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Research Article

A single-cell epigenetic model for paternal psychological stress-induced transgenerational reprogramming in offspring[†]

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Abstract

Experimental evidence shows that parental psychological stress affects the long-term health of offspring in an inheritable fashion. Although epigenetic mechanisms, including DNA methylation, miRNA, and histone modifications, are involved in transgenerational programming, the underlining mechanisms of transgenerational inheritance remain unsolved. Here, we present a single-cell-based computational model for transgenerational inheritance for investigating the long-term dynamics of phenotype changes in response to parental stress. The model is based on a recent study that has identified the imprinted sperm gene *Sfmbt2* as a key target, and incorporates crosstalks among drastically different time scales in mammalian development, including DNA methylation, transcription, cell division, and population dynamics. Computational analysis of the model suggests a positive feedback to DNA methylation in the promoter region of sperm *Sfmbt2* gene that provides a possible mechanism to mediate the parental psychological stress reprogramming in offspring. This approach provides a modeling framework for the understanding of the roles that epigenetics play in transgenerational inheritance.

Summary Sentence

A positive feedback to DNA methylation in the promoter region of an imprinted gene is a possible mechanism to mediate the parental psychological stress reprogramming in offspring.

Key words: developmental, reprogramming, epigenetics, DNA methylation, genomic imprinting, transgenerational inheritance.

Introduction

The groundbreaking work by David Barker shows that metabolic diseases including diabetes and cardiovascular diseases can be induced during embryo development by insults such as undernutrition during pregnancy [1,2]. The Barker hypothesis has evolutionally changed our view as to how these diseases develop [3]. Importantly, growing evidence shows that these diseases programmed during embryo development by parental stresses, such as maternal stress

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during pregnancy or paternal stress before mating, affects later-life health not only in F1 but also future generations (F2 and beyond) [4–8]. Thus, the transgenerational inheritance nature of metabolic diseases sounds an even more astounding alarm for these diseases as the biggest global public health threats [9,10].

Epigenetic mechanisms, including alterations in histone modification [11,12], RNA molecules such as various noncoding RNAs (i.e. microRNAs (miRNAs) [13–17], and DNA methylation [7,18–20], are involved in transgenerational programming [10,21]. The levels of histones and their post-translational modifications in sperms can be modulated by environmental factors. Rats with liver fibrosis were enriched for the histone variant H2A.Z and H3K27me3 at the promoter of peroxisome proliferator-activated receptor γ in sperm, and displayed multigenerational epigenetic adaptation of the hepatic wound-healing response [11]. Traumatic experience in early life and chronic social instability during adolescence and early adulthood are known to produce long-lasting effects for future psychiatric disorders and affect future generations [22,23]. Traumatic stress in early life can alter mouse miRNA expression, and behavioral and metabolic responses in the progeny; injection of sperm RNAs from traumatized males into fertilized wild-type oocytes can reproduce behavior alterations in the resulting offspring [16].

Environmental insults during embryo development can result in alterations in genome-wide DNA methylation [18,24–26]. Altered DNA methylation in specific genes is associated with phenotype changes in offspring. For instance, undernutrition during pregnancy alters DNA methylation of *Lxra* gene in F1 males, which influences liver lipogenic gene expression in F2 mice [7]. Paternal psychological stress induces sperm *Sfmbt2* promoter hypermethylation to downregulate liver miR-466b-3p in F1 mice; this further upregulates a key glucose producing enzyme phosphoenolpyruvate carboxykinase (PEPCK) to promote hepatocyte glucogenesis and hyperglycemia [27]. Thus, changes in gene expression due to altered DNA methylation is a route for the transgenerational inheritance of developmentally programmed diabetes in offspring. However, a key question as to how changes in DNA methylation are propagated through generations remains to be unanswered.

Significant DNA methylation reprogramming occurs during early embryo development in mammals [28–32]. Upon fertilization, DNA methylation marks represent an epigenetic barrier in mammalian development, and hence need to be restored and subsequently rebuilt with the commitment to a particular cell fate during development. Two waves of genome-wide DNA demethylation take place in early mouse embryo development. The first occurs following fertilization when paternal pronucleus undergoes rapid demethylation in zygote [33,34], followed by a passive loss of DNA methylation marks in the maternal genome over subsequent cell divisions [35]. The first low point of global methylation occurs in blastocyst approximately at embryonic day 3.5 (E3.5), followed by the reestablishment of DNA methylation patterns in inner cell mass (ICM) cells [31,32,36]. At approximate E6.5, cells either continue to develop toward a somatic fate or are specific as primordial germ cells (PGCs). Somatic fated cells acquire distinct methylomes according to their lineage but maintain high global levels of DNA methylation. PGCs initiate a second wave of comprehensive DNA demethylation, which is complete approximately at E12.5 [28]. Then, PGCs reestablish unique gamete-specific methylome during gametogenesis [28]. During fetal reprogramming, DNA methylation at imprinting control regions (ICRs) in gametes is stably maintained during embryo development [37]. Additionally, intracisternal A particles (IAPs) make up the sequence class that seems most highly protected against demethylation in zygote and PGCs [30].

Computational models have been widely used to study DNA methylation kinetics at CpG sites [38–41], genetic regulatory networks [42], cell population dynamics [43], and multicellular systems [44]. However, no models exist for transgenerational inheritance. With many molecular processes in fertilization and embryo development remaining mostly unknown, DNA methylation in fetal programming adds further complexity to the computational modeling. New model development is needed for dissecting the interplay between DNA methylation and gene regulatory networks for transgenerational inheritance at single cell level.

