Genes and environments, development and time

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A now substantial body of science implicates a dynamic interplay between genetic and environmental variation in the development of individual differences in behavior and health. Such outcomes are affected by molecular, often epigenetic, processes involving gene–environment (G–E) interplay that can influence gene expression. Early environments with exposures to poverty, chronic adversities, and acutely stressful events have been linked to maladaptive development and compromised health and behavior. Genetic differences can impart either enhanced or blunted susceptibility to the effects of such pathogenic environments. However, largely missing from present discourse regarding G–E interplay is the role of time, a "third factor" guiding the emergence of complex developmental endpoints across different scales of time. Trajectories of development increasingly appear best accounted for by a complex, dynamic interchange among the highly linked elements of genes, contexts, and time at multiple scales, including neurobiological (minutes to milliseconds), genomic (hours to minutes), developmental (years and months), and evolutionary (centuries and millennia) time. This special issue of PNAS thus explores time and timing among G–E transactions: The importance of timing and timescales in plasticity and critical periods of brain development; epigenetics and the molecular underpinnings of biologically embedded experience; the encoding of experience across time and biological levels of organization; and gene-regulatory networks in behavior and development and their linkages to neuronal networks. Taken together, the collection of papers offers perspectives on how G–E interplay operates contingently within and against a backdrop of time and timescales.

gene–environment interplay | timing | biological embedding of experience | critical periods | gene regulation

Biological embedding occurs when early experience changes an individual's biology (e.g., stress or immune system responses) affecting their subsequent development, health, and behavior. The interplay between our genetic predispositions and the environments we experience (G–E interplay) is an essential consideration for the biological embedding of experience, as there are differences in the extent to which individuals are responsive to their experiences (1, 2). The 2012 PNAS volume "Biological Embedding of Early Social Adversity: From Fruit Flies to Kindergartners" summarized evidence, for example, that exposures to traumatic, adverse events in very early development can have disproportionately potent effects on health and disease for individual lives. Increasingly apparent are the larger susceptibilities, among organisms in multiple species, to pathogenic and supportive encounters during the fetal, infant, childhood, and adolescent periods of development (3–6).

Time and timing also appear to play crucial but not yet fully explored roles in guiding societal, developmental, and neurobiological responses to the conditions of early life. A potential for trauma within childhood environments is a well-known correlate of the historical epochs and geographical contexts into which children are born, with the Holocaust and other genocides, the Dutch Hunger Winter, the Ontario Ice Storm, and periods of famine having profound, lasting effects on the health and development of exposed cohorts and their progeny (e.g., refs. 7 and 8). For the most part, the reality of how genes and environments interact within a temporal frame of reference at multiple levels of scale has been neglected: For example, 1) a synaptic/neural circuit level in millisecond intervals of time; 2) transcriptional responses to experience in minutes to hours; 3) developmental systems in days to years; and 4) the intergenerational and evolutionary adaptedness of differentially susceptible phenotypes

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over centuries and millennia (Fig. 1). This theme and the ability to capture individual developmental trajectories with repeated, timed (and often intensive) assessments—not simply with population averages—is critical for enhancing prediction in models of disease and behavior. Along with experimentation within and across species, a focus on time and timing in studies of G–E interplay can begin to move social and behavioral epigenetic research from correlation to causation.

Time, however, is a concept even more challenging than the not inconsiderable provocations of "gene" (G) and "environment," (E), which themselves bear complexities with which science has long contended. Einstein conceived of time not as a separate, distinguishable aspect of physical reality, but rather as an intrinsic feature of a multidimensional universe (9). Human cultural history is itself deeply suffused with two contrasting visions of time: "Time's arrow," invoking the linearity and directionality of biblical and other historical narratives, and "time's cycle," reflecting the recursive, unchanging atemporality of collective human experience (10, 11). The two poles of this dichotomy summon both: 1) Day-to-day encounters with the sequential, ordered irreversibility of real-world events; and 2) either the timeless universality of enduring human values and traditions or a repetitive, cyclical patterning of human history. It is instructive to ask how time and timing, at multiple, layered levels of scale, may constitute an essential element in the operation of genes and environments. G–E interplay, viewed from the perspective of timing, may offer a deeper, more heuristic framework for understanding how aversive and supportive environments interact with genetic variation to undermine or protect human health throughout the course of development.

