

Meeting Report

Driven together by entropy

The tendency of large complexes to congregate drives the organization of the genome and other cellular structures, according to Peter Cook (University of Oxford, UK).

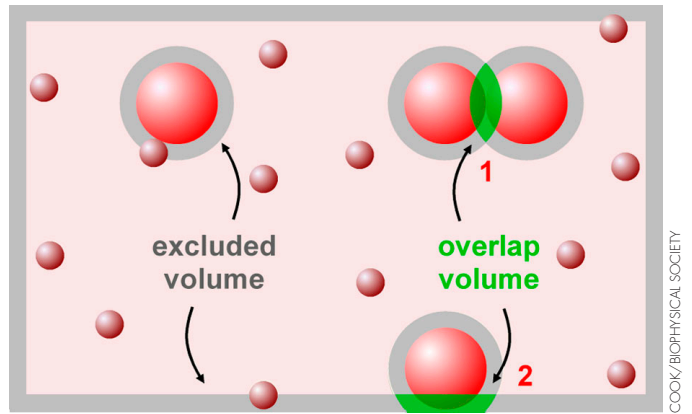
In a crowded, enclosed space (such as the cell), the aggregation of two large spheres increases the system's entropy by giving lots of little molecules more room to move around. In physics, this effect is known as depletion attraction. Using mathematical modeling, Cook and colleagues found that this attraction explains the looping of DNA found naturally in cells.

The model is based on transcription and replication complexes that are spaced along DNA like beads on a string. Measurements of the attraction between the "beads" suggest that the entropy gained by their clustering is enough to cover the energy costs of looping the DNA string between them.

In vivo, the effects are seen as the clustering of active genes and the looping of highly transcribed genomes. As predicted, in slow-growing cells, which have little replication and transcription, polymerases did not cluster.

According to Cook, other large cellular structures are always trying to congregate. The cell must therefore fight the depletion attraction. "Otherwise, everything would become one big blob," he says.

This entropic effect might explain why the size of stable



Clustering of large complexes gives smaller ones more room to move.

complexes is limited to that of a ribosome. It also explains why larger structures, such as the cytoskeleton, are made by loosely associated subunits that constantly turn over. "The cell is spending energy," says Cook, "to maintain the structure." Energy is similarly expended to keep pools of vesicles from fusing into one by turning over their surfaces. Cook explains, "if the vesicles are continually being broken up into smaller pieces that quickly diffuse away, the attraction becomes smaller." **JCB**

Reference: Marenduzzo, D., et al. 2006. *Biophys. J.* 90:3712–3721.

Genetic postal codes

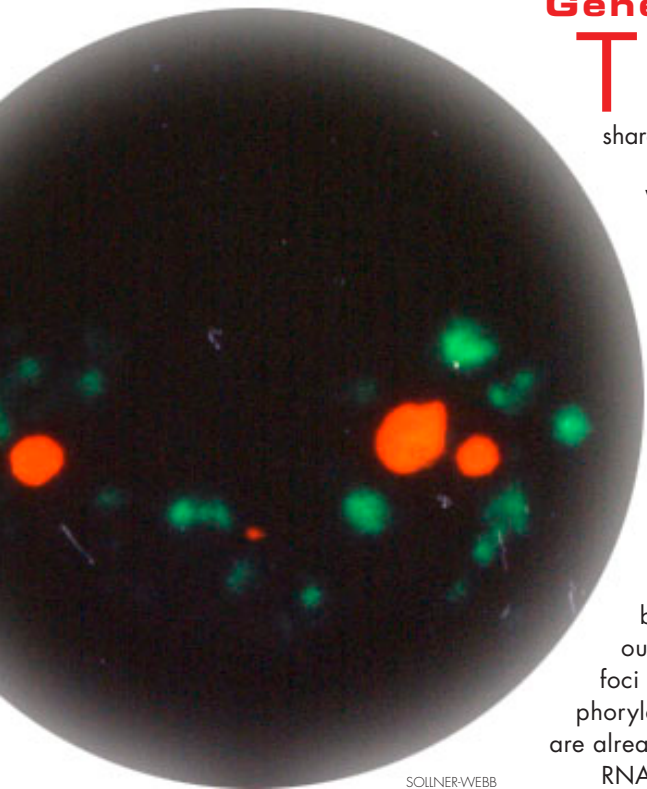
Transcriptional control elements are like zip codes for genes, based on new findings presented by Barbara Sollner-Webb (Johns Hopkins University, Baltimore, MD). Thousands of copies of transfected plasmids find a common area to inhabit if they share the same zip code, sorted away from plasmids with different zip codes.