In a long-term reprogramming process like paternal psychological stress-induced type 2 diabetes in offspring [27], how temporary stimulation-induced epigenetic changes are propagated through cell division? How such changes are maintained despite significant DNA methylation alterations during early embryo development? How the changes in germ line cells affect the phenotype of somatic cells? How the changes be maintained through generations despite random effects at each cell cycle? Here, we proposed a single-cell-based computational approach to model the dynamics of offspring reprogramming. The model is based on an imprinted sperm gene *Sfmbt2* and incorporates crosstalk between DNA methylation and molecular pathways in reprogramming during embryo development and long-term effects on cell division in a life cycle. We validated a positive feedback to DNA methylation in *Sfmbt2* promoter that produces sustained epigenetic changes after temporary stress.

Materials and methods

Model formulations and numerical scheme of the model simulations are detailed in Supplemental material.

Model simulations are performed using C++, and plots and data analysis are produced using MATLAB8 (Mathworks, Natick, MA, USA). The C++ source code can be downloaded from GitHub at https://github.com/jzlei/FetalProg.

Results

An epigenetic and multiple time scale single-cell model on transcriptional inheritance

Paternal psychological stress hypermethylates mouse sperm *Sfmbt2* gene promoter; this is transmitted to stress-F1 mice to inhibit transcription of the 10 intron of *Sfmbt2* for producing miR-466b-3p, which results in increased PEPCK translation. Increased PEPCK ultimately promotes hepatic gluconeogenesis and hyperglycemia in F1 mice [27]. In addition, there are enriched repressive histones (i.e. H3K9 and H3K27) and reduced active histone (H3K4) in the *Sfmbt2* promoter in stress-F1 mice.

Sfmbt2 is an imprinted gene that is expressed preferentially from the paternal allele in early embryos and later extraembryonic tissues in mice [45,46]. Hence, one can track the expression of the paternal allele *Sfmbt2* to investigate the expression of miR-466b-3p in response to temporary stimulation like paternal stress.

Based on these observations as summarized in Figure 1A, we developed a single-cell-based computational model focusing on epigenetic regulations in paternal psychological stress-induced reprogramming in offspring. In the model, the tissue phenotype is represented by the transcription level of *Sfmbt2*, which shows cell-to-cell variation due to random kinetics in DNA methylation at the gene promoter in each cell along with cell divisions. Therewith, the model integrates different scale dynamics, including DNA methylation kinetics and their effects to gene expression inside a cell, random

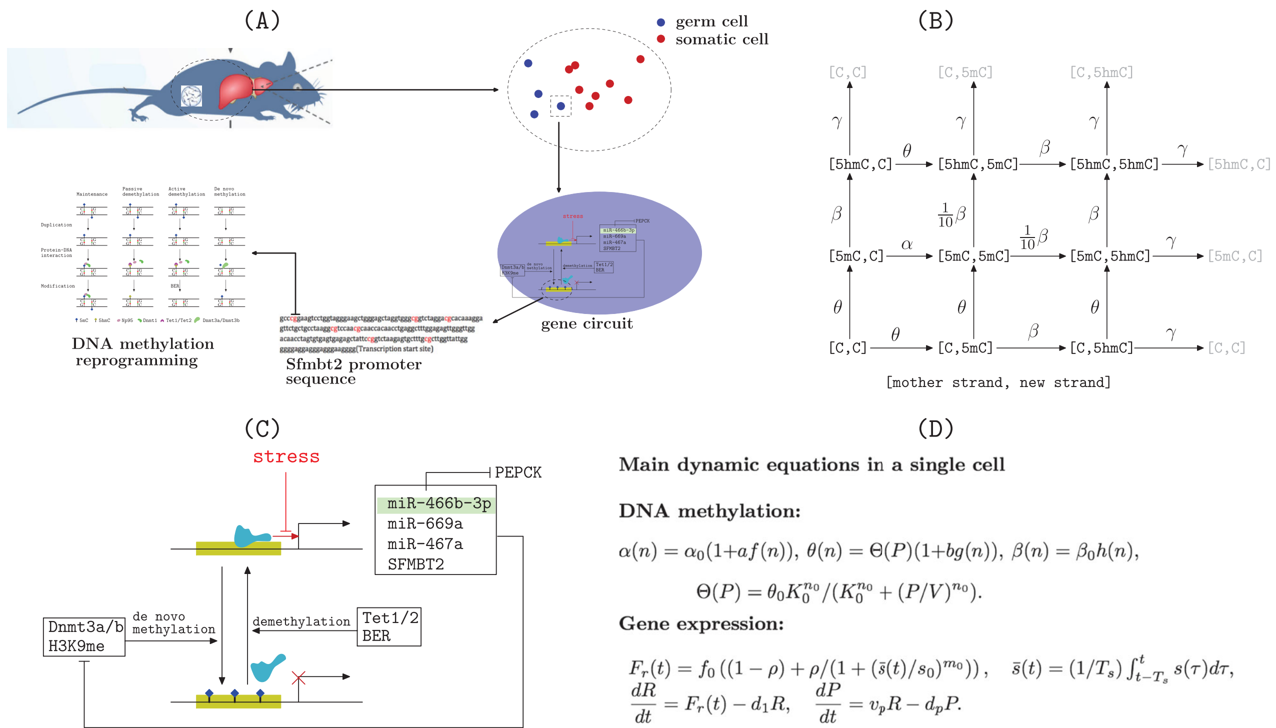

Figure 1. Illustration of the model. **(A)** The multiscale model of *Sfmbt2* gene transcription in mice. **(B)** State transition of a CpG site. Each square bracket shows the state of a pair of CpG site (the two "C" on two strands). Arrows show the dynamics of methyl group transformation, with reaction rates on the arrows. **(C)** Proposed gene circuit for transcription regulation of the gene *Sfmbt2*. **(D)** Summary of formulations of DNA methylation and gene expression dynamics in a single cell. The reaction rates of DNA methylation maintenance (α), oxidation (β), and de novo methylation (θ) depend on the division number (n) starting from fertilization. The de novo methylation is a decrease function of protein concentration. The mRNA transcription rate is a decrease function of the average DNA methylation level ($\bar{s}(t)$) at the gene promoter (see Supplementary material, Sec. S1-S4).

