CYTOGENETICS

New Methods for Expanding The Chromosomal Paint Kit

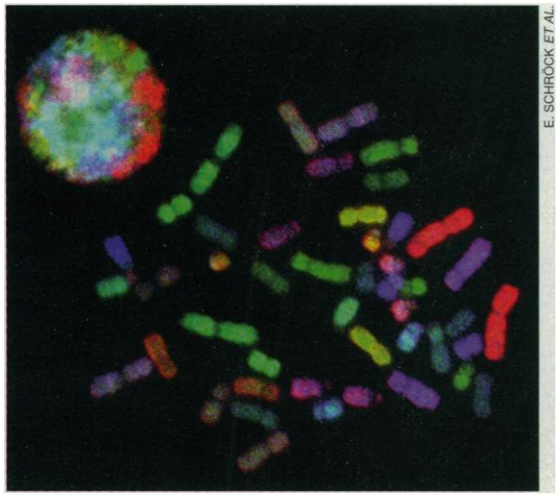
Sometimes an artist can create great paintings with just a few colors, as Picasso did in his blue period. But the cytogenetics "artists" trying to study human and other chromosomes by tagging them with different colors don't have the luxury of creating masterpieces from a limited palette: They need two dozen different shades to paint each chromosome its own distinctive color, and there haven't been enough colors in their paint kit to do the job—until now.

Two teams, one led by Thomas Ried and Evelin Schröck of the National Center for Human Genome Research (NCHGR) in Bethesda, Maryland (see p. 494), and the other by David Ward of Yale University, have devised a clever trick for effectively creating enough shades of color to tag each human chromosome with a distinct hue. They do this by using different dye combinations to label the chromosomes, whose individual labeling patterns are then detected by computer analysis.

Cytogeneticists and genome researchers are excited about the possible applications of the new methods. "The main power of these techniques is their ability to detect subtle chromosome changes. ... They are a major step forward," says Joseph Gray of the University of California, San Francisco. The methods will be useful in basic research for detecting chromosome abnormalities in cancer cells, as well as for chromosome mapping and gene localization. And in the clinic, the new techniques may lead to improved methods for detecting the chromosomal defects that cause Down syndrome and other congenital abnormalities, as well as to better techniques for diagnosing cancer and monitoring patients' responses to therapy.

Both the Ried and Ward teams took as their starting point an older chromosome-staining technique known as FISH (for fluorescent in situ hybridization). In FISH, a fluorescent dye is added either to a piece of DNA that binds to a specific chromosomal site or, in a variation called chromosome painting, to a series of DNA pieces that bind to sites all along a particular chromosome. But FISH can't be used to study all the human chromosomes simultaneously because there aren't enough fluorescent dyes with sufficiently different colors to mark individually all 22 somatic chromosomes plus the two sex chromosomes.

To get around this problem, both teams used a combinatorial approach, labeling the painting probes for each chromosome with a different assortment of fluorescent dyes (fluorochromes). Because the total combinations given by a number of dyes (N) is $2^N - 1$, as few as five dyes can give enough combinations to produce probes individually labeled for each human chromosome. When these probes are then hybridized to a set of chromosomes, Ward says, "the result is that each chromosome gets labeled with a different fluorochrome combination." There is a catch, however: The fluorochrome colors aren't distinct enough for the unaided human eye to distinguish which combination a chromosome carries.



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In living color. The micrograph shows combinatorily stained human chromosomes; at upper left is a nucleus with the chromosomes in a more extended configuration.

The two teams solved this problem in different ways. Ward and his colleagues used a series of filters, each of which transmits only light of the wavelength range emitted by one fluorochrome. The stained chromosomes are then examined, using each filter separately to show individual fluorochrome locations. A computer program then combines the data and displays each chromosome as if it were stained with a distinct color. "We take separate pictures and combine them into one," Ward explains. (The results appear in the April issue of *Nature Genetics*.)

In contrast, Ried, Schröck, and their colleagues use the same type of interferometer that astronomers use to measure the light spectra emitted by distant stars, to determine the full spectrum of light emitted by each stained chromosome. "If you want to detect the nature of color, you can do nothing more precise than measure the entire spectrum," Ried says. Then, like Ward's group, the NCHGR team uses a computer program to display all the chromosomes, each with its own unique color. The Ried team's approach has the advantage of being able to image all the chromosomes simultaneously, but the equipment's cost—about $80,000—makes it twice as expensive as Ward's.

Researchers are already applying the techniques to one major problem: analyzing the chromosomal abnormalities (karyotyping) in cancer cells. Neither FISH nor an older technique, in which all the chromosomes are stained with a single dye that produces characteristic banding patterns on the various chromosomes, is up to this job. That is especially true for solid cancers, which have multiple abnormalities, including deletions, additions of whole or partial chromosomes, and exchanges of material between different chromosomes (translocations). "There are so many, they are impossible to figure out by standard banding methods. People just gave up," says NCHGR director Francis Collins. And FISH could be very tedious, requiring analysis with as many as 20 painting probes.

But early results using Ried's technique, called spectral karyotyping, are "miraculous," says cytogeneticist Janet Rowley of the University of Chicago, who is collaborating with his team. In cells from some leukemia patients, the researchers could not only pin down a chromosomal abnormality they knew existed but hadn't been able to define—the addition of what turned out to be part of chromosome 2 to chromosome 15—but they also picked up several such translocations that they didn't even know were present, all in one analysis. "Older FISH techniques only got answers to questions you specifically asked, like how many chromosome 8s are there? This way, you do it all in one hybridization," Rowley says.

Experiments such as these will help researchers understand the genetic changes leading to the cancer, and may also provide guides to therapy, including ways to diagnose cancer and track patients' responses to treatment. The hope is that the new procedures might even be automated, although that may be tough to accomplish for cancer cell karyotyping, given its complexity. Automating karyotyping for detecting birth defects may be more feasible, as the changes are much less extensive and would likely be extensively used. Ried points out that diagnostic karyotyping is now performed about 400,000 times a year in the United States and Canada, mostly for pre- or postnatal diagnosis. If automated karyotyping does become possible, it would be a prime example of "art" that is practical as well as aesthetic.

–Jean Marx