The Mitochondrion in the Germline and Early Development

Edited by
Justin C. St. John
The Role of the Mitochondrion in Sperm Function: Is There a Place for Oxidative Phosphorylation or Is This a Purely Glycolytic Process?

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We review here the current knowledge related to the metabolic pathways used by spermatozoa to meet their high demands for ATP. This is discussed with special emphasis on one of their key roles, motility. We believe that the controversy among glycolytic and oxidative phosphorylation supporters is artificial and, as it happens in many other cell types, the source of ATP is multiple and depends on external inputs. © 2007, Elsevier Inc.

I. Introduction

Which is the major biochemical pathway for the supply of energy that mediates sperm motility? Is this glycolysis or oxidative phosphorylation (OXPHOS)? This is a long-lasting debate in the field of gamete research (Ford, 2006). We believe that the underlying cause of this never-ending dispute is not the lack of sufficient body of experimental data, but that the question itself does not make sense. To understand this statement, we can slightly modify the question by substituting “sperm motility” by “muscle contraction.”

1 These two authors act together as senior authors.
Firstly, we should be aware that the requirements for sperm motility and muscle contraction are a basal ATP supply and an adequate rate of replacement for the hydrolyzed ATP when it is needed. Following our comparison with muscle, it is well established that several overlapping metabolic pathways contribute to the supply of ATP and that the preponderance of a given pathway at a particular time point depends on a variety of parameters, amongst others: a carbon source and oxygen supply, rate of lactate clearance, and so on. In fact, most cell types are capable of both glycolysis and OXPHOS, and they rely on one or other pathway as the major ATP supplier according to external inputs.

Secondly, glycolysis and OXPHOS are interdependent metabolic processes. Thus, glycolyzable substrates reach OXPHOS through glycolysis; however, for ATP production, anaerobic glycolysis is not very efficient and because of that, it is mainly used under hypoxic conditions or when glycolytic substrates are very abundant. Whenever oxygen is not limiting and the availability of glucose is restricted, cells rely on the highly efficient OXPHOS to cover their demands of ATP.

In this chapter, we focus our attention on the following aspects. (1) Genetic and molecular programs aimed to keep and maintain OXPHOS-competent mitochondria during differentiation of the gamete. (2) The available data supporting the argument that the spermatozoon is capable of degrading glucose to CO$_2$ and H$_2$O through glycolysis, tricarboxylic cycle, and OXPHOS. (3) The current knowledge on substrate availability (carbon source and oxygen) to spermatozoa throughout their race to reach the oocyte. (4) The evidence demonstrating that sperm motility is impaired whenever the glucose catabolic route (either glycolysis or OXPHOS) is impaired.

II. Spermiogenesis: Removing Everything But the Essential

To fulfill its role, the spermatozoon requires a tightly packed haploid genome to reach the target and deliver its load. Thus, a refined differentiation program is implemented. Sperm maturation is therefore an amazing and exquisite exercise of economy relinquishing every structure unnecessary for the unique aim of the male gamete, namely, bringing together the paternal contribution to the maternal one.

Sperm structure is simple and well defined, a head and a tail. In the head, the acrosome contains hydrolytic enzymes needed to fuse and deliver the condensed nucleus inside the ovum. The tail or flagellum includes the mid-piece tightly packed with mitochondria and the principal piece, both required for the sperm motility. In support of this, three different types of defects in flagellum structure have been described in decapitated spermatozoa. In two of them the mitochondrial sheath is absent or incompletely
developed. These flagella contain a certain amount of cytoplasm in the proximal end but motility is low or nonexistent. In the third type, however, the mitochondrial sheath is perfectly formed and, the motility of these flagella is very high. Therefore, complete differentiation of the midpiece is required for motility (Baccetti et al., 1984; Holstein et al., 1986; Perotti et al., 1981; Toyama et al., 1995). Moreover, the tail length correlate with the fraction occupied by the midpiece as does mitochondrial volume with flagellum length or beating frequency (Cardullo and Baltz, 1991). Not surprisingly then, asthenozoospermic humans have smaller midpieces with lower number of mitochondrial gyres and anomalous organization of the organelles (Mundy et al., 1995).

