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Heritable Epigenetic Changes Alter Transgenerational Waveforms Maintained by Cycling Stores of Information

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Our view of heredity can potentially be distorted by the ease of introducing heritable changes in the replicating gene sequences but not in the cycling assembly of regulators around gene sequences. Here, key experiments that have informed the understanding of heredity are reinterpreted to highlight this distortion and the possible variety of heritable changes are considered. Unlike heritable genetic changes, which are always associated with mutations in gene sequence, heritable epigenetic changes can be associated with physical or chemical changes in molecules or only changes in the system. The transmission of cycling stores along the continuous lineage of cells that connects successive generations creates waves of activity and localization of the molecules that together form the cell code for development in each generation. As a result, heritable epigenetic changes can include any that can alter a wave such as changes in form, midline, frequency, amplitude, or phase. Testing this integrated view of all heritable information will require the concerted application of multiple experimental approaches across generations.

1. Introduction

From single cells that divide into similar daughter cells to complex organisms that mate and generate similar organisms, living systems largely preserve their form and function from one generation to the next. This similarity across generations is observed at many stages of development. Yet, during development, both form and function change dramatically within each generation. Therefore, the lineage of cells that connects generations needs to preserve or recover the information for generating entire organisms through nearly reproducible development. Cells of this lineage copy the replicating stores of sequence information in the genome at each cell division and recreate the cycling stores of regulatory information in the arrangements of molecules at the start of each generation. Together, these two forms of heritable information make up the cell code for building an organism.[1]

For organisms to evolve, cell codes need to change. A change introduced into any living system can formally result in three possible responses across generations: dilution, restoration, or maintenance (**Figure 1**). Dilution is the expected default option for any change that is not recognized by evolved mechanisms. To actively restore the prior state, living systems need to recognize the change using evolved sensors and engage negative feedback mechanisms that reduce the change. To actively maintain the new state, living systems need to similarly recognize the change but engage positive feedback mechanisms that amplify the change.

The similarity of organisms from one generation to the next suggests the presence of robust mechanisms for preserving similar molecular arrangements across generational boundaries. This transgenerational homeostasis[1] likely relies on the ability of organisms to recover from changes. For example, DNA repair pathways can restore the original sequence after mutation and thus preserve replicating stores of heritable information. Such homeostatic control is also necessary for all molecules or arrangements of molecules that persist as cycling stores of heritable information because they are exposed to environmental perturbations and molecular motions that are inherently stochastic. Changes that overcome homeostatic mechanisms that act on either store of heritable information could alter cell codes and drive evolution.

Here, I consider the heritable changes we have been able to induce thus far and the diversity of heritable changes that can occur in principle to expand the conception of heredity in living systems.

2. A Unit of Heredity Is More than the Changeable Part

Inferred molecular causes of heritable changes have shaped our understanding of all heritable information. The earliest experiments on peas led to the idea that there could be a particulate basis for heredity.[2] At the time of these early experiments, however, the molecular identities of such particles were not known but their possible plurality and transience were acknowledged.

> *The attribution attempted here of the essential difference in the development of hybrids to a permanent or temporary union of differing cell elements can of course only claim the value of an hypothesis.*
> —Gregor Mendel

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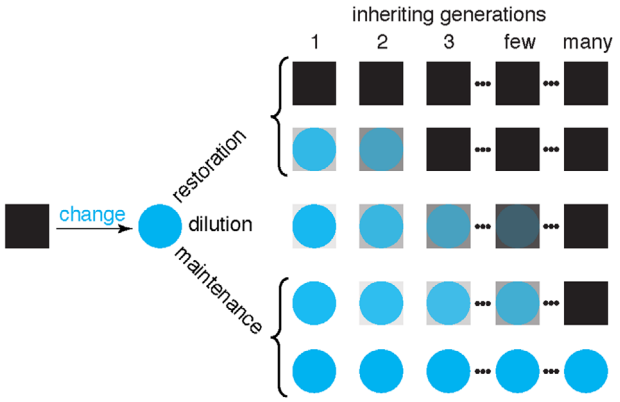


Figure 1. Changes in heritable information need to overcome restoration and engage maintenance. Any change (blue circle) of a process (black box) introduced in one generation is expected to be diluted across generations in the absence of evolved mechanisms that recognize and respond to the change. Mechanisms that act through negative feedback loops could accelerate the recovery from change (restoration). Mechanisms that act through positive feedback loops could delay the recovery from change (maintenance).

2.1. DNA Is Not Enough

Early cytological investigations associated a single trait, sex, with the physical presence or absence of particular chromosomes,[3,4] suggesting that the physical basis of other traits may also be similarly localizable. The use of X-rays and γ-rays to induce chemical modifications enabled the generation of many mutants and their analysis led to the general acceptance of the chromosome theory of heredity,[5] which postulated that individual units of heredity physically reside on a chromosome like beads on a string. However, the chromosome is simply the site of a changeable part and not the whole unit of heredity.

Building on such inferred localization, the heritable material was proposed to be an aperiodic crystal,[6] which popularized the focus on the changeable part rather than the whole unit of heredity. In apparent support for this focus, the ability of a heat-killed virulent strain of *S. pneumoniae* to transform an avirulent strain into a virulent one[7] suggested that a heat-resistant molecule was being taken up as the "transforming principle". Experiments with purified molecules suggested that this principle was DNA.[8] However, these results only show that the entire cell code of an avirulent strain can be modified through the physical addition of DNA and not that the added DNA alone is sufficient for causing virulence in all contexts.