partition of methylation marks and methylation maintenance during cell division, and tissue growth due to cell division. The model consists of three major components, including DNA methylation kinetics, gene regulatory circuit for *Sfmbt2* transcription, and single-cell-based developmental dynamics.

DNA methylation kinetics model the methylation/demethylation dynamics of CpG sites that are associated with methylation maintenance during DNA replication, demethylation, and de novo methylation [30–32]. Seven CpG sites are reported in the promoter sequence of *Sfmbt2* under the influence of paternal stress [27]. The dynamical processes of each CpG site was described as Markov process of the kinetic transitions between nine states [s^1, s^2], with s^i the state of one cytosine (C, 5mC, or 5hmC) (Figure 1B). The kinetic rates are dependent on the enzyme activities of nuclear protein 95 (NP95 or UHRF1), DNA (cytosine-5)-methyltransferases (DNMT1 and DNMT3a/3b), ten-eleven-translocation proteins (TET1/2/3), or the base excision repair pathway (see Supplementary material, Sec. S1).

The genome-wide DNA demethylation events during early mammalian embryo development coincide with the changes in the concentration of the enzymes DNMT1, DNMT3a/3b, and TET1/2/3 [31,32]. In our model, time-dependent functions for the concentrations of DNMT1, DNMT3a/3b, and TET1/2/3 proteins were selected to mimic the reprogramming dynamics of DNA methylation during development, so that the kinetics reaction rates are changeable depending on cell division number starting from fertilization (Figure 2, also see Supplemental material, Sec. S2 and Figure S3).

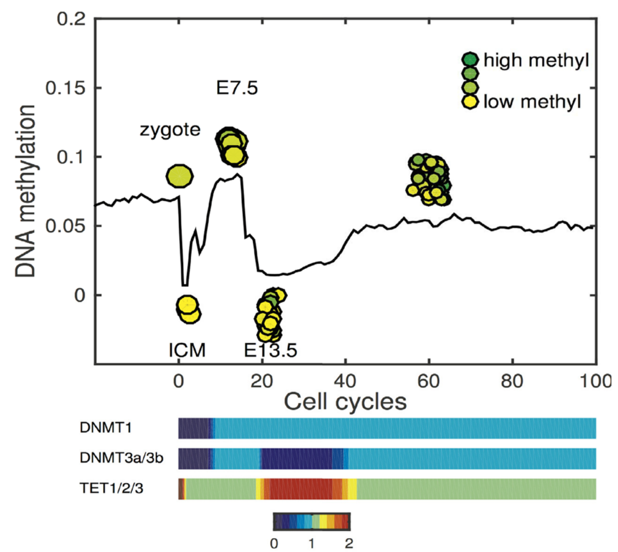

Figure 2. Simulations on fetal reprogramming during early embryo development. Black curve shows the average DNA methylation level of PGCs in simulation at the given time point measured by cell cycles. Sample cell states are shown at four time points: zygote, ICM (2 cycles), E7.5 (12 cycles), E13.5 (20 cycles), and PGC (60 cycles). Time-dependent changes in DNMT1, DNMT3a/3b, and TET1/2 proteins in PGCs are shown by color bars at the bottom (see Supplementary material, Sec. S2) and other parameter values were taken from Supplementary material Table S1.

