Chapter 3

Gluconeogenesis: A Metabolic Pathway in Eukaryotic Cells such as Cellular Slime Molds

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Abstract

Dictyostelium discoideum or cellular slime mold is simple eukaryotic microorganism, which generally grows in forest soil and decaying leaves. This amoeba feeds on bacteria and grows as single cells. The development of *Dictyostelium discoideum* is simpler than that of mammalian cells. It uses many of the same signals that are found to function in higher eukaryotic organisms like plants and animals. *Dictyostelium discoideum* is an excellent system in which to study metabolic pathways which are simpler than that of the complex systems like mammalian system. Glucose is metabolized in glycolysis to yield pyruvate and lactate and further metabolized in the tricarboxylic acid cycle. Glucose can be polymerized into glycogen in addition to glycolysis process. In a metabolic pathway, the generation of glucose from certain non-carbohydrate carbon substrates is called gluconeogenesis. In *Dictyostelium discoideum*, glucose is synthesized by the breakdown of pyruvate. Glycogen phosphorylase and amylase break down glycogen to form glucose. Glycogen synthase and glycogen phosphorylase are the key enzymes for the regulation. Both the enzyme equally regulated the process simultaneously, so that when one is activated, the other is deactivated. During gluconeogenesis, glucose is synthesized from pyruvate but sometimes during this process, three enzymes, glucose-6-phophatase, fructose-1,6-bisphosphatase, and phosphoenolpyruvate carboxykinase catalyze an irreversible reaction.

Keywords: gluconeogenesis, eukaryotic system, *Dictyostelium discoideum*

1. Introduction

An amoeba is very interesting organism to study because it grows as single cells and develops as multi-cellular organisms. They present a range of developmental processes which can be used to study of any molecular pathways like glycolysis or gluconeogenesis pathways [1]. During evolution, the amoebozoa generated a large number of species which goes through the similar developmental stages, from unicellular to multi-cellular stages [1–4]. The well-characterized amoebozoan species is *Dictyostelium discoideum,* which is easy to study as compared to mammalian cell [5–7]. *D. discoideum* uses many similar signals and contains similar pathways that are presented in plants and animals [1]. *D. discoideum* or cellular slime mold is simple eukaryotic microorganism which generally grows in forest soil and decaying leaves. This amoeba feeds on bacteria and grows as single cells [2, 3]. The development of *D. discoideum* is simpler than that of mammalian cells.

2. The gluconeogenesis process in eukaryotic cell

Gluconeogenesis is a process by which carbohydrate is synthesized from non-carbohydrate precursors like oxaloacetate and pyruvate (**Figure 1**). In the first step of the gluconeogenesis process, oxaloacetic acid is synthesized from pyruvic acid. On the other hand, in the citric acid cycle, oxaloacetic acid reacts with acetyl-CoA. So, at low concentration of acetyl-CoA and high concentration of ATP, gluconeogenesis proceeds. Gluconeogenesis starts in the mitochondria of the cells. In the first step, carboxylation of pyruvate occurs by pyruvate carboxylase enzyme and it forms oxaloacetate by using one ATP molecule. Oxaloacetate is reduced to malate by using NADH. After this step, the remaining steps of gluconeogenesis process occur in the cytosol. In the next step, malate is oxidized to oxaloacetate using NAD^+. Oxaloacetate is first decarboxylated, and after that, it is phosphorylated by using the enzyme, PEP carboxykinase, and one GTP. In the next step, PEP converted into 2-phosphoglycerate, 3-phosphoglycerate and then 1,3-bisphosphoglycerate by the enzyme enolase, phosphoglycerate mutase and phosphoglycerate kinase, respectively. In the next step of this reaction, 1,3-bisphosphoglycerate converts into glyceraldehyde 3-phosphate by the enzyme glyceraldehyde phosphate dehydrogenase. Now, the glyceraldehyde 3-phosphate converts into fructose 1,6-bisphosphate via two ways: one is direct conversion and another through the intermediate component called dihydroxyacetone phosphate. In the next step, fructose 1,6-bisphosphate converts into fructose 6-phosphate, using an enzyme, fructose 1,6-bisphosphatase, one water molecule, and releasing one phosphate. This step is the rate-limiting step in gluconeogenesis process. Glucose-6-phosphate is formed from fructose 6-phosphate followed by glucose by the enzyme glucose-6-bisphosphatase. The reaction of the glucose formation occurs inside the endoplasmic reticulum, specifically in the lumen, where glucose-6-phosphate is hydrolyzed and produces glucose and releases an inorganic phosphate [8].