Although phages and the bacterial cells they infect form joint living systems that are more complex than an uninfected cell, the simplicity of phages provided an opportunity to test whether the protein or the DNA from the phage was sufficient for producing more phage. Selective labeling of DNA or protein in phages revealed that the entry of DNA into bacterial cells was sufficient for the production of more phage.[9] The "genetic material" thus deduced is only sufficient for making more phage when added to bacteria and not by itself in all contexts. In other words, the phage DNA contributes to the heritable information in the cell code of an infected cell but by itself does not contain all the information required for making a phage.

With the suggestion that one gene regulates one chemical reaction,[10] the elucidation of the crystal structure of DNA[11] and the formulation of the "central dogma"[12,13] to explain the flow of sequence information among DNA, RNA, and protein, the physical localization of units of heredity on DNA and the apparent centrality of DNA were cemented. However, if the central dogma is misunderstood as sufficient explanation for the timely production of proteins and by extension everything else in the cell from DNA, the one-dimensional DNA sequence becomes erroneously regarded as the sole source of the information needed for the development of the three-dimensional organism over time.

2.2. Cell Code Is Enough

We now appreciate that heritable changes can be either genetic—caused by mutations in DNA sequence—or epigenetic—caused without mutations in DNA sequence. Yet, only molecular changes that are stable across generations continue to be appreciated as heritable information (chemical mutation in DNA sequence, physical alterations in protein folding, chemical modifications of DNA, histones, etc.). The unchanged arrangements of molecules with which such changeable parts form joint units of heredity are ill-defined and underappreciated.

Exclusive focus on the changeable parts can blind us to heritable changes in a living system that occur without accompanying chemical or physical changes in any particular molecule. For example, the cortical arrangement of cilia in *Paramecium tetraurelia* can be heritably changed in response to a transient perturbation of their orientation because new rows of cilia are made using previous rows as templates[14] (see Box 1 in ref. [1] for a summary). Where is the information for the cortical arrangement of cilia stored? It is stored in the relative geometry of the previous rows of cortical cilia within the system. Thus, changes in cortical arrangements could occur without modifying particular molecules—not all changes in the system need to leave a molecular scar.

Conversely, not all molecular scars need to reflect a significant change in the system. Ignoring the homeostatic context within which molecules exist can result in 1) undue emphasis on a particular molecular change while ignoring potentially compensatory changes and 2) the attribution of cause to particular "epigenetic marks" or molecules while ignoring the accompanying cellular and organismal contexts that can alter their interpretation.

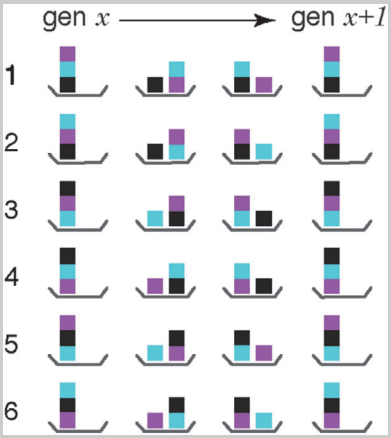
These shortcomings can be overcome by explicitly including cycling stores of heritable information. In modern parlance, the units of heredity originally imagined as "cell elements" can be thought of as having two parts: gene sequence that is part of the replicating store and gene regulators that are arranged as part of the cycling store. Genetic changes alter gene sequences and epigenetic changes alter the arrangement of gene regulators. Genetic changes can be recognized through the chemical changes in the sequence of bases in the genome. In contrast, epigenetic changes can be associated with correlated chemical changes in a molecule (e.g., histone modifications), correlated physical changes in a molecule (e.g., protein misfolding), or correlated changes in the system without any physical or chemical changes in molecules (e.g., cortical inheritance).

Box 1
Analogies for Information in Cycling Stores

A few analogies could help clarify the kinds of information held in cycling stores.

Tower of Blocks: Consider three different colored blocks stacked on top of each other in a tray. Because there are six possible vertical sequences (1 through 6 in Figure I), the maximal information stored in each vertical sequence is ≈2.6 bits ($\log_2 6$, also see ref. [15]). Let the blocks be moved at each time step in the same sequence for each set such that each move changes the arrangement of the blocks in the tray. Despite the continually changing arrangement of the blocks, executing the same series of moves can recover the initial configuration of the tower of blocks, and thus the stored information, in each case. Analogously, continually changing arrangements could preserve information by periodically returning to similar configurations within the lineage of cells that connects successive generations.

Figure I. Analogy for the preservation of information despite changing arrangements in cycling stores of information. The order of blocks in different arrangements (1 through 6) is transmitted from one generation (gen *x*) to the next (gen *x+1*) through a series of operations where the relative order changes but the original order is ultimately recovered.



