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The Role of NADH Redox Metabolism and Mitochondrial Complex I in Embryonic Development: A Focus on Maternal and Neonatal Care

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Abstract

The quiescent to transcriptionally active embryonic transition is one of the most impressive biological developmental biology changes. The major feature of this process is the complex interconnection between mitochondrial metabolism, especially NADH redox processes and Complex I activity and the development of transcriptional competence via embryonic genome activation (EGA). This is an extensive review of mechanistic interactions among mitochondrial energy metabolism, redox signaling and chromatin remodeling which together coordinate the maternal-to-zygotic transition. We discuss the effects of changes in NADH/NAD+ ratio in epigenetic change, especially the control of sirtuins and other enzymes dependent on NAD+, and developmental progression through a metabolic checkpoint of Complex I activity. Moreover, we mention the impact of reactive oxygen species (ROS) production by Complex I in cellular signaling pathways which control cell fate decisions, pluripotency maintenance, and differentiation. Through recent developments in single-cell metabolomics and live imaging technology, the subsequent levels of spatial and temporal heterogeneity of mitochondrial activity during early embryogenesis have been shown, which does not fit the extant models of homogeneous metabolic reprogramming. The review is a synthesis of existing knowledge regarding the role of mitochondrial metabolism, especially NADH redox metabolism and Complex I activity, in being a key regulator of embryonic genome activation and cellular state transitions, which have

implications to assisted reproductive technologies, regenerative medicine, and our developmental disorder biology.

Introduction

Initial embryonic developmental phases are marked by a major change in cellular metabolism, transcription, and developmental potential. After the fertilization process, the newly created zygote has to deal with a complex set of molecular and metabolic events that eventually define the developmental program necessary to form the organism (Peng et al., 2022). In particular, embryonic genome activation (EGA) is one of the most important transitions, as it is the stage when the embryonic genome takes over the developmental process by taking over maternally inherited factors. Such a shift is not a genetic phenomenon but is closely linked to the dramatic alteration of cellular metabolism, that is, in the mitochondrial compartment (May-Panloup et al., 2021).

The mitochondria, which are commonly referred to as the powerhouse of the cell can fulfill a variety of roles that go way beyond the production of ATP. These organelles are found to be central integration sites of metabolism, redox, calcium, and apoptotic regulation. During early embryonic development, mitochondria are reorganized dramatically, and they change their state of relative inactivity inherited in oocytte to highly active organelles that aid the high energy and biosynthetic needs of fast cell division and differentiation (He & Maheshwari, 2023). This metabolic shift is coordinated by the shifts in the mitochondrial membrane potential, the ability to oxidatively phosphorylate, but, most importantly, the redox state of the major electron carriers like NADH (Fisher et al., 2025).

NADH (reduced nicotinamide adenine dinucleotide) and its oxidized form NAD + are one of the most common and functionally ubiquitous redox couples in cell metabolism. The NAD +/ NADH ratio is not only a marker of cellular metabolic condition but also an essential controller of a wide variety of enzyme activities, including those activating epigenetic regulation (Thompson et al., 2024). The proper maintenance of NAD+/NADH ratios is especially important during early embryogenesis, when metabolic flows in the glycolysis pathway, tricarboxylic acid (TCA) cycle and oxidative phosphorylation have to be tightly regulated to respond to developmental requirements(He et al., 2023).

Complex I (NADH: ubiquinone oxidoreductase) is the first and the biggest electron transport chain enzyme complex, which lies at the core of mitochondrial NADH oxidation. Complex I is the mechanism which transfers the electrons out of NADH to ubiquinone and at the same time it pumps protons over the inner mitochondrial membrane, which helps in the generation of the electrochemical gradient that leads to the production of ATP (Peng et al., 2021). In addition to being an essential bioenergetics player, Complex I is a key location of reactive oxygen species (ROS) production and important in the redox signaling of cells. Complex I activity and integrity have now been found to play a major role in cellular state transitions, including those during embryonic development, cellular differentiation, and metabolic reprogramming (Rodríguez-Cano et al., 2020).

The recent years have been characterized by an avalanche of interest in metabolic control of cell fate decisions, which is in part due to the understanding that metabolism is not merely a byproduct of cellular programming, but a participant in the determination of developmental outcomes. The idea of so-called metabolic checkpoints has developed, where particular metabolic conditions are needed to pass through developmental transitions. The role of mitochondrial metabolism, and in particular: the role of the Complex I and the dynamics of NADH redox metabolism, is a highly important regulatory node in this framework between cellular energy state and transcriptional and epigenetic programs (Rodríguez-Cano et al., 2020).

The convergence of the metabolism of NADH with the activity of Complex I as well as with embryonic genome activation demonstrates an interesting phenomenon of the metabolic-epigenetic interaction. The direct connection between cellular redox state and chromatin modifications and gene expression is directly mediated by NAD+ dependent enzymes, especially sirtuin family of deacetylases. Modification in NAD+/NADH ratios during early development can thereby spread out to modification in histone acetylation, DNA methylation, and eventually transcription activity. Likewise, Complex I produces ROS which can be used as signaling molecules that regulate the transcription factor activity, chromatin accessibility, and cell fate choice(Harvey, 2019).

This review will serve as a detailed analysis of the functions of NADH redox metabolism and mitochondrial Complex I activity in embryonic genome activation and cell maintenance. These metabolic processes will be discussed in relation to their molecular processes involved in transcriptional regulation, developmental control of mitochondrial activity during early embryogenesis and the overall implications to the understanding of developmental biology and disease. With a combination of the most recent breakthroughs in the fields of metabolomics, developmental biology and chromatin biology, we offer a single model of how metabolism can influence developmental potential and cellular identity.

Fundamentals of NADH Redox Metabolism and Complex I Function

NADH: Structure, Function, and Cellular Distribution

Nicotinamide adenine dinucleotide occurs in cells in two oxidation states; the oxidized form (NAD+) and the reduced form (NADH). These two nucleotides are oriented together as a pair by their phosphate groups, where one of the nucleotides has an adenine base and the other one has a nicotinamide base. The redox activity of NAD + / NADH is concentrated in the nicotinamide moiety, which may alternatingly accept or release a hydride ion (H -), two electrons and one proton. The involvement of NAD+/NADH in the hundreds of redox reactions that take place in cells is based on this basic chemical reaction (Chandel, 2021).

The NAD+ and NADH distribution is compartmentalized in the cell, and separate pools are present in the nucleus, cytoplasm, and mitochondria. The highest concentration of NAD+ is found in the mitochondrial matrix which ranges between 200-500 μM, in comparison with cytoplasmic concentrations of 50-100 μM. Notably, a significant difference in the ratio of NAD+/NADH across compartments is that the cytoplasm is in a rather oxidized condition (NAD+/ NADH ratio of 60-700) and the mitochondrial matrix is more reduced (NAD+/NADH ratio of 7-8). The difference in these redox states represents the various metabolic activities within each compartment with far reaching consequences on enzyme processes and signaling pathways (Sies et al., 2024).

NAD+ is synthesized by de novo via tryptophan, and salvage via nicotinamide and nicotinic acid. The most common pathway to NAD + in mammals is the salvage pathway, catalyzed by nicotinamide phosphoribosyl transferase (NAMPT). The pathway is essential in ensuring NAD+ homeostasis since NAD+ is constantly depleted by NAD+-dependent enzymes such as sirtuins, poly (ADP-ribose) polymerases (PARPs) and CD38. The regulation of NAD + production and breakdown together with the rate of NADH oxidation via the electron transport chain dictate the cellular level of NAD +/NADH ratio and therefore affect many metabolic and regulatory processes (Goswami, 2025).

NADH Generation: Metabolic Pathways

Various metabolic pathways produce the NADH and all of them help to oxidize nutrients with the aim of extracting energy. The process of glycolysis in the cytoplasm involves producing two molecules of NADH per mole of glucose; this is done by

reaction with the glyceraldehyde-3-phosphate dehydrogenase reaction (Yang et al., 2025). Nevertheless, most of the NADh production takes place in the mitochondrial matrix where acetyl-Coa undergoes oxidation in the tricarboxylic acid (TCA) cycle. In the process of the acetyl-CoA in the TCA cycle, three molecules of NADH are produced as a result of the combined activity of isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase. Also, NADH is formed during fatty acid 2-oxidation and each cycle yields one molecule of FADH2, and one molecule of NADH (Qin et al., 2025).

NADH is also produced by the pyruvate dehydrogenase complex which is the place of intersection between glycolysis and the TCA pathway as it converts pyruvate to acetyl-CoA. This response takes place on the matrix of mitochondria and is a pivotal control of glucose metabolism. Phosphorylation-dephosphorylation controls the activity of pyruvate dehydrogenase, with the pyruvate dehydrogenase kinases (PDKs) inhibiting the complex and the pyruvate dehydrogenase phosphatases (PDPs) simulating the activity. This rule enables cells to adjust glucose oxidation according to the nutrition status and energy requirement and this regulation directly affects the NADH levels in the mitochondrion(Qin et al., 2025; Wang et al., 2026).

In addition to these main metabolic pathways, NADH can be produced in amino acid catabolic process, one-carbon metabolism or other specialized metabolic pathways. The variety of NADH-producing pathways indicate the hub position of the role of this redox cofactor in cellular energy metabolism and offers a variety of regulatory sites where the cellular level of NADH can be altered. In early embryonic development the relative roles of the various NADH-producing pathways change drastically which is indicative of the change in dependence on maternal stores to active nutrient use and production (Xie et al., 2020).

Mitochondrial Complex I: Structure and Mechanism

Mitochondrial Complex I (also called NADH: ubiquinone oxidoreductase, about 45 protein subunits in mammals) is the largest respiratory chain protein complex, which has a molecular mass of more than 1 MDa. The enzyme takes an L-shaped form whereby the peripheral arm is hydrophilic and sticks out to the mitochondrial matrix and the inner membrane arm is a hydrophobic one that sticks inside the inner mitochondrial membrane. This structure represents the two roles of Complex I; electron transfer between NADH and ubiquinone and the pumping of protons across the inner membrane (Parey et al., 2020).