"That endogenous genes have specific locations is not so surprising," said Sollner-Webb. "But why are ribosomal genes [for instance] inside the nucleolus? What gets them there?" Her group's findings, although using exogenous DNA, may offer the best explanation so far.

During transient transfection experiments, the group noticed that tens of thousands of copies of a plasmid went to the same location in the mammalian nucleus. The locale was promoter specific. Plasmids containing RNA polymerase (RNAP) I promoter sequences were found only in nucleoli, whereas those with RNAP III promoter sequences formed perinucleolar foci. RNAP II promoters took the plasmid DNA to nucleoplasmic foci, but different promoter sequences resulted in different foci.

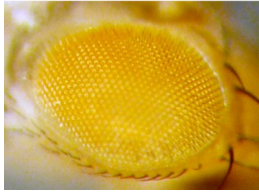
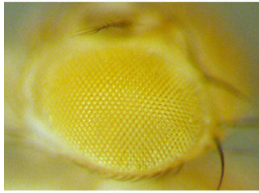
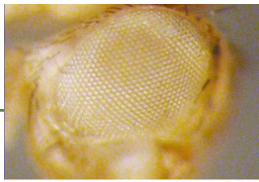
The zip codes seem to be read by transcription factors. As even untranscribed plasmids localized in this manner, polymerase activity is not necessary. The perinucleolar localization required just an 18- or 26-base-pair region that binds to TFIIIA or TFIIIC, respectively. These RNAP III factors are found throughout the nucleus, but Sollner-Webb hypothesizes that the subset in these perinuclear foci is special, either because it is bound to DNA or because it is modified (by phosphorylation, for example) to favor DNA binding. Most RNAP I transcription factors are already concentrated in the nucleoli.

RNAP I transcription factors are probably abundant enough, based on published estimates, for each plasmid to have its own copy. Life might be more complicated for less abundant RNAP II factors. The modularity of RNAP II promoters suggests that multiple sequence elements and transcription factors might be necessary. **JCB**



SOLLNER-WEBB

Plasmids with an RNAP I promoter (red) sort to nucleoli, away from plasmids with an RNAP II promoter (green).



CAVALLI/ELSEVIER

The silencing of eye color (top) is lost in RNAi mutants (bottom two panels).

RNAi and Polycombs cooperate

The RNA interference (RNAi) machinery holds together copies of silencing elements from multiple genes, as described by Giacomo Cavalli (CNRS, Montpellier, France). This group has reinforced developmental gene silencing.

The silenced state of a developmentally regulated homeotic gene is maintained by chromatin-modifying Polycomb group (PcG) proteins. PcG response elements (PREs), the DNA sequences that recruit PcG proteins, are self locating: PRE-containing sequences from multiple genes travel long distances to cluster

with each other, and this association enhances the transcriptional silencing.

The silencing that accompanies heterochromatin formation in yeast requires RNAi components. Cavalli's group has found that PcG-mediated euchromatin silencing also depends on RNAi proteins. Silencing of multiple PRE-containing genes was relieved in fly mutants lacking RNAi proteins such as AGO1 and Dicer-2. PcG proteins were still recruited to their targets, but clustering was lost.