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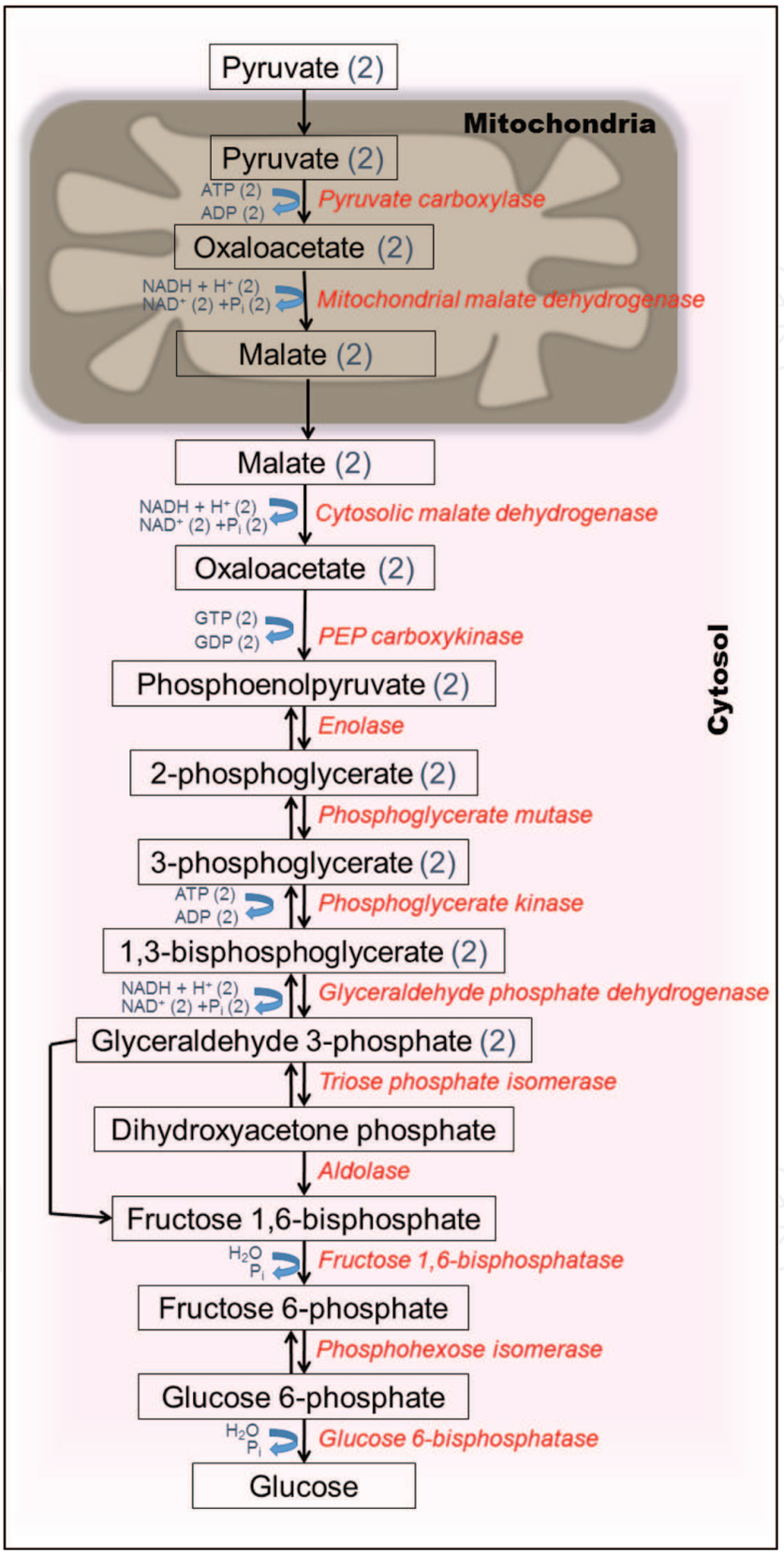

Figure 1. Gluconeogenesis process in the eukaryotic cell.