The NADH binding site is found in the peripheral arm that has a chain of iron-sulfur clusters which form an electron transfer chain of about 90 Angstroms long between the NADH binding site and ubiquinone binding pocket. NADH oxidation takes place at one flavin mononucleotide (FMN) prosthetic group and transfers two electrons to a succession of seven iron-sulfur clusters referred to as N1a, N1b, N2, N3, N4, N5, and N6a. These groups promote long-range electron transfer between the protein, eventually reducing ubiquinone to ubiquinol on the interface between the peripheral and membrane arms (Guo et al., 2018).

The proton pumping complex is located in the membrane arm of Complex I, which has four antiporter-like subunits (ND2, ND4, ND5 and probably ND6) which translocation of protons in the matrix to the intermembrane space (Zhang & Li, 2019). There has been extended interest in the coupling mechanism between electron transfer and proton pumping, which has recently been the focus of structural and computational studies, which indicate a conformational-driven model. According to this model, conformational changes in the periphery arm driven by redox are passed on to the membrane arm by a set of structural components which cause proton translocation. On the oxidation of every NADH, Complex I moves four protons across the inner membrane, which amounts to about 40 percent of the total proton-motive force produced by the respiratory chain (Djurabekova et al., 2024).

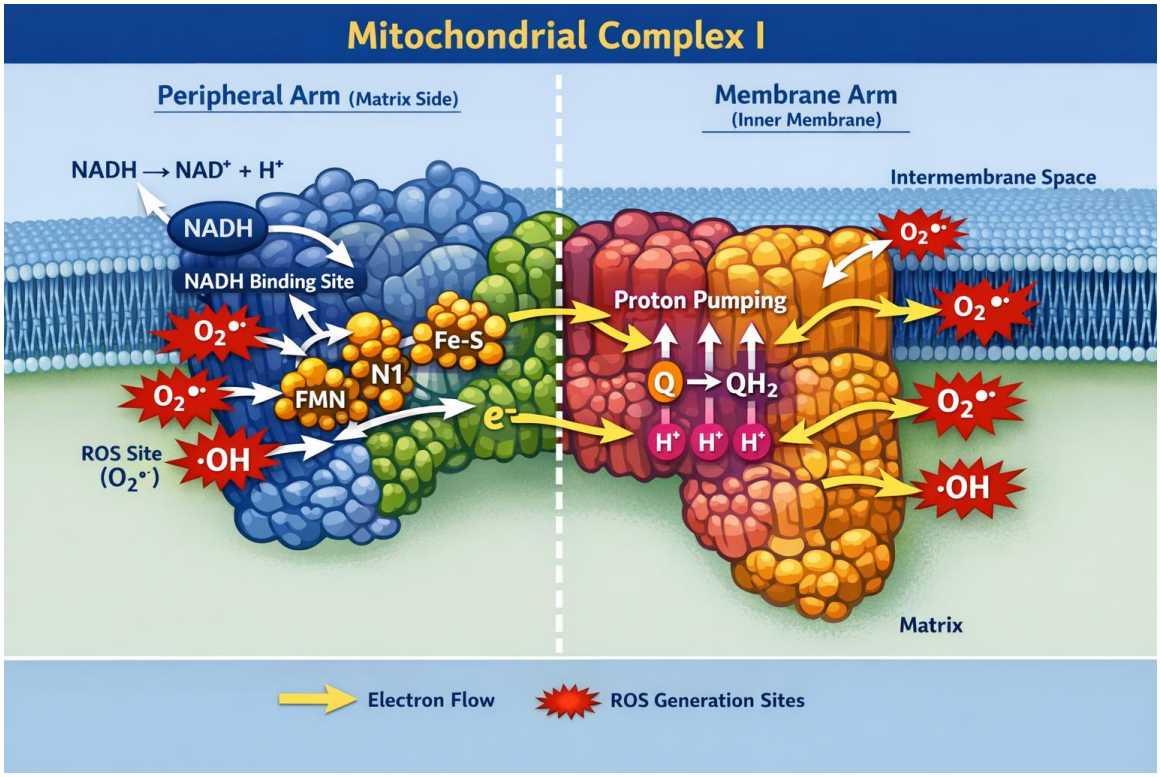

Figure 1: Mitochondrial Complex I structure showing the peripheral arm (matrix side) with NADH binding site and Fe-S clusters, and the membrane arm with proton pumping machinery. ROS generation sites and electron flow pathways are indicated.

Figure 1 shows the composition and the role of Mitochondrial Complex I, which is the important enzyme of the electron transport chain. The NADH binding site and a set of flavin mononucleotide (FMN) and iron-sulfur (Fe-S) groups are present in the peripheral arm located on the matrix side and this transfers electrons obtained by NADH. These electrons proceed to the ubiquinone (Q) in the complex to form QH 2 and it is this flow of electrons that triggers the membrane arm to pump over protons (H +) across the inner mitochondrial membrane into the intermembrane space creating the proton gradient that drives the production of ATP. It also shows the location of reactive oxygen species (ROS) generation sites where electron leakage may result in the production of superoxide and hydroxyl radicals suggesting possible sources of oxidative stress in mitochondria.

Complex I as a Source of Reactive Oxygen Species

Besides aiding in energy transduction, Complex I is also an important source of the reactive oxygen species (ROS) in mitochondria. The generation of ROS by Complex I is mainly attained by the partial reduction of molecular oxygen with electrons present on the flavin site or iron-sulfur clusters to generate superoxide anion (O2•-). The rate of ROS generation is affected by various factors, such as the reduction state of NAD pool, the proton-motive force and concentration of ubiquinone (Hadrava Vanova et al., 2020). When the ratio of NADH and NAD+ is high and the membrane potential is high, the resident time of electrons at Complex I is high, which increases the chances of leaking electrons to oxygen (Mailloux, 2020).

Complex I has the capability to produce ROS by forward and reverse electron transfer (RET). The forward electron transfer production of ROS is involved in the normal production of NADH oxidation, whereas the Ret is a backwards electron transfer involving the transfer of electrons between ubiquinol to Complex I normally found under conditions of high succinate and high membrane potential. RET-mediated ROS production may be significantly greater than forward electron transfer, and as such is a very significant factor to consider in some metabolic conditions. Complex I

produces superoxide, which is mostly released into the mitochondrial matrix, which is quickly changed into hydrogen peroxide by the manganese superoxide dismutase (MnSOD) (Onukwufor et al., 2019).

Though overproduction of ROS may cause oxidative damage, a regulated production of ROS carries significant signaling roles. Mitochondrial ROS has the potential to oxidative modulate cysteine residues of target proteins, change the transcription factor activity, and alter different signaling pathways. ROS generated by Complex I in embryonic development have been found to play a role in cell fate choice, cell differentiation and developmental transition timing (Dubouchaud et al., 2018). The two-sided features of ROS as harmful species and signaling molecules require a high regulation of their production and consumption during early embryogenesis.

Embryonic Genome Activation: Molecular Mechanisms and Regulation

The Maternal-to-Zygotic Transition

One of the most extreme cellular reprogramming processes in biology is the maternal-to-zygotic transition (MZT), during which the developmental control of the embryo is transferred to the new embryonic genome. After fertilization, early embryonic development is initially thematically dependent on mRNAs, proteins and other factors stored in the oocyte during oogenesis. This maternal program promotes initial few cell divisions and in the course of this process, the embryonic genome is largely transcriptionally silent. Embryonic genome activation (EGA) or zygotic genome activation (ZGA) is the species-specific activation of the embryonic genome, which takes place at the 1-2 cell stage in mice, 4-8 cell stage in humans, and at the mid-blastula transition in zebrafish and Xenopus (Kojima et al., 2025).

EGA is divided into two stages: a minor wave of genome activation, which starts just after fertilization and results in the transcription of a small number of genes, and a major wave that leads to the large-scale transcriptional activation of embryonic genome. The small wave generally entails activation of genes that play a role in chromatin remodeling, control of cell cycle as well as preparation of major EGA. The great wave is a huge transcriptional upsurge, where thousands of genes are transcriptionally active during a developmental range. This biphasic pattern of activation seems to be conserved in a variety of species, implying that there are some basic biological principles of genome activation (Chen et al., 2023).

Along with the zygotic genome activation is the degradation of maternal transcripts, also known as maternal RNA clearance. This clearance is mediated in several ways such as microRNA-mediated silencing, decay regulated by deadenylation, and special RNA-binding proteins targeting the destruction of maternal mRNAs. A combination of zygotic genome activation and maternal transcripts eradication promotes a seamless change in developmental regulation and eradication of possible contradictions between maternal and zygotic programs (Brantley & Di Talia, 2024). The developmental arrest of either EGA or maternal RNA clearance may result in failures of the transition, which underscores the importance of the transition.

Chromatin Remodeling and Epigenetic Reprogramming

A large-scale chromatin remodeling is needed to activate the embryonic genome, where highly condensed, transcriptionally repressive gametophyte-inherited chromatin is converted into a more open and permissible transcriptionally active state. Such restructuring encompasses remodeling across several scales of chromatin organization, including histone modification, nucleosome placement to more complicated chromatin structure. After fertilization, the paternal genome is subjected to a rapid protamine-histone exchange, but the paternal genome, which is already histone-organized, is subjected to massive remodelling of histone transitions (Liang & Wan, 2026).

Histone acetylation is one of the most dynamic alterations in early development and global alterations in acetylation patterns take place within hours of fertilization. Transcriptional activation is connected to histone acetylation by several different mechanisms: acetylation neutralizes the positive charge of lysine residues leading to reduced histone-DNA associations and increased chromatin accessibility; acetylation binding sites on the surface of bromodomain-binding proteins stimulates transcription; and other repressive marks are antagonized by acetylation (Hussain et al., 2025). The elevated histone acetylation levels seen in EGA are a product of the concerted efforts of the histone acetyltransferases (HATs) and the decreased activity of the histone deacetylases (HDACs) (Cui et al., 2025).