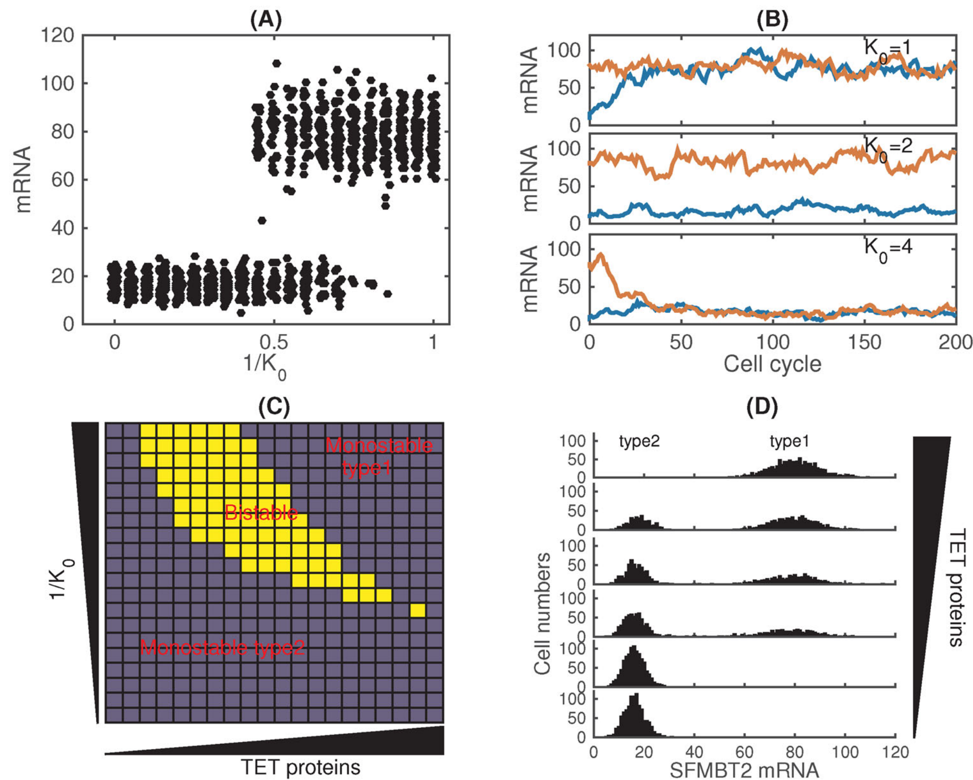

Figure 3. Bimodal cell phenotype. **(A)** Dependence of the stationary state mRNA with the EC50 (K_0) in the regulation of DNA de novo methylation. **(B)** Time courses of mRNA levels with different initial conditions and under various K_0 values. **(C)** Bifurcation diagram with changes in TET proteins (β_0) and K_0 in the regulation of DNA de novo methylation in the positive feedback. Yellow region marks the bistable region; the two blue regions mark the two monostable regions with low and high *Sfmbt2* transcripts, respectively. **(D)** Histograms of SFMBT2 mRNA copy number with different levels of TET proteins. Type 1 and type 2 cells are marked with high and low SFMBT2 mRNA levels, respectively. Parameters were taken from Supplementary material Table S1. In (C) and (D), β_0 varies from 0 to 4, and $K_0 = 2$ in (D).

DNA methylation at promoter region often associates with transcription repression, such as gene silencing and heterochromatin [47]. Moreover, the protein Sfmbt2 contains a MBT (malignant brain tumor) domain that binds to mono- and dimethylated lysines on histone tails to affect a variety of chromatin processes [48]. There are enriched histones H3K9me3 in the *Sfmbt2* promoter in stress-F1 mice [27]. H3K9me3 can direct de novo DNA methylation via Dnmt3a/3b [49]. We made a major assumption that *Sfmbt2* transcription products repress the activity of de novo DNA methylation (Figure 1B, see Supplemental material, Sec. S3). Hence, there is a positive feedback of *Sfmbt2* to its own expression through de novo DNA methylation.

The single-cell-based hybrid model tracks cell population dynamics with expression kinetics of *Sfmbt2* in each individual cell, and the kinetics of DNA methylation and transcription are described with biochemical reactions in Figure 1B and C. In cell population dynamics, each generation begins from only one cell—the zygote, and the cell number doubles after each division (Supplementary material Figure S2). All cells are grouped into either somatic cells or PGCs at division 15 after fertilization. At the end of one generation, a germ cell was randomly selected to produce the zygote of next generation (Supplementary material, Sec. S4). This study intended to investigate transgenerational inheritance upon parental stresses. We took 200 divisions as a lifespan of an individual from zygote to adult for simulating a long process in fetal programming. Nonetheless, from the simulations results, the system achieves homeostasis after 60 divisions and the final result is quit insensitive with the division number used in simulations. The procedure can be extended to any generations. Two examples from F0 to F3 are shown in Supplementary material Figure S4.

Main equations of the model are summarized at Figure 1D, and are detailed at Supplemental material.

Bimodal transcription mediated by the enzyme activities for DNA methylation

When male mice are subjected to psychological stress before mating, their stress-F1 offspring show a different phenotype (type 2 diabetes) compared to control-F1 mice; obvious alterations in DNA methylation in *Sfmbt2* promoter and its transcription occur in both early embryo and liver cells in stress-F1 mice [27]. These observations suggest alternative *Sfmbt2* transcription in stress vs control F1 mice. We asked whether the proposed DNA methylation and transcriptional regulation are able to yield the observed bimodal gene expression. To this end, we assumed that the DNA de novo methylation rate depends on gene transcription product and mediates the EC50 (50% effective concentration) K_0 in Equation 2. For each K_0, we simulated the regeneration of a group of cells with randomly assigned initial condition, and measured the final transcription level in each cell at homeostasis. The homeostasis mRNA count depended on K_0 differently with two thresholds (Figure 3A). All cells showed low expression when $1/K_0$ was small (Figure 3B, $K_0 = 4$), and high