Gene–Environment Interplay

Much has been written about relations between genes and environments and their effects on health, well-being, and behavior (for reviews, see refs. 12–14). Historically, these writings were rooted in arguments over whether nature (genes) or nurture (environments) were responsible for individual differences in behavioral traits. After the debunking of this nature–nurture dichotomy, discussions turned to the idea that both genes and the environment contribute additively to individual differences in risk and behavior and that the size of the contributions depended upon the outcome of interest. Statistical analyses of large populations later revealed the importance of G–E interactions and G–E correlation in human behavior genetic research (14–16). G–E interactions occur when genetically different individuals exhibit different responses to environmental variation. G–E correlations are when there is a genetic predisposition for an individual to choose or alter the rearing environment or when a child's genetic variation influences how people respond to that child (e.g., how a naturally athletic child may be treated differently from a nonathletic child). The term "gene–environment interplay" expands on G–E interactions and correlations by postulating a dynamic complexity in

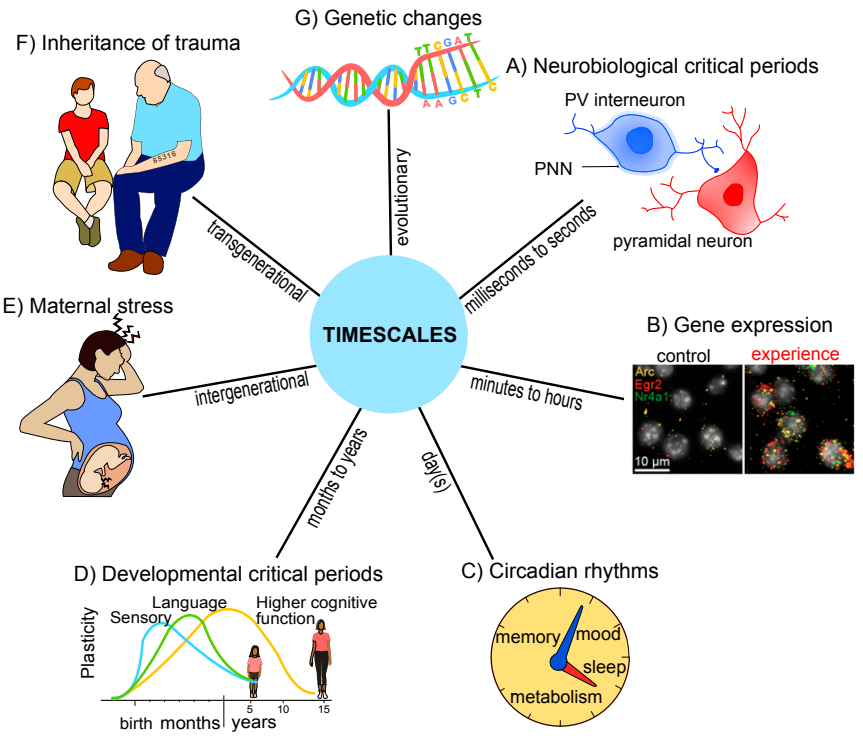

Fig. 1. Pictoral representation of time scales in G–E interplay. (*A*) At the cellular level, the opening and closing of critical periods in brain plasticity act at the millisecond to second time scale (the parvalbumin-positive [PV] interneurons of the PV cell innervates the pyramidal neuron, the perineuronal net [PNN] gradually forms around PV cells) (45). (*B*) An experience of cocaine induces the expression of multiple IEGs in the mouse dorsal striatum; ~40 Å images show expression of the following IEGs: Activity-regulated cytoskeleton-associated protein (Arc), early growth response 2 (Egr2), and nuclear receptor subfamily 4 group A member 1 (Nr4a1) in control vs. 1 h following acute cocaine (73). Reprinted from ref. 73. (*C*) Circadian clock genes generate circadian rhythms that affect many biological processes, including critical periods, sleep, metabolism, mood, and memory (45). (*D*) Critical periods in brain plasticity development are found within and across sensory, language, and higher cognitive domains. Image credit: Charles A. Nelson (Harvard University, Boston, MA). (*E*) Biological embedding of maternal stress in the developing fetus. (*F*) Transgenerational inheritance of trauma in grandchildren of holocaust survivors. (*G*) From an evolutionary time scale, the genome is a repository of changes in DNA sequence. Image credit: Sydney Gram (University of Toronto, Toronto, ON, Canada).

the reciprocal relation between genes and environment (1, 2, 14, 17–19).

Before the availability of full genome sequences, G–E interaction was studied one or two genes at a time using a candidate gene approach. As costs decreased and technologies improved, the sequencing of individual genomes, including human genomes, became possible, thereby enabling genome-wide investigation of how genes contribute to variation in a complex trait of interest, such as behavior or liability to risk (20). This was also a welcome development as it became clear that many studies of single genes were underpowered and not reproducible (21). Genome-wide association studies (GWAS) are now used to address genetic contributions to variation in a trait by identifying genetic variants, such as single-nucleotide polymorphisms (SNPs) that are statistically associated with trait variation. When SNPs are located near a gene, then it is postulated that the gene contributes to the variation under investigation.