The patterns of DNA methylation are also subject to extensive remodelling during early embryonic development. Global DNA demethylation in mammals in the preimplantation embryo removes most of the methylation that was deposited during gametogenesis. This is demethylated by a combination of passive dilution during replication of the target DNA and by active processes involving the TET (teneleven translocation) family of dioxylases. TET oxidizes 5-methylcytosine into 5-hydroxymethylcytosine and oxidized forms thereby helping to demethylate DNA (Peng et al., 2023). The re-programming of the DNA methylation marks is essential in inducing developmental competence and enabling the embryonic genome to react to developmental signals.

ATP-dependent enzymes involved in chromatin remodeling complexes are important in creating chromatin accessibility during the EGA process. Embryonic genome activation has been implicated in several remodeler families such as SWI/SNF, ISWI and CHD complexes. Such complexes are able to slide, eject or reorganize the nucleosomes forming the accessible chromatin landscape required to bind transcription factor and recruit RNA polymerase II. Temporal regulation of chromatin remodeling activity with transcriptional activation indicates complex regulatory components involve the combination of multiple signals to guarantee appropriate developmental timing (Cipriano et al., 2024).

Transcription Factors and Pioneer Factors in EGA

Embryonic genome activation is coordinated by a particular group of transcription factors, most of them being maternally inherited or early zygotic genes. The pioneer transcription factors are among these and have particularly significant roles since they are capable of binding the target sites of condensed chromatin initiating local chromatin remodeling (Asami & Perry, 2025). Pioneer factors are able to bind their binding sites to the nucleosomal DNA, recruit chromatin remodeling complex and create accessible chromatin domains, which in turn permit binding of other transcription factors (Vendrell et al., 2025).

Multiple transcription factors have been found to play an essential role in EGA in mammalian embryos, among them, OCT4 (also termed POU5F1), SOX2 and NANOG, which constitute the main pluripotency network. Although these factors are familiar with the preservation of pluripotency at the subsequent stages of development, they also have a role in the creation of transcriptional competence during the early genome activation. Moreover, species-specific aspects cause genome activation: in mice, DUX (double homeobox) has become a strong suppressor of minor EGA, triggering a transcriptional program of the 2-cell stage. Like its human ortholog, DUX4 also promotes the transcription of cleavage-stage selective genes (Facioli, 2025).

TEAD4 (TEA domain transcription factor 4) is another key player in early mammalian development as it is necessary to define the initial cell lineage specification event giving rise to trophectoderm and inner cell mass (Yang et al., 2024). TEAD4 is a regulated activity controlled by the components of the Hippo signaling pathway and reacts to cell position and contact cues. Developmental

transcription factor networks interact with signaling pathways to enable embryos to organize the synchronized production of transcription with cell-cell communication and positional inputs, generating adequate developmental patterning(Latham, 2024).

Metabolic-Epigenetic Coupling: NAD+ and Chromatin Modifications

NAD+-Dependent Enzymes: Sirtuins and PARPs

The relationship between epigenetic regulation and cellular metabolism can be best exemplified by the NAD+ regulated enzymes that actively modify the chromatin and control gene expression. Sirtuins, which is a conserved family of NAD+-dependent protein deacetylases, is an important mechanistic intermediate between the cellular level of NAD+/NADH ratio and histone acetylation state. Mammals have seven members of the sirtuin family (SIRT1-7), which are localized in various cell compartments: SIRT1, SIRT6 and SIRT7 are located in the nucleus; SIRT3, SIRT4 and SIRT5 mainly in the mitochondrion; SIRT2 is mainly in the cytoplasm. All sirtuins activate deacetylation of lysine residues requiring NAD + at the expense of NAD + and generating nicotinamide, O-acetyl-ADP-ribose, and the deacetylated product (Z. Wang et al., 2018).

The sensitivity of sirtuins to cellular metabolic state is exquisitely sensitive to the dependence of the activity of these proteins on NAD + as a cofactor. Since most sirtuins have a physiological range ranging of NAD+ concentrations in their Km, alterations in the NAD+/NADH ratio have a direct effect on the activity of sirtuins. Under nutrient-rich conditions, when the ratio of NAD+/NADH is low, sirtuin activity is suppressed, and more histone acetylation and permissive chromatin conditions occur. In contrast, when energy becomes depleted or oxidized away through oxidative metabolism, the high ratios of NAD+/NADH will promote the activity of the sirtuin, which in turn will activate histone deacetylation (Gao et al., 2024). This energetic-dependent transcriptional adjustment is made possible through this metabolic coupling, in which cells in response to energetic status alter their transcriptional programs.

The most studied nuclear sirtuin, SIRT1, deacetylates histone H3 lysine 9 (H3K9) and histone H3 lysine 14 (H3K14) and histone H4 lysine 16 (H4K16), which are modifications that tend to result in active transcription. Other than histones, SIRT1 also deacetylates many other transcription factors and regulatory proteins, such as p53, FOXO family members, PGC-1 2, and NF- -B, and has been shown to affect a variety of cell processes such as metabolism, resistance to stress, and inflammation (Baran et al., 2023). SIRT1 has also been involved in maintaining the self-renewal and differentiation balance in early development and appropriate SIRT1 activity is needed to support normal embryonic development (Y. Wang et al., 2018).

The other large group of NAD+ depleting enzymes and their far-reaching impact on the chromatin structure and gene expression are poly (ADP-ribose) polymerases (PARPS). There are 17 members of the PARP family in humans and the most common and well characterized ones are PARP1 and PARP2. These enzymes participate in the transfer of units of ADP-ribose of NAD+ to protein targets and generate poly(ADP-ribose) chains which may significantly change protein activity and protein-protein interactions. Specifically, PARP1 is involved in the process of DNA damage repair, chromatin remodelling and transcriptional regulation in critical ways. PARP1 is activated on binding to breaks in strands of DNA, and is massively self-modified and surrounding proteins with poly (ADP-ribose), attracting the DNA repair machinery, and affecting the local chromatin structure(Tan & Doig, 2021).

The excessively high level of NAD+ usage linked to PARP activation can significantly affect cellular NAD +NADH ratios and, as such, respond to sirtuin function, a type of metabolic cross-talk among NAD+ -dependent enzymes. Under widespread activity of DNA damage or hyperactivity of the PARP, NAD+ levels can drop to destabilize the bioenergetics status of cells and activity of sirtuins. During

early embryogenesis when both the repair of DNA and chromatin remodeling are highly active the equilibrium between PARP and NAD+ may be of particular importance to preserving the correct epigenetic states and developmental fidelity(Groslambert et al., 2021).

Impact of NAD+/NADH Ratio on Histone Modifications

The NAD+/NADH ratio has a significant effect on the topography of histone modification by direct and indirect action. As explained, sirtuins have a direct connection between NAD+ and histone deacetylation whereby their functions are regulated by alterations of NAD+/NADH ratio. But cellular redox state has a broader effect on histone modifications than the activity of sirtuins, and includes the activation of histone acetyltransferases (HATs) and the accessibility of acetyl-CoA, the acetyl-histone donor molecule(Berthiaume et al., 2019).

All histone acetyltransferases require acetyl-CoA as the obligate substrate and histone acetylation directly depends on its nuclear/cytoplasmic concentration. Acetyl-CoA production is closely connected with cell metabolism as it is produced in several pathways such as ATP-citrate lyase (ACLY) in the cytoplasm and the nucleus which breaks down citrate transported by mitochondria, or pyruvate dehydrogenase in mitochondria. The presence of these enzymes is dependent on the redox condition of the cell, and NAD+/NADH ratio determines the availability of a substrate and the regulation of the enzyme. One example is that high glycolytic flux can increase cytoplasmic pyruvate and acetyl-CoA concentration, and oxidative metabolism can affect the phenomenon of citrate export out of mitochondria (Yuan et al., 2020).

It has been shown through recent research that remarkable spatial and temporal heterogeneity in histone acetylation happens during early embryonic development and is correlated with metabolic state changes. The process of a switch between the pyruvate and lactate oxidation and the consumption and biosynthesis of glucose is accompanied by the changes in the histone acetylation patterns. Such metabolically-mediated changes in chromatin acetylation have the capacity to affect the timing and magnitude of genome activation, which connects developmental progression to metabolic development (Bozdemir & Uysal, 2023). The idea of epigenome metabolic instruction implies developmental transitions are not passively accompanied by an evolutionary change of central metabolism but might contribute to developmental transitions occurring through epigenetic changes(Sivanand et al., 2018).

In addition to acetylation, the NAD+/NADH ratio has a role in influencing other histone changes in a number of ways. As an example, histone methylation requires S-adenosylmethionine (SAM) as a methyl donor, and SAM metabolism is linked with the NAD+ metabolism by one-carbon metabolism. Also, the cellular redox state affects flavin adenine dinucleotide (FAD)-dependent histone demethylases LSD1 and LSD2, as well as the activity of the Jumonji C domain-binding (JMJC) family of histone demethylases, which use 02-ketoglutarate as a cofactor. Generation of α-ketoglutarate during the TCA cycle is strictly interrelated with the generation of NADH which introduces one more bridging between the metabolism in mitochondria and changes in the chromatin(Pladevall-Morera & Zylicz, 2022).