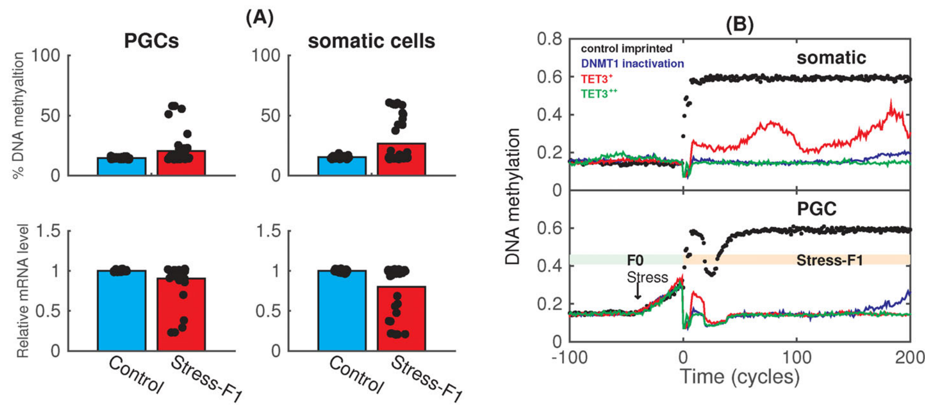

Figure 4. Paternal stress-induced transgenerational reprogramming. **(A)**. Simulations of transgenerational reprogramming. Upper panel: percentages of DNA methylation in the gene promoter in germ and somatic cells. Bottom panel: relative mRNA levels in PGCs and somatic cells. Bars show the average of 40 independent runs in each situation, and dots are the values for each individual trial. Results for control (blue bar) and stressed (red bar) F1 offspring are shown. **(B)** Dynamics of DNA methylation at somatic cells (upper panel) and PGCs (bottom panel) after paternal stress. Here, four conditions for DNA demethylation during early embryo stage are considered: imprinted gene with constant DNMT1 activity (control, black), DNMT1 inactivity during early embryo (DNMT1 inactivation, blue), upregulation of TET3 proteins at cycles 1∼2 [TET3^{+} (green) or TET3^{++} (blue)] (see Supplementary material, Sec. S6). The reduction factor was taken as $RF = 0.2$.

expression when $1/K_0$ was large (Figure 3B, $K_0 = 1$), and the homeostasis mRNA counts showed bimodal phenotype with either low or high expression when $1/K_0$ is at intermediate level (Figure 3B, $K_0 = 2$). This result suggests that a proper level of feedback strength is able to ensure the bimodal gene expression after stress perturbation in the renewal of stem cells.

The transcriptional and post-translational regulations of TET proteins are essential for establishing DNA methylation patterns [32] as TET proteins are main enzymes to induce DNA demethylation through iterative oxidation of 5mC [32,50–53]. We asked how TET proteins affect the bimodal gene transcription upon the feedback through DNA methylation. We changed the levels of 1/K_0 and TET proteins and examined homeostasis of gene transcription. Bimodal expression was seen when the $1/K_0$ was above certain threshold and TET protein was taken at a proper intermediate level (Figure 3C). Taking an intermediate value $K_0 (K_0 = 2)$, gene expression transited from low monostable to high monostable transcription when TET protein varied from low to high levels, and the mRNA level showed bimodal distribution under intermediate level TET proteins (Figure 3D). Thus, the fine tuning of TET protein levels is essential for the bimodal gene expression after stress perturbation. In simulations, we took the parameter values to ensure the bimodal expression so that there were two possible phenotypes, including type 1 with high *Sfmbt2* transcription and type 2 with low transcription, and the control-F0 was with type 1 before stress.

Maintenance of bimodal transcription by DNA methylation accounts for the reprogramming in stress-F1

To examine transgenerational inheritance of developmental reprogramming after paternal psychological stress through the proposed computational model, we introduced a stress to F0 at 2 to 30 cycles before mating, and measured the dynamics of both DNA methylation and transcription levels of germ cells and somatic cells (Supplementary material Figure S4). During stress, we introduced a reduction factor (RF, $0 < RF < 1$) so that the *Sfmbt2* transcription rate reduced by a factor RF. The reduction factor measures the overall effect of decreasing in transcription in sperm during stress. The value of RF represents the strength of stress. Consequently, the stress resulted in a temporary decrease in mRNA level and an increase in DNA methylation. In stress-F1, the average DNA methylation showed coexistence of two phenotypes in both germ cells and somatic cells, either maintained at low level as control-F0 or switched to high methylation level. Consequently, the average mRNA level showed either type 1 with high transcription or type 2 with low transcription (Figure 4A, Supplementary material Figure S5). To identify the phenotype changes in an adult, we measured the relative average mRNA level of somatic cells with respect to the control situations, which showed well separation among different sample trials. Here, we took a threshold of 0.5 for the relative mRNA level as a criterion of phenotype changes.

Since paternal psychological stress perturbs sperm gene expression, we asked how F0 sperm variance affects the probability of phenotype changes in offspring. To this end, we alternated RF to examine the resulting phenotypes in stress-F1. The probability of phenotype changes at stress-F1 increased with stress strength (Figure 5A). During fertilization, one sperm is randomly selected to form a zygote; hence each individual begins from a zygote with specific mRNA and DNA methylation levels. The phenotype of somatic cells of an adult is usually associated with the states of the zygote and its early developing embryo, which serves as the initial condition for later development. To test this dependence, we varied mRNA and DNA methylation levels at zygote and examined the phenotype of somatic cells after 100 cell divisions. The probability of having a type 2 phenotype mainly depends on the mRNA level at zygote; lower mRNA level yields a likelihood to develop into phenotype 2 (Figure 5B). Taken $RF = 0.2$, for instance, 8 in 40 simulation individuals showed phenotype 2 in stress-F1. All individuals with phenotype 2 displayed lower *Sfmbt2* transcription at zygote and latter development (Figure 5C), consistent with the bistability that type 2 gene expression is maintained through a positive feedback of transcription to DNA methylation. These results show that paternal stress alters *Sfmbt2* transcription in zygote to induce developmental reprogramming in stress-F1.

