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Review

A New Role for SMCHD1 in Life's Master Switch and Beyond

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Structural maintenance of chromosomes flexible hinge-domain containing protein 1 (SMCHD1) has emerged as a key regulator of embryonic genome function. Its functions have now extended well beyond the initial findings of effects on X chromosome inactivation associated with lethality in female embryos homozygous for a null allele. Autosomal dominant effects impact stem cell properties as well as postnatal health. Recent studies have revealed that SMCHD1 plays an important role as a maternal effect gene that regulates the master switch of life, namely embryonic genome activation, as well as subsequent preimplantation development and term viability. These discoveries mark SMCHD1 as a major regulator linking developmental processes to adult disorders including a form of muscular dystrophy.

A New Role for SMCHD1 in Life's Master Switch and Beyond

Building an embryo is much like building a house. Both are complex systems, and both require components to be built in the appropriate sequence. For example, turning on the main power supply to a home would make little sense if the electrical circuitry has not been installed. Similarly, activating gene transcription in the embryo would make little sense if there is no way for it to be regulated. That regulation is powered by the creation of a transcriptionally repressive chromatin state, a process that is fundamental to the very process of a new life emerging. Recent studies have revealed a novel role in this essential repressive process for the broadly acting transcriptional repressor, SMCHD1 [1,2]. SMCHD1 is best known for its role in promoting DNA methylation and gene silencing, and also has apparent roles in X chromosome inactivation, imprinted gene regulation, and autosomal gene cluster repression [3]. The discovery of the role of SMCHD1 in controlling early embryonic processes provides crucial new insight into the mechanisms that program early embryo genomes for development, and establishes novel links between these early processes and human disorders.

Turning 'On' the Master Switch

Early genome reprogramming processes are complex (Figure 1, Key Figure). Fertilization brings together two highly specialized genomes from two highly differentiated cell types. After fertilization, the two parental sets of chromosomes decondense, and undergo extensive chromatin restructuring and epigenetic changes [4–7]. Passage through the first round of DNA replication in mouse embryos creates a transcriptionally permissive state in which transcriptional enhancers are not required for a high rate of transcription [8,9]. To this end, transcription activation of an Hsp70.1 promoter-driven luciferase reporter transgene [10] was observed following passage through the first S phase in mice [8,9,11]. Additional nuclear transfer studies demonstrated a transition from a transcriptionally nonpermissive state to a permissive state as embryos reached the late one-cell stage. Two-cell stage nuclei were transplanted to enucleated early- or late-stage one-cell zygotes. After 4 h of culture the production of the prominent two-cell stage-specific 'transcription-requiring complex', a group of transiently synthesized peptides at approximately 70 kDa molecular mass was assayed that account for nearly 4% of protein synthetic activity and that served previously as a prominent marker of EGA1 [12]. Only late-stage zygote recipients supported expression of the transcription-requiring complex. Other studies demonstrated that subsequent passage through the second round of DNA replication is accompanied by the establishment of a transcriptionally repressive chromatin state in which transcriptional enhancers are necessary to drive the elevated rate of gene transcription [4,9,11,13,14].

Thus, the first two rounds of DNA replication are essential to establish the ability to execute transcription in a well-controlled manner. Multiple waves of tightly controlled embryonic genome activation (EGA 1–4, Figure 1) and gene silencing occur as the embryo progresses through cleavage divisions,

Highlights

The first burst of mouse embryonic genome activation (EGA1) is short-lived, and must be initiated and terminated in a timely manner for maximum embryo viability.

A new pathway has recently emerged that controls the onset and termination of EGA1 by the transcriptional activator, developmental pluripotency associated gene 2 (DPPA2), and by the transcriptional repressor, SMCHD1.

Following EGA1, a transcriptionally repressive state is established to allow thousands of other genes to be regulated correctly. Oocyte- and embryo-expressed SMCHD1 is poised to play a significant role in establishing this repressive chromatin state.

In humans, mutations in *SMCHD1* contribute to facioscapulohumeral muscular dystrophy and Bosma arhinia microphthalmia syndrome. These effects suggest that at least part of this regulatory pathway functions during later development.