Figure 2: NAD⁺/NADH in Cellular Metabolism and Epigenetic Regulation

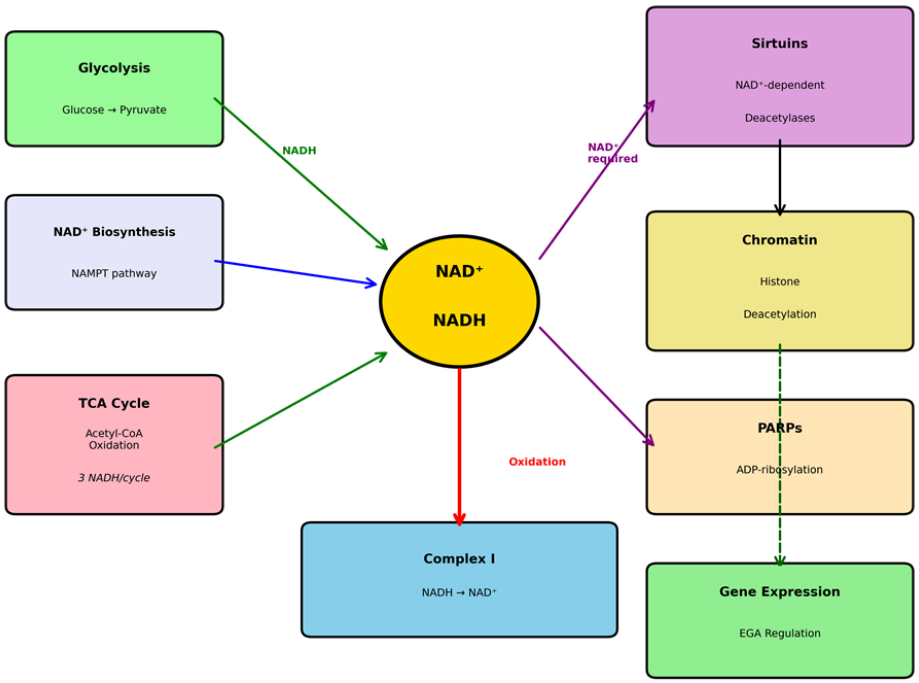

Figure 2: Integration of NAD⁺/NADH metabolism with cellular processes. NADH is generated through glycolysis, TCA cycle, and other pathways, then oxidized by Complex I. NAD⁺ serves as a cofactor for sirtuins and PARPs, linking metabolism to chromatin regulation and gene expression.

DNA Methylation and Redox-Sensitive Enzymes

DNA methylation, the addition of methyl groups to cytosine bases (in the CpG dinucleotide in mammals), is one of the most basic epigenetic marks which play key roles in gene regulation, genomic imprinting and genome stability. The cellular metabolism regulates the activity of the relevant enzymes that create and do away with DNA methylation, providing further connections between redox status and epigenetic control. DNA methyltransferases (DNMTs) make use of S-adenosylmethionine as a methyl donor, linking the process of DNA methylation to one-carbon metabolism and, indirectly, to NAD + metabolism via a pathway known as the methionine cycle as well as the folate metabolism(Sundar et al., 2022).

Active DNA demethylation is caused by the family of DNA dioxygenases known as TET, with especially close ties to the metabolism and redox potential of cells. TET enzymes promote the sequential oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) which undergo DNA demethylation. This catalytic activity needs the presence of α-ketoglutarate as a cofactor and Fe(II) as a cofactor and thus TET enzymes are susceptible to the presence of TCA cycle intermediates and iron position in the cells. Furthermore, oxygen and the redox state of the cell may affect TET activity since the iron center requires being in the reduced Fe(II) form in order to catalyze a reaction (Li et al., 2025).

In early embryonic development TET -mediated DNA demethylation is important in epigenetic reprogramming. After the fertilization, the paternal genome completes active demethylation led by TET3 whilst the maternal genome completes passive, replication-dependent demethylation. Such difference in the kinetics of these parental genomes demethylation must be tightly controlled in terms of TET3 activity and localization. Considering that TETs require the presence of α-ketoglutarate that is

produced in the TCA pathway with NADH, modifications to mitochondrial metabolism and the level of NAD+/NADH ratio may potentially affect the rate and degree of DNA demethylation in the initial stages of preimplantation development (Jakubek et al., 2023).

The fact that both DNA methylation and demethylation machinery rely on metabolites that are closely related to cellular energy metabolism indicates that metabolic influencing may have long-term consequences on epigenetic programming. In fact, experiments on multiple systems have shown that metabolite availability during critical developmental periods can result in long-term changes in the DNA methylation state, and this change may cause long-term effects on gene expression and phenotype (FC Lopes, 2020; Thakur & Chen, 2019). Such metabolic-epigenetic coupling is of significance to developmental programming and offspring well-being in the context of assisted reproductive technologies and in vitro embryo culture, in which the metabolic conditions could not be comparable to those in vivo(Yu & Li, 2024).

Mitochondrial Dynamics during Early Embryogenesis

Mitochondrial Transitions from Oocyte to Blastocyst

The drastic changes that mitochondria undergo in the change of oocyte to blastocyst reveal the changing metabolic needs at the beginning stages of development. The number of mitochondria in mature oocytes is in the range of 100,000 to 200,000, which is far higher than that of normal somatic cells. These mitochondria however exist in a relatively dormant state, that is, of low membrane potential, low activity of oxidative phosphorylation, and low production of ATP by the electron transport chain. Rather, oocytes use glycolysis and oxidation of pyruvate, lactate and amino acids mainly to generate energy(Kirillova et al., 2021). This metabolic arrangement assists in allotropic production of ROS and the developmental competence of the oocyte throughout the lengthened interval of meiotic arrest.

After fertilization, the mitochondria slowly switch this dormant position to that of energetic organelles. In this reactivation, there are enhancements in membrane potentials, the activity of the electron transport chain, and the rise in the level of ATP. This mitochondrial activation is timed in close relationship with the great wave of embryonic genome activation, and indicates that there is coordinated regulation of metabolic and transcriptional programs. Mitochondrial membrane potential rises significantly in mouse embryos between the 2-cell and 4-cell stages which are associated with significant EGA. In the same case, in human embryos, mitochondrial activation is found during the 4-8 cell stage, once again, with significant genome activation (Hashimoto et al., 2017).

During preimplantation, there is also a dramatic change in morphology of mitochondria. Mitochondria in oocytes and during the early cleavage-phase of the embryo tend to have a spherical shape (poorly formed cristae) and are, therefore, less able to handle oxidative stress. With further development, mitochondria acquire larger cristae that represent increased oxidative phosphorylation in mitochondria. At the blastocyst level, the mitochondria in the cells of the inner cell mass and trophectoderm have developed well-developed cristae and is more elongated like active metabolic cells. These morphological modifications accompany the functional differentiation of mitochondria and their progressive role in energy generation by cells (Santos, 2022).

Another important factor in mitochondrial dynamics in early development is the replication of mitochondrial DNA (mtDNA). In contrast to nuclear DNA, which is rapidly replicated in every cell division of early embryogenesis, the development of preimplantation suppresses the replication of the mitochondrial DNA to a great extent. This suppression causes successive dilution of copy number of mitochondrial DNA during embryo division so that the number of mitochondria in individual cells in the

blastocyst stage are significantly lower than the number of mitochondria in the oocyte. The biological importance of this repression of replication of the mitochondrial genome is not fully comprehended, but can be associated with the necessity to eradicate the presence of mutant copies of the mitochondrial genome by a bottleneck effect, or to keep the metabolism of the cell in the quiescence phase until the proper developmental stages(Coticchio et al., 2025).

Regulation of Mitochondrial Metabolism During EGA

Embryonic genome activation is associated with a metabolic shift that entails significant shifts in the nutrient uptake and energy generation pathways. At early cleavage-stage levels of embryogenesis, a high glycolytic activity despite the availability of oxygen termed the Warburg-like metabolism, is observed in early embryonic cells, as is observed in cancer cells. This metabolic arrangement promotes the rapid biosynthetic needs of cell division as well as could be one of the ways of reducing oxidative stress. Nevertheless, embryos near, and once they experience significant EGA, their metabolic change to further oxidative metabolism ensues, increasing glucose oxidation in the TCA cycle and electron transport chain(Savy et al., 2025).

There are several mechanisms that control this metabolic transition and they are at transcriptional, post-transcriptional and post-translational level. Gene expression of mitochondrial proteins augments significantly during EGA, accompanied by co-ordinated augmentation of elements of the electron transport chain, enzymes of the TCA cycle and mitochondrial ribosomal proteins. Part of the mechanism of this transcriptional program is facilitated by the peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 2) and associated transcriptional coactivators which drive mitochondrial biogenesis and oxidative metabolism (Bai et al., 2025). The nuclear respiratory factors (NRF1 and NRF2) are important transcription factors that activate nuclear genes that encode mitochondrial proteins.

The post-translational modification especially the phosphorylation has significant roles in the process of mitochondrial metabolic enzyme regulation during early development. Reversible phosphorylation regulates the pyruvate dehydrogenase complex that connects glycolysis with the TCA cycle, where the phosphorylation of the complex suppresses it and the dephosphorylating of the complex activates it. In early stages of development, alterations in the balance between the activity of PDK and PDP help to metabolic shift towards greater glucose oxidation. Equally, the electron transport chain complexes also undergo various post-translational modifications to regulate their functions in response to the metabolism and signaling signals(Karwi et al., 2019).

Mitochondrial activation and genome activation could be coordinated, implying that the metabolic condition can be a checkpoint to development. In fact, blocking or delaying normal EGA by experimental perturbation of mitochondrial activity suggests that defective mitochondrial metabolism is incompatible with this developmental transition. On the other hand, the activation of oxidative metabolism at an early stage of cleavage embryos may result in impairment of developmental competence, implying that the temporal transition of metabolism should be strictly regulated(Jellusova, 2020). These findings confirm the idea of metabolic gates or checkpoints that also make sure that the developmental transitions are made only when the relevant metabolic conditions are reached.

