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A third-generation method reveals cell lineage ancestry

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Abstract

A combination of proximity ligation and *in situ* hybridization enables visualization of epigenetic marks in single cells.

Epigenetics is the study of information heritable during cell division other than DNA sequence per se. As such, epigenetics is an essential pathway to understanding the mechanism for the development of multicellular organisms with distinct cell types and the diseases that involve disruption of those developmental pathways. These include obesity, cardiovascular disease, neurodegenerative disease and cancer. Moreover, tissues do not exist in a state of homogeneity; rather, each tissue exhibits considerable variability within itself, in phenotype and responsiveness. Yet our approach to the study of mammalian epigenetics almost always involves analysis of homogenized tissues, or at least many fractionated cells from a given tissue. And our approach to medical diagnostics and treatment is almost entirely geared toward analysis of mean values and mean phenotypes, such as the ‘cancer cell’ or ‘atherosclerotic lesions’. Gomez *et al.*¹ now provide a powerful lens for visualizing such epigenetic variation in single cells and applying this tool to cardiovascular disease.

I teach my students not to read News and Views articles, or the abstract of the original paper, until they have looked at the figures; so in that spirit I ask you to examine, at the risk of not finishing this piece, Figure 1c of the article by Gomez *et al.*¹ This shows a specific activated chromatin mark at a specific gene—that is, a dimethylation mark at lysine 4 on histone H3 (H3K4me2) on *MYH11*, a gene whose expression is specific for smooth muscle cells (SMCs)—as a small pink dot at the end of the arrows. What is very cool is that we can see that the modification occurs only in SMCs that line the small blood vessels in this atherosclerosis model. Now jump to Figure 4 panels e and f. Using the same methodological trick, the authors show that this same epigenetic mark, which tags SMCs, is part of experimentally induced atherosclerotic lesions, even though conventional staining for SMCs with antibodies against the gene products of *MYH11* and *ACTA2* could not reveal this because the genes are downregulated. The authors know that they are examining SMCs because they went to the trouble of engineering a lineage-specific transgenic tag, but now that they know that H3K4me2 on *MYH11* is specific for SMCs, they no longer have to use such a transgene to examine the SMC-lineage contribution to human pathology. They do just that in Figure 5b, showing again that the epigenetic mark tags SMCs in human atherosclerotic lesions even though conventional gene-product immunostaining is negative.

COMPETING FINANCIAL INTERESTS

The author declares no competing financial interests.

This means that pathologists may have underestimated the role of SMCs in human atherosclerosis; the current study provides support to investigators studying SMC therapeutic targeting for this disease² and, more importantly, provides a method for studying the effects of such intervention directly on the targeted tissue itself rather than the more complex end phenotype of heart disease.

So what is the method in the paper? It is a variation of the proximity ligation assay (PLA), which the current authors call, non-euphoniously, ISH-PLA, because it involves *in situ* hybridization (ISH) added to the PLA. The PLA was first developed by Ulf Landegren to detect protein interactions, and in its original version it used two DNA aptamers selected for their targeting of a given pair of proteins³. When the aptamers were close enough because of target-protein interaction, they could ligate to a linker, and then the ligation products could be detected by PCR. In a subsequent version, the signal was enhanced by rolling circle amplification, which allowed *in situ* analysis; hence its name of proximity ligation *in situ* assay, or P-LISA. Gomez *et al.*¹ adapted the latter method by using a biotinylated probe targeting the gene of interest, in this case *MYH11*, and another probe targeting the chromatin modification, namely H3K4me2 (Fig. 1a). They conjugated PLA probes to rabbit anti-biotin and mouse H3K4me2 primary antibodies. In theory, then, the method can be applied to any gene–chromatin mark pair. I am excited by the potential application of the method to visualize higher-order chromatin modifications of genomic regions, such as large organized chromatin K-modifications⁴ or lamina-associated domains⁵, which are thought to change during development^{4,6}, epithelial-mesenchymal transition⁷ and cancer⁶. Such experiments might be combined with optical methods for visualizing the relationship of these modified regions to the nuclear membrane in cell populations. Another promising application of the new method is in examination of the cellular heterogeneity of epigenetic marks (Fig. 1b). Epigenetic information may be represented not only in the mean, but also in the variability of the mark, allowing for greater plasticity of a tissue's response to the environment. Such epigenetic heterogeneity has been observed indirectly^{8–10}, but using this method, it could be observed directly.

The current study also shows the far-reaching contribution of inventors, who are often underappreciated by the scientific community, except for by other inventors. The current paper is a third-generation invention. The first-generation method in this case was obviously PCR itself. But for the second generation, PLA, the credit goes to Landegren, a soft-spoken and generous Swedish 'methods guy' at Uppsala University. He also invented padlock probes¹¹, which, along with their variants, lie at the heart of methods for capture-based genome sequencing strategies. I'm not sure that the current American funding system would appreciate Professor Landegren, as study sections generally reward biology over technology, but I, for one, am extremely grateful for his contributions.

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Figure 1. ISH-PLA and one of many potential applications. **(a)** General scheme of ISH-PLA. **(b)** A possible application includes a dual-color ISH-PLA for two chromatin marks. Users could then test for cellular heterogeneity of epigenetic marks (yellow and red dots) in normal and cancer cells.