.

Miller and Nasmyth (1984) showed that passage through S phase was required for heterochromatin to be established in budding yeast. But Kirchmaier and Rine (2001) and Li and Gartenberg (2001), while confirming the need for an S-phase event, found that DNA replication was not required.

Whatever the outcome, Lima-de-Faria’s initial result remains unassailable. It also led others to consider DNA replication not as a simple, monolithic process, but as something that was complex and potentially regulated. **JCB**

Howard, A., and S.R. Pelc. 1953. *Heredity*. 6:261–273.
Kirchmaier, A.L., and J. Rine. 2001. *Science*. 291:646–650.
Li, Y.-C., et al. 2001. *Science*. 291:650–653.
Lima-de-Faria, A. 1959. *J. Biophys. Biochem. Cytol.* 6:457–466.
Meselson, M., and F.W. Stahl. 1958. *Proc. Natl. Acad. Sci. USA*. 44:671–682.
Miller, A.M., and K.A. Nasmyth. 1984. *Nature*. 312:247–251.
Taylor, J.H. 1960. *J. Biophys. Biochem. Cytol.* 7:455–463.
Taylor, J.H., et al. 1957. *Proc. Natl. Acad. Sci. USA*. 43:122–128.



LIMA-DE-FARIA

Only heterochromatin is labeled in this spermatocyte.

The nucleolar origin of rRNA

The nucleolus was identified early on as a site that made a lot of RNA (Caspersson and Schultz, 1940); later that RNA was shown to have metabolic dynamics distinct from those of chromosomally-derived RNA (McMaster-Kaye and Taylor, 1958). But the function of RNA made in the nucleolus was obscure. Two papers from Jan-Erik Edström (Edström, 1960; Edström et al., 1961) gave the first clues.

Edström’s approach was simply to look at the base compositions of different populations of RNA. He was not the first to do so. Vincent (1952) had found that base compositions did not match up for nucleolar and cytoplasmic RNAs. But he

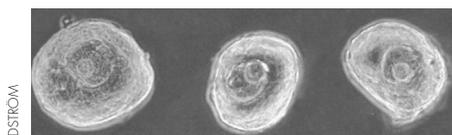
had used bulk isolation of full-grown starfish oocytes to gather material. Edström opted instead to use young, growing oocytes isolated via microdissection. His first subject was oocytes from spiders—“a good choice of materials,” he says, “because the building was old and there were plenty of them along the walls”—with the later study using starfish oocytes.

Both studies came to essentially the same conclusion: nucleolar and cytoplasmic RNA had similar base compositions, whereas nucleoplasmic RNA was distinct (high A/U and low G/C, like DNA). Thus nucleolar RNA might be the precursor of cytoplasmic RNA, as nuclear RNA was known to move to the cytoplasm.

This was all that Edström could say in 1960, but by 1961 he was emboldened and enlightened by the discovery of mRNA (Brenner et al., 1961; Gros et al., 1961). The later abstract (Edström et al., 1961) stated that “our data favor a nucleolar origin for the stable part of the ribosomal RNA and a nucleoplasmic one for the unstable

part (the messenger RNA).” This idea was explained further in the discussion: “The base analyses indicate...that in case there is a high relative contribution of nucleoplasmic RNA [to the cytoplasmic pool] it is necessary that this RNA should have a considerably shorter life than that [from] the nucleolus. The nucleoplasmic RNA could thus very well fit into the role of messenger RNA.” Shortly afterwards, Perry (1962) used inhibitors to prove that nucleolar RNA was, indeed, the obligatory precursor of cytoplasmic ribosomal components. **JCB**

Brenner, S., et al. 1961. *Nature*. 190:576.
Caspersson, T., and J. Schultz. 1940. *Proc. Natl. Acad. Sci. USA*. 26:507–515.
Edström, J.-E. 1960. *J. Biophys. Biochem. Cytol.* 8:47–51.
Edström, J.-E., et al. 1961. *J. Biophys. Biochem. Cytol.* 11:549–557.
Gros, F., et al. 1960. *Nature*. 190:581.
McMaster-Kaye, R., and J.H. Taylor. 1958. *J. Biophys. Biochem. Cytol.* 4:5–11.
Perry, R.P. 1962. *Proc. Natl. Acad. Sci. USA*. 48:2179–2186.
Vincent, W.S. 1952. *Proc. Natl. Acad. Sci. USA*. 38:139–145.



EDSTRÖM

RNA from nucleoli (dark stain in center of oocytes) has a similar base composition to cytoplasmic RNA.

A pathway for secretion

Location, location, location. It has been, and remains, one of the best initial clues to understand the function of a protein or process. The same was true for those trying to localize protein synthesis in the 1950s and 1960s, but their experiments came with a twist. This early in the history of cell biology, the localization effort was not far behind, or even inspired, the definition and naming of the locations.

The cells of choice were pancreatic or liver cells that were furiously churning out secreted proteins. Littlefield et al. (1955) made a promising start by showing that radioactive amino acids accumulated first in the detergent-resistant particles of the microsomes (i.e., the ribosomes) before moving into the membranous portion. The authors felt confident to state that "the cytoplasmic ribonucleo-protein particles are the site of initial incorporation of free amino acids into protein."

Siekevitz and Palade (1958) came to a similar conclusion, and extended the work to show that the label continued on from the microsome fraction to reach the pancreatic zymogen granules. The same sequence held true for individual purified secretory proteins (Siekevitz and Palade, 1960).

