Bell *et al. Genome Biology (2024) 25:320*
https://doi.org/10.1186/s13059-024-03461-x

Genome Biology

REVIEW

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Chromatin-based memory as a self-stabilizing influence on cell identity

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Abstract

Cell types are traditionally thought to be specified and stabilized by gene regulatory networks. Here, we explore how chromatin memory contributes to the specification and stabilization of cell states. Through pervasive, local, feedback loops, chromatin memory enables cell states that were initially unstable to become stable. Deeper appreciation of this self-stabilizing role for chromatin broadens our perspective of Waddington's epigenetic landscape from a static surface with islands of stability shaped by evolution, to a plasticine surface molded by experience. With implications for the evolution of cell types, stabilization of resistant states in cancer, and the widespread plasticity of complex life.

Background

"*Everything in epigenetics is circular*"—Amos Tanay, EMBL Chromatin and Epigenetics, 2019.

The formation of distinct, stable cell types from a single genome is a remarkable achievement of biological evolution. The noise due to low molecule numbers, the microscopic scale of molecular processes, the diversity of macromolecules, and the interconnectedness of regulatory interactions presents a persistent challenge to the stability of a cell [1–3]. Yet for the most part, cell types in complex multicellular organisms are specified correctly and function appropriately to achieve successful development and reproduction.

The remarkable stability of cell types and organisms captured the attention of Waddington as early as the 1950s, when he famously proposed the analogy of an "epigenetic landscape" to describe cell type specification during development [4]. In this analogy, cells are visualized as balls rolling down the canals carved into a landscape shaped by the genotype, as they proceed towards their final fate. Waddington was struck by the ability of development to proceed appropriately in the face of perturbations, which he described with the term, canalization, now more commonly referred to as robustness [5]. At the time, the underlying basis for this robustness was inaccessible to Waddington,

as little was understood about the molecular basis of cell and developmental biology [6]. Now with our much deeper understanding of the principles and mechanisms of cell type specification and maintenance, it seems an appropriate time to revisit this analogy and update our view of how cell types are specified, and stability is achieved.

On the most fundamental level, stability requires memory of prior states and a way for the system to assess whether it is deviating from the appropriate state. Therefore, stable systems, like cells or organisms, must have evolved mechanisms to remember. In eukaryotic cells, there are two memory systems that are considered most important for maintaining cell identity. These are network-based memory (*trans* or global memory) and chromatin-based memory (*cis* or local memory) [7–9]. The cooperative activity of these two memory systems is what is often referred to as "epigenetics"—heritable maintenance of gene activity states independent of changes in the underlying DNA sequence [10]. Importantly, for cis or trans memory to influence cell identity, they must feed information back into the system as an input through a type of circular logic, known as feedback [11]. This feedback can come in two different forms. Negative feedback, where increased output has a negative influence on future output, and positive feedback, where increased output amplifies future output. Negative feedback helps buffer away from homeostasis, while positive feedback enables systems to bifurcate and establish two divergent states, i.e., bistability [11]. As we will explore below, these feedback loops are prevalent at both the network and chromatin level and help to specify and stabilize cell types [8, 9].

Despite an increasing appreciation of chromatin-based mechanisms in cell type specification, most of our conceptual models are based on the idea that GRNs are the primary force regulating cell type identity and stability [12–17]. Here, we will explore the traditional view of cell type specification, before exploring how chromatin memory contributes to the stabilization of cell types and how a deeper appreciation for this role of chromatin alters our perspective of cell type specification.

The network basis of stability

In multicellular organisms, different genes are expressed in different cell types, directed by complex gene regulatory networks (GRNs). These GRNs are coordinated by combinations of transcription factors that recognize and activate specific genes [17]. The traditional view in cell and developmental biology is that GRNs specify and stabilize different cell types [18–20]. As a result, the properties of GRNs have been investigated for decades, pioneered by the early work of Stuart Kauffman [21], who famously created a minimal model of a gene regulatory network, in which he randomly connected a large number of genes. Through this analysis, he demonstrated that islands of stability, known as "attractor" states [21, 22], emerge naturally as a consequence of randomly connecting nodes in the network. This suggested that cell type stability could be an expected and emergent outcome of connecting large numbers of genes into a gene regulatory network. Despite this insight, it remained unclear whether the stability produced by these random connections was really sufficient to maintain identity in the highly complex and messy environment of a real cell.