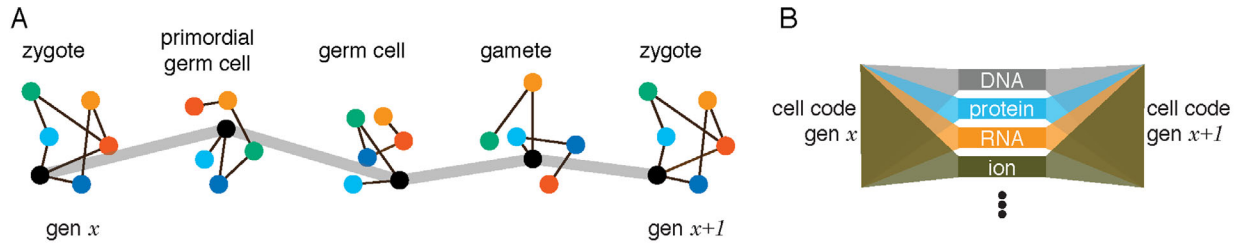
Water in Container: Imagine water in a container. Let this be the start of generation one from which we proceed to the next generation by following a series of instructions. First, freeze the water in the container. Second, break the container leaving the block of ice intact. Third, pour plastic around the ice forming the container again. Fourth, melt the ice. Now we have water in the container again—the next generation. In our minds, we went from the shape in one generation to the shape in the next generation. Yet, where did the information for the shape of the container originate? It originated in our minds. Where was the information for the shape stored throughout the life cycle? It was transferred from one store to another, from container, to ice, to container. Cycling stores have been similarly transmitting form and function since the origin of life with the information being transferred continually among different kinds of molecules. Furthermore, distortions in the transfer of information could be introduced at any step, for example, as warm plastic melts some ice in step three above. Such reductions in the fidelity of the transmitted information would similarly occur when sensors require levels of measured entities to cross thresholds for detection.[15]

Patterns in Air: Imagine someone juggling three differently colored objects—red, green, and blue. The objects can form two different patterns in the air that continually cycle: red, then green, then blue or red, then blue, then green. What does an observed pattern indicate? It reflects the sequence in which the objects were thrown up in the air by the juggler. The larger the number of objects, the more distinct patterns can be generated by shuffling the sequence in which the balls are thrown in the air (for *n* objects, there are (*n*-1)! patterns). Cycling stores similarly reflect the sequence of past regulatory events that have led to the current pattern and indicate the future patterns that will ensue.

As suggested by the strict interpretations of landmark experiments highlighted here, DNA is simply the part of the heritable information in the cell code that could be easily changed using the experimental approaches of the time. The linear DNA sequence cannot account for all the information needed for the development of an organism. The cell code, on the other hand, includes cycling stores and therefore can account for all the information needed for the development of a three-dimensional organism over time in a given environment (see refs. [1, 15]).

3. Cycling Stores of Information Encode Time in Space

Organisms progress along paths of development propelled by past regulatory events. These past events are reflected in the current composition and arrangement of molecules that in turn will influence the future. For example, the expression of a transcription factor in the past could have resulted in the production of a suite of proteins and RNAs that are localized to different parts of the cell, forming a spatial arrangement. Such arrangements



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heredity	genome sequence	cycling stores of information				
		small RNA	protein folding	chemical modification	histone modification	ion
change	mutation	abundance	prion	phosphorylation	methylation	influx
restoration	repair	degradation	chaperone	phosphatase	demethylase	efflux pump
maintenance	polymerase	polymerase	structural template	feedback kinase	methyl transferase	gated channel

Figure 2. Cycling stores enable transmission of heritable information through many interdependent channels. A) An illustration of cycling stores of information. Regulators (circles) and regulatory interactions (lines) within bottleneck stages change (e.g., traced for black) along the lineage of cells connecting successive generations (zygote of generation *x* to primordial germ cell to germ cell to gamete to zygote of generation *x+1*). B) Schematic of interdependent channels that transmit cell codes. C) Table showing specific mechanisms of change, restoration, and maintenance for different molecular stores of information. Heritable changes can be transmitted through multiple molecular channels as long as specific mechanisms of restoration are overcome and mechanisms of maintenance are engaged.

that recur during the bottleneck stage between successive generations form cycling stores of heritable information (**Figure 2**A) that have been evolving since before the origin of life.[1] Cycling stores of information thus encode the sequence of developmental events for each generation in the spatial arrangement of regulatory molecules within the bottleneck stage (see **Box 1** for illustrative analogies).

4. Heritable Information Is Distributed among Many Kinds of Molecules

The astounding spatial complexity of current cells reflects the storage of information about the very distant past and for driving very complex development. This vast information is distributed across many different molecules in the cell codes of each extant organism (Figure 2B).[1] Molecules like DNA can be used as replicating stores of information because of their ability to store linear sequence and serve as templates for replication, but also as part of cycling stores of information because of their interactions with other molecules. Molecules like proteins that catalyze reactions and interact with other molecules are part of the cycling stores of information but can also be used as replicating stores of information if they can fold into alternative states that serve as templates for replication (e.g., prions). Molecules like RNA can store sequence information like DNA as well as fold into complex shapes like proteins, and therefore can be used as part of both replicating and cycling stores of information. Small molecules like ATP or ions cannot be used as replicating stores of information, but can be part of cycling stores of information through their accumulation at different concentrations in different spatial domains. Collectively, most of the information in all these stores must be recreated with a period of one generation for the preservation of form and function in successive generations that is observed in all living systems.