Localization moved from biochemistry to cytology with Caro and Palade (1964), who used electron micrographs to trace radioactive leucine as it moved through the endoplasmic reticulum, then the Golgi, and finally to zymogen granules. The Golgi had been destroyed by the earlier biochemical fractionations,

so this was its first appearance in the pathway.

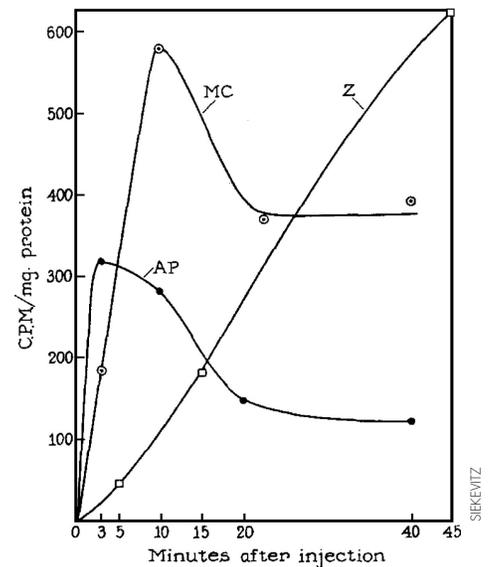
All these *in vivo* studies were hampered by overly long pulse and chase times. Thus it remained formally possible that label was not moving from one compartment to another, but accumulating in the different compartments independently and at different rates. Jamieson and Palade (1967a,b) used *in vitro* tissue slices so that pulse times could be more precisely controlled; thus they demonstrated that there was indeed a flow of protein from one compartment to the next. As the autoradiography became more exact, Jamieson and Palade (1971) could also conclude that the proteins were inside rather than outside the Golgi cisternae, and thus the proteins were not traveling through the cytoplasm as some had suggested.

"The evidence was overwhelming" for this basic secretory pathway, says Siekevitz, and it was generally accepted. These early results from studies with secretory cells had turned up a model that was good at explaining secretion.

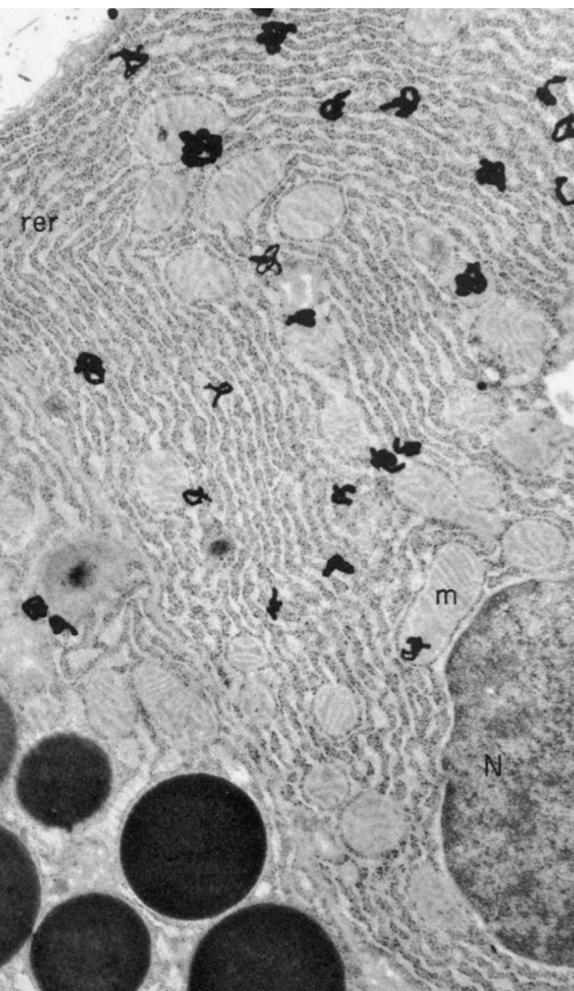
But the story of protein synthesis itself remained incomplete—not least because of the inexact nature of biochemical fractionation. "Even with the corrections envisaged," said Siekevitz and Palade (1958), "...it seems unlikely that the decrease in microsomal radioactivity [over time] could balance the increase in counts in all the other cell fractions. It follows that incorporation of amino acids into proteins, and presumably protein synthesis, is not necessarily restricted to microsomes."

The other half of the equation was not filled in until the report of Ganoza and Williams (1969). They made the correlation between, on the one hand, membrane-bound ribosomes that made secreted proteins and, on the other, nonmembrane-bound ribosomes that made nonsecreted proteins. **JCB**

- Caro, L.G., and G.E. Palade. 1964. *J. Cell Biol.* 20:473–495.
Jamieson, J.D., and G.E. Palade. 1967a. *J. Cell Biol.* 34:577–596.
Jamieson, J.D., and G.E. Palade. 1967b. *J. Cell Biol.* 34:597–615.
Jamieson, J.D., and G.E. Palade. 1971. *J. Cell Biol.* 50:135–158.
Littlefield, J.W., et al. 1955. *J. Biol. Chem.* 217:111–123.
Siekevitz, P., and G. Palade. 1958. *J. Biophys. Biochem. Cytol.* 4:557–566.
Siekevitz, P., and G. Palade. 1960. *J. Biophys. Biochem. Cytol.* 7:619–630.
Ganoza, M.C., and C.A. Williams. 1969. *Proc. Natl. Acad. Sci. USA.* 63:1370–1376.



Radioactive leucine is incorporated first into "attached particles" (AP; ribosomes) before entering the general microsomal content (MC) and then zymogen granules (Z).



Labeled proteins start off in the ER.