Until recently, there has been little if any functional follow-up of the actual role a given SNP or gene plays in the trait variation under study (22). Replication of gene–trait associations identified in GWAS and their functional analyses continues to be critical. GWEIS (genome-wide by environment interaction study) brings G–E interaction analyses into GWAS, but GWEISs are uncommon (23). One challenge is to develop models that will enable multilayered measurements within environments to be incorporated into GWEIS designs. More detailed quantification of environmental/experiential factors and their timing, modeled with machine-learning approaches, should soon reflect with greater acuity the many experiential contexts that affect the lives of individuals. Functional genomics techniques—including DNA and RNA sequencing, epigenomics, and experimental manipulations using animals and cell culture—have been used together to elucidate the mechanistic bases of G–E interplay as described in the current volume.

One replicated GWEIS finding is that a SNP in *PRKG1*, a cGMP-dependent protein kinase gene, interacts with lifetime trauma exposure to affect alcohol misuse (24, 25). How this SNP might influence *PRKG1* is unknown, but animal models can be used to address this question directly (26). Another example of a G–E interaction with early life experience involves the *FKBP5* gene, a regulator of the stress-neuroendocrine system that is induced by glucocorticoid receptor activation (27). Several well-replicated SNPs in *FKBP5* are known to interact with early life adverse events to affect risk and resilience for specific psychiatric disorders, including posttraumatic stress disorder and depression (28, 29). This interaction involves differential expression of *FKBP5* in response to glucocorticoid receptor activation and an epigenetic modification of allele-specific *FKBP5* through DNA demethylation. *FKBP5*–childhood trauma interactions are referred to in several papers in this volume (30–32).

Allele-specific epigenetic marks may provide a mechanism for some G–E interactions (14, 27, 33). In Teh et al. (34), almost 1,500 variably methylated regions were identified from the genomes of 237 neonatal umbilical cord samples, along with environmental factors that included maternal smoking, depression, and high body mass index. Results showed that variation in DNA methylation was best accounted for by genes and G–E interaction, but not environment. Specifically, genes accounted for ∼25% and G–E interaction 75% of the overall variation in DNA methylation, while the effect of the environment alone was not significant. These results suggest the need for studies that integrate analyses of both DNA sequence and DNA methylation. The findings of Teh et al. (34) are bolstered by Czamara et al. (35), who investigated the relative contributions of prenatal environmental factors and SNPs on DNA methylation at variably methylated regions in neonatal blood, in four independent cohorts (n = 2,365).