Over the years a more detailed and nuanced view of GRNs has emerged. GRN wiring is not random but has evolved a highly organized structure. The structure is largely hierarchical, hub-like, and modular, as convincingly argued by Eric Davidson, who explored

GRNs in a variety of developing organisms [19]. Under his model, master regulator transcription factors sit on top of a hierarchical network, cooperating to regulate "batteries" of effector genes that perform the necessary functions of the cell. These master regulators are themselves very tightly regulated, through autoregulatory and cross-regulatory interactions that involve extensive positive and negative feedback loops [19, 23]. This hierarchical, hub-like, modular network structure centralizes regulatory potential on a small number of highly connected and very tightly regulated genes, which helps to reduce crosstalk between network components and de-constrains the evolution of new stable networks [19, 24, 25]. Over the years, computational modeling and experimental approaches have largely validated this conceptual model of GRN structure [24, 26–32].

To summarize, the network-perspective considers cell types as the product of the regulators they express [12, 17]. Cell types are thought to represent points of stability on a static epigenetic landscape that is shaped by the genotype of the organism [4, 14]. The reality, however, is that cellular memory is not only encoded in GRNs—it is also encoded into chromatin. Chromatin-based mechanisms are inherently flexible and adaptive, and as a result, are not only shaped by evolution, but also by experience. As we discuss below, the plethora of feedback loops involved in chromatin regulation suggest that it has evolved as a general mechanism to help stabilize cell identity. Therefore, rather than finding pre-existing islands of stability (defined by the GRN), cell types may also be able to self-stabilize through the use of chromatin-based feedback mechanisms [33]. This perspective alters our view of the epigenetic landscape from a static structure shaped by evolution to a plasticine structure molded by experience.

Contribution of chromatin-based memory to identity and stability

The fact that "*everything in epigenetics is circular*" is a known frustration for anyone who has attempted to deduce the mechanism of a chromatin-based process. The extensive feedback loops involved in chromatin regulation render attempts to define causative relationships and the order of events at chromatin almost impossible. Chromatin is awash with positive and negative feedback loops suggesting that it plays an important role in mediating memory and cell type stability [8, 33–41]. Below, we have provided specific examples of chromatin memory mechanisms (Table 1) and refer the reader to the following review for more detailed and specific examples of chromatin memory mechanisms [42]. We have defined chromatin as the full polymer of DNA and histone proteins; therefore, any modifications added to the DNA or proteins of chromatin are considered as potential sources of chromatin memory.

The clearest example of the role for chromatin memory in stabilizing cell types is euchromatin and heterochromatin. Euchromatin is enriched for active genes and is largely open and accessible to regulatory proteins, while heterochromatin is gene-poor and closed, preventing access. Heterochromatin locks away large parts of the genome, increasing the energy required for activity, which helps to prevent aberrant activation of inappropriate genes [19, 66–68]. Euchromatin and heterochromatin can be broadly viewed as a bistable system, each of the states is initiated and maintained by self-reinforcing positive feedback loops [69–71]. The stability of this memory is reflected by the stable maintenance of X-chromosome silencing throughout the organism's life.

