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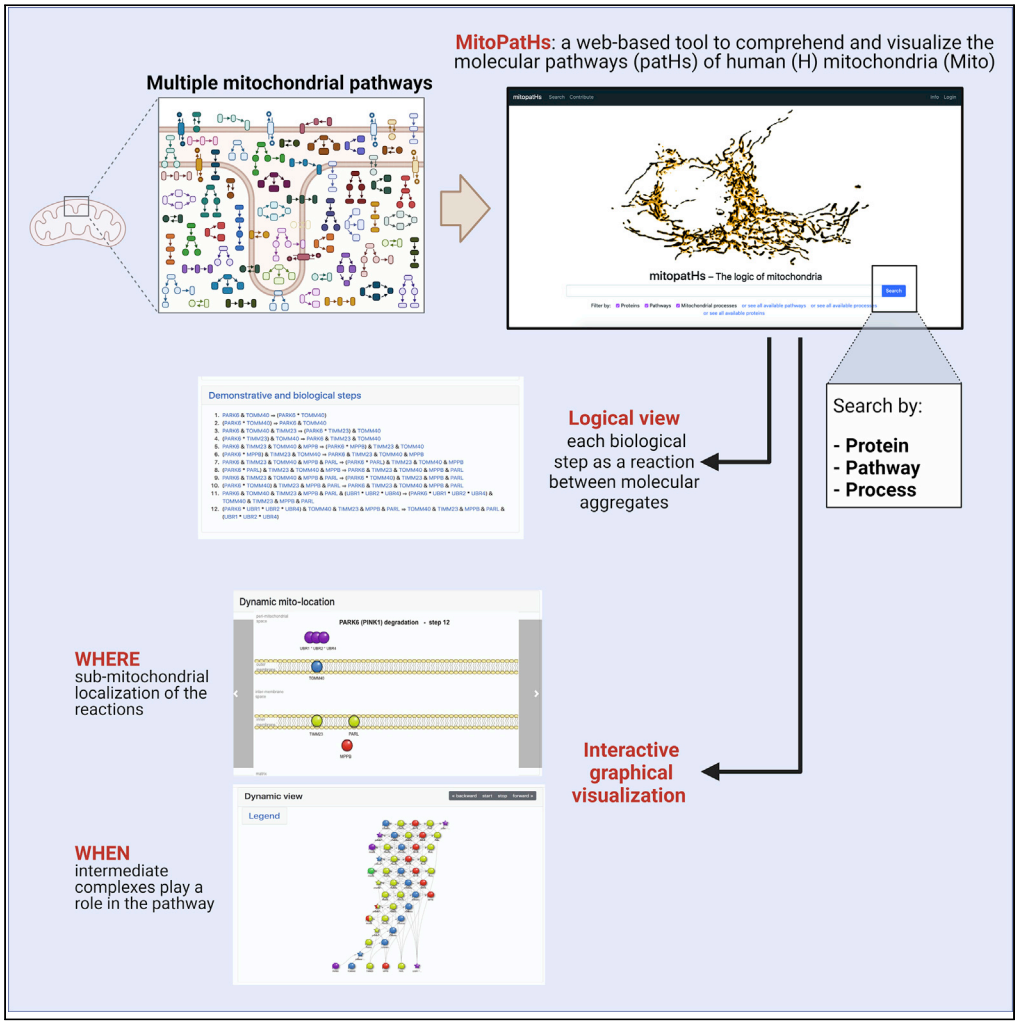

Saverio Marchi,
Marco Zanella,
Paolo Pinton,
Silvia Crafa,
Giovanni Boniolo

s.marchi@univpm.it (S.M.)
crafa@math.unipd.it (S.C.)

Highlights

We present *MitopatHs*: a new tool for exploring mitochondrial pathways

A rigorous logical framework manages mitochondrial pathways as logical deductions

A graphical visualization allows us to conceptualize the entire process step-by-step

The *MitopatHs*' user can scrutinize a pathway by navigating through its reactions

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MitopatHs: a new logically-framed tool for visualizing multiple mitochondrial pathways

Saverio Marchi,[1,6,7,*] Marco Zanella,[2,7] Paolo Pinton,[3,4] Silvia Crafa,[2,*] and Giovanni Boniolo[5]

SUMMARY

Mitochondria are key organelles inside the cell that house a wide range of molecular pathways involved in energy metabolism, ions homeostasis, and cell death. Several databases characterize the different mitochondrial aspects and thus support basic and clinical research.