Notably, mitochondria are not only maintained in the fully differentiated spermatozoon but they also undergo substantial programmed modifications throughout spermatogenesis. They modify their cellular localization, morphology, and metabolism. Thus, spermatogonial organelles are oval in shape with lamellar cristae and are located around the nucleus (De Martino et al., 1979). However, mitochondria are totally condensed at the late pachytene state and a few cristae occupy most of the mitochondrial volume (De Martino et al., 1979). During this process, mitochondria elongate and divide to produce small clusters of round organelles randomly distributed in the cytoplasm (De Martino et al., 1979). Individual mitochondria are close to the plasma membrane in spermatids. Throughout spermatid maturation, mitochondria develop convolved cristae, the intracrista space is reduced and some of them move to the flagellum. The remaining undifferentiated mitochondria and the rest of cellular organelles are lost to the residual body. In testicular spermatozoa, mitochondria are wrapped around the flagellum and the cristae and matrix form a concentric system (Baradi and Rao, 1979; Cieciera and Klimek, 1988; De Martino et al., 1979).

All these structural changes during spermatogenesis mirror metabolic differentiation. Thus, some OXPHOS subunits such as cytochrome c and subunit VIb-2 of the cytochrome c oxidase (COX) are exclusively expressed in germinal cells (Hess et al., 1993; Huttemann et al., 2003). Moreover, COX activity is higher in spermatozoa than in pachytene spermatocytes (De Martino et al., 1979) and, as expected, mitochondrial membrane potential increases by up to sixfold throughout spermatogenesis because this potential is dependent on OXPHOS activity (Petit et al., 1995; Saunders et al., 1993).

These observations contrast with those from erythrocyte differentiation. These cells do not require mitochondria for their functional role. Therefore, they simply eliminate them. Then, why does a cell that dramatically reduces its volume by removing any superfluous structure retain very specialized mitochondria? Mitochondria contain their own genome, the mitochondrial DNA (mtDNA), which encodes essential genes for OXPHOS function
(Enriquez et al., 1999). Therefore, it is possible that spermatozoa retain their mitochondria to deliver a full set of male genetic information, including mtDNA, to the oocyte. However, it has been demonstrated that mitochondria and their mtDNAs are committed to degradation within the zygote or embryos (Sutovsky et al., 1999). In normal circumstances, mtDNA is only maternally inherited (Giles et al., 1980). Therefore, delivering mtDNA to the oocyte is not the purpose for retaining mitochondria in sperm cells.

**III. Sperm Mitochondria Are Required for Functional Purposes**

Mitochondria host critical metabolic pathways. They regulate the levels of important intracellular second messengers, such as calcium and reactive oxygen species, and they are also involved in the control of apoptosis (Kroemer et al., 1997). Other important mitochondrial biochemical pathways are the Krebs cycle and the OXPHOS system. These biochemical pathways are critical for the integration of cell metabolism. Thus, they connect sugar, fatty acid, and amino acid catabolism with anabolic pathways like pyrimidine synthesis, urea cycle, and so on. However, spermatozoa are terminal cells and mitochondrial anabolic pathways are likely not very relevant. OXPHOS is not only 15 times more efficient than anaerobic glycolysis for ATP production but it also increases the number and variety of suitable substrates. Therefore, considering the efficiency of the energy supply, OXPHOS as a support for sperm motility would be the best explanation for understanding the strong investment in mitochondria during spermatogenesis.

**A. OXPHOS Is a Major Pathway for Sperm Motility**

Analysis of the implications for OXPHOS in the supply of energy for sperm motility is straightforward. First, there is a great variety of OXPHOS-specific inhibitors and their effect on flagellum activity and ultimately on sperm motility is easy to evaluate. Second, OXPHOS biogenesis depends on the maintenance and expression of mtDNA as well as well-defined nuclear-encoded genes (Fernandez-Silva et al., 2003). Then, genetic approaches are also attainable.