Table 1: Metabolic Characteristics During Preimplantation Development

Developmental Stage	Primary Metabolic Pathways	Mitochondrial Characteristics
Oocyte / 1-Cell	Pyruvate oxidation, Glycolysis, Lactate	Quiescent, Low membrane potential, Spherical

	oxidation	morphology
2-Cell (mouse) / Early Cleavage	High glycolysis, Beginning oxidative metabolism	Increasing membrane potential, Developing cristae
4-8 Cell / Morula	Enhanced glucose oxidation, Active TCA cycle	Active OXPHOS, Well-developed cristae, Increased ATP production
Blastocyst	Oxidative phosphorylation dominant, Biosynthetic pathways	High membrane potential, Elongated morphology, Maximal respiratory capacity

OXPHOS = oxidative phosphorylation; TCA = tricarboxylic acid

Table 1 summarizes changes which take place in preimplantation development in metabolism and mitochondria. During the oocyte and 1-cell stage, the central energy source is primarily pyruvate, glycolysis, and lactate oxidation and the membrane potential and spherical shape of mitochondria are relatively low. In early cleavage or 2-cell phase, there is increased activation of glycolysis and onset of oxidative metabolism together with an increase in mitochondrial membrane potential and initial cristae formation. Metabolism changes to a basal glucose oxidation rate, active TCA cycle, and active oxidative phosphorylation (OXPHOS), cristae are well-formed, and ATP production is raised at the 48 cell and morula stages. At the blastocyst level, oxidative phosphorylation prevails, facilitating rapid growth and biosynthesis and the mitochondria are highly membrane potential, long-shaped and have a maximum respiratory ability, which is a sign of complete metabolic maturation.

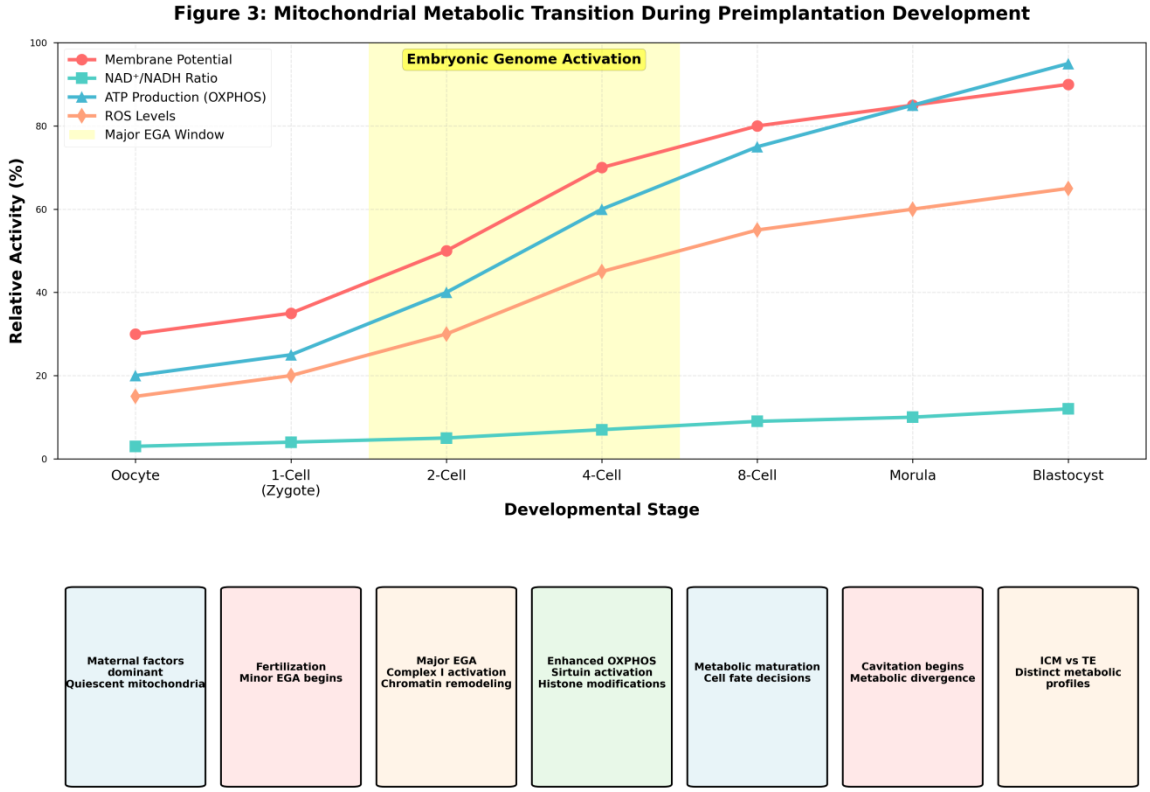

Figure 3: Temporal changes in mitochondrial parameters during preimplantation development. Mitochondrial membrane potential, NAD^+/NADH ratio, ATP production, and ROS levels all increase progressively, with major transitions occurring during the embryonic genome activation window.

The Figure 3, display the metabolic shift of mitochondria in the development of preimplantation embryos, which reveal an increase in mitochondrial activity, that goes on progressively between the oocyte stage and the blastocyst stage. Early phases depend primarily on maternal low-metabolism mitochondria, but in the embryonic activation of the genome (EGA), as noted in the central shaded area, membrane potential, oxidative phosphorylation (OXPHOS) ATP generation, and NAD + /NADH ratio are significantly increased, indicating an increase in mitochondrial activity. Meanwhile, ROS levels also rise with time, which means that there is increased metabolic activity. The bottom panel is a summary of the important developmental milestones, such as fertilization, genome activation, chromatin remodeling, metabolic differentiation, and the ultimate differentiation of inner cell mass (ICM) and trophectoderm (TE) with divergent metabolic niches. On balance, the figure indicates a progressive increase in the activity and specialization of the mitochondrial activity in the development of the embryo.

Complex I Function in Embryonic Development

Complex I Activity and Developmental Competence

A role of Mitochondrial Complex I in the development of embryos has turned into a crucial factor of embryonic developmental competence with several areas of evidence showing that appropriate Complex I activity is the key to successful preimplantation development. Experiments testing embryos with impaired Complex I activity, either by genetic mutation, by pharmacological inhibitors, or by environmental stressors, all show developmental defects such as slowed genome activation, poor blastocyst development and impaired viability (Liu et al., 2024). These observations not only define Complex I as a passive source of energy-giving enzyme but also an active part of developmental control.

The need of the Complex I activity seems to be especially sharp at the time of embryonic genome activation. Early embryos treated with rotenone, which is a selective Complex I inhibitor, develop on the arrests at major EGA or earlier, unable to activate the embryonic genome normally by transcription (Ibarra-Gutiérrez et al., 2023). In a similar manner, embryos with mutations in Complex I subunits tend to have delayed or slow activation of their genome, and have some corresponding defects in expression of early embryonic genes. Such results indicate that the activity of Complex I is an important signal or a metabolic condition needed by the transcriptional machinery to trigger the transcription of the genome (Heinz et al., 2017).

Complex I and its effects on developmental competence are multidimensional. To begin with, Complex I activity dictates the speed of NADH oxidation and, in turn, the cellular NAD+/ NADH ratio. This ratio, as mentioned above, has far-reaching consequences on the activity of sirtuins and histone acetylation that directly affect the structure of the chromatin and transcriptional potential. Second, Complex I activity has an impact on membrane potential and ATP generation in the mitochondria, which has an effect on the cellular energy status and activity of ATP-dependent chromatin remodeling complexes. Third, generation of Complex I-mediated ROS can have signaling roles which can modulate transcription factor action and development (Xiong et al., 2012; Zhang & Xu, 2025).

Interestingly, an excesses of insufficient Complex I activity can impair development implying that there is some optimal range of Complex I functionality in relation to normal embryogenesis. Excessive ROS generation and oxidative stress, potentially causing cellular macromolecular damage and impaired development, can be caused by hyperactivity of Complex I, e.g., due to excessive availability of substrates or loss of other regulating factors (Paloviita et al., 2026). On the other hand, lack of adequate Complex I activity helps to hinder energy production as well as it might not allow formation of the right ratios of NAD+/NADH. This demand of a balanced Complex I

activity indicates the accuracy of the regulation of the metabolism of mitochondria in early development.

ROS Signaling and Cell Fate Decisions

The reactive oxygen species produced by mitochondrial Complex I play both potentially harmful oxidant roles and a potent role as intermediate signalers of cell fate choice in embryonic development. Signaling roles of ROS have been more and more appreciated over the past years, and regulated ROS generation is now known to control a wide range of cellular activities such as proliferation, differentiation and cell death. There should be a balance in the level of ROS during early embryogenesis that is neither too high nor too low because too high will lead to oxidative damage and too low cannot support the required signaling functions (Khacho & Slack, 2018).

Gene expression can be affected in a multitude of ways by complex I-derived ROS. The product of the superoxide dismutation process, hydrogen peroxide (H_2O_2), may also enter membranes and oxidatively react with cysteine residues on transcription factors to alter their DNA-binding or protein-protein interactions. As an illustration, numerous redox-sensitive transcription factors such as NRF2, NF- -B, AP-1, all have critical cysteine residues, the oxidation state of which affects their transcriptional activity. On early development, the expression of the genes that are related to antioxidant defense, metabolism, and specifying cell fate may be affected due to ROS-mediated control of these factors(Brillo et al., 2021).

The first cell fate choice during mammalian development is also affected by ROS which is the segregation of inner cell mass (ICM) versus the trophectoderm (TE) lineages. It has been found out that cells of trophectoderm lineage have a higher level of ROS, which is associated with their more oxidative metabolic character, than ICM cells. The contribution of this ROS differential to lineage specification can be due to effects on transcription factors and signaling pathways. It was demonstrated that the Hippo signal pathway that is an important aspect of ICM/TE specification by regulating TEAD transcription factors is affected by cellular redox state, suggesting a possible pathway by which Complex I-derived ROS affects cell fate determination(Venditti & Di Meo, 2020).

The production of ROS at early stages of development is highly controlled in nature and there are certain time-dependent surges of ROS at particular developmental stages. An example is that a burst of ROS generation has been described during the period of genome activation in a number of species, implying that this oxidative signal could be involved in the initiation or promotion of transcriptional activation. Not only mitochondrial Complex I, but also NADPH oxidases and other systems of ROS formation are the sources of this developmental ROS burst. However, mitochondrial ROS especially that of Complex I pose a quantitatively important fraction of total cellular ROS and are placed at an advantage to combine developmental signaling with metabolic status (Chakrabarty & Chandel, 2021).