Here we present MitopatHs, a web-based data set that allows navigating among the biochemical signaling pathways (PatHs) of human (H) mitochondria (Mito). MitopatHs is designed to visualize and comprehend virtually all types of pathways in two complementary ways: a *logical view*, where the sequence of biochemical reactions is presented as logical deductions, and an *intuitive graphical visualization*, which enables the examination and the analysis of each step of the pathway. MitopatHs is a manually curated, open access and collaborative tool, whose goal is to enable the visualization and comprehension of complicated molecular routes in an easy and fast way.

INTRODUCTION

In addition to the well-known role played in oxidative phosphorylation and energy metabolism, mitochondria are involved in a large number of processes taking place at their inside, including ion homeostasis, amino acids metabolism, protein import and sorting, and cell death (Galluzzi et al., 2012; Rizzuto et al., 2012). The multi-tasking nature of mitochondria originates from over 1,000 proteins that are located in different sub-mitochondrial districts and participate in multiple pathways for carrying out the crucial organelle functions (Pfanner et al., 2019). Mutations in genes encoding mitochondria-located proteins or alterations of the mitochondrial signaling systems have been widely associated with different pathological contexts, including aging and age-related defects, metabolic and cardiovascular diseases, neurological or muscle-related disorders, as well as cancer (Giorgi et al., 2018; Taylor and Turnbull, 2005). Therefore, easy access to knowledgeable mitochondrial data is of interest to a wide research community. Indeed, dissecting the molecular routes that coordinate the multiple mitochondrial activities is a key step to define how a specific mitochondrial dysfunction could represent the leading pathogenic mechanism or contribute to worsening certain diseases.

In the last years, several computational solutions have been created to characterize the different mitochondrial features and thus support basic and clinical research. Most of them consist of web inventories of the mitochondrial proteome, which include annotations of intra-mitochondrial localization and process involvement (Cotter et al., 2004; Rath et al., 2021; Smith and Robinson, 2019), whereas others are aimed to collect the human mitochondrial DNA variations (Lott et al., 2013). A recent tool termed mitoXplorer hosts the mitochondrial interactomes for 4 model organisms with in-depth description and annotation of its components, helping analyze expression dynamics and mutations of mito-genes in data coming from RNA-seq or similar techniques (Yim et al., 2020). However, no published datasets that include the specific characterization of multiple mitochondrial pathways and their association with different processes are available to date. Indeed, information about different mitochondrial reactions can be extracted only through generic pathway databases, such as Reactome (Jassal et al., 2020), KEGG (Kanehisa et al., 2017), or HumanCyc (Romero et al., 2005).

In this study, we present MitopatHs (https://web.math.unipd.it/mitopaths/), a mitochondrial-centric data set that allows navigating among the biochemical signaling pathways (PatHs) of human (H) mitochondria (Mito). MitopatHs is a manually curated, open access, and collaborative web tool, which allows the interactive search and graphical navigation through mitochondrial data. Information can be found by specific

[1]Department of Clinical and Molecular Sciences, Marche Polytechnic University, Ancona, Italy

[2]Department of Mathematics, University of Padua, Padua, Italy

[3]Department of Medical Sciences, Section of Experimental Medicine, Laboratory for Technologies of Advanced Therapies (LTTA), University of Ferrara, Ferrara, Italy

[4]Maria Cecilia Hospital, GVM Care & Research, Cotignola, Ravenna, Italy

[5]Department of Neuroscience and Rehabilitation, University of Ferrara, Ferrara, Italy

[6]Lead contact

[7]These authors contributed equally

*Correspondence: s.marchi@univpm.it (S.M.), crafa@math.unipd.it (S.C.)

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protein, by category of mitochondrial process and by genetic mutation. All the mitochondrial pathways collected in MitopatHs are displayed in two complementary ways: (i) a *logical view*, where the sequence of biochemical reactions is presented as a logical deduction, and (ii) an *intuitive graphical visualization*, which enables the examination and the analysis of each step of the pathway both in terms of spatial and temporal information.