Mitochondrial activity correlates with sperm motility (Gopalkrishnan et al., 1995). Thus, normozoospermic individuals show higher sperm motility and vitality and also a higher mitochondrial activity than oligozoospermic individuals. Motility and sperm concentration are improved when mitochondrial
membrane potential ($\Delta \psi_m$) is high (Donnelly et al., 2000; Marchetti et al., 2002; Troiano et al., 1998). Moreover, a high $\Delta \psi_m$ increases the in vitro fertilization (IVF) rate. These results suggest that in humans, the analysis of mitochondrial $\Delta \psi_m$ is the most sensitive test to determine sperm quality and that sperm motility is associated with the functional status of mitochondria (Marchetti et al., 2002). Similar results have also been obtained for other species such as rat or ram (Auger et al., 1989, 1993; Evenson et al., 1982; Gravance et al., 2001; Kramer et al., 1993; Ronot and Auger, 1990; Windsor, 1997).

Indirect biochemical analysis also provides strong support for the dependence of sperm motility on OXPHOS. Oxygen consumption and sperm motility correlate (Ford and Harrison, 1981; Halangk et al., 1990). This correlation was also found in cells with modified metabolism by using metabolic inhibitors (Halangk and Bohnensack, 1986). Cyclic nucleotides and phosphodiesterase inhibitors stimulate respiration and increase sperm motility (Garbers et al., 1971). On the other hand, the higher transmission rate of mouse spermatozoa with the mutant allele $t^a$, an allele of the T locus transmitted in a non-Mendelian frequency, is accompanied with a lower NADH/NAD$^+$ ratio, an increase in oxygen consumption and higher motility (Ginsberg and Hillman, 1974).

Mitochondrial $\Delta \psi_m$ and oxygen consumption depend on the activity of the mitochondrial electron transport chain (ETC) and the ATP synthase (both constituting the OXPHOS system). The four respiratory complexes (I–IV) form the ETC. The activity of all four respiratory complexes is higher when sperm motility increases (Ruiz-Pesini et al., 1998). Moreover, the use of drugs that specifically inhibit individual respiratory complexes dramatically decreases sperm motility. In particular, rotenone, an inhibitor of respiratory complex I depleted ATP and decreased sperm motility in humans (de Lamirande and Gagnon, 1992; Halangk et al., 1985a; Rikmenspoel, 1965; Ruiz-Pesini et al., 2000). Rotenone, but not glycolytic inhibitors, reduces the fertility of cervically inseminated sheep, suggesting that mitochondrial respiration plays an important role in the penetration of the cervix by the spermatozoa (Windsor, 1997). Antimycin A, an inhibitor of the respiratory complex III, promotes similar effects on sperm motility (Ford and Harrison, 1981; Krzyzosiak et al., 1999; Ruiz-Pesini et al., 2000). Nitric oxide (NO) by acting on ferreproteins of the ETC is able to decrease ATP levels and affect sperm motility (McKinney et al., 1995; Weinberg et al., 1995). Inhibition of complex IV activity either by NO (Brown, 1995) or by KCN strongly reduces sperm motility (Halangk and Bohnensack, 1986; Pascual et al., 1996; Ruiz-Pesini et al., 2000). Gossypol that inhibits respiration also decreases sperm motility (Breitbart et al., 1989; Kim et al., 1984). To complete the picture, oligomycin, a drug that directly blocks ATP synthesis by mitochondria, negatively affects sperm motility (Dreanno et al., 1999; Halangk et al., 1985a).
Four of the five enzymatic complexes that constitute the OXPHOS system are partially encoded by mtDNA. Thus, mutations in mtDNA genes that impair the expression of one or more proteins encoded in the mtDNA can promote diseases in humans. Since these diseases are very often severe, not very much attention has been paid to the sperm quality of patients with mitochondrial diseases. Systematic analysis on patient fertility has allowed reporting that a single deletion (Kao et al., 1995) or multiple deletions within the mtDNA are associated with diminished fertility and motility of human sperm. Moreover, dysfunction in the proper adjustment of the sperm mtDNA level either in terms of depletion (Kao et al., 2004) or excess amounts (Diez-Sanchez et al., 2003a; May-Panloup et al., 2003) seems to be detrimental to motility. Folgero et al. (1993) investigated the quality of sperm in a patient suffering from mitochondrial encephalopathy with lactic acidosis and stroke (MELAS) syndrome who harbored the mtDNA A3243G mutation. They were able to demonstrate reduced sperm motility in this patient (Folgero et al., 1993). This observation was corroborated in a second MELAS patient (Spiropoulos et al., 2002). More interestingly, it has been shown that some human mtDNA population variants can influence sperm motility (Montiel-Sosa et al., 2006; Ruiz-Pesini et al., 2000). We have previously proposed that some of these variants could decrease OXPHOS coupling efficiency, and subsequently affect sperm motility (Ruiz-Pesini et al., 2004).

