

Identifying the role of complex I in organohalide respiration of *Firmicutes*

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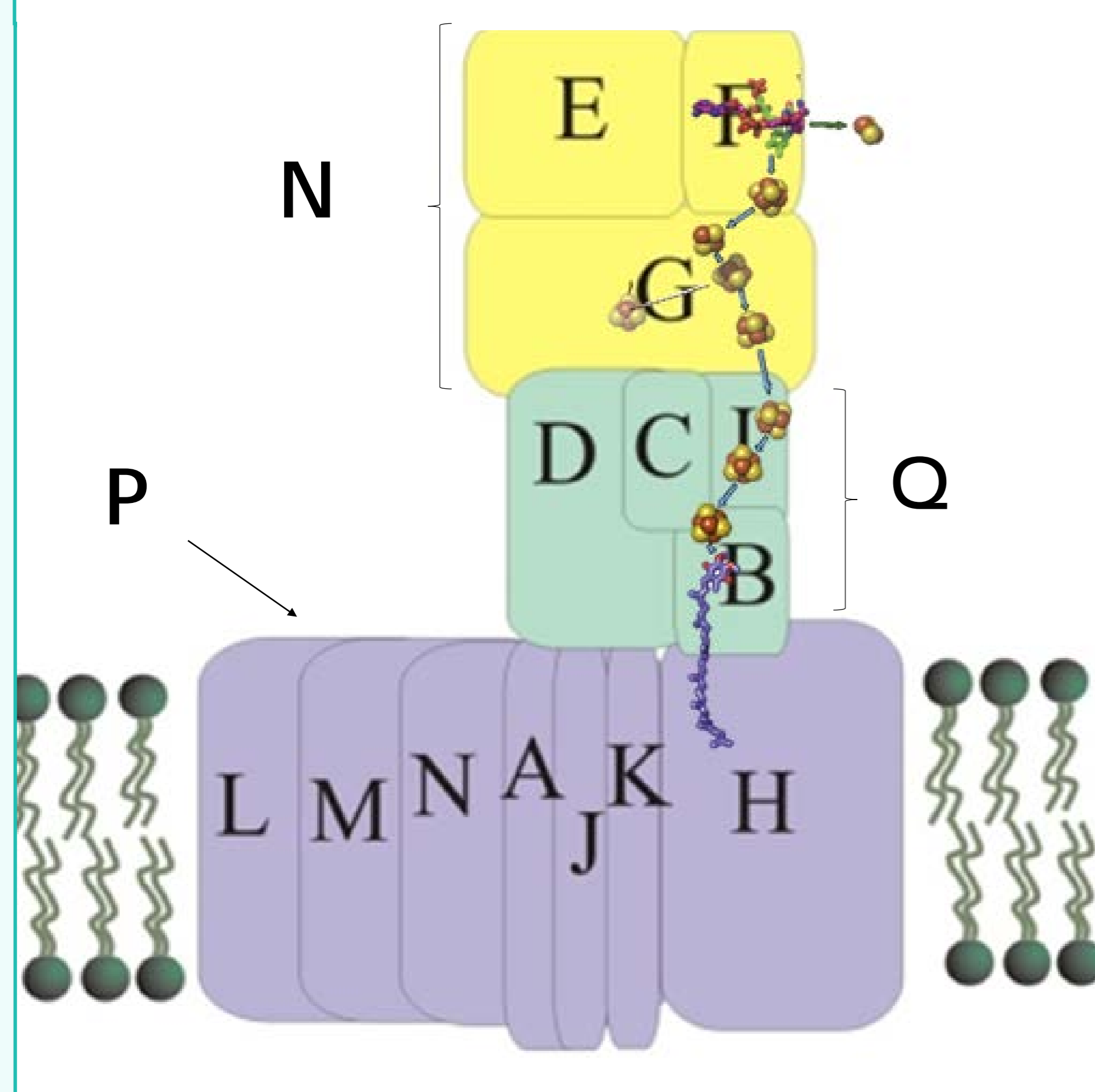
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1. Context

Organohalide compounds are used as terminal electron acceptors in a bacterial energy conserving process called **organohalide respiration (OHR)**.

Even though the enzymes playing key roles in OHR have been identified, the complete picture of the **respiratory chain is still lacking**. **Complex I** has been identified in genomic and proteomic studies in obligate OHRB and could be an important player in the process. Genomes from *Firmicutes* generally encode an **11-subunit version** of the complex, **lacking the NADH dehydrogenase (N) module** which suggests the use of an alternative electron donor to complex I.