The core unit of MitopatHs is *Zsyntax*, a logical system that allows us to manage a molecular pathway as a biological theorem, whose proof is given as a logical deduction that precisely encodes the sequence biochemical steps involved in the pathway (Boniolo et al., 2010). This logical view provides for a pathway a description that is precise, clear, and compact enough to easily grasp the meaning of the mitochondrial processes and connect them with other related biochemical processes. Moreover, it provides the basis for the automatic generation of the graphical data presentation. The distinctive data visualization allows the researchers to easily visualize and traverse the entire process step-by-step, also by considering the locations of the involved molecules in the sub-mitochondrial compartments (outer membrane, inter-membrane space, inner membrane, matrix), thereby immediately grasping many molecular details, going beyond what is available elsewhere, such as KEGG, Reactome or HumanCyc.

Besides web users searching for mitochondrial data, the tool has been created with a second class of intended users that is data contributors. Any experimental scientist is invited to submit a new pathway or a comment to already available data, through a dedicated web page. To check the correctness of biological data and its formalization in *Zsyntax*, the submitted mitochondrial process is revised and its graphical view is automatically generated. Our goal is the construction of knowledgeable information in a collaborative style, indeed using the rigorous logical framework underlying data organization, virtually all mitochondrial processes, such as the assembly of multi-protein complexes, ion transport, protein degradation, or post-translational modifications (i.e. phosphorylation) can be dissected and analyzed, enabling the comprehension of a complicated molecular route in an easy and fast way that clearly shows *what* happens, *when* it happens, and *where* it happens.

RESULTS

Design strategy

A key choice of MitopatHs is to not display too much information: our reasoning behind this strategy is to design a user-centered interface and a data visualization style that is simple, compact, and uniform. Additional information about proteins and pathways are provided utilizing external links to well-known databases (es. each protein is linked to its entry on Gene Ontology, and each pathway is appropriately referenced) while keeping the info in MitopatHs simple and focused on the mitochondrial functionality.

Compared to other generic (non-mitochondrial-centered) tools (i.e. KEGG, Reactome, HumanCyc), MitopatHs offers additional value both in terms of *data organization* and *data visualization*. The cornerstone of the data organization is the logical language *Zsyntax*, through which a single molecular pathway is managed as a formal theorem, whose proof can be scrutinized by navigating through the sequence of biological steps involved in the pathway, organized and encoded as logical deductions. Indeed, a single mitochondrial pathway is dissected in a series of reactions that are described with a level of detail and precision that is not available in, e.g., Reactome. Moreover, the logical encoding is exploited to automatically generate spatial and temporal description and animation of the data. Indeed, a pathway can be also visualized in its spatiality (i.e., positions of the molecules involved in the different mitochondrial compartments) and temporality (the series of biological steps composing the pathway), allowing the fast and exhaustive comprehension of all the molecular events that occur for the generation of the final product, including the interactions between the different subunits, their intra-mitochondrial localization and the role of accessory factors. To enhance the readability and provide access to additional biochemical information, each pathway is enriched with a short textual description and appropriate citations, as well as links to other generic databases. Finally, the collaborative nature of this data set advocates the inclusion of comments about the pathway's formalization that can be submitted by contributors.

How to use MitopatHs

The homepage of MitopatHs is displayed as a simple search engine, focused on mitochondrial information. By default, the string entered in the form by the user is searched in the list of proteins, in the list of pathways, and the list of categories of mitochondrial processes. To restrict the search to the list of mitochondrial