In principle, changing any store of information can impact heredity and development (Figure 2C). Such changes include mutations in genome sequence that overcome DNA repair and are replicated by DNA polymerases; increases in small RNA abundance that overcome RNA degradation and catalyze production of more small RNAs through the recruitment of RNA-dependent RNA polymerases; altered protein folding of prions that overcome refolding chaperones and template similar folding of other proteins; chemical modifications such as the addition of phosphate groups to a substrate by a kinase that overcome phosphatases and recruit feedback kinases that similarly phosphorylate more of the same substrate; histone modifications such as methylation that overcome demethylases and recruit methyltransferases that similarly methylate other histones; and increased influx of small molecules or ions that overcome efflux pumps and activate ligand-gated channels that import the same small molecule or ion. When two or more such kinds of molecules are coupled into a single positive feedback loop, complex heritable changes could occur. For example, a kinase could phosphorylate a channel that imports an ion that activates an enzyme that modifies histones to promote the expression of the same kinase. Nevertheless, in every case, heritable changes simply need to overcome mechanisms for restoration and engage mechanisms for maintenance (Figure 2C).

5. The Path from Genotype to Phenotype Is through Cycling Stores of Information

Cells provide a complex and dynamic context within which molecular changes can propagate. Within a cell, the "central dogma"[11–13] is simply one route for the transmission of change.

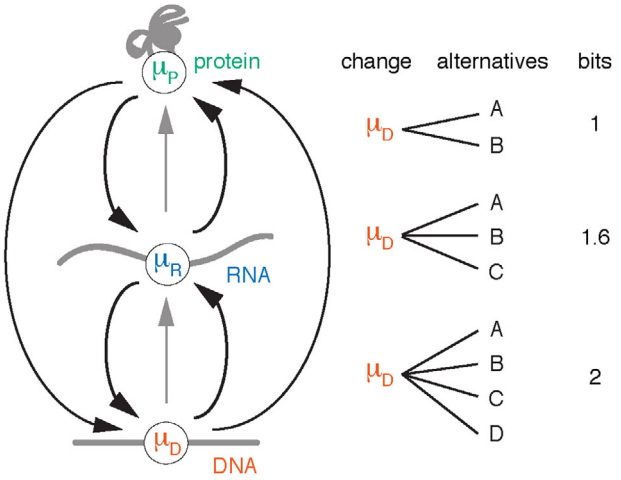

Figure 3. Mutation and Meaning. Left: Impact of changes in molecules—illustrated here with the key macromolecules DNA, RNA, and protein—is contingent on cellular context. Straight arrows indicate propagation of changes through the familiar "central dogma" and curved arrows indicate propagation of changes through the arrangements of molecules. Right: Alternative phenotypes supported by the same DNA sequence reveal information in cycling stores. Every change in DNA sequence that can result in two alternative states uncovers one bit of information in cycling stores.

Yet, the central dogma is often misunderstood as the DNA sequence dictating the RNA sequence and the RNA sequence dictating the protein sequence. In reality, the existence of processes such as alternative RNA splicing and RNA editing means that the DNA sequence constrains possible RNA sequences that accumulate in a cell. Similarly, the existence of processes such as protein splicing, post-translational modifications, and pro-protein cleavage means that the RNA sequence constrains possible proteins that accumulate in a cell. In short, DNA proposes, cell disposes.[1]

Ignoring the importance of cellular context can result in misconceptions about living systems and potentially grave errors in medical judgment. For example, the Merriam-Webster dictionary defines phenotype as "the observable properties of an organism that are produced by the interaction of the genotype and the environment". This definition ignores the varied contexts provided by different cell types that can support the same genome sequence and respond differently to different environments. Furthermore, it falsely implies that the phenotype can be derived from just the genotype if the environment is held constant.

The impact of any change in an entity within a cell depends on the sensors and the relevant properties being sensed in that cell.[15] This contextual nature of the consequence of any change is evident when we attempt to enumerate the possible impacts of a mutation in DNA (μ_D in **Figure 3** Left). For simplicity, consider DNA, RNA, and proteins as the only kinds of molecules within a cell. A single mutation in DNA could alter physical interactions with an unrelated RNA or protein. If the mutated DNA is transcribed into RNA, then the mutation could alter the corresponding RNA as well (μ_R in Figure 3 Left). This change in an RNA could also disrupt physical interactions with unrelated parts of DNA or an unrelated protein. If the mutated RNA is translated into a protein, then the mutation could alter the corresponding protein as well (μ_P in Figure 3 Left). This change in a protein could also disrupt physical interactions with unrelated parts of DNA or an unrelated RNA. Thus, while the central dogma provides a simple linear route for the propagation of change within a cell, the web of physical interactions provides a multitude of branched and cyclical routes. This complexity is likely increased when chemical reactions and interactions with all other types of molecules within the cell (ions, sugars, lipids, etc.) are included. Thus, any attempts to construct a causal story to explain how an alteration within a cell resulted from a change in the DNA sequence have to deal with the variety of possible paths from mutation to meaning in the cell (Figure 3 Left).

