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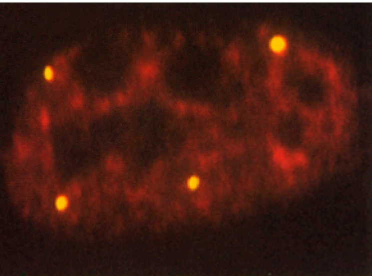
The Hunting of the snRNP

The protein–RNA complexes known as snRNPs are the cell's editors. In 1992, researchers knew from many in vitro studies that different snRNPs band together to form the "spliceosome," which splices premessenger RNA molecules to form functional mRNAs. But they didn't know where in the nucleus snRNPs congregated. Using a new technique, Angus Lamond (now at the University of Dundee, UK) and colleagues tracked snRNPs to two mysterious structures, the interchromatin granules and the Cajal bodies (formerly coiled bodies). Follow-up work suggests that both objects are important for snRNP production and activity.



CARMO-FONSECA

Foci rich in snRNPs (red) also contain the coiled body protein coilin (green).

When cell biologists first detected snRNPs using immunofluorescence, they observed glowing "speckles" strewn around the nucleus. Although this technique could reveal the presence of snRNPs, the antibodies initially available couldn't determine which kinds of snRNP mustered in a particular location. So Lamond and his team added another ingredient: antisense probes that could discriminate among different snRNPs. The combination of antisense and antibodies revealed that "not all speckles were equal," says Lamond. Some, which the researchers dubbed "nuclear foci," glowed brighter (Carmo-Fonseca et al., 1991a,b).

The next year, Lamond's group confirmed the existence of two kinds of speckles and pinned down their identities (Carmo-Fonseca et al., 1992). Electron microscopy studies from David Spector's group at the Cold Spring Harbor Lab in New York had suggested that antibodies against snRNPs detected the structures known as interchromatin granules. Lamond's group, then at the European Molecular Biology Laboratory, had access to one of the first confocal microscopes, allowing them to analyze combinations of labels and test this possibility. When the team tagged cells with antibodies against snRNPs and against the granules, the staining patterns corresponded, confirming that some speckles were granules.

However, the antigranule antibody didn't cling to the bright foci, suggesting that they were different. The researchers suspected that they were Cajal bodies. Another group at the Scripps Research Institute in California had just discovered antibodies in autoimmune patients that recognize a Cajal body protein called coilin (Raska et al., 1991). When Lamond's lab tagged nuclei with antisense strands and this anticoilin antibody, labeling overlapped, showing that snRNPs were loitering in the Cajal bodies. Huang and Spector (1992) obtained similar results around the same time. These findings "gave us a much higher resolution picture of what was going on [in the nucleus]," says Lamond.

But they didn't explain why snRNPs were parking in the Cajal bodies and interchromatin granules. Sleeman and Lamond (1999) provided one clue by showing that snRNPs that have just entered the nucleus gather in the Cajal bodies. The snRNPs then travel to the interchromatin granules and acquire their finishing touches, maturing into functional particles (Jády et al., 2003). The granules may serve as storage depots for inactive snRNPs, says Lamond, while active snRNPs likely bind to pre-mRNA molecules at the genes themselves. Their dispersal around the nucleus probably accounts for the diffuse glow researchers noted in some labeling experiments, says Lamond. **ML**

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ECM determines fate

Biologists long thought that the extracellular matrix (ECM) provided only support and protection for cells. But from the early 1980s on, Mina Bissell of the Lawrence Berkeley Laboratory in California contended that the ECM was a prime influence on cells, transmitting signals that direct gene expression and differentiation (Bissell et al., 1982). A 1991 paper from her lab (Streuli et al., 1991) clinched the case for this view, showing that single mammary cells growing in ECM could fashion a milk protein without stimulation from other cells. Later studies from her group identified the signal-sending component of ECM and revealed how it affected cancer cells.

By 1991, Bissell's lab and others had demonstrated that mammary cells reared on basement membrane, the ECM underlying epithelial layers, form bulbs—just like those that abound in breast tissue and exude milk proteins such as β-casein (Li et al., 1987; Barcellos-Hoff et al., 1989). By contrast, cells nurtured on plastic or collagen did neither, unless they could synthesize and assemble their own basement membrane.

But because cells growing in these cultures were in contact with each other, the possibility remained that cell–cell interactions, not ECM signals, provoked differentiation, Bissell recalls. So instead of growing the mammary cells at high density, her group embedded them in basement membrane gels, leaving many cells with no close neighbors. Most single cells in these gels oozed β-casein, the researchers reported (Streuli et al., 1991), but no single cells embedded in collagen did. By adding antibodies that jammed one of the integrins, the surface receptors that receive messages from the ECM, Bissell slashed β-casein output in the basement membrane gels. But blocking other surface proteins that don't heed the ECM had no effect. "It was the first clear demonstration of the role

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The kinetochore uncoiled

Bill Brinkley had first described the kinetochore in the 1960s. He saw, by electron microscopy, a trilaminar, proteinaceous disc structure that flanked the centromere (Brinkley and Stubblefield, 1966). But further details were obscure for another 20 years.