Table 1 Representative examples of feedback mechanisms on chromatin

Chromatin state	Enzyme	Known feedback loops	Refs
Active positive feedback			
Euchromatin/open chromatin	Various enzymes	Active chromatin is established by transcription factors, which recruit enzymes that remove nucleosomes and deposit acetylation, increasing chromatin accessibility. This promotes further transcription factor and RNA Pol2 occupancy	[43]
H2AK120ub	RNF20/40	Deposited co-transcriptionally. Promotes DOT1L activity to promote further transcription	[44]
H3/4Kac	CPB/P300	Deposited by CBP/P300. Promotes further CBP/P300 occupancy through bromodomain-dependent binding	[45]
H3K4me3	MLL/SET	Deposited co-transcriptionally. Promotes TAF3 binding to trigger further transcription, recruitment of SETD1 and H3K4me3	[46]
H2BK120ub	UBE2A, RNF20/40	Deposited co-transcriptionally. Stimulates activity of ASH2L which deposits further H3K4me3	[47]
Active cross-antagonism			
H3K4me1/2/3	MLL/SET	Blocks DNMT occupancy to prevent DNA methylation	[48]
Nascent RNA	RNA Pol2	Blocks PRC2 occupancy to prevent H3K27me3	[49–51]
H3K27ac	CBP/P300	Sterically blocks H3K27me2/3	[52]
H3K36me2/3	SETD2	Deposited co-transcriptionally. Prevents PRC2 activity	[53]
SWI/SNF	SMARCA2/4	Antagonizes the function of PRC2	[54]
Repressive positive feedback			
DNA methylation	DNMT1	Recognizes hemi-methylated DNA and deposits DNA methylation	[55, 56]
DNA methylation	DNMT1/3	Promotes recruitment of SUV39H to trigger H3K9me3	[57]
H3K27me3	PRC2	Triggers PRC2 spreading to deposit further H3K27me3	[58, 59]
H3K27me3	PRC2	Promotes recruitment of PRC1 through CBX proteins to stimulate further reinforcement of repression	[60]
H2AK119ub	PRC1	Promotes PRC2 activity to further reinforce repression	[61]
H3K9me3	SUV39H1/2	HP1a recognizes H3K9me3 to recruit SUV39H1/2, leading to further H3K9me3	[62, 63]
Repressive cross-antagonism			
Heterochromatin	Various	Blocks transcription factor and RNA Pol2 access	[64]
H3K27me2/3	PRC2	Sterically blocks H3K27ac	[52]
DNA methylation	DNMT1/3	Blocks transcription factor binding	[65]

This type of large-scale chromatin memory is so strong that it can even be retained upon major changes in cell state. For example, during iPSC reprogramming, cells that transition from a fully committed cell type into a pluripotent cell state retain memory of their cell of origin [72–78]. This memory is retained in the form of heterochromatin signatures, such as H3K9me3, lamin-B1, and CpH methylation [79], which are known to participate in self-sustaining positive feedback loops (Table 1). Only through transient reprogramming through a "naïve" developmental state can this chromatin memory be fully erased, and a genuinely reprogrammed iPSC line be derived [79]. This

memory is unlikely to be restricted to iPSC reprogramming and may also be retained in cases of trans-differentiation, or more subtle changes in state.

Chromatin-based memory also manifests at the scale of individual genes. Perhaps the most well-defined example of gene-specific memory is the MLL/Trithorax–Polycomb axis [34, 80, 37, 81, 82]. In metazoan organisms, the MLL–Polycomb axis has become one of the dominant mechanisms of gene regulation and involves a diverse array of different proteins [83]. MLL complexes are associated with activation, while Polycomb complexes (PRC1 and PRC2) are associated with repression. Both sets of complexes participate in complex positive feedback loops. For example, PRC2 is activated by the histone modification that it deposits, which increases its ability to spread to adjacent regions [34, 80, 82]. Both axes are also mutually antagonistic and therefore, once established, help prevent transition to the alternative state [34, 80]. These features facilitate the establishment of a gene-specific, bistable system and are thought to underpin the ability for MLL and Polycomb to mediate epigenetic memory [34, 80].

To illustrate the importance of Polycomb in maintaining memory and cell type stability, a recent study transiently perturbed the function of PRC2 in mammalian cells and observed whether there were permanent changes to cell identity [84]. Upon restoring PRC2 function, they discovered that approximately 30% of the genes that changed upon transient inhibition did not return to their initial state upon restoring PRC2 function. Disruption of their *cis*-based memory of repression caused these genes to enter an active state and, once active, they no longer possessed the necessary information to re-establish repression. Their initial repression was therefore the result of prior regulatory signals that were recorded in the chromatin state. Similar findings have also been demonstrated in *Arabidopsis* and *Drosophila* [34, 81, 85–88] and even in reductionistic synthetic biology systems [89, 90].

Overall, this suggests that the cell state is not only influenced by *trans* factors. It is also influenced by *cis* chromatin state. As a result, cell states are specified by a combination of *cis* and *trans* regulatory influences, both of which contribute to cell type stability in different ways.

How chromatin-based feedback loops could stabilize changes in identity

To understand how these feedback loops on chromatin help to stabilize different cell states, it is helpful to compare and contrast with a counterfactual scenario in which only *trans* or network properties contribute to stability. To do this, we can visualize a cell's state by taking a cross-section of Waddington's landscape, where movement in either direction from the initial stable state (attractor basin) can be interpreted as a change in identity. In a purely network-based view, when we apply an external signal that pushes the cell away from its initial state, if the signal is not strong enough to push the cell into an adjacent attractor, then upon removal of the signal, the cell will return to its initial state. Some minor gene expression changes may be retained; however, for the most part, unless another attractor state is reached, the original cell state should be restored.