Time, Timing, and Timescales in Biology

There are both ultimate (why?) and proximate (how?) questions in biology, and the concepts of timing and timescales are relevant to both. Darwin visualized evolution, for example, as a "tree of life" representing evolved relationships among species over time, thus addressing "why" questions regarding the ultimate origins of phylogenesis (36, 37). Natural selection results in DNA sequence differences among individuals, populations, and species (38). Changes in population genetic variation over time, for example—whether within long, millennial expanses of time or the short intervals involved in laboratory experimentation—is a fundamental precept of Darwin's evolutionary vision. G–E interactions that predict phenotypic variation are influenced by the pace and duration of change over time (39, 40). Fundamental questions in ecology, such as why two species coexist or how species conservation strategies can be employed across fragmented habitats, also require considerations of time and space (41).

From a more proximate perspective, time and space are also critical elements in biological processes—such as cell division, metabolism, development, neural signal transmission, and gene expression—which figure prominently in "how" questions regarding behavior and risk (42). The dynamic interactions underlying these processes change over time and are guided by environments that are internal and external to the organism. Examples of time-regulated processes are the opening and closing of critical periods in brain plasticity (43, 44) and the biological periodicities of rapid eye-movement (REM) and non-REM sleep (i.e., ultradian rhythms), sleep–wake cycles (circadian), and seasonal hibernation and migration behaviors (i.e., circannual) (45). Development itself is also, by definition, a temporal process in which the time required for any given developmental stage can be stretched or shortened depending upon environmental factors. Nutritional and socioemotional factors, for example, can influence pubertal timing (46) in such a way that developmental time becomes untethered to chronological time (47). Environmental exposures (e.g., socioemotional adversity or environmental toxins) occurring during sensitive windows in development can have a long, enduring reach into adulthood, affecting lifespan health and well-being (48).

Cellular systems involved in memory also act at different timescales (30, 49). Neuroscience research, for example, is addressing multiple questions related to time. How do prior experiences that exist on different timescales collectively alter synaptic plasticity, itself governed by processes that occur at the millisecond level and longer? How is the timing of synaptic and network activity governed? How are specific memories encoded and retrieved days, months, and years later? The time frames involved in these processes can operate at the diverse speeds involved in action potential propagation (i.e., milliseconds to seconds), in the signaling of gene-expression changes in the nucleus (minutes), or in the transport of gene products to other cellular locations (minutes to hours) (49). How these activity-dependent changes are modulated by oscillatory brain activity, which itself may be experience-dependent, is described in Reh et al. (45). Finally, changes in gene expression can have longer timescales (days to weeks) (30), and epigenetic modifications can

result in transcriptional changes over much longer periods of time (months to years) (31).

Time and G–E Interplay

There is also substantial evidence for a temporal moderation of both genetic and environmental influences. As summarized by Golombek et al. (50), genetically encoded biological clocks appear to conform to many biological processes to regular periodicities, ranging from circadian timing to microsecond processing and seasonal rhythms. Mood disorders, for example, may be traceable in part to misalignments between external temporal patterning (e.g., shift work) and internal rhythms (e.g., temperature, circadian gene expression, and sleep). Recent work by Horvath and Raj on an "epigenetic clock" (51) has demonstrated how the passage of time is linked to aging-related shifts in epigenetic marks, and in the present volume a pediatric "clock" that accurately estimates DNA methylation age is described (52).

Such temporal organization also seems to play a role in both adaptive and maladaptive responses to timed environmental events and cycles, with time moderating the effects of potentially pathogenic environmental exposures. Participants first exposed to child maltreatment during early childhood, for example, had depression and posttraumatic stress disorder symptoms with twice the severity compared to those exposed during later developmental stages (53). In a sample of girls from the National Comorbidity Survey Replication-Adolescent Supplement, trauma during puberty conferred higher risk for diagnoses of anxiety disorders, while prepubertal trauma was significantly associated with diagnoses of depressive disorders (54). In species as varied as fruit flies and human children, early life adversity has longer lasting effects on adulthood than adversity experienced in adulthood alone (e.g., refs. 51, 55, and 56).

