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Beyond ATP Synthesis: Could the Histidine-Rich ATP6 Extension Contribute to Thermal Homeostasis in the Glacier Ice Worm?

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ABSTRACT

The glacier ice worm (*Mesenchytraeus solifugus*) is adapted to an exceptionally cold environment, and the molecular mechanisms supporting its bioenergetic stability under these conditions remain incompletely understood. Its mitochondrial ATP synthase subunit ATP6 contains an unusual histidine-rich C-terminal extension, and experimental studies using a bacterial model system have associated this extension with increased ATP synthesis activity. We propose that the extension may have a broader physiological significance beyond ATP production, potentially contributing to thermal homeostasis by influencing mitochondrial energy conversion and dissipation. Two non-exclusive mechanistic possibilities are considered. First, the extension may modify ATP synthase function and thereby indirectly influence mitochondrial processes involved in cellular thermal balance. Second, it may alter the relationship between proton transfer, ATP synthesis, and energy dissipation, potentially affecting the amount of energy released as heat. Importantly, increased proton conductance or mitochondrial uncoupling would not necessarily reduce heat production; depending on the mechanism, these processes may instead increase it. The hypothesis therefore does not presume a particular direction of change in heat generation. Rather, it predicts that the ATP6 extension may influence the relationship between ATP synthesis, respiratory activity, proton conductance, and heat production under cold-adapted physiological conditions. We outline experiments to distinguish these possibilities, including measurements of ATP synthesis, oxygen consumption, membrane potential, proton conductance, and heat production across controlled temperatures. This framework provides a testable basis for investigating whether the ATP6 extension contributes to thermal homeostasis in addition to its reported association with enhanced ATP synthesis.

Keywords: *Mesenchytraeus Solifugus*; ATP6; ATP Synthase; Cold Adaptation; Thermal Homeostasis; Mitochondrial Uncoupling; Proton Conductance; Energy Dissipation

Introduction

The glacier ice worm, *Mesenchytraeus solifugus*, is an unusual annelid adapted to life in glacier-associated environments. Its ability to maintain cellular function under persistently cold conditions raises questions about how its energy metabolism is regulated and how mitochondrial activity contributes to physiological stability. [1] reported temperature-dependent changes in ATP, ADP, and AMP levels in the ice worm, including increases in ATP and adenylate energy charge under particular cooling conditions. These observations suggest that adenylate homeostasis is an important component of its cold-associated physiology. However, intracellular ATP concentration reflects the balance between ATP synthesis and consumption and cannot, by itself, establish the rate of ATP production or the mechanisms regulating energy expenditure. A potentially important molecular feature is the unusual histidine-rich C-terminal extension of mitochondrial ATP synthase subunit ATP6. ATP6 is a component of the membrane-embedded Fo sector of ATP synthase, where it contributes to proton translocation coupled to ATP synthesis. [2] characterized the distinctive evolutionary features of ATP6 in glacier ice worms.

Subsequently, [3] demonstrated increased ATP synthesis activity when the ice-worm-specific extension was introduced into the bacterial ATP6 orthologue AtpB in *Escherichia coli*. Building on these findings, [4] investigated naturally occurring extensions and engineered variants in a bacterial model system, suggesting that histidine resi-

dues may influence proton-transfer behavior. These findings provide a basis for investigating the functional significance of the extension. Nevertheless, enhanced ATP synthesis in a bacterial system does not establish its complete physiological role in native ice-worm mitochondria. In particular, the available evidence does not determine whether the extension influences only ATP synthase activity or also affects broader aspects of mitochondrial energy conversion. We therefore propose that the histidine-rich ATP6 extension may contribute to the thermal homeostasis of the glacier ice worm in addition to facilitating ATP synthesis. This hypothesis focuses on two possible routes: an indirect effect on cellular processes involved in thermal balance, and an effect on the relationship between ATP production and mitochondrial energy dissipation.

Central Hypothesis: ATP6 May Contribute to Thermal Homeostasis Beyond ATP Synthesis

Our central hypothesis is that the histidine-rich C-terminal extension of ATP6 may have an additional role in the cold adaptation of *M. solifugus* by influencing mitochondrial energy conversion and, potentially, thermal homeostasis. The extension consists of additional amino acid residues within ATP6; it is not a separate protein-coding sequence for an independent cooling or uncoupling protein. Accordingly, any proposed effect would most plausibly arise from how the extension changes ATP6 structure, interactions, proton-transfer properties, or ATP synthase function, with possible downstream consequences for mitochondrial physiology.

We propose two mechanistic possibilities.