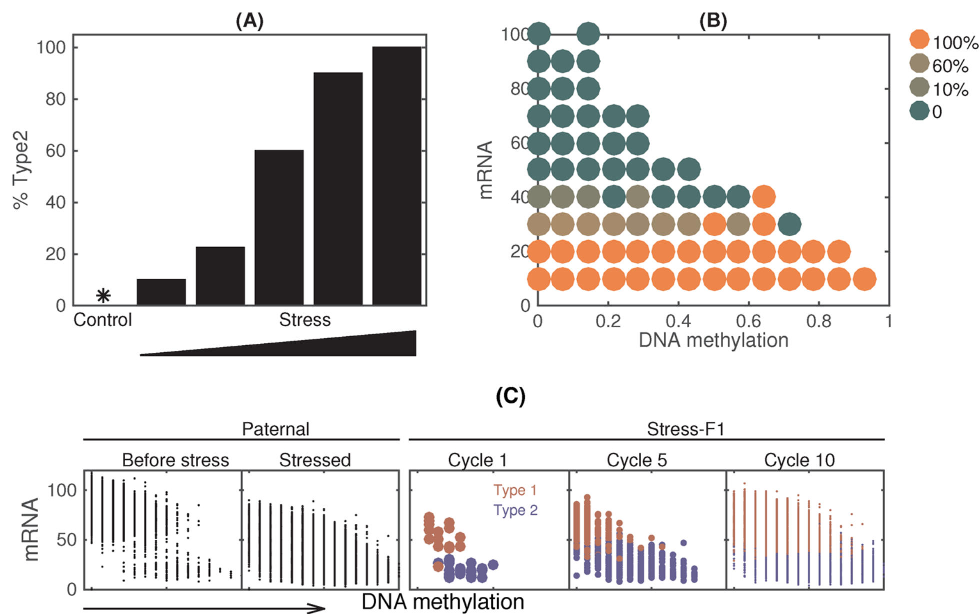

Figure 5. Transgenerational phenotype switches. **(A)** The probability of having a type 2 phenotype in F1 offspring increases with the strength of parental stress. Here, RF varies from 0 to 0.5. **(B)** Percentage of type 2 phenotype in adult at different initial conditions measured by the levels of DNA methylation and mRNA of the gene. Here, RF = 0.2. **(C)** Temporal dynamics of DNA methylation and mRNA expression. Each dot represents a state from a single cell. Data for stress-F1 are obtained from 20 independent trails. Each trail is classified as type 1 or type 2 according to the phenotype at adult (200 cell cycles from the zygote).

DNA demethylation in early embryo weakens stress-F1 reprogramming

In mammals, the first wave genome-wide DNA demethylation occurs at fertilization and during preimplantation, resulting in *re*-establishment of global DNA methylation patterns so that most parental stress-induced changes in germ cells are erased. However, imprinted genes are protected from reprogramming in early embryo and resets in PGCs [31]. We then asked how *re*-establishment of DNA demethylation in early embryos affects paternal stress-induced developmental reprogramming in offspring. In an early embryo, global demethylation of the parental strain can be induced with methyl dilution due to DNMT1 inactivation or active demethylation through TET3. We took RF = 0.2 in paternal stress, and varied the DNMT1 activity or TET3 concentration during preimplantation to examine the response in stress-F1.

To investigate the effects of DNA demethylation in early embryo, we performed model simulation with varying conditions, including imprinted gene (control), nonimprinted gene with DNMT1 inactivation, and nonimprinted gene with TET3 upregulation at early embryo. For an imprinted gene (control), DNMT1 and TET3 activities persisted at normal level during early embryo. In the case of nonimprinted gene with DNMT1 inactivation, DNMT1 activity dropped to an extreme low level immediately after fertilization, maintained low for two to three cycles, and then restored to normal approximately at eight cycles (Figure 2). For the case of nonimprinted genes with upregulated TET3 activity, we increased the coefficient for TET3 activity (b_1) to twofold or fivefold of the normal level (see Supplementary material, Sec. S6). To minimize the effects of various initial states at zygote, we compare individual runs obtained from each condition with the same DNA methylation at zygote (Figure 4B). DNA methylation in PGCs increased from low (0.1) to immediate level (0.3) after stress, and showed different dynamics under different conditions. In control condition (imprinted genes), DNA methylation increased to reach a high methylation state (0.6) in both PGCs and somatic cells, implying the reprogramming in stress-F1 and the possibility of transgenerational reprogram in F2. However, under conditions of nonimprinted genes, DNA methylation dropped to extreme low level at two-cycle stage (0.05), followed with an increase in 3 to 10 cycles. In somatic cells, the average DNA methylation levels are either low (0.1–0.2) or slowly increase to high methylation in later development, showing the resistance of F1 reprogramming. Moreover, under nonimprinted conditions, the DNA methylation levels of PGCs in stress-F1 restored the low level as in F0, and hence prohibit further reprogramming in F2. These results indicate that DNA demethylation in early embryo can eliminate the effect of parental stress by the *re*-establishment of low methylation patterns.

Increases in CpG sites in gene promoter decrease the probability of stress-F1 reprogramming

In the positive regulation circuit (Figure 1C), transcriptional products repress de novo methylation to activate gene transcription. The transcription rate depends on DNA methylation through a mean field approximation, which is given by average methylation of each CpG site in the gene promoter over a period of response time (Equation 3). Thus, the number of CpG sites in the promoter region affects the fluctuation of gene transcription. Differentially methylated regions (DMRs) are often involved in transgenerational inheritance and the number of CpG sites in a DMR normally ranges from a few to 30 [7,19,54]. We asked how CpG number in the proposed positive feedback circuit affects paternal stress-induced phenotype switch in offspring.

We altered CpG number from 5 to 28, and investigated how the stochasticity of gene expression and cell variability depend on CpG number under unstressed condition. Decreasing the number of CpG sites did not change average transcription, but slightly increased the fluctuation of gene expression at a single-cell level (Figure 6A).