The antioxidant systems have important roles in the regulation of ROS and in the prevention of oxidative damage of embryos. There is also the maintenance of the ROS homeostasis by superoxide dismutases (SOD1 and SOD2), catalase, glutathione peroxidases and thioredoxin systems. These antioxidant enzymes themselves are developmentally regulated and alterations in antioxidant capacity are correlated with alterations in mitochondrial ROS generation (Lin & Wang, 2021). The normal ROS dynamics of early development stage can be perturbed by genetic manipulation of antioxidant systems or environmental stress, and disrupt the normal embryonic viability, establishing the relevance of each system in preserving the normal oxidative homeostasis (Deluao et al., 2022).

Integration of Metabolic and Transcriptional Programs

Metabolic Checkpoints in Development

Metabolic checkpoints have become a concept that has provided a unifying theory on the role of cellular metabolism in developmental progression. Metabolic checkpoint may be described as a developmental transition which falls into the achievement of a given metabolic state and blockage or delay of the transition is seen when proper metabolic condition has not been attained. This understanding places metabolism as a proactive guardian of the developmental transitions regulating how and when a developmental transition occurs and which developmental transitions are fidelitous (Wei et al., 2025). During early embryogenesis there seem to be several metabolic checkpoints that allow adequate supplies of energetic and biosynthetic resources to be met before transitioning to the next developmental stage.

The embryonic genome activation transition can be considered a vivid illustration of a metabolic checkpoint. All normal genome activation necessitates adequate mitochondrial function, the right NAD+/NADH ratios, and sufficient generation of energy as discussed in this review. Disturbance of these metabolic parameters experimentally slows down or inhibits EGA and reinstatement of correct metabolic parameters can save development. These molecular processes of sensing of metabolic state and transduced information to transcriptional machinery in this are NAD + - dependent enzymes, energy-sensing kinases, including AMPK, and nutrient-responsive pathways, including mTOR (Zhao et al., 2021).

AMPK (AMP-activated protein kinase) is the central metabolic sensor which reacts to changes in the cellular AMP/ATP ratio. When the energy stress conditions occur, AMPK is activated and phosphorylates many of its downstream targets to reduce energy homeostasis, such as the activation of catabolic and suppression of anabolic pathways. AMPK activity in early embryos should be tightly controlled to avoid the improper activation that may cause the inhibition of the biosynthetic mechanisms needed to support the rapid division of cells. On the contrary, total lack of AMPK activity impairs the embryo to react to energetic stress (Schulz & Harrison, 2019). Another connection between energy status and gene expression is direct regulation of chromatin modifications by phosphorylation of histone modifying enzymes and transcriptional coactivators by AMPK.

The other important mechanisms of checkpoint in metabolism is the mTOR (mechanistic target of rapamycin) pathway, which is a system of two complexes: mTORC1 and mTORC2, the mTORC1 is very sensitive to nutrient availability and energy status. In embryonic development, the mTOR activity has to be properly balanced to promote the fast biosynthesis needed to divide the cell and prevent overuse of resources that might jeopardize subsequent embryonic development. The dysregulation of mTOR signaling during early embryos may lead to developmental defects, which is why it is increasingly important to pay attention to the correct nutrient sense and metabolic regulation(Hussein et al., 2020).

Metabolic Heterogeneity and Cell Fate

In the recent years, the resurgence of single-cell metabolomics and live imaging has shown that early embryos contain surprising metabolic heterogeneity, refuting developmental metabolic reprogramming. Mitochondrial membrane potential, NAD +/NADH ratios, ATP, and metabolic pathway usage may differ significantly among individual cells of the same embryo. This metabolic heterogeneity is associated with and could influence cell fate choices, indicating that metabolic condition is a determinant and a result of cell identity (Hu et al., 2019).

The initial lineage segregation during mammalian differentiation, which is the formation of inner cell mass and trophectoderm, is linked with unique metabolic profiles. Trophectoderm cells show the presence of more mitochondrial membrane potential, more oxidative phosphorylation, and more ROS than the inner cell mass

cells. These metabolic differences are developed in the morula phase and enhanced in the blastocyst. Interestingly, manipulation of cellular metabolism in experiments can affect lineage specification, where the enhancement of oxidative metabolism can support trophectoderm fate and the inhibition of oxidative metabolism can support inner cell mass fate (Ghosh-Choudhary et al., 2020).

The pathways between metabolic heterogeneity and cell fate specification are connected through a variety of pathways. Variations in NAD+ /NADH ratios among cells might result in a difference in sirtuin activity and hence different histone acetylation patterns, which may determine accessibility of lineage-specific genes. Alterations of the level of ROS are capable of differentially initiating redox-sensitive signal pathways, which influence the functioning of transcription factors and the expression of genes. Moreover, the metabolic heterogeneity affects the activity of the metabolite-sensing signaling pathways like mTOR and AMPK, which themselves control transcription factors being used in the determination of cell fate (Ly et al., 2020).

It is still not completely understood where the metabolic heterogeneity of early embryos originates but probably includes both stochastic and deterministic elements. Mitochondrial asymmetric segregation during cell division may also be one of the causes of metabolic variation in daughter cells. Metabolic heterogeneity can also be induced by positional effects, e.g. exposure to oxygen or paracrine pronouncements in cells adjacent. Moreover stochastic differences in gene expression especially of metabolic enzymes may be scaled up to large metabolic differences between cells (Stegen & Carmeliet, 2024). The nature and outcomes of metabolic heterogeneity is a major developmental biology frontier with potential implications on regenerative medicine and insights into the cell fate determination.

Implications for Assisted Reproductive Technologies

Culture Conditions and Metabolic Programming

These discoveries into mitochondrial metabolism and embryonic genome activation have significant implications on assisted reproductive technologies (ART), when embryos are prepared in vitro within defined conditions that can vary significantly to the in vivo setting. The embryonic metabolism can be dramatically altered by culture media composition, oxygen tension and other environmental influences which could have developmental competence, epigenetic programming and offspring health issues in the long term. The knowledge of the effect of culture conditions on the functionality of mitochondria and NADH redox metabolism gives possibilities to optimize ART procedures and enhance their results (Gomez Romero & Boyle, 2023; Stegen & Carmeliet, 2024).

Over the decades of ART practice, culture media formulations have been developed to a greater extent, and the contemporary media are tailored to accommodate particular developmental levels. Early cleavage-stage embryos are usually grown in media that has got pyruvate and lactate as primary energy sources, which is a replica of the composition of the oviduct fluid. The high glucose levels in blastocyst-stage culture media are to meet the high biosynthetic needs of the blastocyst-stage. Nevertheless, the exact levels of nutrients and the proportions among the various energy sources are capable of modulating the mitochondrial metabolism, the NAD+/NADH ratios, and, eventually, the genome activation and developmental course (Nimmakayala et al., 2021).

Another important parameter of culture that has a significant impact on mitochondrial metabolism is oxygen tension. Whereas atmospheric oxygen (~20% has long been employed in embryo culture, the amount of oxygen found in physiological levels in the female reproductive tract is considerably less (~5% or lower). This may lead to oxidative stress due to high oxygen culture, which may produce high levels of mitochondrial ROS and change developmental programming. In fact, many studies

have established a better embryo development and less oxidative stress in low oxygen culture temperature (Sylvester et al., 2025). Effects of oxygen on the functioning of Complex I and on the production of ROS seem to be of special significance, because the given complex is highly sensitive to oxygen supply and is a significant source of ROS.

The culture condition metabolic effects could further have long-term implications on the development of the epigenetic programming and not necessarily limited to immediate developmental outcomes. Animal model research has shown that the conditions of the embryo culture may change the DNA methylation profile, histone alterations, and gene expression, some of which may be maintained into adulthood and even passed on to new generations. With the links between mitochondrial metabolism, NAD+/NADH ratios and epigenetic alterations, as made in this review, it is reasonable to suspect that metabolic disturbances caused by culture may spread to permanent epigenetic alterations (Block & El-Osta, 2017). These metabolic-epigenetic connections can be applied to understand how culture can be utilized to reduce undesirable epigenetic changes.

Table 2: Impact of Culture Conditions on Mitochondrial Function and Embryo Development

Culture Parameter	Effects on Mitochondria	Impact on NAD+/NADH	Developmental Outcomes
High O_2 (20%)	Increased ROS, Enhanced OXPHOS	Elevated NAD^+/NADH ratio	Oxidative stress, Altered epigenetics
Low O_2 (5%)	Reduced ROS, Moderate OXPHOS	Balanced NAD^+/NADH	Improved development, Normal epigenetics
High Glucose	Substrate excess, Potential ROS increase	Variable, depends on utilization	Advanced metabolism, Possible glycation
Pyruvate/Lactate	Optimal substrate for early stages	Maintained ratio	Physiological development

OXPHOS = oxidative phosphorylation; ROS = reactive oxygen species

Table 2 draws attention to the way in which various conditions of embryo culture affect the mitochondrial activity and developmental results. Excessive oxygen (20%) enhances the mitochondrial oxidative phosphorylation (OXPHOS), but also, it increases the production of reactive oxygen species (ROS), which elevates the NAD/NADH ratio and could cause oxidative stress and epigenetic changes, which may harm development. Low oxygen conditions (5%), in contrast, mimic physiological conditions better, inhibit ROS production, stabilize the NAD 3+/NADH ratio, and promote healthier embryo development and normal epigenetic activity. High glucose levels may result in excess supply of substrates, which in some cases might result in ROS and redox imbalance and may result in a metabolic imbalance and glycation effects, but may initially give a false impression of an accelerated metabolism. In the meantime, pyruvate and lactate supplementation, which proteins like embryo favor during the early developmental stages, serves in the redox homeostasis and more physiological and stable development of embryos.