1. **Possibility 1:** An indirect contribution to thermal regulation. The extension may modify ATP synthase function in ways that influence mitochondrial energy availability, respiratory activity, or other cellular processes relevant to thermal balance. Under this possibility, the extension would not itself encode a cooling protein. Instead, its effects on a central component of mitochondrial energy conversion could influence the operation of downstream physiological mechanisms.
2. **Possibility 2:** Regulation of energy partitioning and dissipation. The extension may alter the relationship between proton movement, ATP synthesis, and the dissipation of proton-motive energy. Depending on the direction and magnitude of this effect, it could influence how much energy is conserved in ATP and how much is dissipated through other pathways, including pathways that generate heat.

These possibilities are related but experimentally distinguishable. Neither has yet been established as a physiological function of the extension in the native ice worm. The proposed hypothesis is therefore that the extension may contribute to thermal homeostasis, not that it has already been demonstrated to function as a dedicated thermoregulatory element.

Why ATP Synthesis Alone may not Explain the Complete Physiological Role

ATP synthase uses the proton-motive force across a membrane to drive ATP synthesis. The resulting relationship between proton movement, ATP production, and respiratory activity depends on the functional state of ATP synthase, membrane properties, proton conductance through other pathways, and cellular metabolic demand. An increase in ATP synthesis activity may reflect improved proton-transfer kinetics or altered enzyme function. However, this finding alone does not establish whether overall oxidative phosphorylation becomes more efficient, whether energy dissipation changes, or whether heat production is altered. Likewise, elevated ATP concentrations do not necessarily demonstrate increased ATP synthesis. They may reflect increased production, reduced utilization, altered adenylate interconversion, or a combination of these processes. These distinctions are central to interpreting the ice-worm phenotype. The reported increase in ATP synthesis activity in a bacterial model provides evidence that the extension can influence ATP synthase function under the tested conditions. It does not establish whether the extension changes coupling efficiency, proton conductance, heat production, or thermal tolerance in native mitochondria. We therefore propose that a complete assessment of the extension should go beyond ATP synthesis measurements and examine how ATP production relates to respiration, proton-motive force, and energy dissipation.

Could the ATP6 Extension Influence Thermal Homeostasis?

For an organism adapted to a persistently cold environment, maintaining cellular function requires an appropriate balance between energy supply, metabolic demand, and the physical effects of temperature on biochemical processes. It is therefore reasonable to investigate whether unusual mitochondrial protein features contribute to this balance. The histidine-rich ATP6 extension is a candidate for such investigation because of its reported association with altered ATP synthesis activity and the proposed involvement of histidine residues in proton transfer. Histidine side chains can participate in protonation–deprotonation reactions, although their actual contribution depends on their local structural and chemical environment. The extension's sequence and conformation could therefore influence the behavior of ATP synthase in ways that merit direct testing. One possibility is that the extension primarily improves ATP synthesis while maintaining effective coupling between proton translocation and ATP production. If so, the energetic benefit could help support cellular activity at low temperatures without requiring an independent thermoregulatory function.

A second possibility is that the extension also modifies energy partitioning. Such an effect could alter the relationship between respiratory activity, ATP synthesis, and heat production. The physiological consequence would depend on the direction of the change.

For example, more efficient coupling could reduce the respiratory expenditure required for a given amount of ATP synthesis, whereas increased proton leak or uncoupling could dissipate more energy and potentially increase heat production. This distinction is important for the present hypothesis. If the proposed physiological benefit is to limit unnecessary warming or prevent excessive heat generation, increased uncoupling would not automatically provide the expected mechanism. In many contexts, uncoupling increases the fraction of energy dissipated as heat rather than conserving it in ATP.

Thus, an uncoupling-related mechanism should be considered a testable alternative, not assumed to be a cooling mechanism. We propose that the ATP6 extension may contribute to thermal homeostasis by influencing mitochondrial energy conversion, either through more efficient ATP synthesis, altered energy partitioning, or context-dependent effects on these processes. Whether these changes affect heat production, the stability of the organism's physiological temperature or tolerance to temperature fluctuations remains to be determined experimentally. Schematic representation of the proposed hypothesis that the histidine-rich C-terminal extension of mitochondrial ATP6 may contribute to thermal homeostasis in glacier ice worms (Figure 1). ATP6 is a component of the membrane-embedded F_0 sector of mitochondrial ATP synthase, which participates in proton translocation coupled to ATP synthesis. The unusual histidine-rich extension may potentially influence temperature-sensitive molecular interactions, membrane stability, and mitochondrial function under cold conditions. These proposed effects could contribute to thermal homeostasis in glacier ice worms. The mechanisms illustrated remain hypothetical and require experimental validation. The figure was generated using ChatGPT (OpenAI) and subsequently reviewed and verified by the author. The author takes full responsibility for the scientific accuracy and final content of the Figure 1.