This striking variation in phenotypic expression over time may point more usefully to a "probabilistic epigenesis," involving substantial uncertainties and random effects (57). Such dependable uncertainties may be a source of the unreliability in observations of G–E interplay (20) and a further indication of the importance of time and stochasticity in the development of individual variability (58, 59). Although analyses of G–E interplay have been mostly conducted within the space of conventional statistics, the stochastic, indeterminate nature of these relations may be more appropriately addressed using large datasets and machine-learning strategies (60). Furthermore, while parametric, linear, tests of association are the familiar and established territory in the analysis of G–E interplay, nonlinearities are legion within biological systems and often account for the robustness of phenotypes to perturbations in environmental exposures or genetic variation (61). For example, the relation of early adversity experiences to physiological sensitivity to stressors has been characterized as a U-shaped curve, in which children reared in both exceptionally high- and low-adversity contexts had the highest levels of autonomic and adrenocortical reactivity (62). More generally, GWASs on complex quantitative traits have increasingly turned to examine nonlinearities associated with G–G and G–E interactions (63). Such biological curvilinearities in the interactive relations among genes, environments, and time may be analogous to the curvilinearity in space–time foundational to contemporary physical science (9).

Both the past/future asymmetry of linear time (time's arrow) and the recursive patterning of cyclical time (time's cycle) are present in how we conceptualize the biological embedding of experience. Traces of the past are left in our present, but the future is all but unknowable. This linear ordering of time is also the basis for inferences of causality in both the physical sciences and biology: Causes are antecedent to effects; effects follow causes. The possibility of temporal cycles also appears in the biological sciences (64). Hypotheses surrounding "recursive causality"—the idea that every biological effect in living systems feeds back in some manner to its original cause—have been advanced (65). Furthermore, individual differences in early life human brain development may involve cyclical modifications of synaptic circuitry (66), and recursive, maladaptive interactions between children and their social environments may foster the emergence of developmental psychopathology (67). Thus, causal inference in research addressing associations among genetic variation, environmental conditions, and development may be especially sensitive to considerations of time and timing,

Content of the Special Issue

The series of papers comprising this special PNAS issue advances an agenda for future research on "genes and environment, development and time" and reflects recent discoveries by contributors to this volume. These include: 1) A debunking of two assumed truths about critical periods in development, namely, that they are fixed within chronological windows and irreversible; 2) a shift in research on G–E interplay from an initial assumption that epigenomic variation is largely sculpted by the "environment" to a more sophisticated model in which gene expression and epigenetic modification are dependent upon genomic context (DNA sequence); 3) a higher resolution picture of the environments that influence biological embedding and the emergent genes and pathways involved; 4) evidence that past experiences prime the genomic response to future experiences; 5) increased reliance on the network concept, at different time scales of operation; and 6) the successes in translating research insights bidirectionally between animal and human.

Furthermore, the set of papers yield direct insight, explicitly or implicitly, into the operation of time and timing in the context of G–E interplay. Collectively, the papers argue—we believe persuasively—that G–E interplay research must now incorporate time at multiple scales (38). Timing increasingly emerges as a fundamental element in the epigenesis of developmental events, and each of the assembled papers recapitulates and illustrates this reality in some manner. The volume contains four perspective papers and eight original research papers, which are highlighted below.

Reh et al. (45) discuss processes involved in the onset and closure of critical periods of brain plasticity across multiple timescales. Their paper describes the central role of the network of parvalbumin-positive, inhibitory interneurons that shape excitatory–inhibitory balances within successive regions of cortical circuitry. The molecular events determining the timing of critical periods and the perturbations of development attending various exposures span multiple temporal scales, ranging from milliseconds in the case of neuronal oscillations to generational or even intergenerational lifespans in the case of epigenetic processes. The Reh et al. paper discusses how critical periods, although conventionally viewed as static and unchanging in time, can be altered by experience-dependent epigenetic processes and by pharmacologic agents and genetic manipulations. Understanding the normative development and function of these parvalbumin-positive, inhibitory interneuron-mediated processes can provide insight into mental illness and brain injury.