Now, let us imagine the analogous situation, in the presence of chromatin-based memory. When we push the cell in a particular direction, as changes in gene expression begin to occur, feedback loops regulating the *cis* chromatin state of these genes will begin to take effect. If the signal is removed before the change chromatin state is

reinforced, then the cell will simply return to its initial state [37]. However, if the signal is sustained, genes that are downregulated may begin to be silenced by Polycomb, or locked away in heterochromatin, while genes that are activated may become more accessible and easier to activate in the future (Fig. 1). In other words, genes may switch between bistable states making the transition back to the alternative state more difficult. As a result, a state that was initially unstable could become stabilized.

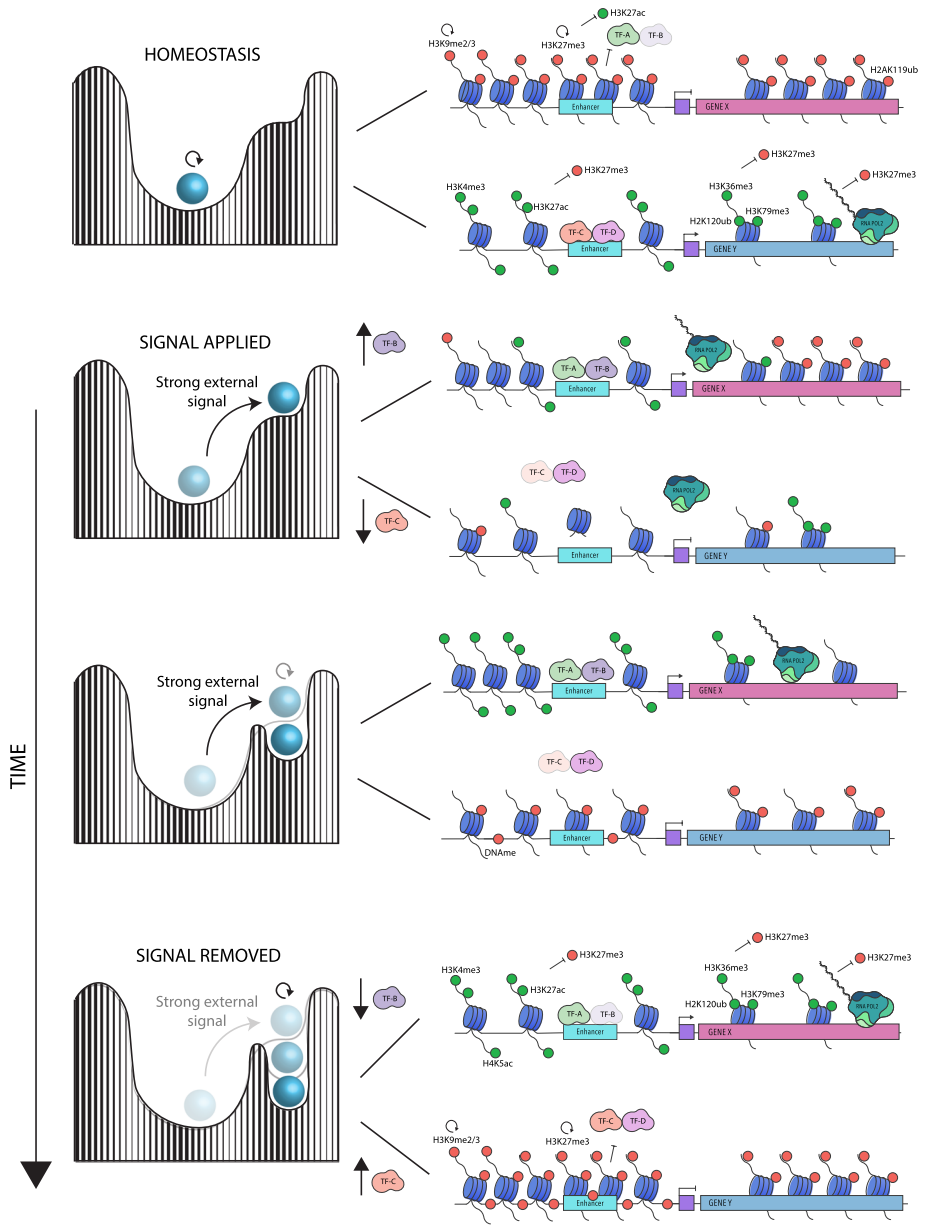

Fig. 1 Schematic representation of the stabilization of new cell states. (Left) Cross-section of Waddington's epigenetic landscape during the stabilization of a new cell state upon application of an external signal. Ball represents the cells' gene expression state over time. (Right) Corresponding changes occurring at particular loci to stabilize the new epigenetic state over time. Chromatin-based memory results in gene X and gene Y transitioning between bistable states, resulting in differential response to the same *trans* environment before and after application of an external signal. Components of this figure were created with Biorender

The adaptive nature of chromatin regulation causes genes that were driving the cell back to the attractor basin to become harder to activate, while genes that were activated in response to the signal easier to maintain. The net overall result could be that a new stable state is produced, a state that was unstable when the initial stimulus was applied and would not have become stable based on the GRN alone. This simple example demonstrates the power of feedback loops acting locally on chromatin to provide a general mechanism for self-stabilization of new cell states.

Implications of chromatin feedback for cell type evolution and development

This view of chromatin memory as an adaptive, self-stabilizing influence on cell state can help to explain the establishment and maintenance of stable cell types. Here, we explore two important contexts in which this is relevant: development and cancer.

The process of development requires the specification of multiple cell types from a single genome. Since gene number does not scale linearly with genome size or organismal complexity, it appears that instead of evolving new genes, organisms generally create new cell types by using unique combinations of the existing set of genes [91]. This creates a challenge, as each new combination must be compatible, and not interfere with the other combinations expressed from the same genome. As cell diversity increases, this challenge becomes increasingly difficult. Many network properties, such as modularity, help to enable the functioning of these combinatorial regulatory systems [19, 91]; however, chromatin memory could provide a generic and flexible solution.