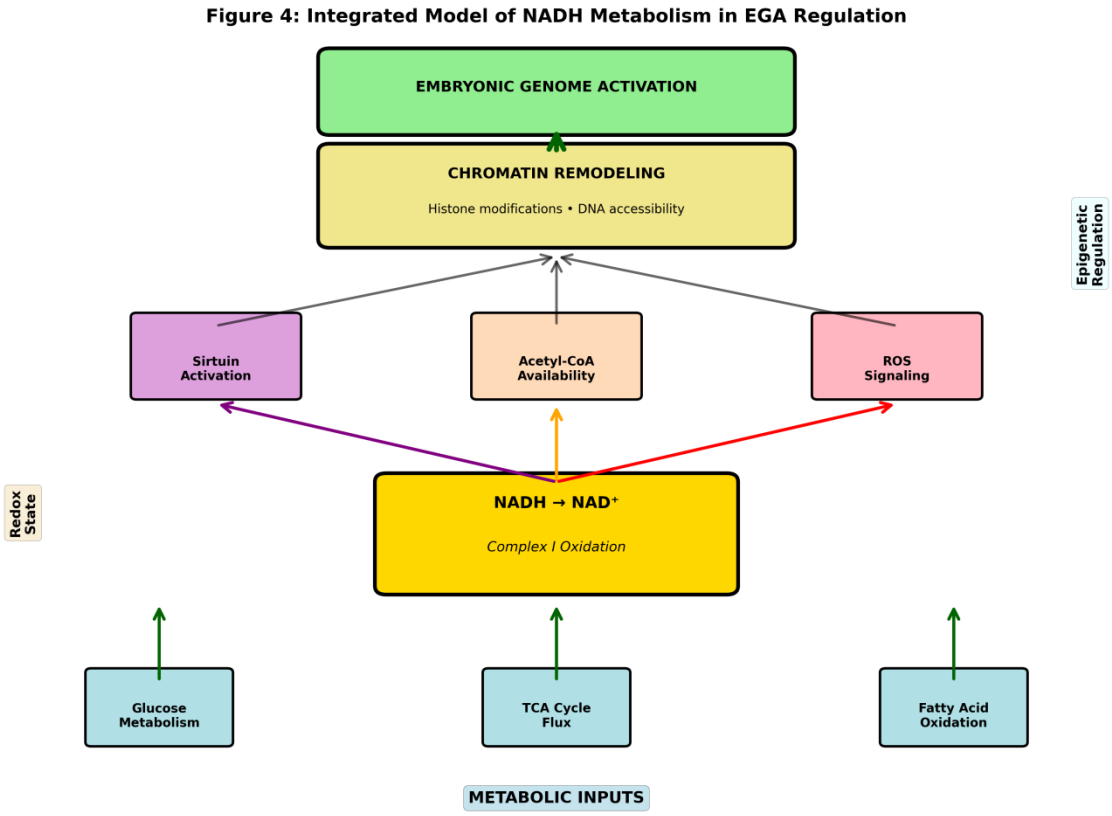

Figure 4: Integrated model of NADH redox metabolism in embryonic genome activation. Metabolic inputs generate NADH, which is oxidized by Complex I to NAD^+. This regulates sirtuins, acetyl-CoA availability, and ROS signaling, converging on chromatin remodeling.

Figure 4 depicts a combination of models which illustrate that NADH metabolism controls embryonic genome activation (EGA) by mitochondrial and epigenetic pathways. The metabolic processes including glucose metabolism, TCA cycle activation, and fatty acid oxidation produce NADH, which is oxidized to NAD + by mitochondrial Complex I, to define the cell redox status. This NAD + availability affects essential regulatory pathways, comprising of the activation of sirtuin, the availability of acetyl-CoA, and signaling of reactive oxygen species (ROS), which are all linked to chromatin remodeling by changing histones and opening up DNA. The resultant effects of these epigenetic modifications are the activation of the embryonic genome, which couples cellular metabolism to the direct influence of the genes and their early developmental establishment.

Non-Invasive Assessment of Embryo Metabolism

The identification of metabolism as one of the main determinants of the developmental competence has led to the desire to devise non-invasive techniques of assessing embryonic metabolic status. These methods might offer helpful data in the process of embryo selection in ART without limiting viability. The time-lapse imaging systems have been modified to measure metabolic parameters such as mitochondrial membrane potential by use of fluorescent probes, pyruvate consumption by analysing the media, and even to measure autoflourescence of NAD(P)H and flavins as indicators of the metabolic state (Gardner & Leese, 2026).

Non-invasive window into cell redox States NAD(P)H and FAD have intrinsic fluorescence, which does not require exogenous labels. The optical redox ratio is the NAD(P)H/FAD fluorescence ratio that is proportional to the cellular NAD+/NADH ratio and indicated metabolic activity. Even deeper information on the metabolism can

be provided by advanced imaging methods such as fluorescence lifetime imaging microscopy (FLIM) that is able to differentiate between free and protein-bound NAD(P)H. The use of these technologies on preimplantation embryos has demonstrated stage specific patterns of metabolic activity and has established metabolic identifiers of developmental competence (Motiei et al., 2020).

Another non-invasive method used to measure embryonic metabolism is in the form of a metabolomic profiling of the spent culture media. The researchers can deduce the metabolic activity and the use of metabolic pathways by quantifying the loss of nutrients and accumulation of the byproducts of metabolism in the media. The method used in studies has determined metabolic markers of successful implantation and live birth, which may have clinical implications. Nonetheless, there exist difficulties in normalization of such measurements in various systems of cultures and the identification of the most predictive metabolic parameters of developmental potential (Alizadeh Moghadam Masouleh et al., 2025).

The creation of artificial intelligence and machine learning strategies has a potential to incorporate a variety of metabolic and morphological parameters to determine the quality of embryos. In the future, non-invasive embryo selection criteria can possibly be created by training algorithms on large datasets that combine metabolic measurements, time-lapse imaging measurements, and clinical outcomes (Eldarov et al., 2022). These strategies may assist in selecting embryos with the best mitochondrial activity and metabolic phenotypes that may enhance the success rates of ARTs and also minimize the number of embryos that should be transferred.

Disease Implications and Therapeutic Opportunities

Mitochondrial Diseases and Developmental Disorders

Mitochondrial-based mutations, such as Complex I subunits, lead to serious cases of developmental disorders and premature embryonic death. The total incidence of mitochondrial diseases is about 1/5000 individuals, and the disease may be manifested either by isolated organ dysfunction or severe multi-system disorders. Numerous mutations that lead to mitochondrial diseases cannot be sustained in embryonic stages, which causes early termination of pregnancy or birth defects. The knowledge of the functions of mitochondrial metabolism in the activation of embryonic genome and development process sheds some light on the developmental implications of mitochondrial impairment (Cabello-Pinedo et al., 2024).

Complex I deficiency is the most widespread respiratory chain disease with the prevalence of about 30-40 percent of cases of mitochondrial diseases. Complex I deficiency is characterized by a broad range of symptoms in patients such as encephalopathy, cardiomyopathy, liver disease, and Leigh syndrome. Most Complex I mutations are embryonic, and animal models with impaired Complex I activity have developmental arrest at best and serious structural abnormalities at worst. The stage of developmental stage of the embryonic lethality is usually associated with the severity of the mutation and the remaining Complex I activity, and complete loss-of-function mutations are usually associated with early developmental arrest (Nõmm et al., 2019).

The causation of developmental failure in mitochondrial disease is the metabolic and signaling pathway impairment as reported in this review. Loss of Complex I activity inhibits NADH oxidation and disrupts NAD+ /NADH ratios, impacting on NAD+ - dependent enzymes such as sirtuins. This perturbation of metabolism may pass on to disturbed histone acetylation levels and dysregulated gene expression (Amorim et al., 2022). Also, mutated Complex I activity leads to impaired ATP generation, which may interfere with energy-dependent activities such as chromatin remodeling and transcription. The failure to adequately activate embryonic genome can be a significant bottleneck to developing embryos with severe mitochondrial dysfunction.

Recent developments of mitochondrial replacement therapies show some possible ways of how mitochondrial disease can be prevented. Maternal spindle transfer and

pronuclear transfer are some of the techniques that can be used to transfer the nuclear genome into a donor oocyte or zygote with wild-type mitochondria when the oocyte or zygote has mutant mitochondrial DNA (Zong et al., 2024). These methods have already been effective in the prevention of transmission of mitochondrial disease both in animal models and early human trials. The knowledge of the metabolic inputs necessary to initiate embryonic genome activation has been used to optimize such processes, so that the metabolic competence of embryos manipulated in this way remains intact to allow their normal development.

Metabolic Interventions and Developmental Reprogramming

The closer relationships between metabolism and maintenance of the cellular state are indicative of the fact that metabolic interventions have a potential to to affect developmental outcome and cellular reprogramming effectiveness. Metabolic transitions are one of the most important issues in the context of somatic cell reprogramming to induced pluripotent stem cells (iPSCs). Pluripotent stem cells are more glycolytic in metabolism whereas somatic cells are usually very dependent on oxidative phosphorylation as a source of energy. Reprogramming efficiency can be boosted by interventions that facilitate this metabolic transition either by acting on mitochondrial activity or by acting on NAD+ metabolism (Tain & Hsu, 2022).

One of the therapeutic modalities that could be applied in development and regenerative medicine is the NAD+ supplementation or the increase in the NAD+ production. Elevating cellular NAD+ levels with the use of nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN) precursors of NAD+ has been found to be beneficial in several different settings, such as aging, neurodegeneration, and metabolic disease. Regulation of NAD+ levels in early embryos may have the potential of affecting the timing or efficiency of genome activation, but it would be necessary to carefully consider such interventions since proper ratios between NAD+ and NADH are extremely important to normal development(Nüsken et al., 2018).

Another type of metabolic intervention that has possible developmental implications is sirtuin activators or compounds that directly or indirectly stimulate the activity of sirtuins via NAD +-promotion. The effects of resveratrol and analogs on the cellular aging process, metabolism and stress resistance are studied. Sirtuin activation may have an effect on chromatin remodeling, epigenetic programming, and developmental competence in oocytes and early embryos. The exact effects of sirtuin regulation at the early development stages are however worth investigating as too much and too little sirtuin activity may actually affect the normal developmental process (Li & Kang, 2024).