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Key Figure

Structural Maintenance of Chromosomes Flexible Hinge-Domain Containing Protein 1 (SMCHD1) Plays Two Key Roles in the Early Embryo, Terminating the First Wave of Gene Transcription and Establishing a Transcriptionally Repressive Chromatin Structure That Enables Gene Regulation

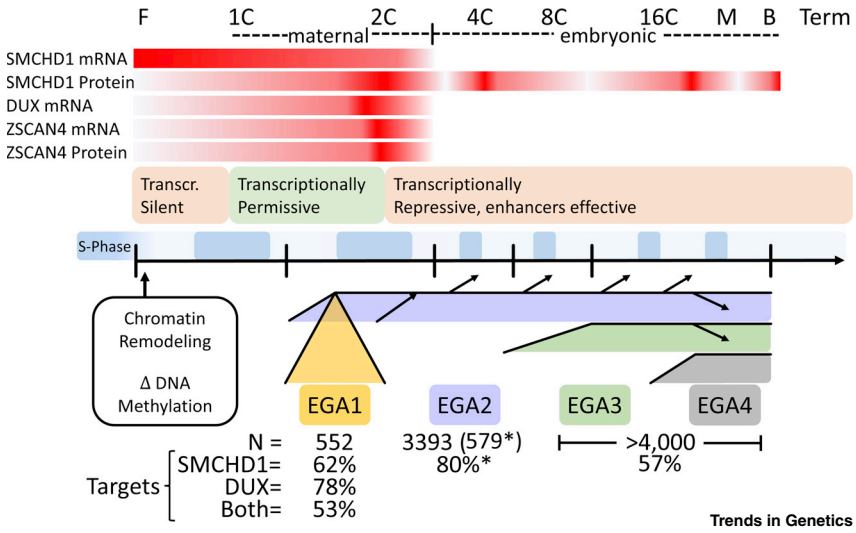

Figure 1. *Smchd1* mRNA is expressed as a maternal mRNA, and SMCHD1 protein displays periodic peaks in nuclear content with the correct timing to fulfill these roles (red bar). After fertilization, embryos transition from a transcriptionally silent state to a permissive state and then to a repressive state, in conjunction with passage through the first two rounds of DNA replication, associated chromatin remodeling, and changes in DNA methylation. Multiple waves of genes are transcriptionally activated [embryonic genome activation (EGA)1, 2, 3, and 4] and sets of genes are up- or downmodulated during development (denoted by upward and downward pointing arrows). EGA1 refers to a set of genes transiently induced during the early two-cell stage. SMCHD1 terminates EGA1 and is itself a regulator of a large fraction (62%) of EGA1 genes. Thereafter, SMCHD1 modulates the expression of a large fraction of EGA2 genes during the two-cell stage, and likewise modulates the expression of a large fraction of EGA3 and EGA4 genes during later stages (indicated by percentages). SMCHD1 is a potential regulator of a large fraction of genes that are also regulated by DUX, but some putative SMCHD1 targets are not regulated by DUX, and only 53% of EGA1 genes are regulatory targets of both. These early SMCHD1 actions contribute to embryo viability to term, suggesting that these early events have a long-term impact on later stages of development. Abbreviations: 1C/2C/4C/8C/16C, one-, two-, four-, eight-, and 16-cell stages; M, morula; B, blastocyst. * Denotes EGA2 genes with limited expression at the 2-cell stage, but higher levels at later stages; 80% are putative targets of SMCHD1.

compaction, and blastocyst formation. This basic series of events is shared across species, although differences in timing relative to cleavage are seen [15].

The initial low level of transcription observed after the first round of DNA replication in mouse embryos is followed by an initial burst of a set of transiently transcribed genes (denoted here embryonic genome activation 1, EGA1) (Figure 1). This EGA1 wave coincides with a vast degree of rapid change in the pattern of protein synthesis, including the prominent transcription-requiring complex [16]. Additional genes are activated at the early or mid two-cell stages and show sustained expression

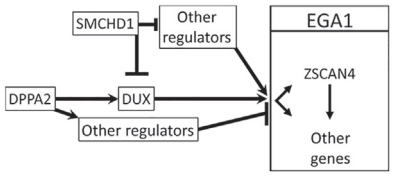

Figure 2. Structural Maintenance of Chromosomes Flexible Hinge-Domain Containing Protein 1 (SMCHD1) Participates in a Newly Discovered Pathway Regulating Embryonic Genome Activation (EGA)1.
SMCHD1 negatively regulates *DUX* (activated by DPPA2) and other EGA1 genes such as *ZSCAN4*. Incomplete overlap between putative DUX and SMCHD1 target gene sets indicates potential roles for other regulators on EGA1 genes, perhaps including other regulators downstream of SMCHD1. DPPA2 may also work through downstream effectors other than DUX. At least part of this pathway is cyclically recapitulated in embryonic stem cells.