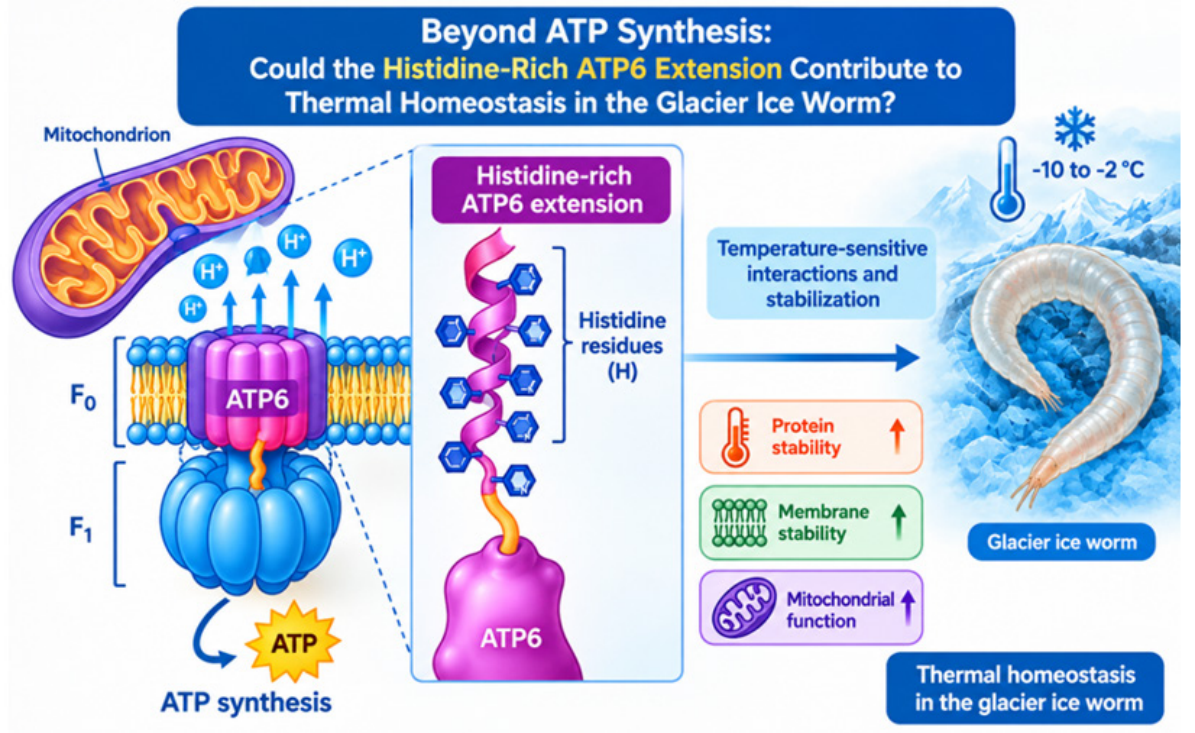


Figure 1: Hypothetical role of the histidine-rich ATP6 extension in thermal homeostasis in the glacier ice worm.

Alternative Mechanistic Models

Three models can be used to organize the hypothesis and guide experimental testing.

Model A: Enhanced ATP Synthesis with Preserved Coupling

In this model, the histidine-rich extension facilitates proton transfer through ATP synthase and increases ATP synthesis activity while preserving effective coupling. Its primary contribution to cold adaptation would be improved ATP availability or more efficient energy conversion, without a separate effect on heat production. This model is consistent with the possibility that the extension's functional effect is principally bioenergetic rather than directly thermoregulatory.

Model B: Altered Coupling and Energy Dissipation

In this model, the extension changes the relationship between proton movement, ATP synthesis, and respiratory activity. The resulting change could influence how much energy is conserved as ATP versus dissipated through other pathways. The direction of the effect cannot be assumed in advance. Increased coupling efficiency could reduce the respiratory energy required for a given ATP output, whereas increased proton leak could increase energy dissipation and potentially heat production. Demonstrating a change in coupling would therefore not, by itself, establish a heat-limiting or cooling function.

Model C: Context-Dependent Effects on Mitochondrial Energy Conversion

In this model, the extension's functional consequences vary with temperature or other physicochemical conditions, such as pH and membrane potential. Its effects on ATP synthesis and energy dissipation might therefore differ under cold-adapted conditions and during temperature changes. This model is particularly relevant to the proposed role in thermal homeostasis because it predicts that the extension's effects may not be fully characterized by measurements performed under a single condition. The three models are not necessarily mutually exclusive. The extension could enhance ATP synthesis while also exerting context-dependent effects on energy partitioning. Experiments in physiologically relevant systems are required to determine which model, if any, best explains its role in the ice worm.