When a new cell type first evolves, it is not likely to be highly stable. Due to the tinkering nature of evolution, the regulatory connections within the new GRN are unlikely to have been fully optimized to minimize crosstalk with other GRNs. Chromatin-based self-stabilization could help an initially low-stability cell type to become stabilized, if the cell type can persist for long enough for positive feedback loops acting locally at specific genes to "lock-in" the set of expressed genes. The longer the cell remains in a particular state (through intrinsic or extrinsic signals), the more stabilized it becomes and thus analogous to the hypothetic example above (Fig. 1), an initially unstable cell type becomes stable. This self-stabilizing function of chromatin may play a key role in reducing the constraints for the evolution of new cell types by allowing networks to function appropriately with less initial optimization.

Based on this view, we would expect that in many cases, perturbations of chromatin regulation would not necessarily have major phenotypic effects, instead resulting in a general destabilization of cell type identity. Several recent studies provide support for this view [92–97]. In fact, many perturbations of chromatin regulators display similar phenotypes as other proteins implicated in regulating robustness, in that they are only visible in suboptimal environmental conditions [94, 97]. Additionally, mutations in chromatin regulators are widely observed in cancers, where they are not essential for cell growth, but their loss has been proposed to destabilize cell type identity and promote phenotypic plasticity [98–102]. These findings support the idea that chromatin regulation acts as a flexible and adaptive stabilizing mechanism [94, 103].

Implications for cancer evolution

In recent years, it has become increasingly apparent that cancer cells adapt to therapeutic pressure, not only through mutations, but also through non-genetic mechanisms [99]. Non-genetic changes are likely to also be important in the initiation and evolution of treatment naïve cancer [104]; however, most current work has focused on non-genetic adaptation to therapeutic pressure [99]. Non-genetic adaptation provides a perfect case study for the stabilization of new cell states in response to external signals, as these resistant states are not physiological and therefore could not have evolved stable networks across evolutionary time. Consequently, it remains unclear how these states can be produced and stabilized in the absence of genetic changes.

In an attempt to explain this mystery, some have proposed that perturbations, such as cancer treatment, push cells across the epigenetic landscape into new stable attractor states [12, 22, 99, 105–107]. In this conceptual model, cancer cells are thought of as aberrant states sitting adjacent to the normal developmental landscape, and upon therapeutic pressure, these cells are pushed into alternative aberrant states. However, there are now numerous examples demonstrating that non-genetic adaptation can be a gradual and continuous process that does not involve the discrete transitions in identity that would be expected by this model. For example, adaptation of melanoma to BRAF inhibitors results in a gradual acquisition of the resistance phenotype, which the authors described as being "burnt in" by the therapeutic pressure [108]. Likewise, resistance to PARP inhibitors in ovarian cancer, or ALK inhibitors in NSCLC, has been demonstrated to occur through a continuous adaptive process towards the stable resistance state, with no clear mechanism provided to explain the stabilization of these states [109, 110].