Genetically modified mice also illustrate the relevance of OXPHOS to sperm motility. Thus, a testis-specific cytochrome c null mouse has been generated (Narisawa et al., 2002). These mice produce properly differentiated spermatozoa. Unfortunately the lack of OXPHOS activity in these spermatozoa was not demonstrated. Despite that, and when compared to wild-type sperm, the proportion of immotile spermatozoa is greater, more than 50%. This would categorize such mice as asthenozoospermic when assessed according to human semen analysis criteria (WHO, 1999). Moreover, sperm from mutant mice contain reduced level of ATP and showed a fourfold decrease efficiency for IVF assays (Narisawa et al., 2002). Interestingly, despite their evident impairment in motility, these males are still fertile. To properly analysis this, we have to keep in mind that fertilization in laboratory animals is assessed without competition between males (one single male with several females) and that mouse females are naturally highly fertile, with multiple ovulations. In this way, conditions that result in a subfertile or infertile human couple would still allow fertile mice. Unfortunately, no estimation of the in vivo fertility efficiency (as average size of the litters, rate of pregnancy failures after copulation, and so on) for these mice is available.

A second mouse model provides additional insights into the role of mitochondria in sperm function. Trifunovic et al. (2004) developed a homozygous knock-in in mouse that expresses a proofreading-deficient version of polymerase gamma (POLG), the nuclear-encoded catalytic subunit of mtDNA-specific
polymerase. These animals manifest an mtDNA mutator phenotype with a substantial increase in the level of mtDNA mutations as well as deletions. They develop and grow normally up to the age of 25 weeks. After that an impressive phenotype of premature aging is expressed. Very interestingly, a reduction in fertility of mtDNA-mutator mice of both sexes was found. However, all young females were fertile and became prematurely infertile probably as part of the general premature aging phenotype. To the contrary, young and still healthy males were virtually infertile, only 1 litter from 8 males bred with 16 wild-type females (Trifunovic et al., 2004). In humans, POLG has been proposed to have a role in male infertility (Jensen et al., 2004; Rovio et al., 2001), although this association has been questioned by others (Aknin-Seifer et al., 2005; Brusco et al., 2006). All theses observations indicate that pathological genetic alterations of OXPHOS-related genes impair sperm quality and particularly motility.

IV. Is Glycolysis Required for Sperm Motility?

Recent reports present evidence that glycolysis is needed for sperm motility. However, since ATP production from glucose involves both glycolysis and OXPHOS, it is necessary to establish whether the ATP needed for sperm motility can have a pure glycolytic origin (fermentation). This has been proposed because: (1) the absence of mitochondria at the end of the sperm tail and the potential difficulty in meeting satisfactorily flagellum ATP demands at the tail and (2) some published observations interpreting their findings as evidence that OXPHOS is not required for sperm motility.

The delivery of ATP at the appropriate rate to the dynein ATPase along the flagellum is a logistic problem that it is not fully understood. It has been proposed that physical forces that originate cytoplasmic waves may facilitate the ATP supply to the end of tail. These forces would be derived from the flagellum’s movement itself. In addition, specific metabolic shuttles such as adenylate kinase and phosphoglycerate kinase would contribute to the ATP supply (Ford, 2006). Both mechanical and biochemical contributions will promote rapid diffusion of mitochondrial ATP from the midpiece to the tail.

Miki et al. (2004) provide the strongest evidence for the importance of glycolysis for sperm motility. They generated a knockout mouse for the sperm-specific isoform of glyceraldehyde 3-phosphate dehydrogenase (GAPDHs), a glycolytic enzyme. Homozygote knockout males were infertile and had profound defects in sperm motility, exhibiting sluggish movement without forward progression (Miki et al., 2004). Motility was not abolished by the absence of glycolysis, only about 3% of sperm showed progressive motility immediately after removal from the epididymis, and this was not maintained after 2 hours incubation. However, up to 60% of spermatozoa from GAPDHs−/− mice
kept some nonprogressive movement even after 4 hours incubation. Very interestingly, the authors highlighted a characteristic flagella bending in the middle piece that was not propagated effectively along the principal piece of the sperm tail. In other words, flagella beating seemed to be initiated at the proximal tail but was not propagated along the flagellum (Miki et al., 2004).