Clayton et al. (30) discuss how dynamic patterns of gene expression play a role in the encoding of experience. They update the concept of the genomic action potential (gAP), analogous to

the familiar electrophysiological action potential, which occurs in milliseconds (68). In contrast, the gAP occurs over minutes and is measured as an array of immediate early genes (IEGs) becoming responsive to salient experiential events. Clayton et al. (30) discuss the gAP from the perspective of molecular elements within a brain cell, neural contexts, the encoding of engrams, and cellular proliferation. An example of the gAP at the organismal level is also provided from the perspective of stress responses.

Aristizabal et al. (31) provide a primer on the many molecular processes that can contribute to the biological embedding of experience. These include the methodologically accessible methylation and hydroxymethylation of DNA at cytosine-guanine dinucleotides (CpG sites), as well as structural revisions of chromatin accessibility, posttranslational modifications of nucleosomal histone proteins, noncoding- and micro-RNAs, and more than 150 varieties of RNA base modifications. Future research that takes into account DNA sequence variation, developmental timing, tissue specificity, age, and sex will be required to delineate the molecular mechanisms that function together in the biological embedding of experience. The pairing of longitudinal human cohort studies with experimental animal studies should also facilitate the transition from correlative to causal studies in this young field.

Sinha et al. (69) examine emerging insights into the spatial and temporal aspects of linkages between neural networks (NNs) and gene regulatory networks in the brain. They present a strong case for brain gene regulatory networks (bGRNs), as important substrates of behavior involving gene-expression changes in hundreds to thousands of genes within a neuron in response to the environment. NNs comprise circuits of neurons transmitting electrochemical signals from one neuron to another and integrating experiential stimuli to orchestrate an organism's behavior. bGRNs act at different (but sometimes interacting) levels of organization than NNs and on different timescales. NNs act in milliseconds to seconds, while bGRNs affecting gene expression and epigenetic changes arise over minutes to days. A role for developmental GRNs (dGRNs) in the interplay between NN's and bGRNs is also considered.

The aging or cellular weathering associated with the passage of time in individual lives depends on cumulative exposures to adversity. As a model for prenatal stress exposure, Provençal et al. (32) expose a human fetal hippocampal progenitor cell line to glucocorticoids. Exposure early in neurogenesis results in lasting changes in DNA methylation, altering the set point for future transcriptional responses to stress. Such early priming of neural responses with glucocorticoids exposure could contribute to individual differences in vulnerabilities to stress later in life.

Temporal response latencies figure prominently in the paper by Dason et al. (70). They focus upon the role of the *foraging* gene (*for*), which encodes a guanosine 3′,5′-cyclic monophosphate-dependent protein kinase (PKG) in nociceptive-like escape responses among *Drosophila melanogaster* larvae. The paper shows that nociceptive-like response latency (curling and rolling of the larva) during threat is faster in one genetic variant of *for* (rover) than the other (sitter). Dason et al. use optogenetics and transgenic manipulations to trace these behavioral differences to variation in gene expression from *for*'s pr1 promoter among neurons of the ventral cord, and show that prior activation of the pr1 circuit during development suppresses the nociceptive-like escape response.

Measures of individuality are important for trajectories of child development when reliance on population means cannot be used to predict individual susceptibilities. Nonetheless, little is known about developmental, genetic, environmental, and stochastic contributions to individuality. Honegger et al. (71) investigate the biological basis of individuality in the odor responses of *D. melanogaster.* They measure individual variability using repeated measures of the same olfactory response behavior over time and map neural activity in the brain. The author's find, when comparing individual flies, that the same odor stimulus can result in different behavioral responses and different brain activities. Transgenic and pharmacological manipulations reveal that neuromodulators and sets of neurons in the fly brain's olfactory region directly modulate behavioral variability and that this modulation is flexibly dependent on the environment.