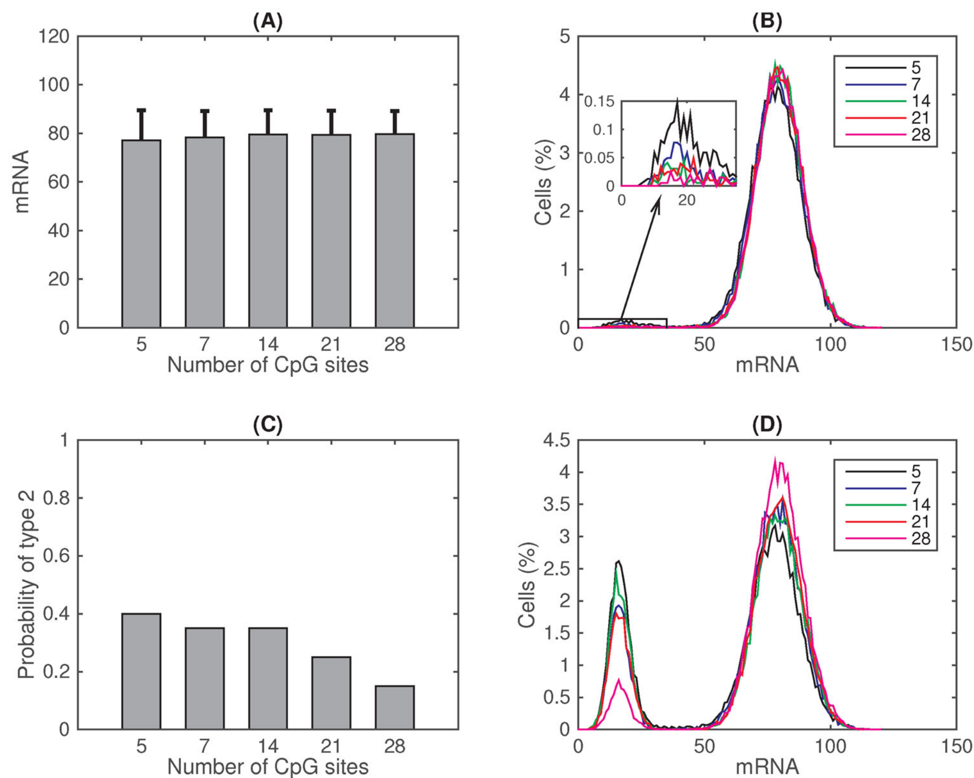

Figure 6. Effects of the number of CpG sites in the promoter region. **(A)** Dependence of the average mRNA level in mature germ cells on the number of CPG sites. Error bars show the deviations. **(B)** Distribution of mRNA counts of germ cells at 100 cycles. Inset zooms in the low mRNA region. **(C)** Probability of paternal stress induced phenotype changes at F1 offspring. **(D)** Distribution of mRNA counts of somatic cells at 100 cycles after fertilization at stressed F1 offspring. All results are calculated with the number of CpG sites varied from 5 to 28 as shown in the figures. Here RF = 0.2.

Consequently, bimodal phenotype in germ cells was seen when CpG numbers were in accordance with an increase in fluctuation in gene expression (Figure 6B). Next, we induced paternal stress, same as the simulation protocol in previous simulations, and calculated the probability of having phenotype 2 F1 offspring. In a case of five CpG sites, there was a slightly higher probability to have phenotype 2 in stress-F1, confirmed by the distribution of mRNA levels among somatic cells (Figure 6C and D). However, the dependence between the tendency of phenotype change in stress-F1 and CpG number is complex; further studies of the issue are required with approaches of both computation and experiments.

Discussion

Herein we developed a novel single-cell-based computational model to address a key unanswered question as to how offspring phenotypes are originated from the dynamics of DNA methylation-induced gene expression in response to paternal stress. Our model incorporates DNA methylation, sperm *Sfmbt2* gene expression, and cell divisions in a life cycle. The model integrates biochemical reactions of DNA methylation dynamics at a single-cell level with cell-to-cell variation along with individual development and transgenerational inheritance. We have identified a feedback to DNA methylation for paternal stress-induced reprogramming in offspring. The core circuit in a cell is the positive feedback of gene transcription through repression of de novo DNA methylation by the transcription products. Although direct evidence of the feedback regulation in *Sfmbt2* is missing, there are decreased SFMTB2 expression and enriched H3K9 methylation in the *Sfmbt2* promoter in stress- vs control F1 mice [27]. H3K9 trimethylation results in direct de novo DNA methylation via Dnmt3a/3b [49]. Hence, a possible route to form the positive feedback is through inhibition of *Sfmbt2* transcription production to H3K9 methylation at its own promoter.

In our model, the development of germ cell and somatic cell populations is considered at single-cell resolution in which each generation begins from zygote, followed by either symmetric or asymmetric cell divisions in each cell cycle; the state of each cell is tracked in simulations (Figure 1, Supplementary material Figure S2). The molecular mechanisms for fertilization and early cell fate decision are largely unknown; it is difficult to track the dynamics of gene transcription at early embryos. Nevertheless, the current study was aimed to investigate *Sfmbt2* gene, which is paternally imprinted in mice so that its expression is preferentially from the paternal allele [55]. The methylation level in *Sfmbt2* promoter is well conserved from sperm at stress-F0, embryo at stress-F1, and liver at stress-F1 after paternal psychological stress [27]. While limiting our discussion to the paternal imprinted gene circuit, we expect that the transgenerational transcriptional dynamics is predicable despite the yet unclear fertilization process.