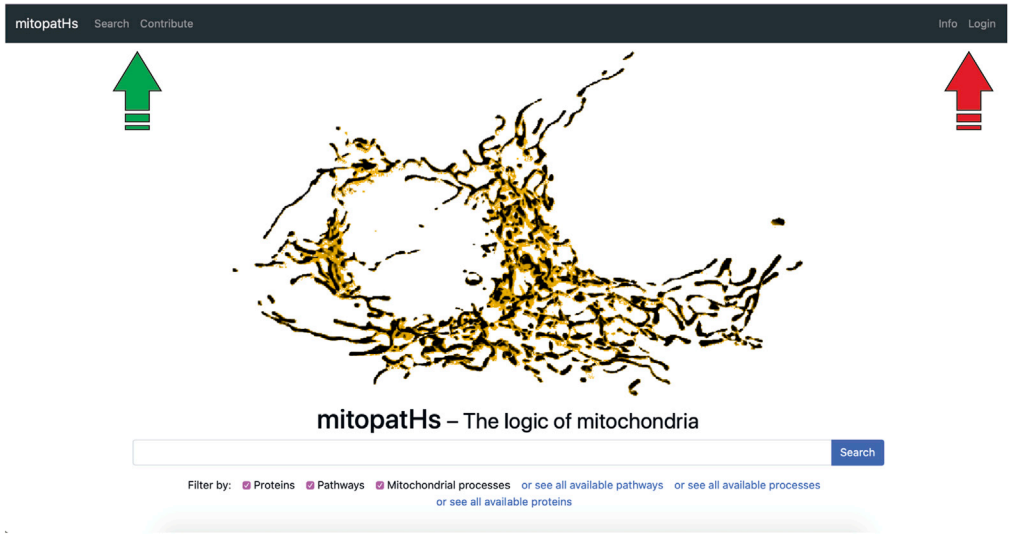

Figure 1. The Mitopaths homepage

The header bar offers a point of contact for the users. The "info" button on the top right corner (red arrow) provides basic explanations about MitopatHs, together with the full description of an example pathway. On the top left corner (green arrow), the user can suggest comment or a new pathway after logging in and previous registration. The "search" tool allows discriminating between simple proteins, molecular pathways, or mitochondrial processes. Alternatively, a user can directly go to the entire list of pathways or processes by clicking on the "browse pathways" or "browse processes" button, respectively.

proteins, it is sufficient to un-select the buttons corresponding to pathways and processes. Alternatively, a user can directly go to the list of pathways (resp. process categories) by clicking on the "see all available pathways" (resp. processes) links (Figure 1). Basic explanations about MitopatHs can be accessed through the "info" web page (Figure 1, red arrow), which also links to a web page that describes how to read and encode a pathway using *Zsyntax*, relying on the Uniporter pathway as an illustrative example.

Any pathway can be accessed from the homepage by searching for the name of the pathway or one of the involved proteins. Details about the selected pathway are displayed on a single web page, according to a common and simple format organized into several boxes. The top of the page displays the logical formalization of the pathway, based on *Zsyntax*, providing a useful *static* overview of the whole process. The box named "Theorem" summarizes the initial and the final stage of the pathway, while the box named "Demonstrative and biological steps" details the chain of logical and biochemical reactions that lead from the initial to the final state. Textual information with references and comments are displayed in a dedicated box, while a different box contains a link to the corresponding category of the mitochondrial process.

The graphical information about the *dynamics* of process formation is displayed downwards and can be navigated through an automatic slow animation or manually, using buttons to move backward and forward. This kind of data visualization enables an interactive and user-centered investigation (Figure 2). Indeed, it is possible to focus on a specific biochemical reaction, or the role of a specific mitochondrial protein and its mutations, or on a specific sub-mitochondrial compartment, and move through a pathway both in a top-down and bottom-up mode.

How to contribute to MitopatHs

The links "Contribute" and "Login" (Figure 1, green arrow) can be used to submit a new mitochondrial pathway or a data comment already available in MitopatHs. In particular, after registration, the user can enter its proposal for a new pathway through a simple form or by uploading a PDF file. S/he is encouraged to use *Zsyntax* to express the sequence of steps corresponding to the new pathway (with the helpful information provided through the "Info" page). However, the suggested pathway will go through a revision to check both biological correctness and *Zsyntax* conformance. If accepted, the new process is added to the MitopatHs search engine, together with the name of the contributor. Our Scientific Reviewer Team consists