3.The developmental stages of *Dictyostelium discoideum.*

About 80 years ago, Ken Raper isolated *D. discoideum* from the forest floor at North Carolina [9]. He observed that when the cells had a depletion of food, they aggregated into mounds [9]. John Bonner showed that when the cells were in the starving condition, they secreted a chemical which acts like chemoattractant and the cells responded by moving up the gradient [10]. After 20 years, Konijn et al. showed that the chemoattractant was cAMP [11]. After this discovery, *D. discoideum* considered as a model organism to study chemotaxis and developmental biology. The connection between cell signaling pathways and biochemical pathways was established by using this organism. The pathways for cAMP synthesize, the surface receptors for cAMP and many other cell signaling to biochemical and molecular biological techniques were established by using *D. discoideum* [12–15]. The developmental stages of *D. discoideum* started from slug-shaped structures and go till the formation of the fruiting body [1, 2, 16]. Pre-spore and pre-stalk cells at the slug stage formed spores and stalk cells in fruiting bodies, and it was also found that pre-stalk cells were at the front of the slugs, and pre-spore cells were all in the back [2, 16]. There were 20-fold differences between the size of slugs and the total number of individual cells in each slug. This variation showed that there was some intracellular signal which determines the proportions of pre-spore and pre-stalk cells. Soderbom and Loomis showed that the pre-spore cells synthesize an inhibitor which inhibits pre-spore differentiation, and the pre-spore cells were resistant to this inhibitor [2, 17, 18]. This mechanism was responsible for size invariance of the slugs [18]. The amoeba went through three cycles when it was facing starvation [7].

3.1. The microcyst

Encystment was a very common process to amoebae, but it was not known for *D. discoideum*. In microcyst stage, each cell elaborated into a two layered cellulose coat and went to the dormant stage [7].

3.2. The macrocyst

In this stage of the sexual cycle, cells of two mating types fused [2]. Under wet condition, the macrocyst form, which had three layered cellulose coat at maturity. After fusion, the cells formed giant cells which had at least two nuclei or many nuclei. This fused structure attracted other amoebae by chemotaxis to cAMP. The endocytes were formed by engulfing these cells, and after that the giant cells produced meiotic offspring [7]. Macrocysts were formed from endocytes including hundred of cells.

3.3. Fruiting bodies

The fruiting body was formed through complex and polarized cell movements. In this stage, cells were not engulfed to form endocytes because one cell was recognized by the other cell. For the formation of fruiting body of *D. discoideum,* cells did chemotaxis and cells were more elaborated and involved a relay mechanism. But this mechanism either suppressed or did not

exist during the macrocyst formation. Fruiting body formed by aggregation of one lakh cells. In this case, cells were adhesive in nature and moved among each other and able to distinguish between cAMP and other molecules [7].

During mid-developmental stage, *D. discoideum* can choose between two different pathways, one is from the finger stage, it can directly precede to culmination, or it can fall over to form a phototactic, migratory slug. This migratory stage is important for cells to find an appropriate site for fruiting body formation [19]. Slugs prefer dark and low ionic strength environment [20]. Two cell types were there inside the slug which implied that they were connected with the signaling system [7]. Twenty percentage cells died for the formation of the stalk of the fruiting body, and eighty percentage cells survive by the formation of spores. **Figure 2** represents the developmental stages of *D. discoideum*.