Raymond Zinkowski planned to change that. When he joined Brinkley's lab at the University of Alabama, Birmingham, as a grad student in 1986, "only a handful of labs were seriously investigating the centromeric region of chromosomes," says Zinkowski. "It just looked like a parking place for a kinetochore handle to move chromosomes."

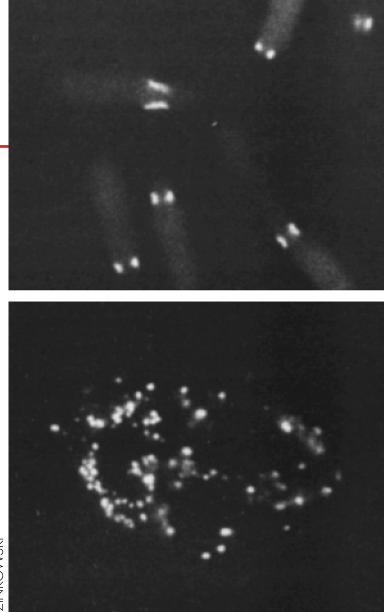
Zinkowski became interested when studies using autoantibodies from scleroderma patients identified kinetochore-associated proteins (Earnshaw and Rothfield, 1985). Brinkley already held a notion that the kinetochore might be organized as repeat subunits. The Indian muntjac, a small Asian deer, had been identified as the mammal with the lowest number of chromosomes—a mere seven (Wurster and Benirschke, 1970). These cells with very few but large chromosomes gave Brinkley an easy target for examining kinetochore structure. He found what appeared to be compound, segmented structures. These may have evolved from fusions of the 46 chromosomes of an almost identical cousin, the Chinese muntjac (Brinkley et al., 1984). Also, Indian muntjac prekinetochores stained with the scleroderma autoantibodies in interphase showed more than seven discrete spots arranged in threadlike arrays.

Zinkowski treated G1 muntjac cells with a combination of hydroxyurea and caffeine, which breaks up the chromatin and induces cells to enter mitosis prematurely. The "pulverized chromatin" wandered from the spindle but the multiple kinetochores still made functional attachments to the spindle. What's more, the seven Indian muntjac kinetochores now appeared as 80–100 kinetochore subunits (Zinkowski et al., 1991).

In addition, when Brinkley and Zinkowski stretched out metaphase centromeres by hypotonic treatment and then stained kinetochore proteins using IF, they saw a repeating subunit pattern. It was similar to the less-organized interphase staining pattern.

The staining patterns, together with the kinetochore subunits found in G1 cells, argued for discrete, preformed protein subunits. Those subunits, the authors concluded, were separated by stretches of centromeric DNA that came together into coils as chromosomes condensed.

The model went against the current thinking that centromeres recruited kinetochore proteins into a solid disc structure after condensation (Jokelainen, 1967; Ris and Witt, 1981; Rattner, 1986). Brinkley says the model holds up in other stretched chromosome studies (Haaf and Ward, 1994) and in studies of budding yeast and worm centromeric proteins that appear to form subunits before chromosome condensation is complete (Meluh and Koshland, 1997; Moore et al., 1999). **KP**


ZINKOWSKI
Modular kinetochores (top) come to pieces after premature mitotic entry (bottom).

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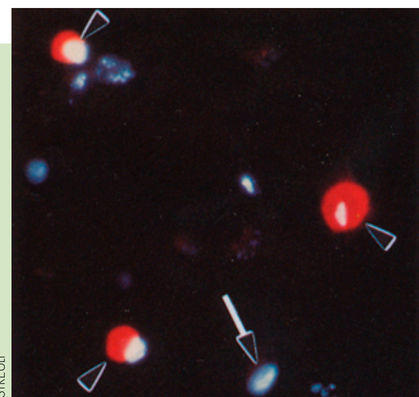
STREULI
ECM prompts embedded mammary cells to produce β-casein (red).

of an ECM receptor in the regulation of specific gene expression," says Bissell.

In subsequent studies, Bissell and colleagues followed the signaling pathway from the cell's exterior to the nucleus. They showed, for example, that laminin is the basement membrane protein that prods the cell to activate the β-casein gene (Streuli et al., 1995). At the membrane, two kinds of integrins and the protein dystroglycan work together to recognize laminin (Muschler et al., 1999). The β-casein promoter contains a sequence that responds to ECM directives (Schmidhauser et al., 1992; Myers et al., 1999). Bissell's lab has also probed how ECM signaling influences cancer, showing that tumor cells sport extra integrin receptors and that blocking the receptors with an antibody tames the malignant cells (Weaver et al., 1997). **ML**

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