Experimental Predictions and Falsifiability

The hypothesis can be tested through experiments comparing the native ATP6 extension, appropriate shortened or altered variants, and suitable controls. Initial mechanistic experiments may use a heterologous model, but validation in a physiologically relevant mitochondrial system would be essential.

First, ATP synthesis and oxygen consumption should be measured together under matched conditions. These measurements would establish whether increased ATP output is accompanied by proportional changes in respiratory activity. Second, membrane potential and proton conductance should be assessed independently.

Direct proton-flux measurements, with appropriate controls, would help determine whether the extension affects proton transfer and whether any observed effect can be attributed to ATP synthase rather than other membrane pathways. Third, coupling efficiency should be evaluated using integrated measurements of ATP production and respiration. Differences in ATP synthase abundance, protein expression, membrane integrity, enzyme stability, and background activity must be considered when comparing variants. Fourth, experiments should be performed across a controlled temperature range. This would reveal whether the extension's effects on ATP synthesis, respiratory activity, or coupling are temperature-dependent. Fifth, heat production should be measured directly using calorimetry or another validated method. ATP synthesis, oxygen consumption, and proton conductance alone cannot establish the direction or magnitude of heat production. Any claim that the extension contributes to thermal homeostasis would also require evidence that the measured effects are physiologically relevant in the intact organism.

The hypothesis generates four principal predictions:

1. **If the extension primarily enhances ATP synthesis,** ATP production should increase without a substantial independent change in coupling efficiency or heat production. This outcome would favor Model A.

2. **If the extension changes energy partitioning,** the relationship between ATP synthesis, oxygen consumption, and proton conductance should differ reproducibly between the extension-containing and control systems. This would support a broader bioenergetic role, although further measurements would be needed to determine its consequences for heat production.

3. **If the extension has context-dependent effects,** differences in ATP synthesis, coupling, or heat production should vary systematically with temperature or other controlled physicochemical conditions. Such effects would need to be distinguished from changes in protein abundance, membrane integrity, or experimental artifacts.

4. **If the extension contributes to thermal homeostasis,** changes in mitochondrial function should be linked to a measurable and physiologically relevant thermal outcome in the native organism or an appropriate model of its mitochondrial physiology. Without this link, the evidence would support a bioenergetic effect but not a demonstrated thermoregulatory function.

Conversely, if coupling efficiency, proton conductance, and heat production remain unchanged across relevant conditions, the hypothesis of an additional role in energy partitioning or thermal homeostasis would be weakened. If the extension increases ATP synthesis but does not produce any independent thermal effect, its established role may be adequately explained by enhanced ATP synthase activity.

Significance and Limitations

This hypothesis proposes that the unusual ATP6 extension of the glacier ice worm may have physiological significance beyond its reported association with increased ATP synthesis. Its potential importance lies in connecting a distinctive protein sequence with two broader questions: how mitochondrial energy conversion is adapted to extreme cold, and whether changes in this process influence thermal homeostasis.

The hypothesis also emphasizes the need to distinguish ATP synthesis from coupling efficiency, proton conductance, and heat production. These are related aspects of mitochondrial physiology, but none can be inferred conclusively from another without appropriate

measurements. Several limitations must be acknowledged. The extension's proposed contribution to thermal homeostasis has not been demonstrated directly. Findings from a bacterial model cannot fully reproduce the structural, regulatory, and metabolic environment of native ice-worm mitochondria. In addition, the existence of a histidine-rich extension does not, by itself, establish a proton-transfer mechanism or a specific effect on heat production. Accordingly, the proposed role should be regarded as a testable hypothesis rather than an established function. Its validity depends on experiments that link the extension to measurable changes in mitochondrial energy conversion and, ultimately, to a physiologically relevant thermal outcome.

Conclusion

The histidine-rich C-terminal extension of ATP6 in the glacier ice worm has been associated with increased ATP synthesis activity in a bacterial model system. However, its complete physiological significance in the native organism remains unresolved. We propose that the extension may contribute to cold adaptation not only through its effect on ATP synthesis but also through a potential influence on mitochondrial energy partitioning and thermal homeostasis. Two broad possibilities merit investigation: an indirect effect on cellular processes relevant to thermal balance, and a change in the relationship between ATP production and energy dissipation. The hypothesis does not assume that the extension encodes a separate cooling or uncoupling protein, nor does it assume that uncoupling would reduce heat production. Instead, it predicts that the extension may influence mitochondrial energy conversion in ways that could have thermal consequences under particular physiological conditions. Simultaneous assessment of ATP synthesis, oxygen consumption, membrane potential, proton conductance, and heat production across controlled temperatures would help determine whether the extension acts primarily as a facilitator of ATP synthesis or has a broader role in the thermal physiology of the glacier ice worm.

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