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EDITORIAL

# Epigenetic "Transgenerational" Inheritance

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Conrad Waddington (1905–1975) was a brilliant British developmental biologist, paleontologist, geneticist, embryologist, and philosopher who laid the foundations for systems biology, epigenetics, and evolutionary developmental biology.[1] He had to be all that to manage those 3 challenging disciplines. In his spare time, he also praised Marxism as a "profound scientific philosophy."[1] In the late 1930s, Waddington focused on *Drosophila* mutations. He discovered mutations that affected cell phenotypes and wrote his first textbook of developmental epigenetics, a term that meant at that time the external manifestation of genetic activity. Waddington introduced the concept of canalization, namely the ability of an organism to produce the same phenotype despite variation in genotype or environment. He also identified a mechanism he called genetic assimilation, which would allow an animal's response to environmental stress to become a fixed part of its developmental repertoire, and then he went on to show that the mechanism worked. At the time, the role of DNA was unknown. Today, we view Waddington's epigenetic landscape as a metaphor for how gene regulation modulates development.

Article, see p 1082

The evolution of traits is based on continuous variability, and natural selection is determined by those most fit. However, the idea that acquired traits such as "reaching for the juiciest fruits from the highest trees directly lead to a longer neck development," as Lamarck proposed, has continued appeal. Alfred Wallace independently developed the ideas of variability and natural selection. His article on the subject was jointly published with Darwin's article in 1858 (Darwin's book came in 1859). Wallace emphasized environmental pressures on varieties and species, forcing them to become adapted to their local conditions, a bit of a lamarckian view. Such a state of affairs could lead populations in different locations to diverge. Today, we define epigenetics as the study of heritable phenotypic changes that do not involve alterations in the DNA sequence. Covalent modifications, handling of RNA transcripts, microRNAs, mRNA regulation, prions, structural inheritance, nucleosome positioning, histone variants, and genomic architecture all play a role.[2] Thus, even we Darwinists must accept Waddington's epigenetics that can explain so much.

## CLAIMED EVIDENCE

Barker demonstrated a striking correlation between low birth weight and death resulting from cardiovascular disease later in life. The appearance of hypertension and cardiovascular disease in 50-year-old survivors of the Dutch famine underscored the in utero origins of cardiovascular disease.[3] The in utero famine stress has numerous elements, but one standout is a possible role for epigenetics.[4] For instance, Einstein et al[5] obtained cord blood and observed alterations in cytosine methylation in neonates after intrauterine growth restriction. They also observed and identified specific loci that are targeted for dysregulation of DNA methylation, in particular the hepatocyte nuclear factor 4α (*HNF4A*) gene. A mechanistic reproducible animal model would be a great advance. In such a model, subsequent inheritance of the phenotype could be tested. We may now have such a model. Can cataclysmic events be imprinted into the human genome without altering the DNA sequence?

Cao and colleagues[6] report in this issue of *Circulation* that when prenatal lipopolysaccharide (endotoxin) was administered to pregnant dams (shock stress), dams

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even of the fifth generation of offspring developed salt-sensitive hypertension. The investigators found that the Ras-related C3 botulinum-toxin substrate-1 (*Rac1*) gene is upregulated and that this product activates mineralocorticoid receptor signaling. The prenatal lipopolysaccharide evidently triggered reactive oxygen species, and the reactive oxygen species upregulated histone demethylase lysine-specific demethylase 3B. In their report,[6] the authors provide an interesting schematic model of how they believe their findings work. The model is not reiterated here; rather, I focus on the downstream molecular mechanism known from the cancer field (Figure). Upregulation of lysine-specific demethylase 3B led to a low level of H3K9me2. H3K9me2 is an epigenetic modification to the DNA packaging protein histone H3. As a result, *Rac1* was upregulated across generations (if forever remains to be seen). Let us march through these data to determine whether we remain convinced.

The investigators exposed pregnant dams (F0) to prenatal lipopolysaccharide, resulting in increased blood pressure in F1 animals. Urinary sodium excretion was reduced (ignore balance arguments, please). Elevated blood pressure was sustained in F2 and F3, and even in F4 and F5. Sufficient telemetric blood pressure recordings were performed.[7] Next comes the renal transcriptome (obviously with enrichment analyses and other analyses). Then gene picking identified *Rac1*, the protein product of which is a GTPase (a molecular switch). The node is responsible for numerous things, including mineralocorticoid receptor regulation. It turns out that Rac1 signaling is important in mineralocorticoid receptor signaling in renal and cardiac disease. The investigators even used an inhibitor of RAC1 protein in their studies and showed that blood pressure was reduced.

## CHECKING THE DATA

The readout is blood pressure in 20-g mice.[8] Some telemetry measurements in subsets were obtained. Next is the sodium story. The authors state that urinary sodium measurements were conducted in 24-hour collection procedures. These technologies are far more taxing than any single-cell sequencing exercise. Furthermore, the authors performed old-fashioned pressure natriuresis experiments. A rightward shift in pressure natriuresis is the hallmark of salt-sensitive hypertension. The use of spironolactone to ameliorate this state of affairs also inspires confidence. Measurements of renin-angiotensin-aldosterone components and total-body sodium measurements would have provided further evidence.