Artoni et al. (72) develop an approach for early detection of neurodevelopmental spectrum disorders, in this case, autism spectrum disorder (ASD), by using a transfer learning experiment across species (mouse to human). Their approach is based on spontaneous arousal fluctuations combined with deep learning and is a possible breakthrough in the early detection of risk for ASD and related disorders, where late diagnosis strongly diminishes intervention efficacy. The research attends to critical periods in developmental time and illustrates the utility of methods—in this case, the use of convolutional neural networks—that allow for the detection of nonlinear functions in biological systems.

Gonzales et al. (73) investigate the cellular distribution of IEG expression after an acute exposure to cocaine in mouse striatal neurons. The induction and decay of IEG expression is used as a marker to encode recent experience. They investigate the timing and spatial distribution of IEGs in the neuronal ensembles and find spatially defined clusters characterized by consistent and robust expression of many IEGs. The authors suggest that the existence of clusters of neurons in response to acute cocaine experience may be a general principle for responses to other types of experience.

George et al. (74) show that social isolation in the highly social zebra finch affects gene expression in the brain and that this is correlated with an increase in DNA methylation of a subset of those differentially expressed genes. Hundreds of genes located in higher forebrain centers were involved in social communication when birds were isolated in a sound chamber overnight, compared to when they were paired with a same-sex partner in the chamber. Changes in circulating corticosterone levels were not sufficient to explain the genomic response.

Sanz et al. (75) show that the response of the rhesus macaque immune system is affected by current social conditions and a biological memory of past conditions. A history of social subordination in female rhesus monkeys changes the blood gene-expression responses to experimentally induced bacterial and viral challenges. Pathogen exposure, type, and social history all affect immune cell gene expression. The authors also found that a history of social subordination reduces sensitivity to present-day social conditions. Their paper provides a compelling example of the biological embedding of social experience over long timescales.

Rivenbark et al. (76) demonstrate, in a British birth cohort of mono- and dizygotic twins, how the passage of developmental time can alter associations with mental and physical health endpoints. They find evidence for a "status syndrome" at 18 y of age, in which subjective estimates of family social position in the community are significantly predictive of multiple indicators of mental health and developmental well-being, even with controls for objective socioeconomic status and family environment. The

relative lack of such associations with subjective social position assessed at 12 y of age underscores the manner in which relations among social environmental and health measures can change longitudinally over developmental time.

The aging or cellular weathering associated with the passage of time in individual lives is not absolute but dependent upon cumulative exposures to adversity, and perhaps other factors. McEwen et al. (52) develop a pediatric-buccal-epigenetic (PedBE) clock tool by using the DNA methylome to measure the biological age of children ages 0 to 20 y taken from 11 distinct cohorts. They find an array of methylation scores at 94 CpG dinucleotide sites highly predictive of chronological age. Positive deviation from predicted age (suggesting more advanced weathering of cells) is associated, in a separate sample and analysis, with ASD.

Taken together, the collection of papers herein weaves together a sturdy if incomplete fabric of evidence for how time and timing are critical to developing our understanding of G–E interplay in the biological embedding of experience. Time, it increasingly appears, is an essential element in plumbing and understanding how genes and environments operate together to shape probabilistically the trajectories of individual lives. This elegance and complexity, added to the pullulating and enticing story of how human differences arise, call to mind the work and thinking of two memorable, now departed, progenitors of the Canadian Institute for Advanced Research Child and Brain Development Program: Fraser Mustard and Clyde Hertzman (77, 78). Both were convinced that the experiences and exposures of early life, and especially the timing of such events, were the elemental building blocks of human potential, for good or ill, for success or failure, for health or misfortune. It is that discerning insight that has guided and continues to mark the developmental science to which this collection of papers presents a compelling, if promissory, note.

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