This picture of change propagation within cells makes explicit how cycling stores of information that arise through physical interactions between many kinds of molecules provide context within a cell. Any change in DNA sequence that can result in two different outcomes in a cell reveals one bit of information in cycling stores (Figure 3 Right). The maximal bits of information in the linear genome is easily enumerated and requires only the discovery of the number of different base-pairs and the length of the genome—2L bits for a 4-base genome of length L.[15] In contrast, the maximal information in the arrangement of molecules that can support a given genome is not easily enumerated and requires the exhaustive discovery of all processes within a cell, although its general form can be understood by parsing the contents of the bottleneck stage into an entity–sensor–property system.[15] This staggering complexity precludes any simple derivation of phenotype from genotype. Nevertheless, by assuming a fixed context provided by a particular configuration of the cycling stores of information, we can predict changes in phenotype from changes in genotype. As with any unknown that is assumed to be constant, this assumption may not always be true.

In summary, genotypes can be sufficient for predicting phenotypes but not for constructing phenotypes.

6. Cycling Stores of Information Can Be Changed in Several Ways

Changes that persist for many generations without altering the sequence of a genome need to occur within the rest of the cell code that collectively form cycling stores of information. To appreciate the full range of mechanisms that can result in such transgenerational epigenetic inheritance (TEI), it is useful to consider all the ways in which these stores of heritable information can be changed.

6.1. Changes in Cycling Stores

Cycles propagating across generations form waves along the continuous lineage of cells that connects successive generations. Therefore, they can be changed by altering any aspect of the wave—form, midline, frequency, amplitude, or phase (**Figure 4**A). Consider an RNA that varies in abundance throughout development (black, Figure 4A) and a reference protein that also changes throughout development (grey, Figure 4A). Changes in how the RNA accrues over time can alter the form. Changes in the relative abundance of the RNA compared to the reference

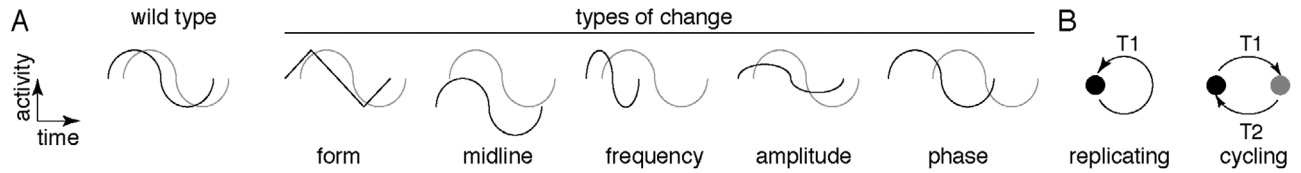

Figure 4. Changes and barriers to change in cycling stores of heritable information. A) All changes to a wave are applicable. Schematic of the variation over time in a changeable component of a cycling store (black) along with that in a reference component of the system (grey). Changes in form, midline, frequency, amplitude, and phase may all be heritable; see text for details. B) Minimal replicating and cycling stores of information have different numbers of thresholds that need to be overcome for a change to be heritable. Entities (filled circles) that serve as templates for their own replication can be changed if the single threshold needed for that entity to be seen as different by the replicating machinery (T1) is overcome. Because cycling stores minimally have two distinct entities, they can only be changed if the thresholds required for each entity to be seen as different by the other (T1 and T2) are overcome.

protein can alter the midline. Coupled changes in the periods of production and degradation can alter the frequency. Changes in the ratio of production to degradation of the RNA can alter the amplitude. Changes in the relative timing of the cycling RNA with respect to the cycling reference protein can alter the phase. Finally, combinatorial changes in multiple aspects can potentially result in complex outcomes. As stated earlier, all such epigenetic changes can be associated with physical or chemical changes in molecules or only with changes in the system.

6.2. Changes in Extra-Genomic Replicating Stores

Cycling stores can include molecules that like the genome are capable of serving as templates for replication and therefore can form extra-genomic replicating stores that are nevertheless collectively arranged as cycling stores. For example, prion proteins that can template the misfolding of other proteins can propagate the misfolded state continually using the same kind of molecule, making it a replicating store of information. Similarly, small RNAs made during RNA interference can result in the sustained production of antisense small RNAs for many generations through the repeated recruitment of RNA-dependent RNA polymerases to the same sense RNAs in every generation. However, unlike mutations in the genome, which are maintained through replication, changes in protein folding or production of small RNA do not always result in maintenance of the changed state.[17,18]

6.3. Thresholds for Heritable Change

Heritable changes to replicating stores and cycling stores need to overcome different numbers of barriers or thresholds (Figure 4B). Heritable changes in replicating stores only need to overcome one threshold—the level that overwhelms the restoration mechanism(s). Once this threshold is overcome and a change is introduced, its perpetuation is the default option. For example, once DNA repair is overcome by a mutation, DNA polymerase will copy the mutation in each cell division and thus perpetuate the change (as long as the mutation did not impair replication). Heritable changes in cycling stores need to overcome at least two different thresholds—the level that overwhelms the restoration mechanism(s) and the level required to recruit the positive feedback mechanism for maintenance. In general, for changes in cycling stores of information to propagate across generations, restoration mechanisms that could be acting at every stage of the cycle need to be overcome. For example, consider the complex loop introduced earlier: a kinase that phosphorylates a channel that imports an ion that activates an enzyme that modifies histones to promote the expression of the kinase. Transmission of this loop by cycling stores of information would result in a certain level of kinase, phosphorylated channel, ion concentration, histone modification, and expression of the kinase in every generation. For changes in the levels of any one of these components to be heritable, the mechanism for restoration of all components need to be overcome, each of which may require different thresholds for propagation to the next step in the chain. As a result, it is more likely for changes in simpler loops of cycling stores to be heritable.

