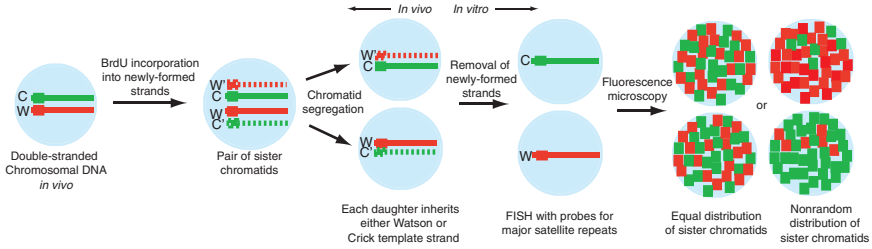
CELL BIOLOGY

Immortal strands versus silent sisters

A method to differentially label each sister chromatid in a cell makes it possible to elucidate segregation patterns after mitosis and should help to pinpoint the mechanism behind nonrandom segregation in certain cell types.

The immortal strand hypothesis, proposed in 1975 by John Cairns, states that adult stem cells selectively retain template DNAs to avoid accumulation of mutations. Peter Lansdorp from the University of British Columbia is one of the scientists who disagree with it. "It was coming up again and again in the stem cell field," he says, "and I had lots of problems with it."

In an essay published in *Cell*, Lansdorp proposed that "future studies should primarily focus on whether asymmetric chromosome segregation does indeed occur." Taking his own words to heart, and searching for a method to address the question of chromosome segregation patterns, Lansdorp turned not to a new technique but to an established method that had been developed over a decade ago by Ed Goodwin, Susan Bailey and Julianne Meyne at the Los Alamos National Laboratory in New Mexico to study the organization of repetitive DNA in chromosomes.



Schematic to study sister chromatid segregation *in vivo*. Reprinted from *Nature*.

The technique, called CO-FISH (for chromosome-orientation fluorescence *in situ* hybridization), is based on strand-specific fluorescence *in situ* hybridization. Cells are first incubated with high doses of the nucleotide analog BrdU for one round of replication, then addition of a DNA dye and UV irradiation help to destroy newly formed DNA strands, leaving the chromosomes single stranded and ready to be hybridized with unidirectional probes against repeat sequences for major satellite and telomere repeats. All chromosomes in the mouse were found to have a similar organization of major satellite repeats relative to telomere repeats, allowing identification of both DNA template strands. On the basis of this observation, Lansdorp planned to use CO-FISH to follow sister chromatids *in vivo*.

His team chose three mouse tissues for their analysis: intestine, because that was the tissue that gave rise to the immortal strand hypothesis, and embryonic stem cells and skin fibroblasts, two control cell types with assumed random segregation. The researchers observed asymmetric segregation in some colon cells but no clear evidence for nonrandom chromatid distribution in the other two cell types.

GENOMICS

A GENOME IN TIME

Addition of next-generation sequencing to an assay of replication timing enables high-resolution genome-scale analyses of multiple cell types.

Over 15 years ago, Scott Hansen at the University of Washington School of Medicine wanted to analyze replication timing for specific loci. He developed an assay that has provided a wealth of information about specific loci and that is still used today. Taking advantage of the ability of next-generation sequencing to extend many analyses to the whole-genome level, Hansen collaborated with John Stamatoyannopoulos's group (and has since joined the lab) to develop the second generation of the replication timing assay.

In this assay, exponentially growing cells are pulse-labeled to 'mark' newly replicated DNA in each cell. The label, 5-bromo-2-deoxyuridine (BrdU), is a base analog that DNA polymerase incorporates in place of thymidine and can be recognized by an antibody to later identify the newly replicated regions. Next, staining with the fluorescent dye DAPI labels the entire cellular DNA, which enables cell fractionation based on the total amount of DNA and thus the cell's stage in the synthesis (S) phase of cell division. Newly replicated BrdU-labeled DNA is then isolated by immunoprecipitation from each fraction.

Previously, researchers analyzed loci of interest one by one, by PCR—a process with limited throughput. Now, Hansen explains, "we basically used the same technique I had been using, except the DNA fractions were [assembled into separate Illumina sequencing libraries and] sequenced."

After a control experiment showed that the data generated with this 'Repli-Seq' approach agreed with the results of PCR-based assay, the researchers went on to compare early- versus late-replicating DNA in multiple cell lines—from fibroblasts to human embryonic stem cells. They performed a high-resolution analysis, examining six fractions per cell line that represented the G1b, S1, S2, S3, S4 and G2 stages of the S phase. Early replication regions were associated with gene density and chromatin accessibility, but a comparison to microarray RNA-level data revealed that gene expression was only moderately correlated with early replication.

"Given what was known," says Hansen, "there was a thinking that most of the genome was going to be replicated at about the same time in different cell types; that only a few spots here and there will change." It turned out, however, that in this analysis of just four cell types, there were replication timing differences in 49% of the human genome. "We expect now that number will

For Lansdorp these results argue against the immortal strand hypothesis. "There is no obvious fixed pattern of sister chromatid segregation," he explains. Instead, he wants to pursue his previously suggested 'silent sister hypothesis', which proposes that non-random segregation of perhaps a few chromosomes could help regulate gene expression and cell fate in adult stem and progenitor cells.