(denoted here EGA2, Figure 1) [1]. Achieving these first two waves of gene activation (EGA1 and EGA2) constitutes the 'master switch of life', and results in a vast change in the array of proteins being synthesized [16] and mRNAs expressed [17–19] relative to oocytes, zygotes, or early two-cell stage embryos. Occurring in concert with widespread degradation of maternal mRNAs, these two waves of gene activation are often taken to signify a switch from maternal to embryonic control of development. The vast change at the levels of both mRNA and protein synthesis signifies a major switch in cellular properties rather than simply replacing maternal with embryonic copies of transcripts.

Disruption of this complex pattern of transcriptional activation and repression leads to immediate or delayed embryo mortality [20,21]. EGA1 is essential for mouse embryo progression beyond the two-cell stage [21]. In addition, correct regulation and termination of EGA1 gene expression is required for efficient development of mouse embryos to the blastocyst stage [20]. It is striking that EGA1 is initiated before the second S phase, and thus before an enhancer-dependent repressive chromatin state is established. Thus, the early embryo faces a unique challenge of initiating and then terminating EGA1 at a time when the ability to regulate gene transcription is still emerging. Achieving the correct temporal pattern of EGA regulation therefore requires a combination of transcriptional activation and repression events taking place in close succession, and at a time when the essential regulatory structure of the genome is being established. Recent studies provide insight into the molecular players responsible for this remarkable and unique process.

What Are the 'On' Switches?

Oocyte-expressed factors that promote EGA have been identified [22–27]. Most recently, oocyte-expressed DPPA2 (developmental pluripotency associated gene 2) was discovered to play an important role in activating EGA1 [27] by activating the expression of a key EGA1 mediator, DUX, which in turn activates zinc finger and scan domain-containing protein 4 (*Zscan4*) and other EGA1 genes downstream of ZSCAN4 [20,27–29]. Mouse *Dux* is, thus far, the earliest zygotically transcribed transcription factor gene so far observed, and an α-amanitin-sensitive increase in *Dux* mRNA expression is detectable before the first cleavage division [1]. This novel pathway (Figure 2) is an exciting finding in which a mammalian oocyte-expressed factor (DPPA2) drives the early transcriptional expression of another key transcriptional mediator (DUX). This pathway is activated even before first cleavage and drives a major event, in this case, EGA1. Interestingly, *Dux* null embryos are viable to term, albeit with reduced numbers of survivors and evidence of some preimplantation defects [30,31]. Likewise, not all EGA1 genes are *Dux*-responsive. This indicates that other factors likely participate in EGA1 but have not been identified (Figure 1).

After Turning 'On' the Master Switch, Molecular Circuit Breakers Enable Transcriptional Control

If the master switch is turned on, then one or more controlling mechanisms equivalent to the set of circuit breakers in a home is necessary to regulate transcription and allow subsets of genes to be expressed. These could include factors that directly inhibit the functions of activating factors, such

as DPPA2, or factors that create the repressive chromatin state. Both are apparently used. An oocyte-expressed factor, E3 ligase enzyme (protein inhibitor of STAT4, PIAS4), sumoylates and destabilizes DPPA2 and DPPA4, providing one means of reducing DPPA2 action, as well as providing a brake on EGA1 by inhibiting activator function [32]. Another oocyte-expressed gene, *Smchd1*, also contributes a crucial repressive function to terminate EGA1, one of the first such negative transcriptional regulators identified to do so, and acts downstream of DPPA2 (Figure 2).

The *Smchd1* mRNA is expressed as a maternal transcript in mouse oocytes and zygotes. However, the amount of SMCHD1 protein expression detected in embryonic nuclei first peaks at ~9 h after the first cleavage division [1], suggesting possible regulation at the level of maternal mRNA translation or SMCHD1 nuclear localization, or both (Figure 1). *Dux* and *Zscan4* mRNAs peak in expression at the early two-cell stage and are rapidly downregulated between 3 and 9 h after cleavage (Figure 1) [1]. ZSCAN4 nuclear protein staining also declines by 9 h post-cleavage (Figure 1) [1], indicating that the temporal regulation of SMCHD1 is consistent with a possible role in repressing DUX expression and EGA1 in two-cell embryos; also consistent with this, SMCHD1 represses *DUX* in somatic tissues [3].