We propose that the adaptive remodeling of chromatin could help to explain the stabilization of these transitionary states and facilitate gradual non-genetic adaptation in response to therapeutic pressure. Due to feedback loops on chromatin, genes that are upregulated by therapeutic pressure become more difficult to repress, while genes that are downregulated become more difficult to activate. As a result, over time, these changes are "burnt in" due to chromatin memory stabilizing this new therapy-resistant, epigenetic state. We speculate that many of the recently developed "epigenetic" therapies [104], which directly target chromatin proteins, may be ideally placed to help erase these chromatin-based memories, and in turn, help prevent or overcome non-genetic resistance [111].

Conclusions

Here, we have argued that chromatin regulation provides an important self-stabilizing role in cell type specification. This perspective helps to reconcile two traditionally conflicting views in the field of epigenetics [7, 12, 112, 113]. One perspective considers trans factors, such as transcription factors and the GRNs they regulate, as the primary factor in cell type specification [12, 17–19]. The other perspective argues that cis factors, such as chromatin regulation, also play a major role in cell fate decisions [114]. Those advocating the GRN view rightly argue that from an informational perspective, transcription factors are required to specifically activate the genes that specify a cell's state [12, 18, 19, 115]. Yet, they often overlook the undisputable functional evidence that chromatin regulation is required

for appropriate cell type specification [92, 116]. Our view is that, despite lacking a direct, instructive role in activating specific gene expression programs, chromatin regulatory mechanisms play a central role in reinforcing and stabilizing cellular state. Through the use of extensive positive feedback loops, chromatin memory in effect imprints the cellular state onto itself, providing the ability for cells to constantly sense and remember past intrinsic and extrinsic signals.

Since chromatin state is dynamic and therefore constantly updated, rather than being restricted to cell states for which a suitable GRN has evolved, chromatin-based memory provides a flexible and general mechanism for stabilizing new states in response to new internal or external conditions [117–119]. We believe this self-stabilizing function not only has a critical role in the evolution of new cell types, but also during the timescale of an individual organism, providing cells with the plasticity to adopt new, non-encoded states. Self-stabilization through chromatin memory removes the need for cells to access points of stability on static epigenetic landscape [12], instead providing cells with ability to create new stable states by constantly adapting their chromatin to suit the particular environment to which they are exposed. Ultimately, this ability stems from the fact that chromatin memory acts in cis to regulate gene activity locally, helping to separate the regulation of individual genes from the global network. Divorcing local and global activity reduces the constraints for establishing a stable cell type, allowing the cell to access a much broader range of stable states [113].

Overall, we propose that the evolution of chromatin regulation as a flexible, adaptive, and local memory system was an important prerequisite to encoding multiple cell types from a single genome and, in turn, enabled the amazing diversity and plasticity of complex life.

Acknowledgements
The authors would like to thank Graham Magor and Scott Berry for their critical input and discussions about the manuscript.

Peer review information
Wenjing She was the primary editor of this article and managed its editorial process and peer review in collaboration with the rest of the editorial team. The peer-review history is available in the online version of this article.

Authors' contributions
C.C.B and O.G conceived of the manuscript. C.C.B, G.J.F and O.G co-wrote and reviewed the manuscript.

Funding
This research was supported by Cancer Council Victoria Postdoctoral fellowship (C.C.B.), ARC DECRA fellowship (C.C.B.) #DE250100618, NHMRC Project Grants #1173711 (G.J.F.), #1146192 (O.G.), #2021063 (O.G.), NHMCR Synergy Grant #2019251 (G.J.F.), Cancer Australia #2003170 (G.J.F.), VCA Mid-Career Research Fellowship (O.G.), and the Mater Foundation (C.C.B., G.J.F.).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Competing interests
G.J.F. is a member of the ***Genome Biology*** editorial board. All other authors declare no competing interests.

Received: 24 July 2024 Accepted: 16 December 2024
Published: 30 December 2024

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