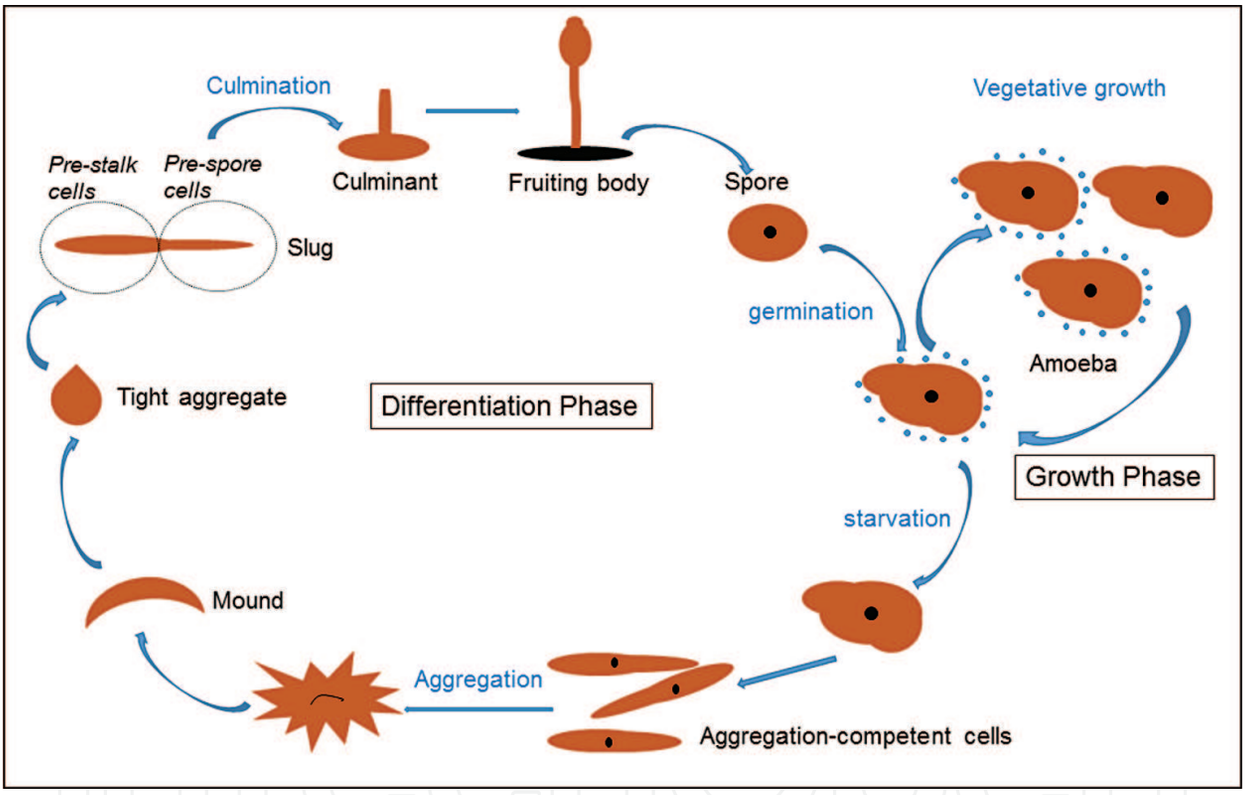

Figure 2. Lifecycle of *Dictyostelium discoideum*.

4. Size of the aggregates of *D. discoideum* is depending upon the gluconeogenesis pathway indirectly

The group size of *D. discoideum* is regulated by a negative feedback pathway mediated by counting factor (CF) which consists of at least five protein complexes [24]. During the early developmental stage, the counting factor (CF) breaking up the big aggregate of cells to the smaller aggregates of about 2×10^4 cells [21]. High levels of CF decrease cell-cell adhesion and

also decrease the amplitude of cAMP and increase random motility. The glucose metabolism is affected by the CF, and it decreases the CF glucose levels [22]. CF decreases the activity of the gluconeogenic enzyme, glucose-6-phosphatase, which decreases the level of glucose in the cell with the high secretion of CF [23, 24]. In that case, if glucose has been added externally then the size of the fruiting bodies get increases [24]. This process alters the intermediates of the metabolic pathways, such as pyruvate and lactate [22]. Jang and Gomer showed that, if the cells exposed to CF, the CF has very small effect on amylase or glycogen phosphorylase, enzymes involved in glucose production from glycogen [24]. On the other hand, it has a huge effect on glycolysis pathway. If the CF is high, then it is inhibited the glucokinase activity, but it does not regulate phosphofructokinase (enzyme responsible for glycolysis pathways). CF showed some effect upon the enzyme involved in a gluconeogenesis pathway such as fructose-1,6-bisphosphatase and glucose-6-phosphatase. The fructose-1,6-bisphosphatase is not regulated by CF, whereas glucose-6-phosphatase is regulated by CF [24].

The size of the terminal structures of *D. discoideum* is depending upon the cell surrounded by the number of cells, so the initiation of development is not started with the significant growth of the cell. The secretion of a protein complex is very important to control the size of the aggregates. Large aggregates initially have higher CF levels which can modify gluconeogenesis, and the other metabolic pathways alters the level of metabolites [1, 24]. After that, the level of CF drops down which increases cell-cell adhesion and because of that the random motility decreases which stabilize the smaller aggregates.