Typical 14-subunit complex I. Schematic representation of the complex composed by three modules. **N**ADH dehydrogenase module in yellow, **Q**uinone module in green and the membrane-spanning **P**roton translocation module in purple (Figure adapted from [1-2]).

2. Objectives

- 1) Is complex I specifically used for OHR energy metabolism ?
- 2) What is the electron donor for the 11-subunit complex I ?
- 3) Is the electron donor varying depending on the source of electrons ?

3. Strategy

- 1) Is complex I specifically used for OHR metabolism ?



Culture of *Desulfitobacterium hafniense* in different growth conditions:

- Pyruvate (fermentation)
- H₂/PCE (OHR)
- Formate/PCE (OHR)
- H₂/fumarate (anaerobic respiration)

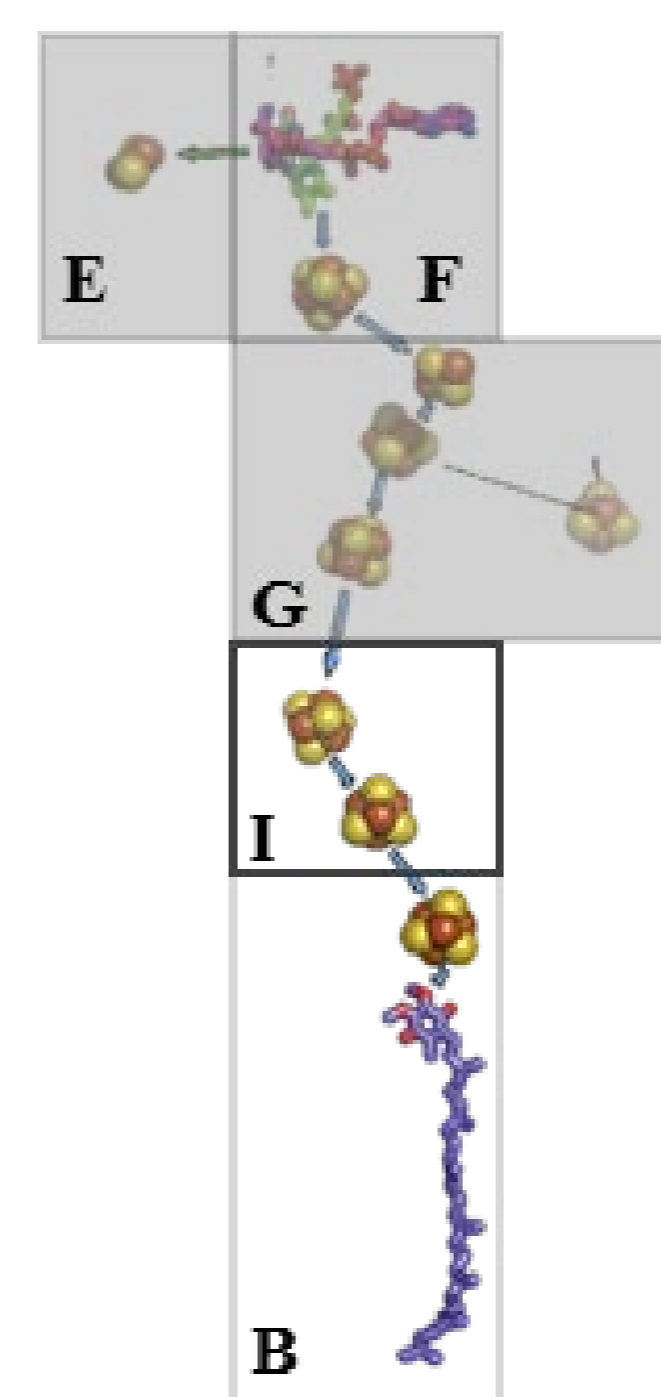
Quantitative proteomics to see if the expression of complex I is dependent on the metabolism in use.

Main Challenges

- Low expression level of complex I observed in the existing datasets
- Overcome the intrinsic proteome dynamic range (abundant S-layer protein)
- SRM approach : which subunit/peptides to target ?

4. Outlook

- 2) What is the electron donor for the 11-subunit complex ?



Protein sample from cultures where complex I is abundant

Co-immunoprecipitation pulling on Nuol subunit

→ Challenge : generation of a polyclonal antibody against Nuol

Complex I redox centers. Complex I subunits involved in electron tunneling are indicated with their Fe-S clusters or FMN cofactors (Figure adapted from [2]).

- 3) Is the electron donor varying depending on the source of electrons ?

Nuol Co-IP with cell extracts cultivated on several conditions.