Model simulations show that the positive feedback through DNA methylation is able to generate bimodal cell phenotype with either high (type1) or low (type2) transcription, and the distribution of transcription levels in a cell population can be adjusted with TET protein levels (Figure 2). The model reveals that paternal psychological stress induces phenotype changes (from type 1 to type 2) in stress-F1 and that the probability of having type 2 at stress-F1

depends on the transcription state of imprinted gene in zygote (Figure 5). DNA demethylation in an early embryo tends to eliminate the effect of parental stress due to the re-establishment of DNA methylation patterns (Figure 4B). We have also investigated the effect of CpG number and found that decreasing the number of CpG sites results in the emerging of type 2 cells under unstressed situation, and higher occurrence of phenotype 2 in stress-F1 (Figure 6).

A main issue in transgenerational programming is how parental epigenetic changes can be maintained in the development of germ cells and preimplantation embryos, in which DNA methylation patterns are reprogrammed genome-wide [32]. In paternal stress-induced hepatic gluconeogenesis in offspring [27], altered DNA methylation of *Sfmbt2* promoter in stress-F0 sperm is found to be well maintained in stress-F1 liver. Similar phenomena of the maintenance of altered methylation in perturbed sperm are also seen in many DMRs [7,19]. It is possible that these DMRs are located in the paternal ICRs, which are protected against both active demethylation in zygote and the ensuing passive loss. From a review of primary research papers using rodents, transgenerational developmental programming often associates with metabolic diseases [4], coincident with the fact that imprinted genes are key regulators of mammalian metabolic processes [45]. In addition, IAPs in both sperm and oocyte are almost completely resistant to demethylation in the early embryo [55–57]. Our model simulations suggest that parental epigenetic changes can be maintained during transgenerational programming when the changes are limited to a gene circuit of those genes covered by ICRs or IAPs.

In paternal stress-induced reprogramming, the first wave demethylation immediately following fertilization in the zygote affects early embryo development; hence, this is important for somatic fated cells in stress-F1 mice. The loss of methylation in the early embryo is the result of a passive mechanism due to the predominant exclusion of DNMT1 and NP95 from the nucleus of early embryonic cells [30,35]. Our study has investigated how changes in the DNMT1 activity alter the occurrence of phenotype 2 in stress-F1. Single-cell sequencing techniques have been recently applied to the cell fate inclination and DNA methylation landscape in early embryos [58,59], which should help clarifying the role of fertilization in transgenerational programming.

Transgenerational programming is a dynamical process of epigenetic changes over generations, during which molecular details are mostly unknown. Experiments are difficult, if not impossible, to track the epigenetic changes over a long period. Computational models are potentially applied to study the process in transgenerational programming by tracking epigenetic changes of each single cell in cell divisions. The proposed model framework, despite simplicity, can be an in silico lab to mimic wet lab experiments. With model simulation, we are able to examine the long-term responses in epigenetic changes to temporal parental stress under different hypotheses, and to predict the probability of transgenerational reprogramming in offspring.

Altogether, this study provides a computational model for the mechanisms that underlies paternal psychological stress-induced programming in offspring. In our model, we focus on transcription dependence of DNA methylation through a mean field approach, which simplifies the yet unclear chemical details as to how DNA methylation regulates complex transcription process such as remodeling of chromosome structure [60,61]. In modeling cell division, methylation markers are assigned randomly to either of the daughter cells, together with the random partitioning of molecules. Since other mechanisms in addition to DNA methylation, especially various sperm RNA molecules such as miRNAs [15,16] and transfer RNA-derived small RNAs [62,63], also play a role in intergenerational inheritance of acquired metabolic disorders under the influence of paternal stress, our model needs to be further studied for better understanding a long-term sophisticated fetal programming process. Nonetheless, this novel modeling approach can be viewed as an in silico laboratory for mimicking wet lab experiments. The combination of model simulation and wet lab experiments may help to address transgenerational reprogramming and to predict the probability of transgenerational reprogramming upon temporal stresses. This work is an initial attempt to introduce mathematical modeling into this topic and thus more work should be done to extend the model to include more details such as molecule partitioning in cell division and the effects of different ways of stochastic partitioning on the long-term cell phenotypes for better understanding the quantitative dependence of transgenerational programming. It is templating that this model framework can be easily extended to study long-term biological processes such as development, stem cell regeneration, and cancer progression, upon further details of gene regulation networks.

Supplementary data

Supplementary data are available at *BIOLRE* online.

Supplemental Information contains Supplemental Experimental Procedures, five figures and one table are available online.

Supplementary Figure S1. DNA methylation reprogramming during DNA replication.

Supplementary Figure S2. Cartoon of cell divisions during in one generation.

Supplementary Figure S3. Time courses in one generation. Results were obtained from an initiation of either high mRNA (left) or low mRNA (right) levels. In each case, 100 sample individuals were calculated. Gray lines are time courses of each individual, and red curves are average over the 100 sample individuals.

Supplementary Figure S4. Simulation results of transgenerational inheritance. Two sample runs are shown at A and B, respectively. In each sample, gray dots are mRNA levels of germ cells (upper panel) and somatic cells (lower panel), red lines are average levels over all cells.

Supplementary Figure S5. mRNA levels of somatic cells in 10 independent individual runs. Gray dots are mRNA levels in each single cell, red lines are average mRNA levels. The time point 0 cycle corresponds to the time point of fertilization.

Supplementary Table S1. Parameters used in model simulations.

Conflict of Interest: The authors have declared that no conflict of interest exists.

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