5. Gluconeogenesis process affected during the differentiation of myxamoebae of the cellular slime mold

Myxamoeba is a naked amoeboid uni-nucleate protoplast that lacks both cilia and flagella. In the life cycle of *D. discoideum*, they had gone through the vegetative state, differentiation state as independent amoeboid cells which called as myxamoebae [25]. If the myxamoebae was grown in different media, with varying carbohydrate content [26], then the chemical composition [27], enzyme composition [28], and physiological behavior [29] got changed inside the cell. After that, if these cells put in the moist condition, the cells formed slug [5, 25]. Carbohydrate content changed inside the *D. discoideum* at different stages of development [30]. So the gluconeogenesis process alters during the carbohydrate conversion process, the energy was coming from cellular protein, RNA, and dry weight to complete this process [25, 30–33].

Wright et al. showed in their kinetic model, the glycogen content of the cell remained constant during the early developmental stage but it decreased when the culmination process occurred [34]. White and Sussman suggested that the glycogen content of axenic cells was small [30]. They also showed that the glycogen initially decreased because of the consumption of the bacterial glycogen for the development of axenically grown myxamoebae. Wright et al. assume in their model, during differentiation of *D. discoideum*, there was no gluconeogenesis process

occurs [34]. Cleland and Coe showed some evidence of the presence of gluconeogenesis process during the differentiation of myxamoebae [31]. However, Hames et al. suggested that initially, cells had low glycogen but during differentiation, the glycogen content increased significantly which depicts that the gluconeogenesis process had a huge effect on the differentiation mechanism of the cell [25, 35].

In the absence of glucose and the presence of very low concentrations of glycogen, myxamoebae grow and degrade all the glycogen during 4 h of development [35]. Glycogen is synthesized during the late developmental stages (5–15 h) and finally broken down by the cell to synthesize saccharide. Hames and Ashworth showed that the amount of glycogen synthesized during the late developmental stage is larger than the glycogen content of the vegetative cells [25]. This glycogen synthesis occurs during gluconeogenesis process when the cellular glucose remains at a constant low concentration. During differentiation, myxamoebal glycogen is not stored, but the gluconeogenesis process still can occur, if the cells initially have a large amount of glycogen.

6. Discussion

D. discoideum is a well-characterized eukaryotic system which grows as single cells and develops as multi-cellular organisms. The development of *D. discoideum* is simpler than that of mammalian cells. They use many similar signals and contain similar pathways that are presented in plants and animals so it is the best biological system to study the molecular pathways like glycolysis or gluconeogenesis pathways, which can correlated with the mammalian system. *D. discoideum* considered as a model organism to study chemotaxis and developmental biology. *D. discoideum* obtained energy through lysosomal degradation and phagocytosis process [36]. Because of that intra-lysosomal nutrient levels are increasing so, the lysosome and the vacuole collect amino acids and other nutrients from cellular components through the autophagy process [37]. This process has been correlated with the gluconeogenesis process. The counting factor (CF) or the protein complex regulates the size of the aggregates of *D. discoideum*, which is indirectly affecting the gluconeogenesis pathway. CF showed some effect upon the enzyme involved in a gluconeogenesis pathway such that fructose-1,6-bisphosphatase and glucose-6-phosphatase. The fructose-1,6-bisphosphatase is not regulated by CF, whereas glucose-6-phosphatase is regulated by CF, which acts as one of the size regulating factor for the aggregates of *D. discoideum*. Large aggregates initially have higher CF levels which can modify gluconeogenesis, and the other metabolic pathways, and also alters the level of metabolites [1, 24]. After that, the level of CF drops down which increases cell-cell adhesion and because of that the random motility decreases which stabilize the smaller aggregates. The glycogen synthesis during the developmental process of *D. discoideum* causes by the gluconeogenesis pathway. During differentiation, myxamoebal glycogen is not stored but the gluconeogenesis process still can occur, if the cells initially have a large amount of glycogen. So gluconeogenesis pathway is very important for the development of the cells, this is one of the best eukaryotic system in which the process can be studied.

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