RNA sequencing uses next-generation sequencing to reveal the presence and quantity of RNA in a biological sample at a given moment, analyzing the continuously changing cellular transcriptome. Here, the authors found *Rac1*. This Rho family member, a small GTPase (switch), has been implicated in renal and cardiac disease involving the mineralocorticoid receptor.[9] The lysine-specific demethylase lysine-specific demethylase 3B has histone H3-methyl-lysine-9 demethylase activity and transcription coregulator activity. This enzyme reduced methylation of the DNA packaging protein histone H3. As a result, the *Rac1* gene was upregulated in the prenatal lipopolysaccharide offspring. Then, the chain of events takes its course. Tempol is a catalyst and chemical oxidant by virtue of being a stable aminoxyl radical. In biochemical research,

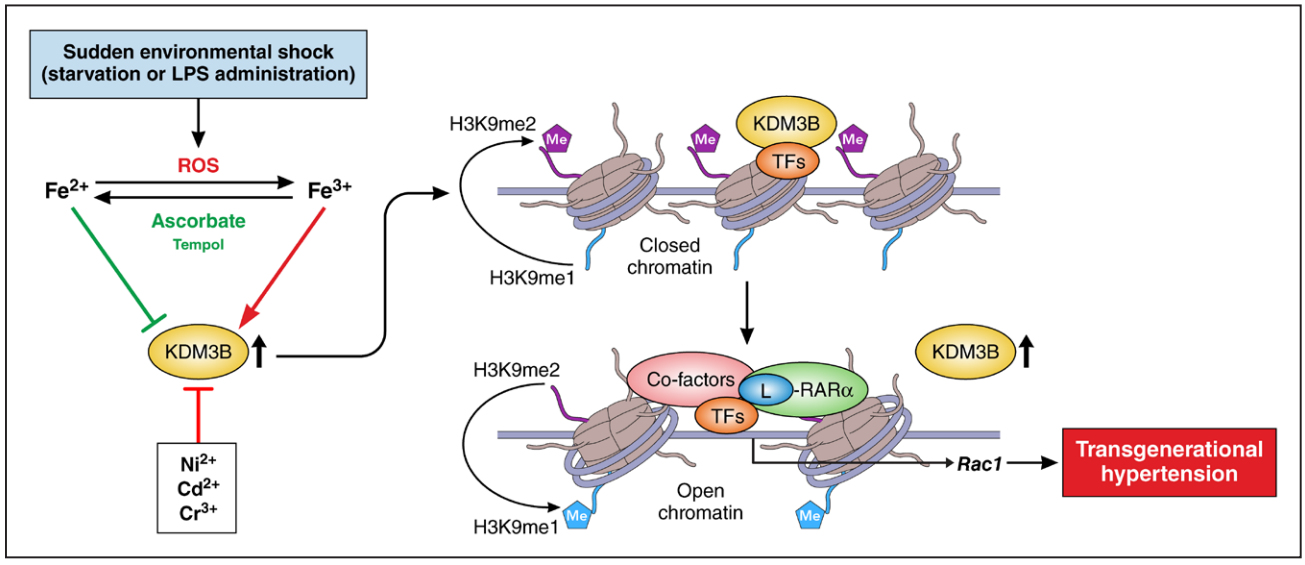


**Figure. Proposed molecular mechanism of transgenerational inheritance of hypertension.**
**Left**, Lipopolysaccharides (LPSs) applied prenatally induce reactive oxygen species (ROS) that indirectly upregulate the lysine (K)-specific demethylase 3B (KDMB3). The histone demethylase is under differential control of ROS, Krebs cycle intermediates, and metals. **Right**, Demethylase 3B catalyzes the demethylation of H3K9me2 residues, which, in concert with transcription factors (TFs), cofactors, and ligands of retinoic-acid receptor α, upregulate Rac1, a known factor inducing hypertension. L indicates RARa ligand; and Me, methylation site.

tempol has been investigated as an agent for limiting reactive oxygen species. We are still at the research stage; no clinician prescribes tempol, and virtually all antioxidant studies in humans yielded unencouraging results.

## RETURNING TO WADDINGTON

We clinicians must deal with overwhelming evidence. Inheritance without changes in the underlying DNA sequence occurs, is common, and is clinically relevant. Now, we are presented a compelling animal model. In addition, we have human cardiovascular data that cannot be denied. Fortunately, technology will help in this regard. In the meantime, we can take solace in Waddington's statement: "We are part of nature, and our mind is the only instrument we have, or can conceive of, for learning about nature or about ourselves."[10] Let clinical trials provide us with the data.

## ARTICLE INFORMATION

### Affiliation

Experimental and Clinical Research Center (ECRC), a joint cooperation between the Max-Delbrück Center for Molecular Medicine (MDC) and the Charité Medical Faculty, Berlin, Germany.

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None.

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