The contribution of SMCHD1 in controlling EGA1 was tested in small interfering (si)RNA knockdown studies using siRNA microinjection at the one-cell stage [1]. Reduction in maternally expressed SMCHD1 led to sustained high expression of *Dux* and *Zscan4* mRNAs [1]. This observation indicates that SMCHD1 protein encoded by maternal *Smchd1* mRNA, and that peaks in nuclear abundance at the mid two-cell stage, is required for the timely termination of EGA1 [1].

Available RNA-seq data for mouse embryos reveal a large set of nearly 3000 characterized genes that are transcriptionally activated within a few hours of the first cleavage division, and >1000 other characterized genes that are activated after the mid two-cell stage (Figure 1); these 3945 genes together comprise the EGA1 and EGA2 gene sets. The EGA1 class encompasses 552 genes that are transiently activated, being expressed more abundantly [false discovery rate (FDR) <0.05, abs($\log_2$foldchange) >2] at the early or mid two-cell stage than at the zygote, late two-cell, or four-cell stages. The EGA2 class includes the remaining 3393 genes.

Using a combination of chromatin immunoprecipitation data, DNA sequence motif analysis, and data on the effects of gene ablation or knockdown, EGA1 genes were categorized as putative targets for DUX, SMCHD1, or both [1]. Most, but not all (78%), of the 552 EGA1 genes were identified as targets of DUX regulation (Figure 1), suggesting that other transcription factors may contribute to EGA1 (Figures 1 and 2). Conversely, only 62% of the 552 EGA1 genes were identified as targets of SMCHD1 (Figure 1), indicating that additional repressors may contribute to the termination of EGA1 (Figure 2). Interestingly, although SMCHD1 is a known regulator of *DUX* in human cells [33,34], a fraction of the EGA1 genes are regulated by SMCHD1 but not by DUX, indicating a possible role for SMCHD1 in repressing EGA1 genes that are activated by factors other than DUX (Figure 2). Overall, 89% of the EGA1 genes are targets for either SMCHD1 or DUX, indicating that other regulators control ~11% of EGA1 genes. These observations indicate that other chromatin regulators must serve as components of the master switch (i.e., activators) or as breakers in the embryonic 'breaker box' that mediates the temporally crucial downregulation of EGA1 genes.

To extend the above analysis and explore the relationships between the actions of *Smchd1*, *Dux*, *Dppa2*, and *Zscan4* on EGA1 genes, we compared lists of putative target genes and associated ingenuity pathway analysis (IPA) canonical pathway and disease/function categories. Putative target genes were identified using a combination of expression effects in gene ablation, overexpression, or knockdown studies in embryos or embryonic stem cells (ESCs), as well as chromatin immunoprecipitation results using ESCs or other cell types, and DNA-binding motif analysis as described [1]. We discovered that DPPA2 displays a much more restricted set of putative target genes than the other three factors, and SMCHD1, DUX, and ZSCAN4 share more EGA1 target genes than any combination involving DPPA2 (Figure 3A). Only ~10% of EGA1 genes were identified as targets of all four regulators. The number of target EGA1 genes identified for the four factors, alone or in combination, indicates that other factors likely contribute to EGA1 gene regulation. Affected IPA categories are