The glue for the PRE clusters seems to be small RNAs. Several species of small RNAs were found that matched the PRE region, and their production depended on the RNAi machinery. Clustering correlates with the presence of small RNAs, but Cavalli has not yet ruled out protein–protein interactions as the cause.

The physical properties of a nanocompartment such as a PRE cluster might help to reinforce silencing. Chromatin rearrangements might be more difficult, or PcG proteins less mobile. Cavalli plans to test the latter theory using GFP-tagged PcG proteins. **JCB**

Reference: Grimaud, C., et al. 2006. *Cell*. 124:957–971.

RNAs regulate rRNAs

RNAs transcribed from between ribosomal genes alter the chromatin structure and thus silence their rRNA-coding neighbors, as discussed by Ingrid Grummt (German Cancer Research Center, Heidelberg, Germany).

Ribosomal genes are in vast excess in the mammalian genome. Of 400 or so copies of rRNA genes, about half are permanently silenced after embryogenesis. Their heterochromatic state is induced by a complex called NoRC, which recruits histone-modifying enzymes to rDNA. When Grummt and colleagues noticed that RNase treatments dispersed NoRC from the nucleoli, they began searching for an RNA component.

The group has since found that the RNAs responsible for targeting NoRC to chromatin originate from spacer regions that separate individual rRNA genes. Like the rRNA genes, the spacer RNAs are transcribed by RNA polymerase I. The RNAs bind to a subunit of NoRC, and this association is necessary for NoRC to grab onto the rRNA chromatin.

The intergenic RNAs are a few hundred base pairs long and are complementary to the rRNA gene promoter. They have the potential to base-pair with the promoter as well as bind to NoRC, with the latter association depending more on RNA secondary structure than on specific sequence. **JCB**

Reference: Mayer, C., et al. 2006. *Mol. Cell*. 22:351–361.

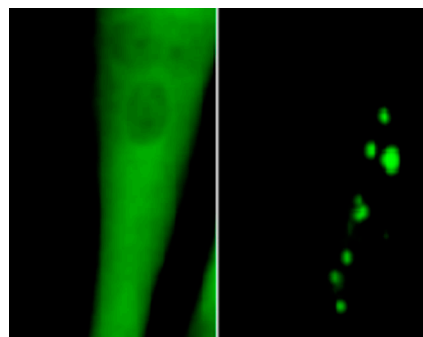
Static for silencing

Oxygen-starved cells conserve their limited energy by shutting down ribosome production. In his talk, Stephen Lee (University of Ottawa, Ottawa, Canada) suggested that cells silence ribosomal genes during hypoxia by locking a ubiquitin ligase in the nucleolus.

In abundant oxygen, this ubiquitin ligase, called VHL, is a free-moving protein that keeps hypoxia-induced factor α (HIF α) levels low. But when cells are using anaerobic metabolism pathways, the resulting decrease in pH somehow causes VHL to stick in the nucleolus, where it cannot curb HIF α .

Until this immobile form of VHL was identified, the only other known

static protein was histone H2B, which silences chromatin. All other tested proteins exchange dynamically. Based on a supposed need for protein exchange during chromatin remodeling, Tom Misteli



At low pH, the locking of VHL (green) in nucleoli (right) silences rDNA.

(NIH, Bethesda, MD) proposed in 2001 that static proteins in general might induce transcriptional silencing.

Now with VHL in hand as a second immobile protein, Lee's group has put Misteli's hypothesis to the test. They found that VHL is indeed required to silence rRNA genes and thereby protect cells from hypoxia-induced death. The return of oxygen is expected to free VHL from the chromatin and restore rRNA synthesis. Lee was still unclear, however, how a ubiquitin ligase—or degradation of its substrate—is able to remodel chromatin to bring about silencing. **JCB**

References: Mekhail, K., et al. 2005. *J. Cell Biol.* 170:733–744.

Misteli, T. 2001. *Science*. 291:843–847.