7. Evidence for Heritable Changes in Cycling Stores of Information

Heritable changes of varying stability that are not associated with mutations in genome sequence have been documented in a variety of experimental systems. As outlined in the introduction (Figure 1), the stability of a change depends on the balance of restoration and maintenance mechanisms that can act across generational boundaries. Changes that cannot be explained by intergenerational mechanisms (e.g., direct exposure in utero, maternal contribution, etc.) have been labeled TEI and can be associated with correlated changes in particular molecules (see ref. [19] for a recent aggregation of many such discoveries). Here, I highlight instances of heritable change that could in principle occur without correlated modifications in particular molecules because overlooking such changes in the pursuit of molecular correlates of TEI could result in a distorted view of heredity.

The best characterized examples of transgenerational inheritance that could occur without changes in particular molecules involve modifications of cellular structures. For example, the amoeba *Difflugia corona* builds a hard shell of silica with a single opening surrounded by pointed projections called "teeth". The numbers of these teeth can be reduced in descendants simply by removing them surgically in a mother cell because daughter cells grow their new teeth in the gaps between the teeth in the mother cell.[20] Similarly, inverted ciliary rows introduced through microsurgery are inherited in a variety of ciliates—*Paramecium tetraurelia*,[14] *Tetrahymena thermophila*,[21] *Stylonychia mytilus*,[22,23] and *Paraurostyla weissei*.[24] These heritable changes in the relative geometry of cortical structures can

be simply explained by each row being made using the previous row as template. Such templating of cortical structures could in principle also result from changes in relative orientation of two distant structures that is transmitted to the cortex through the cytoplasm. One observation consistent with this possibility is the inheritance of changes in the orientation of the oral apparatus in *Tetrahymena thermophila* that can last for up to 300 generations without associated genetic changes.[25] Similarly, cells with two sets of cortical structures that result from aborted cell divisions can transmit these twin structures for many generations in *Leucophrys patula*, *Stentor coeruleus*, *Paramecium tetraurelia*, and *Tetrahymena thermophila* (reviewed in ref. [26]). These heritable twin structures could either have a two-fold rotational symmetry or mirror-image symmetry. Because similar changes can also be induced by genetic mutations, inheritance of cortical structures in ciliates is an example of a phenomenon that can be analyzed to integrate heritable changes that occur with or without molecular changes into a unified model.

In summary, changes in cycling stores of information can in principle be associated with correlated changes in particular molecules or in the arrangements of molecules within the system or in both. Awareness of all such mechanisms of heritable changes promotes the construction of realistic models of heredity in living systems without undue emphasis on any one form of heritable information.

8. Perceived Unknowns Guide Future Research

A clear conception of heredity can help define what is unknown and inspire future research.

Many kinds of molecular changes have been found to correlate with the persistence of induced states across cell divisions or generations. These include changes in histone modification,[27] DNA methylation,[28] protein folding,[29] small RNA,[30] or RNA methylation.[31] However, the detection of a correlated molecule or chemical modification is neither required nor sufficient for explaining how epigenetic changes arise, persist, or dissipate. For example, consider a case of TEI that lasts for five generations and is correlated with the presence of an RNA during some stage of development. An adequate explanation would describe how the RNA accumulates during that stage of development for five generations and then stops accumulating. Accumulation of the RNA during each changed generation cannot itself be the explanation for TEI because it could be only required to execute the changed process in each generation or worse a correlated byproduct. In short, the explanation needs to be in terms of altered transgenerational waveforms maintained by the cycling stores of heritable information that result in periodic accumulation of epigenetic changes that either dissipate eventually or persist forever.

Mistaking genome sequence as sufficient information for the construction of organisms can inflate our successes along the path toward making life. For example, replacing the genome in a cell with a synthetic genome[32] does not amount to creating life as has been articulated before (e.g., in ref. [33]). Similarly, assuming that the genome sequence of extinct organisms like mammoths[34] and Neanderthals[35] is sufficient for "de-extinction" of these ancient organisms ignores the regulatory information in the arrangement of molecules in bottleneck stages that remain unknown.

9. New Metaphors for Heredity and Development

Metaphors can drive or hinder innovation.[36] Two popular metaphors—the genome as blueprint and development as rolling down an epigenetic landscape—have been powerful for progress over the last century but now hinder a clear conception of living systems.

9.1. The Blueprint of Life

The persistent use of "blueprint" as an analogy for the genome perpetuates efforts to understand how the genotype "manifests" as phenotype.[37] Appreciating cycling stores of information[1,15] makes it clear that the derivation of phenotype from genotype is logically impossible even if all environmental conditions are controllable. Rather, what can be understood, through perturbation approaches in model systems and extant differences in populations, are correlations between changes in genotype and changes in phenotype. These correlations can vary depending on the context provided by the cycling stores of information (see Figure 3). Therefore, such correlations observed between organisms in basic research or between humans in medical applications need to be cautiously translated into actionable insights. Why a cell needs a genome is unclear, but our current understanding suggests that the genome is at best an overlapping and potentially scrambled list of ingredients. This list is used differently by different cells to make different ingredients at different times, resulting in cells with different phenotypes that host the same genome.

