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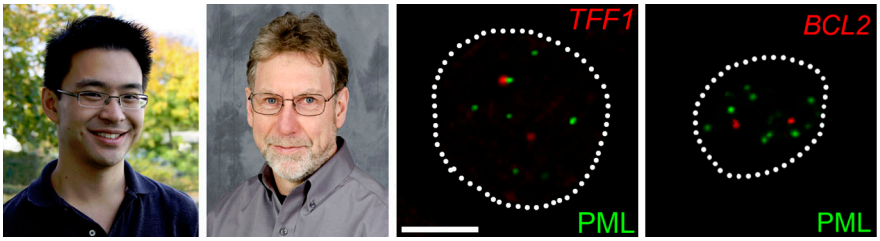
Setting an immuno-TRAP for PML nuclear bodies

Researchers describe technique to identify genomic loci that interact with specific nuclear structures.

Focal Point



Reagan Ching (left), David Bazett-Jones (right), and colleagues (not pictured) describe an unbiased method to identify genomic loci that specifically interact with subnuclear structures like promyelocytic leukemia nuclear bodies (PML NBs). The researchers use an anti-PML antibody to direct the biotinylation of chromatin in close proximity to these mysterious nucleoplasmic particles. By isolating and analyzing the biotinylated chromatin, Ching et al. identify loci that associate with PML NBs in a cell type–specific manner. In situ hybridization can confirm these associations, showing, for example, that the *TFF1* gene (red, center panel) is enriched at PML NBs (green), whereas the *BCL2* locus (red, right panel) shows no such association.

Promyelocytic leukemia nuclear bodies (PML NBs) are nucleoplasmic particles that contain numerous proteins, including the PML protein itself. In patients with promyelocytic leukemia, PML is fused to retinoic acid receptor α, resulting in a loss of PML NBs and a block in promyelocyte differentiation. Yet the precise function of PML NBs remains unclear. To learn more about these particles, Ching et al. identify regions of the genome that associate with PML NBs, using a novel method that could easily be adapted for other subnuclear structures as well (1).

Though PML NBs are found in most somatic cells, their constitution can vary even between particles in the same nucleus. Alongside PML, PML NBs can contain signaling proteins, transcription factors, and chromatin-modifying enzymes, implicating these structures in multiple aspects of genome regulation (2). "When you look at PML bodies by electron spectroscopic imaging, you see a protein-based core with obvious connections to chromatin fibers," says David Bazett-Jones from The Hospital for Sick Children in Toronto. "So the genome and these nuclear bodies are intimately linked in some way."

Indeed, fluorescence in situ hybridization (FISH) studies have shown that PML NBs can interact with specific regions of the genome, including the *TP53* and *MHC* loci (3, 4), but, says Bazett-Jones, "the disadvantage of FISH is that you have to have an a priori idea of what to probe for."

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Bazett-Jones and colleagues, led by graduate student Reagan Ching, therefore wanted to develop an unbiased method to identify genomic loci associated with PML NBs, basing their approach on a technique used previously to identify chromatin regions in the vicinity of an actively transcribing gene (5). Ching et al.'s adapted approach, named immuno-TRAP, involved labeling PML NBs with an anti-PML antibody and a secondary antibody coupled to horseradish peroxidase (1). This enzyme can then conjugate biotin-tyramide onto nearby proteins, including histones, allowing Ching et al. to isolate biotinylated chromatin and identify genomic loci that are close to PML NBs.

Using immuno-TRAP, Ching et al. confirmed the *TP53* locus as a PML NB–associated region of the genome, but they also identified several new loci that are enriched around these nuclear particles. "We pulled out a number of examples, clearly showing that PML bodies have links to specific gene elements," says Bazett-Jones.

The loci associated with PML NBs varied in different cell types and changed in response to cytokine stimulation, suggesting that the particles' interactions may reflect the transcriptional state of the cell. Given that the protein content of PML NBs is also variable, Bazett-Jones thinks that the function of each individual particle may be unique. "Some may activate a set of coregulated genes, whereas other PML bodies may shut genes off," he says. One genomic locus associated with PML NBs in some cell types under certain conditions was the *PML* gene itself, raising the possibility that the nuclear particles can also regulate themselves in some way.

Fully understanding the functions of PML NBs will require characterizing their associations in more detail. In the current study, Ching et al. analyzed their biotinylated chromatin fragments using dot blots of BACMID clones and promoter element microarrays. But in the future, Bazett-Jones says, they plan to deep sequence all the DNA loci enriched at PML NBs, adding that, by using different primary antibodies, researchers can apply the immuno-TRAP approach to identify genomic regions associated with a variety of subnuclear structures, like the nuclear lamina or DNA repair foci.

Once Bazett-Jones and colleagues know which genes are associated with PML NBs, they should be able to ask specific functional questions, such as whether a gene's transcriptional activity is altered in cells lacking PML protein. "Being able to identify genes that associate with PML bodies under different physiological conditions should give us lots of hypotheses to pursue," Bazett-Jones says.

1. Ching, R.W., et al. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201211097
2. Dellaire, G., and D.P. Bazett-Jones. 2004. *Bioessays*. 26:963–977.
3. Shiels, C., et al. 2001. *J. Cell Sci.* 114:3705–3716.
4. Sun, Y., et al. 2003. *Genomics*. 82:250–252.
5. Carter, D., et al. 2002. *Nat. Genet.* 32:623–626.