The GAPDHs−/− mice showed another striking characteristic, a dramatically low level of ATP (10% of control). This was attributed solely to the lack of ATP synthesis by glycolysis and as evidence suggesting that ATP is not produced by sperm mitochondria. However, GAPDHs−/− spermatozoa do maintain the glycolytic reactions that consume ATP. In fact, sperm from mutant mice use ATP to phosphorylate glucose and fructose 6-phosphate in the first steps of glycolysis and they finally accumulate glyceraldehyde 3-phosphate (G3P) by up to fourfold more than controls (Miki et al., 2004). If ATP in spermatozoa is solely produced by glycolysis, what is the source of ATP that generates such abnormal accumulation of G3P? Consequently, an additional source of ATP other than glycolysis has to be used and this source should be OXPHOS. Since GAPDHs and the remaining glycolytic enzymes are located at the fibrous sheath of the flagellum (Bunch et al., 1998) away from mitochondria, the accumulation of G3P also indicates that mitochondrial ATP should diffuse toward the distal part of the flagellum. Interestingly, vanadate, a dynein ATPase inhibitor affects sperm motility and decreases mitochondrial respiration (Halangk et al., 1985b). ADP is the major stimulator of OXPHOS activity. Therefore, inhibition of the dynein ATPase would decrease ADP production and subsequently mitochondrial stimulation, meaning this ADP would return to the mitochondria and would not be consumed in the flagellum by glycolysis.

Therefore, these mutant mice transform glycolysis from an ATP-generating pathway to an ATP-consuming one (Fig. 1). As a result, glycolysis competes against dynein ATPase for the ATP, contributing severely to the motility defect. In fact, the incubation of spermatozoa from GAPDHs−/− in the presence of glucose further reduces ATP concentrations from 10% to 1.9% and eliminates any trace of progressive spermatozoa (Miki et al., 2004). Spermatozoa from many species including human can remain motile in glucose-free media. Interestingly, by using an inhibitor of the GAPDHs, it was found that mammalian spermatozoa were immotile in the presence of glucose but they were motile when glucose was replaced by respiratory substrates (Ford, 2006).

A number of recent papers suggest spermatozoa might be capable of gluconeogenesis and synthesis of glycogen to allow cell motility. Motile spermatozoa consume energy and it would be contradictory that opposite pathways, one catabolic (glycolysis) and the other anabolic (gluconeogenesis and glycogen synthesis), worked simultaneously in the same cell compartment.
Figure 1  Glycolysis. The glycolytic pathway contains two phases: one consumes ATP and the other produces ATP. GAPDHs knockout mouse only maintain the ATP-consuming phase.

and using the same substrate. Moreover, gluconeogenesis consumes three times more ATP than that produced by glycolysis (Pilkis and Granner, 1992). It would be absurd to activate gluconeogenesis to produce glucose for use in glycolysis. However, it would probably be beneficial to activate gluconeogenesis in those scenarios where enough energy obtained from respiration is used in sperm motility and residual levels would be used for energy storage.

V. Substrates Available for the Supply of Energy

A major issue in defining the metabolic process responsible for the ATP production for sperm motility is the availability of the proper substrate. This is very often overlooked in the models proposed to explain the variety of observations available. Thus, glycolytic substrates, respiratory substrates, or both are required to be available for spermatozoa in sufficient amounts and at the right time. Respiratory substrates and oxygen levels (Max, 1992) are
abundant along male and female reproductive tracts to support OXPHOS activity (Fig. 2). The stimulation of Sertoli cells by follicle stimulating hormone (FSH) produces an increase in pyruvate and lactate secretion to the seminiferous epithelium lumen (Jutte et al., 1983; Sylvester and Griswold, 1994) and lactate is the central energy metabolite used by germ cells (Grootegoed et al., 1984; Mita et al., 1982). RNA and protein syntheses, in addition to oxygen consumption of isolated spermatocytes and spermatids, are stimulated by exogenous lactate but not by glucose (Boussouar and Benahmed, 2004). Spermatids possess all enzyme activities for the glycolytic pathway. However, glucose metabolism cannot maintain cellular ATP content, and exposure of isolated spermatids to glucose without other energy substrates soon results in ATP depletion (Grootegoed et al., 1984; Mita et al., 1982). The use of lactate as the main energetic substrate during spermatogenesis might be a mechanism to check a potential deficiency of OXPHOS activity when germ cells are still able to respond because mature spermatozoa are transcriptionally inert (Diez-Sanchez et al., 2003b). Moreover, the mammalian epididymis is rich in lactate but reducing sugars are not present in the epididymal lumen (Jones and Murdoch, 1996).