9.2. The Epigenetic Landscape

Of the many different representations of development considered by Waddington,[38] his conception of a ball rolling down along alternative paths—the epigenetic landscape—has endured as the most popular. This metaphor captures the inevitable downhill progression through which living systems appear to proceed as they develop—starting totipotent and becoming more and more differentiated along different paths. However, this picture distorts the fact that down one of the paths cells reach oocytes that can reach the top of the hill again upon fertilization by sperm or parthenogenetic activation. The successful reversal of apparent differentiation through somatic cell nuclear transfer[39] and induced pluripotent stem cells[40] illustrate the need for losing the idea of development as an inevitable downhill progression. The growing appreciation of the implications of these discoveries have prompted reassessments of differentiation in recent years (e.g., ref. [41]). Perhaps, a better metaphor that highlights both the cyclic nature of living systems and our current lack of understanding of the cycling stores of information is the Penrose stairs[42] (**Figure 5**) made famous in MC Escher's lithograph *Ascending and Descending* where people can walk down upward or equivalently walk up downward.

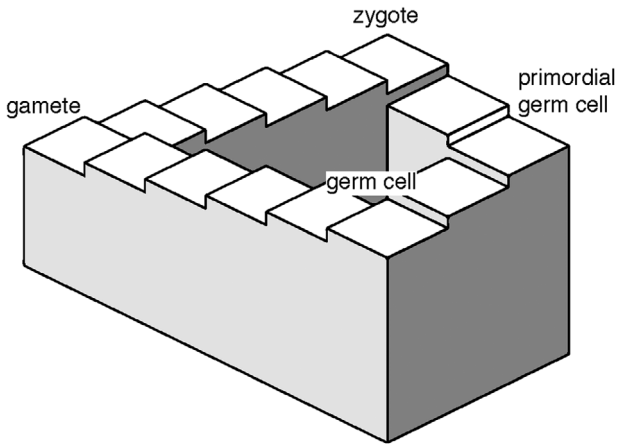

Figure 5. Penrose stairs are a metaphor for heredity and development. People can walk up or down these stairs in a never-ending loop. This perplexing continuity evokes the transmission of information through heredity and development along the continuous lineage of cells that connects successive generations (zygote to primordial germ cell to germ cell to gamete to zygote). The path along the stairs traces the continuity of generations, which can be along the germ lineage for many animals or one cell division cycle for single cells.

10. Multiple Approaches Are Needed to Analyze Cycling Stores of Information

Evolution of cell codes since the origin of life has likely resulted in very complex cycling stores of information even in the simplest of organisms.[1] The large number of components in typical cells requires large-scale approaches (transcriptomics, metabolomics, epigenomics, lipidomics, etc.) to efficiently catalog entities within cells. While these "-omic" approaches can provide selective views of entire living systems, they are not sufficient for the assembly of living systems. To enable the eventual assembly of life from molecules, we also need reductionist analyses that focus on single "cell elements" that can reveal general principles of how heritable information is transmitted as joint replicating and cycling stores. To assemble and understand a variety of such units of heredity in a variety of organisms, multiple complementary approaches need to be applied across generational boundaries. These approaches can be broadly classified as perturbation, visualization, substitution, characterization, reconstitution, and simulation based on the impact each approach has on the living system. Although each approach has its own limitations, careful integration of results from each approach applied to different units of heredity could reveal how the dynamic form and function seen in living systems are encoded as cell elements to collectively form the cell code that is transmitted across generations.

11. Conclusion

Heritable changes and their analyses have been instrumental in constructing models of heredity. Such models constructed thus far likely provide a distorted view because replicating stores are easily changed but cycling stores are not. The information in replicating stores is present in one kind of molecule and thus requires crossing only one threshold for change. The information in cycling stores on the other hand is distributed among two or more kinds of molecules and thus requires crossing two or more thresholds for change. Genetic changes occur in a replicating store and therefore are always associated with mutations in genome sequence. In contrast, epigenetic changes occur in cycling stores and therefore can be more diverse. In principle, changes that result in transgenerational epigenetic inheritance could be associated with changes in molecules, changes in the system, or both, and could alter any characteristic of the waveforms generated upon transmission of cycling stores along the continuous lineage of cells that connects successive generations. An undistorted view of heredity therefore requires the application of a variety of experimental approaches across generations to analyze both replicating and cycling stores of information. Such integrated understanding of all heritable information in organisms of varying complexity could illuminate design principles for assembling molecules into the cell code of any new life that reproduces and evolves.

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Conflict of Interest

The author declares no conflict of interest.

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[1] A. M. Jose, *BioEssays* **2018**, *40*, 1700161.
[2] G. Mendel, in Proc. of the Naturalist Association, Vol. *IV*, Brno, Czech Republic **1866** [Translated in 1901 by William Bateson for Royal Horticultural Society].
[3] N. M. Stevens, *J. Exp. Zool.* **1905**, *2*, 313.
[4] E. B. Wilson, *J. Exp. Zool.* **1905**, *2*, 371.
[5] T. H. Morgan, A. H. Sturtevant, H. J. Muller, C. B. Bridges, *The Mechanism of Mendelian Heredity*, Henry Holt and Company, New York **1915**.
[6] E. Schröedinger, *What is Life?*, Cambridge University Press, Cambridge **1944**.
[7] F. Griffith, *J. Hyg.* **1928**, *27*, 113.
[8] O. T. Avery, C. M. Macleod, M. McCarty, *J. Exp. Med.* **1944**, *79*, 137.
[9] A. D. Hershey, M. Chase, *J. Gen. Physiol.* **1952**, *36*, 39.
[10] G. W. Beadle, E. L. Tatum, *Proc. Natl. Acad. Sci. USA* **1941**, *27*, 499.
[11] J. D. Watson, F. H. C. Crick, *Nature* **1953**, *171*, 737.

