A conundrum in 3-D genome organization and expression?

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ABSTRACT **Recent advances in our understanding of how the genome is folded within the nucleus have included cases in which this positioning correlates with gene expression, either positively or negatively. But is the 3-D location of a gene a cause or an effect of its expression? In this *Perspective* I articulate the problem and then cite as guideposts recent cases where causation has indeed been arguably established. The hope is to critically illuminate this issue for continued consideration in this important, evolving field.**

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There are numerous cases where results require assessment of the degree to which causation of a studied phenomenon has been rigorously established. I had an encounter with the cause-versus-effect conundrum early in my career. Before promoters, enhancers, and transcription factors had been discovered, there were studies that implicated nonhistone chromatin proteins in gene activation. Though I admired this work, I pondered the degree to which cause had been established. In at least the case I studied, it turned out that the implicated proteins accumulated at sites of transcription because they are ones that bind to the nascent RNA, not to the activated DNA (Pederson, 1974).

The notion that a gene's location within the nucleus can influence its expression comes down to us from the classic case of position-effect variegation in *Drosophila*, discovered almost a century ago (Schultz, 1936). In the modern era we have many more cases, especially as chromosome capture technology has revolutionized this field (Dekker *et al.*, 2002; Pederson, 2021). Yet, we should always ask whether the intranuclear location of a gene determines its expression or is the result of it, as this bears, for example, on understanding the establishment of the differentiated state. It has not always been straightforward for this distinction to be made.

Let us consider the following "thought experiment." The so-called Philadelphia chromosome has an ~80% correlation with chronic myelogenous leukemia (CML) (Nowell and Hungerford, 1960), and subsequently it was found that this chromosome had undergone a reciprocal translocation (Rowley, 1973). But had causation with CML been established? Not really. It was only later, when the BCR-ABL1 fusion gene product was shown to be oncogenic, that one could have said this was "demonstrated." Of course, in this case "position" is at minimum the covalent abutment of the two chromosome fragments.

But we might ponder this: when this reciprocal translocation occurs, the locations of other chromosomal loci could in principle also change. The discovery of spatially defined and restricted "gene territories" in the interphase nucleus would not be made for another decade (Cremer *et al.*, 1982), so this caveat was not raised. But it would have been a perfectly valid point. Even today, the possibility that some other gene locus near the Philadelphia chromosome is changed in its location and thus influences the pathogenic process cannot be formally ruled out. Strictly speaking, this issue arises in all cases of reciprocal translocations (Pederson, 2003). Now let's ask whether there have been any compelling cases of causation. It appears that there have been, and they help establish ground rules for assessing other cases ahead.

An astonishing discovery was that the typical spatial arrangement of euchromatin and heterochromatin, with the latter predominantly in the nuclear periphery, is strikingly altered in the nuclei of the retinal outer rod epithelial layer in crepuscular animals (Solovei *et al.*, 2009). Here a positioning of the heterochromatin to the nuclear center actually creates a diffracting lens that enhances visual acuity at low-light levels. This paper is, in my opinion, the most compelling case that the genome's 3-D intranuclear organization is under evolutionary selection. The notion that there is some other parallel event in these retinal cells, yet to be discovered, that enhances

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Abbreviations used: ABL1, a protein tyrosine kinase gene related to the Abelson leukemia oncogene; BCR, breakpoint cluster region; CML, chronic myelogenous leukemia.

or contributes to low-light-level visual acuity seems reductio ad absurdum.

Are there more recent cases where the nuanced edge of cause versus effect has been addressed in the 3-D nucleome field? A study of patients with Covid-based anosmia revealed a non–cell autonomous intranuclear reorganization of the genome including olfactory receptor genes (Zazhytska *et al.,* 2022). From the axis of formal logic, this important finding doesn't prove that the Covid infection caused this, but it comes very close. Consider the less plausible alternative, that a previous global change in the 3-D nucleome (caused by …?) rendered these persons predisposed to Covid infection.

Subsequently, many observed alterations in the 3-D organization of the genome have been shown to correlate with gene expression changes, as has been impressively uncovered in the past 5 years through the National Institutes of Health 4-D Nucleome Initiative as well as by other teams. Many of the most recent of these were reported at the Cold Spring Harbor Laboratory meeting on Genome Organization and Nuclear Function, May 3–7, 2022, where the cohesin-mediated, chromatin loop extrusion concept (e.g., Emerson *et al.,* 2022; Gabriele *et al.,* 2022) was a major focus. But many of these changes in the 3-D nucleome are programmed, that is, under developmental regulation rather than via experimental manipulations, with the correlative effects on gene expression assessed. We do of course have ways to experimentally redirect a gene's location in eukaryotic cells (Finlan *et al.,* 2008; Kumaran and Spector, 2008), and there are more recent, CRISPR-mediated methods tracking of genomic loci in live cells (Ma *et al.,* 2019) and even for inducing heterochromatinization of targeted euchromatic loci (Feng *et al.,* 2020). In a broader context one might think of this issue in terms of the classical genetic phenomenon of epistasis. In the modern era, the elucidation of gene regulatory networks brought this to a deeper level of understanding (Davidson, 2006), and it may be that genome repositioning events as discussed here are also at play. Also to be borne in mind is the fact that the 3-D organization of the genome is cell type–specific within an organism so that the envisioned effects might similarly vary.

It is worth noting that the issue raised in this *Perspective* can in principle operate in *cis* or *trans*, that is, that the changes in intranuclear locations of loci could be on the same chromosome as the "primary" affected locus, or in other chromosomes in the 3-D nucleome. The *cis* case leads to the provocative question of whether gene location affects expression, as either cause or effect, in prokaryotes (i.e., along the single-chromosome genome). This has indeed been revealed in both *Escherichia coli* (Scholz *et al.*, 2019) and *Caulobacter crescentus* (Le *et al.*, 2013; Le and Taub, 2016).

A final point is that although this *Perspective* has emphasized transcriptional changes, we know that DNA replication, repair, and recombination all can be affected by alterations in the 3-D genome (e.g., Gnan *et al.,* 2021, as regards replication). For all these aspects of genome activity, we must push forward to find more ways to experimentally change, and then reverse back to normal, the 3-D location of genomic loci, in constant pursuit of resolving the cause-effect conundrum.

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