Other metabolic intervention is to target mitochondrial ROS production. Mitochondria-targeted antioxidants (e.g., MitoQ or SkQ1) accumulate in mitochondria and are able to decrease the oxidative stress. Such specific antioxidants could be used in old oocytes or under environmental stress, using these antioxidants could help to restore the appropriate level of ROS and developmental competence (Qi et al., 2024). Nevertheless, there would be a high likelihood of harming the body by completely clearing off ROS as these molecules have signaling functions. This is because it is difficult to find the best ROS that promotes signaling functions without causing oxidative damage.

Table 3: Key Regulatory Mechanisms Linking NADH Metabolism to EGA

Mechanism	Metabolic Component	Epigenetic Effect	Impact on EGA
Sirtuin Activity	NAD^+/NADH ratio	Histone deacetylation	Chromatin condensation, Transcriptional

			modulation
PARP Activity	NAD^+ consumption	Chromatin relaxation, DNA repair	Chromatin accessibility
Acetyl-CoA Production	TCA cycle flux, Citrate export	Histone acetylation	Transcriptional activation
TET Activity	α-ketoglutarate availability	DNA demethylation	Epigenetic reprogramming
ROS Signaling	Complex I electron leakage	Transcription factor oxidation	Redox-dependent gene activation
ATP Production	OXPHOS efficiency	Chromatin remodeling complex activity	Nucleosome positioning

EGA = embryonic genome activation; PARP = poly(ADP-ribose) polymerase; ROS = reactive oxygen species; TCA = tricarboxylic acid; TET = ten-eleven translocation; OXPHOS = oxidative phosphorylation

Table 3 provides a summary of the key regulation pathways by which NADH metabolism modulates embryonic genome activation (EGA) either by metabolite-metabolite or metabolite-epigenetic interactions. The regulation of histone deacetylation via the changes in the NAD+/NADH ratio and subsequent regulation of chromatin structure and transcription, regulate sirtuin activity. The NAD+ consuming enzyme, PARP, facilitates the repair of DNA and chromatin relaxation to enhance accessibility to genes. Both TCA cycle metabolic flux and acetyl-CoA production that facilitates acetylation of histones and activates transcription, and α-ketoglutarate that facilitates TET and epigenetic reactivation as well as DNA demethylation. Moreover, mitochondrial Complex I-produced ROS are also signaling molecules that have a redox-dependent effect on the activity of transcription factors. Last but not least, effective ATP generation through oxidative phosphorylation (OXPHOS) promotes chromatin remodeling complexes that reposition nucleosomes, which allow the expression of genes to induce successful EGA.

Future Directions and Emerging Questions

Although the roles of NADH redox metabolism and the activity of the mitochondrial Complex I in embryonic development have been explained in remarkable ways, several questions are still unanswered. New technologies such as single-cell multi-omics, state-of-the-art live imaging, and computational modeling are offering a new era of insight into how development is metabolically regulated, but uncovering new complexity. A number of leading areas of interests are worthy of further exploration to obtain a full picture of the metabolism-developmental coupling.

To begin with, the molecular pathways that detect metabolic condition and relay this signal to transcriptional and epigenetic processes need additional clarification. Even though well-established metabolic sensors are the NAD + dependent enzymes and the energy-sensing kinase, there are likely other mechanisms. It would be desirable to identify more metabolite-responsive proteins, especially those which have a direct effect on chromatin structure or transcription factor activity, in order to understand more about how the two are linked. A sophisticated method of proteomics that will be useful in this aspect will be one that will identify metabolite-protein interactions and post-translational modification caused by metabolites.

Second, more attention should be paid to the spatial arrangement of metabolism in the early embryos. Although cell-cell metabolic heterogeneity has been receiving greater attention, little is known about the subcellular structure of cell metabolism, and how it develops during development. Are the localization of metabolic enzymes and

mitochondria to specific subcellular domains during activation of the genome? What is the effect of metabolic gradients or zones in the embryo? Recent developments in the spatial metabolomics and super-resolution imaging of the metabolic processes should answer these questions.

Third, time dynamics of metabolic transitions in early development need to be finer. Most of the current knowledge is grounded on measurements of populations at discrete developmental stages, and may be very relevant, but metabolic transitions must be continuous and vary significantly among cells. The real-time measurement of metabolic parameters such as the NAD + NADH ratios, mitochondrial membrane potential, and ROS levels in individual cells during preimplantation development would be of great importance to study the dynamics of metabolic changes and their connection to the developmental benchmarks.

Fourth, it is still unfulfilled how the metabolic regulation mechanisms are conserved in the evolution of early development. When compared with a variety of different species the comparisons might reveal basic principles of metabolic-developmental coupling and underscore species-specific adaptations. Are there common metabolic points of activation of the genome in all animals? The question is how species that exhibit diverse reproductive strategies (e.g. oviparous vs. viviparous) control mitochondrial activity early in development? Comparative developmental metabolism is a relatively untapped frontier that could be used to bring forth universal principles.

Fifth, the chronic effects of metabolic disturbances in the early development are worth further research. Although more and more developmental progression effects can be described in relation to a short-term effect of the developmental trajectory, it is still unclear how much a metabolic change during critical windows can impart long-lasting phenotypes. Are the epigenetic changes that result in lasting perturbation of transient metabolic changes in genome activation? Do metabolic treatments in early development prevent or ameliorate diseases in adulthood? Animal-based longitudinal studies and close postnatal follow-ups of people conceived with the help of assisted reproductive technologies will be educative.

Lastly, the translation of the key knowledge into clinical practice is an opportunity and a challenge. Although our comprehensibility of the metabolic control in development has significantly increased, the transformation of such knowledge into better products in assisted reproductive technologies, regenerative medicine, and disease prevention needs to be thoroughly verified and refined. The creation of standard tools of measuring embryonic metabolism, the most favorable culture conditions that would facilitate normal metabolic programming and setting safety and effectiveness of metabolic interventions are significant objectives of translational application.

Conclusions

NADH redox metabolism and mitochondrial Complex I activity in embryonic genome activation and maintenance of cellular state is a paradigm of metabolic-epigenetic interaction during development. This review has integrated existing knowledge of the effects of mitochondrial energy metabolism, via the formation and oxidation of NADH at Complex I, on the chromatin remodelling and transcriptional activation processes that define the maternal-to-zygotic transition. A number of important principles can be identified out of this synthesis.

First, metabolism and development are two aspects that cannot be separated, and the metabolic state has not only been a result of developmental programming but also a real determinant of developmental progression. Controlled by the NAD + /NADH ratio, where Complex I-mediated NADH oxidation plays a central role, chromatin modifications mediated by sirtuins and other NAD-dependent enzymes have direct, direct linkage between cellular redox state and epigenetic programming. This metabolic-epigenetic feedback mechanism is necessary to maintain developmental

transitions to only occur with the proper metabolic conditions established so that there is no likely premature or inappropriate change of cell fate.

Second, mitochondrial Complex I is strategically located in developmental metabolism as it works as an energy transduction machine, a redox regulator, and a signaling center at the same time. Complex I shapes activities of NAD + -dependent enzymes through its oxidation of NADH, improves ATP production and energy-dependent development through its contribution to the proton-motive force, and mediates redox signaling cascades that regulate cell fate decisions. This complex I has a multifunctional property that makes it a critical nexus between development and metabolism.

Third, metabolic transitions undergo a strictly controlled temporal and spatial regulation during the early embryogenesis to facilitate development. These changes in quiescent oocyte mitochondria to active embryonic mitochondria are similar to the activation of the embryonic genome and the processes may be tightly controlled. The interdependence of these processes can be realized by the fact that disruption of one metabolic or transcriptional program can disrupt the other. This is guaranteed by the presence of metabolic checkpoints which should be met before developmental progression can proceed and embryos has the energetic and biosynthetic resources necessary to support the next stage.

Fourth, cell fate decisions and developmental patterning are caused by metabolic heterogeneity in embryos during early development. Instead of considering early embryos as metabolically homogenous, single-cell methods have shown extensive metabolic heterogeneity among cells which are associated with and potentially cause differentiation. This stochastic and deterministic metabolic heterogeneity can offer a mechanism of providing cellular diversity and this could be an underestimated aspect of developmental control.

Fifth, the developmental programming of long-term effects has potential consequences on the environment due to the influence of these factors on the embryonic metabolism. Embryonic mitochondrial activity and NADH redox status may vary under the influence of culture conditions in assisted reproductive technologies, maternal nutrition and metabolism, and environmental stressors. These metabolic perturbations could be transmitted to disturbed epigenetic programming, that has long-term impacts on the expression of genes and phenotype. Indeed there are significant implications of understanding these mechanisms of developmental programming to reproductive medicine and to population health.

Entering the interaction of metabolism and development as discovered in NADH redox metabolism and Complex I activity is one of the key biological principles with far-reaching implications. In addition to early embryogenesis, it is observed that, even with stem cells, cell-differentiation, reprogramming and aging and disease, comparable metabolic-epigenetic coupling events occur. The insights gained through the study of early development can therefore be applied in our conceptualization of these various biological processes and provide some suggestions on the approaches to therapy.

In the future, further research on metabolic control during development is likely to provide not only the basic knowledge of the principles that regulate life but also its practical implementation in the field of reproduction, healing, and prevention of diseases. The paradigm of new technologies that allow measuring and manipulating metabolism in an unprecedented space and time will certainly show new levels of complexity in metabolic-developmental coupling. Nevertheless, the major principle, which is that metabolism and development are closely linked in various ways, e.g. by NAD+-dependent chromatin modification and mitochondrial Complex I activity, appears set to persist.

Finally, it can be concluded that NADH redox metabolism and mitochondrial Complex I activity are important mechanisms of embryonic genome activation

regulation and maintenance of cellular states by their effects on NAD+-dependent enzymes, energy generation, and redox signaling. The molecular insights into these metabolic pathways and their developmental implications offer critical information on the procedures that govern the early embryonic development and give potential solutions to the current assisted reproductive technologies as well as the development of the basic knowledge of the beginning of life.

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