Seminal plasma rich in fructose stays in the vagina and only spermatozoa reach the cervical mucus (Haas and Beer, 1986). Lactobacillus has long been
considered the protective flora in the vagina. Lactic acid produced by this microorganism decreases vaginal pH and acts against vaginal pathogens. Therefore, the vagina is enriched in lactate. Interestingly, in diabetic women, an association has been found between glycemia and prevalence of fungi (Nowakowska et al., 2004). Thus, the levels of glycolyzable sugars are probably low in vagina. Considering the rest of the female reproductive tract, it has been shown that the ewe cervix and uterine washings of swine are poor in glucose and glycolyzable substrates (Jones, 1998; Windsor, 1997). Lactate levels are 15-, 10-, and 38-fold higher than glucose in mouse uterine, oviduct, and follicular fluids, respectively (Harris et al., 2005) and bovine oviductal fluid contains less than 100 μM glucose (Galantino-Homer et al., 2004). All this evidence supports an important role for lactate, a respiratory substrate, as an important energy source for spermatozoa. In agreement with this, there is a testis-specific lactate dehydrogenase isozyme [lactate dehydrogenase C4 (LDH-C4)]. This is first expressed at the beginning of spermatogenic activity in gametogenic cells from preleptotene spermatocytes up to spermatids. It represents the predominant form of LDH in mature spermatozoa and has a dual localization, in the cytosol of spermatocytes, spermatids, and spermatozoa, as well as in the matrix of sperm-type mitochondria (Burgos et al., 1995). Moreover, there is a correlation between LDH-C4, the number of motile spermatozoa, and mitochondrial activity (Sawane et al., 2002). It is lactate, rather than pyruvate, which enters the mitochondria thereby constituting a lactate–pyruvate transport system in these cells for regenerating cytoplasmic nicotinamide adenine dinucleotide (Jones, 1997). Another sperm-specific isoenzyme downstream of the glycolytic pathway is pyruvate dehydrogenase (PDH). Malate increases markedly sperm PDH activity. The differential sensitivity to l-malate appears to be a particular regulatory property of the PDH complex in gametes (Gerez de Burgos et al., 1994). Very interestingly, acetoacetate and β-hydroxybutyrate stimulate sperm motility. Glycolysis inhibitors stop the motility of sperm mediated by glucose but not by ketone bodies (Tanaka et al., 2004). Moreover, a novel mitochondrial succinyl CoA transferase (SCOT-t), required for ketone body metabolism, has been described as being specifically expressed in testicular germ cells and spermatozoa and substitutes for the somatic cell isoform (Tanaka et al., 2002). It should be noted that ketone bodies can only be consumed by the OXPHOS system.

VI. Concluding Remarks

Ejaculated spermatozoa are not under the same homeostatic regulation as the common cell in the body. When ejaculated, they have to survive alone with their own baggage and they have to maximize their chances to fulfill their
function, to reach the ovum. The available evidence indicates that spermatozoae are set to take advantage of a wide-ranging and efficient cohort of metabolic options to survive under different energy sources with OXPHOS being a prominent one. This versatility is critical to ensure fertilization success.

Acknowledgments

We would like to thank Santiago Morales for his technical assistance. Our work was supported by the Spanish Ministry of Education (SAF2003-00103), the Instituto de Salud Carlos III (REDMitoEspaña-G03/011, REDEMETH-G03/054, REDCIENT C03/06-Grupo RC-N34-3, and Research Project FIS-PI-050647), the EU (EUMITOCOMBAT-LSHM-CT-2004-503116), and by the Diputación General de Aragón (Grupo de Excelencia DGA-B55 and Grupo consolidado DGA-B33 and PM-078/2006).

References


1. Energy Metabolism for Sperm Motility


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