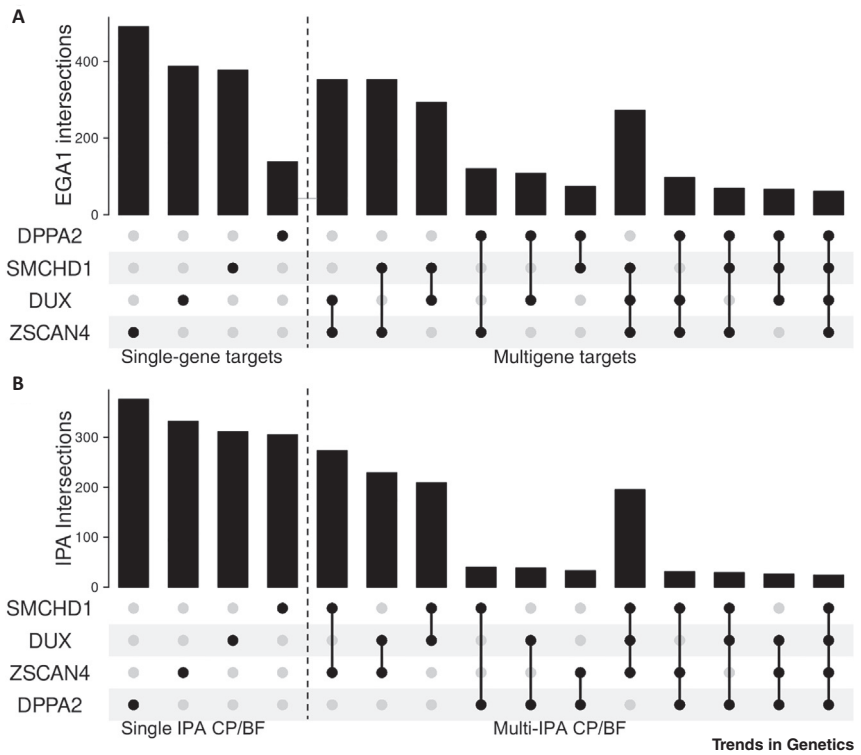

Figure 3. Number and Overlap of (A) Putative Target Genes in Embryonic Genome Activation (EGA)1 and (B) Associated Ingenuity Pathway Analysis Canonical Pathways (CPs) and Biological Functions (BFs) for DPPA2, DUX, ZSCAN4, and SMCHD1.

Lists of putative target genes among the 552 EGA1 genes previously reported [1] were generated for each of the four factors using a combination of chromatin immunoprecipitation data, expression effects reported for ablation, knockdown, or overexpression, and DNA-binding motif analysis as described [1]. The numbers of target genes and associated ingenuity pathway analysis (IPA) categories for each single factor are indicated in the first four bars. IPA categories related to cancer, tumors, and other disease categories deemed unrelated to embryo functions were not included. The number of overlaps in target genes and associated IPA categories for two, three, or all four factors are shown as indicated.

smallest in number for combinations involving DPPA2, whereas a large fraction of IPA categories are shared for SMCHD1, DUX, and ZSCAN4 (Figure 3B), consistent with an apparent regulatory pathway involving these three proteins.

In addition to the EGA1 genes, SMCHD1 could regulate other genes as part of the broader process of establishing the transcriptionally repressive state. A major part of this function would be to control additional genes that are activated during the two-cell stage – the EGA2 gene class. Of the 3393 EGA2 genes, 579 display limited expression in two-cell embryos, but higher levels of expression at later stages, indicating they are 'on' at the two-cell stage but partially transcriptionally repressed. A majority (80%) of these 579 genes are putative targets of SMCHD1. Additional EGA2 genes beyond these 579 are likely also affected by SMCHD1. This observation is consistent with a role for SMCHD1 in establishing the transcriptionally repressive state at the two-cell stage to allow gene transcription to be controlled.

Household Switches – SMCHD1 Regulates Subsets of Genes during Subsequent Cleavage Divisions

The above functions of SMCHD1 in providing brakes on EGA1 and EGA2 are driven by oocyte-derived expression; *Smchd1* mRNA is present in the oocyte, and SMCHD1 is synthesized and peaks in nuclear abundance at the mid two-cell stage [1,2]. Immunofluorescence confocal microscopy

revealed subsequent periodic peaks of SMCHD1 nuclear staining, with peaks at the mid four-cell stage, morula stage, and to a lesser degree at the expanded blastocyst stage [2], indicating embryonic expression that is developmentally regulated in a temporally complex manner. Examining the temporal expression patterns of the EGA2 genes reveals that many of these genes undergo repression after the two-cell stage [1]. Strikingly, SMCHD1 regulates >80% of the EGA2 genes that are downregulated after the two-cell stage. In addition, SMCHD1 regulates 82% of 329 EGA2 genes that are upregulated at the blastocyst stage. Because the trophoblast lineage comprises the majority of the embryo mass at the blastocyst stage, and because *Smchd1* is downregulated by the trophoblast lineage factor CDX2 [2], this upregulation of EGA2 genes most likely reflects a loss of gene repression by SMCHD1 in trophoblast lineage cells.

SMCHD1 continues to regulate genes beyond the four-cell stage, possibly functioning as a 'dimmer switch' that modulates gene expression at later cleavage stages. siRNA knockdown revealed a small number of affected genes at the morula stage [2]. However, the role of SMCHD1 in gene regulation may be broader because the siRNA knockdown was transient, and SMCHD1 expression recovered to ~50% of normal by the blastocyst stage [2]. Of the >12 000 genes displaying different mRNA expression profiles after the four-cell stage, over half (57%) are putative targets of SMCHD1 [1]. These include 2167 genes that increase in expression between the morula and blastocyst stages, which again may represent loss of SMCHD1 repression in the trophectoderm. These observations indicate that SMCHD1 likely contributes to establishing the correct temporal patterns of gene activation and repression throughout the preimplantation period. Given that siRNA knockdown of *Smchd1* reduces blastocyst hatching and term development [2], this ongoing role for SMCHD1 during preimplantation life is important for embryo viability. However, embryonic expression does not seem to fully compensate for early siRNA knockdown, indicating that the oocyte-expressed SMCHD1 may play a crucial role during that early window of time in programming the embryonic genome.