To accumulate evidence for this hypothesis, Lansdorp and his team want to tackle the proposed function of the asymmetric segregation. They plan to design chromosome-specific probes to follow specific sister chromatids and relate their segregation pattern to cell fate. In addition, the scientists want to correlate allele-specific gene expression with chromatid segregation in single cells to confirm the existence of "active" and "silent" sisters. Of course, getting enough material for high-throughput sequencing from single cells is not without its technical challenges and will necessitate the development of sensitive amplification methods.

Once functional evidence for the 'silent sister hypothesis' mounts, Lansdorp believes that studies into the mechanism will also follow. He acknowledges that at present he and his colleagues can only speculate about the mechanism. "In reality we don't have a clue," he says very candidly, adding that different epigenetic marks at the centromeres of the sister chromatids may lead to asymmetric nucleation of microtubules, making a distinction between the active and the silent sister.

Identifying the roles of the sisters could transform how we think about development and stem cell biology.

Nicole Rusk

RESEARCH PAPERS
Falconer, E. *et al.* Identification of sister chromatids by DNA template strand sequences. *Nature* **463**, 93–97 (2010).

go up as we include more cell types," he adds.

And as next-generation sequencing becomes more accessible, others can use Repli-Seq to analyze their cells of interest—other human cell types or cells from other species; all one needs is a reference genome. For a lower-resolution, more cost-effective analysis to look at just early versus late replication, for example, barcoding could be used, enabling analysis in a single lane of sequencing, suggests Hansen, adding: "We think now we can get the basic profiles of the main S-phase fractions in 1–2 lanes of sequencing."

An important advantage of Repli-Seq that will permit analysis of numerous cell populations, including rare ones, is the lower number of cells required—about 2,000 per replication time fraction. Such amounts can be obtained from a tissue sample, and eventually Hansen and Stamatoyannopoulos aim to examine entire lineages. These data might one day be among the reference information for many cell lines; for now the group is negotiating to upload their material into the University of California Santa Cruz Genome Browser, with much more data yet to come.

Irene Kaganman

RESEARCH PAPERS
Hansen, R.S. *et al.* Sequencing newly replicated DNA reveals widespread plasticity in human replication timing. *Proc. Natl. Acad. Sci. USA* **107**, 139–144 (2010).

NEWS IN BRIEF

MICROSCOPY

High-temperature, single-molecule imaging

It is a challenge to image single molecules at temperatures higher than 37 °C because the index-matching fluids used in conjunction with high-numerical-aperture lenses can transfer heat to the lens, causing damage. This has been a limitation for single-molecule sequencing-by-synthesis, preventing the use of thermophilic polymerases. Schwartz *et al.* now describe tiny TiO_2 colloidal lenses that have a high refractive index and are capable of single-molecule imaging at 70 °C, which should improve the efficiency of single-molecule sequencing.

Schwartz, J.J. *et al. Nat. Nanotechnol.* **5**, 127–132 (2010).

SPECTROSCOPY

NMR for membrane-anchored proteins

Membrane-anchored proteins, consisting of a soluble domain and a lipid anchor, are difficult to study by solution NMR spectroscopy without removing the anchor, thus precluding study of its structural role. Valentine *et al.* now report the use of reverse-micelle technology to obtain high-resolution NMR solution structures for two myristoylated proteins. A surfactant shell, through which the lipid extends, encloses an aqueous solution in which the protein is encapsulated, and the whole assembly is solvated in a low-viscosity liquid ideal for NMR spectroscopy.

Valentine, K.G. *et al. Structure* **18**, 9–16 (2010).

IMAGING AND VISUALIZATION

Detecting Fe-S clusters

Proteins containing Fe-S clusters have many essential biological functions, but not much is known about Fe-S cluster metabolism and its role in disease, owing to a lack of tools for imaging Fe-S clusters. Hoff *et al.* now describe a fluorescent probe to detect Fe-S clusters, based on the complementation of two Venus fluorescent protein fragments when glutaredoxin 2 dimerizes upon coordinating a 2Fe-2S cluster. This approach can be used to image Fe-S cluster proteins in both bacterial and mammalian cells.

Hoff, K.G. *et al. Chem. Biol.* **16**, 1299–1308 (2009).

CHEMICAL BIOLOGY

Controlled condensation in cells

Controlling chemical synthesis inside cells is a challenge, owing to problems with biocompatibility and selectivity. Liang *et al.* now report the condensation reaction of 2-cyanobenzothiazole and D-cysteine, which occurs under mild conditions and can take place in living cells under the control of pH, disulfide reduction or enzymatic cleavage. It is promising for use in *in vivo* molecular imaging applications.

Liang, G. *et al. Nat. Chem.* **2**, 54–60 (2010).

CELL BIOLOGY

Delivery with vertical silicon nanowires

Shalek *et al.* demonstrate that DNA, proteins, peptides and small molecules can be delivered into mammalian cells via vertical silicon nanowire arrays. When these nanowires impale the cell membrane, they release their 'cargo' into the cytosol; in all cases shown the introduced molecules carry out their intended functions. No chemical modification or packaging of the delivery agent is needed, and the cells grow and divide normally for several weeks.

Shalek, A.K. *et al. Proc. Natl. Acad. Sci. USA* **107**, 1870–1875 (2010).