[12] F. H. C. Crick, *Symp. Soc. Exp. Biol.* **1958**, *XII*, 138.
[13] F. Crick, *Nature* **1970**, *227*, 561.
[14] J. Beisson, T. M. Sonneborn, *Proc. Natl. Acad. Sci. USA* **1965**, *53*, 275.
[15] A. M. Jose, *J. R. Soc. Interface* **2020**, 20200154 (in press).
[16] M. Cobb, *PLoS* **2017**, *15*, e2003243.
[17] J. Pak, J. M. Maniar, C. C. Mello, A. Fire, *Cell* **2012**, *151*, 885.
[18] R. Halfmann, D. F. Jarosz, S. K. Jones, A. Chang, A. K. Lancaster, S. Lindquist, *Nature* **2012**, *482*, 363.
[19] G. Cavalli, E. Heard, *Nature* **2019**, *571*, 489.
[20] H. S. Jennings, *J. Exp. Zool.* **1937**, *77*, 287.
[21] S. F. Ng, J. Frankel, *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 1115.
[22] G. W. Grimes, S. W. L'hernault, *Dev. Biol.* **1979**, *70*, 372.
[23] G. W. Grimes, E. A. Knaupp-Waldvogel, C. M. Goldsmith-Spoegler, *Dev. Biol.* **1981**, *84*, 477.
[24] M. Jerka-Dziadosz, *Roux Arch. Dev. Biol.* **1985**, *194*, 311.
[25] E. M. Nelson, J. Frankel, L. M. Jenkins, *Development* **1989**, *105*, 447.
[26] J. Frankel, *Eukaryot. Cell* **2008**, *7*, 1617.
[27] A. Jambhekar, A. Dhall, Y. Shi, *Nat. Rev. Mol. Cell Biol.* **2019**, *20*, 625.
[28] M. V. C. Greenberg, Bourc'his D., *Nat. Rev. Mol. Cell Biol.* **2019**, *0*, 590.
[29] R. Halfmann, S. Lindquist, *Science* **2019**, *330*, 629.
[30] L. Duempelmann, M. Skribbe, M. Bühler, *Trends Genet.* **2020**, *36*, 203.
[31] S. Zaccara, R. J. Ries, S. R. Jaffrey, *Nat. Rev. Mol. Cell Biol.* **2019**, *20*, 608.
[32] D. G. Gibson, J. I. Glass, C. Lartigue, V. N. Noskov, R.-Y. Chuang, M. A. Algire, G. A. Benders, M. G. Montague, L. Ma, M. M. Moodie, C. Merryman, S. Vashee, R. Krishnakumar, N. Assad-Garcia, C. Andrews-Pfannkoch, E. A. Denisova, L. Young, Z.-Q. Qi, T. H. Segall-Shapiro, C. H. Calvey, P. P. Parmar, C. A. Hutchison III, H. O. Smith, J. Craig Venter, *Science* **2010**, *329*, 52.
[33] G. F. Joyce, *PLoS Biol.* **2012**, *10*, e1001323.
[34] W. Miller, D. I. Drautz, A. Ratan, B. Pusey, J. Qi, A. M. Lesk, L. P. Tomsho, M. D. Packard, F. Zhao, A. Sher, A. Tikhonov, B. Raney, N. Patterson, K. Lindblad-Toh, E. S. Lander, J. R. Knight, G. P. Irzyk, K. M. Fredrikson, T. T. Harkins, S. Sheridan, T. Pringle, S. C. Schuster, *Nature* **2008**, *456*, 387.
[35] R. E. Green, J. Krause, A. W. Briggs, T. Maricic, U. Stenzel, M. Kircher, N. Patterson, H. Li, W. Zhai, M. Hsi-Yang Fritz, N. F. Hansen, E. Y. Durand, A.-S. Malaspinas, J. D. Jensen, T. Marques-Bonet, C. Alkan, K. Prüfer, M. Meyer, H. A. Burbano, J. M. Good, R. Schultz, A. Aximu-Petri, A. Butthof, B. Höber, B. Höffner, M. Siegemund, A. Weihmann, C. Nusbaum, E. S. Lander, C. Russ, *et al. Science* **2010**, *328*, 710.
[36] D. Gray, M. Macready, *SFI Bull.* **2004**, *19*, 36.
[37] L. M. Zahn, B. A. Purnell, C. Ash, *Science* **2019**, *365*, 1394.
[38] M. Allen, *Contemporaneity* **2015**, *4*, 119.
[39] J. B. Gurdon, T. R. Elsdale, M. Fischberg, *Nature* **1958**, *182*, 64.
[40] K. Takahashi, S. Yamanaka, *Cell* **2006**, *126*, 663.
[41] A. S. Alvarado, S. Yamanaka, *Cell* **2014**, *157*, 110.
[42] L. S. Penrose, R. Penrose, *Br. J. Psychol.* **1958**, *49*, 31.