Outstanding Questions

What are the other maternal effect factors in mammalian oocytes that regulate genome activation and long-term development?

How do these early events in the embryo impact on stem cell formation, organogenesis, and later fetal and adult life?

How do these events relate to the developmental origins of adult health and disease?

Recapitulation of an EGA1-like State in ESCs

One very interesting discovery in recent years is that ESCs recapitulate an early embryo two cell-like or '2C' state [20,28–30,35,36]. DUX drives entry into the 2C state in ESCs as it does in embryos [30]. At any given time 1–5% of ESCs reside within this 2C state [20]. While in that state, ESCs downregulate pluripotency genes such as POU domain class 5 transcription factor 1 (*Pou5f1*, also known as *Oct4*) and manifest totipotency [37]. This suggests that ESCs must exit the 2C state and spend most of their time outside the 2C state so as to be pluripotent. Because SMCHD1 regulates *DUX* and other EGA genes, it is tempting to speculate that SMCHD1 may also play a role in limiting residency time within the 2C state in ESCs, and thereby promote stem cell developmental potential.

Human Developmental Disorders and Diseases Related to SMCHD1 Deficiency in Adults

Haploinsufficiency for *SMCHD1* is associated with autosomal dominant facioscapulohumeral muscular dystrophy (FSHD) in humans. The most common form of the disease (FSHD1) is associated with contraction of the D4Z4 microsatellite [38,39], which when combined with the permissive chromosome configuration leads to stabilized pathogenic D4Z4 transcripts, as well as reduced DNA methylation and increased histone acetylation [40] at D4Z4 repeats, which encode DUX4 [41]. In ~5% of patients (characterized as FSHD2), *SMCHD1* mutation results in haploinsufficiency for SMCHD1 and disease in patients carrying a permissive chromosome 4qa allele [42]. SMCHD1 plays a role in *de novo* methylation of D4Z4 in induced pluripotent stem cells as cells acquire pluripotency [33], indicating a role for SMCHD1 in the normal epigenetic programming of DUX4. SMCHD1 mutations are also associated with another congenital defect, Bosma arhinia microphthalmia syndrome (BAMS), in which mutations are also permissive for *DUX4* expression [33]. Induced pluripotent stem cells from FSHD2 and BAMS patients also display increased expression of DUX target genes, *ZSCAN4*, methyl-CpG binding domain protein 3 like 2 (*MBD3L2*), and tripartite motif-containing protein 43 (*TRIM43*) [33]. Interestingly, imprinting and X chromosome inactivation are not altered in heterozygous *SMCHD1* mutant pluripotent stem cells, and neither FSHD2 nor BAMS are associated with defects in X chromosome inactivation [33]. These observations further illustrate that

heterozygosity for *SMCHD1* mutation exerts selective effects across the genome, and that the role for SMCHD1 in programming embryonic cells is important for avoiding developmental disorders.

Concluding Remarks and Future Perspectives

Recent studies have provided new insight for understanding the essential processes that create the embryonic genome and initiate the developmental program in mammals. SMCHD1 has recently emerged as a major regulator of chromatin, repressing genes across the genome. Its functions have now extended well beyond the initial findings of effects on X chromosome inactivation associated with lethality in female embryos homozygous for a null allele. Indeed, heterozygous *SMCHD1* mutations display autosomal dominance and disorders in humans. Recent studies have revealed essential roles for SMCHD1 in the earliest formative processes that activate the embryonic genome and prepare it to execute the developmental program. These discoveries mark SMCHD1 as a novel maternal effect gene in mammalian oocytes and demonstrate the power of the oocyte to impact on long-term development. Our understanding of the mechanisms controlling EGA1 should grow as we discover more oocyte- and embryo-expressed regulators that create and regulate the early embryonic genome. Future studies (see Outstanding Questions) may reveal additional maternal effect genes that augment the role of SMCHD1. In addition, studies of EGA-like events in pluripotent stem cells as well as embryos may reveal novel mechanisms by which embryonic events, maternal health, and maternal and embryonic environmental factors affect adult health and disease.

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