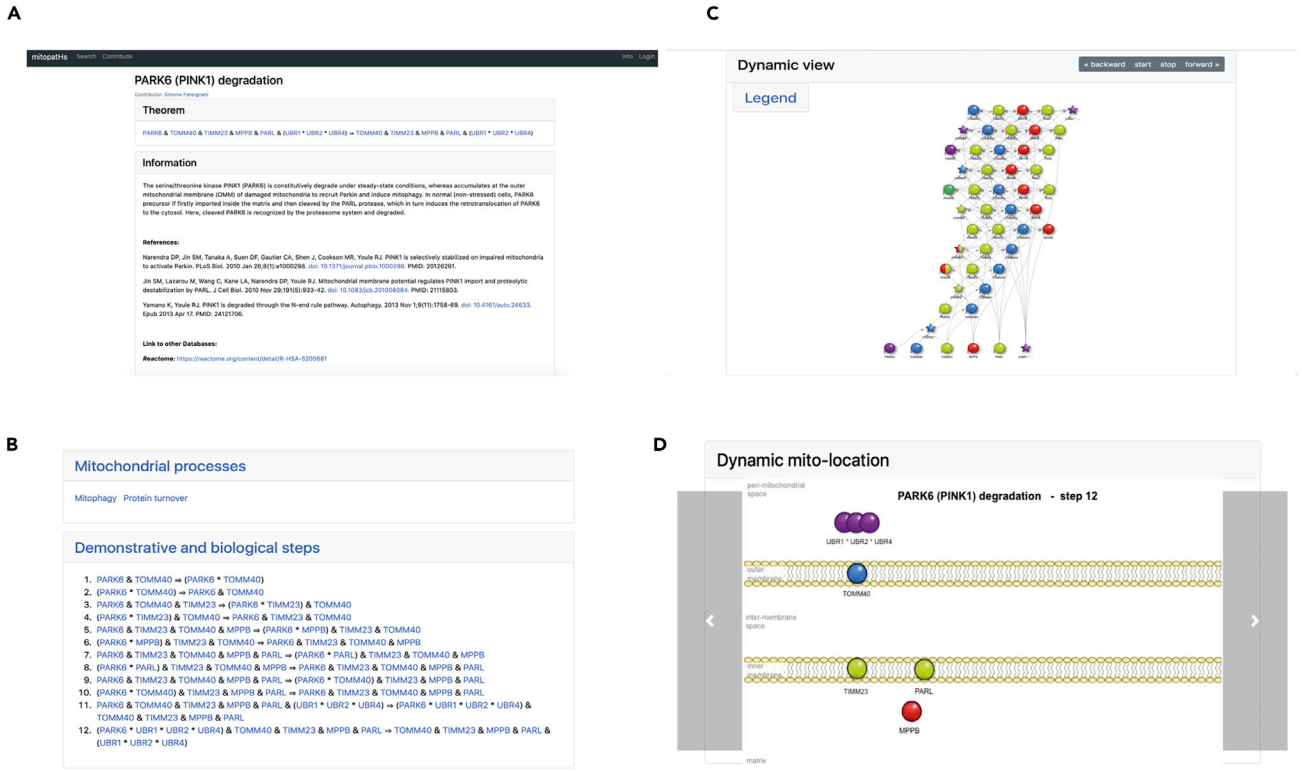

Figure 2. The interface of MitopatHs

Details about the selected pathway are displayed on a single web page. The top of the page displays the logical formalization of the pathway, based on Zsyntax, and a brief description of the process, as well as appropriate references are provided (A) The page also reports the mitochondrial processes in which the specific pathway is involved and the step-by-step reactions (B) The graphical information about the *dynamics* of process formation is displayed downwards and can be navigated through an automatic slow animation or manually, using buttons to move backward and forward (C) Finally, a dynamic mito-location allows visualizing the multiple reactions at different sub-mitochondrial districts (peri-mitochondrial space, outer mitochondrial membrane, inter-membrane space, inner mitochondrial membrane, and matrix) (D).

of internationally recognized researchers that boast consolidated experience in various aspects of mitochondrial biology. The team can be contacted via a dedicated form that can be accessed after the login step. The aim is to foster collaborative practices and exhaustively reporting the multiple (and occasionally opposite) interpretations about the functionalities of some molecular players, to provide an unbiased and comprehensive view on the mitochondrial activities that have been experimentally attributed to a specific protein. For example, see the different pathways described for LETM1 (https://web.math.unipd.it/mitopaths/pathway/Ca2—1—0H—1%20exchange and https://web.math.unipd.it/mitopaths/pathway/K—1—0H—1%20exchange).

Examples of data presentation

To explain how to read the logical information provided by MitopatHs, we illustrate two different kinds of pathways: (1) a protein complex formation (uniplex assembly) and (2) a protein degradation (SMDT1 degradation). In this way, the functionalities showed by our tool can be easily compared to other generic (not mitochondria-focused) pathway databases, such as KEGG or Reactome. A detailed explanation of the *Zsyntax* language is available in the methods section. We just recall that the symbol * is used to denotes the binding of two resources to form a complex, while the symbol & denotes the simple presence of two molecules in the same biological context, and ⇒ represents the occurrence of a biological step.

Protein complex formation

The assembly of the uniporter complex (uniplex) (Kamer and Mootha, 2015) is available at https://web.math.unipd.it/mitopaths/pathway/Uniplex+Assembly.

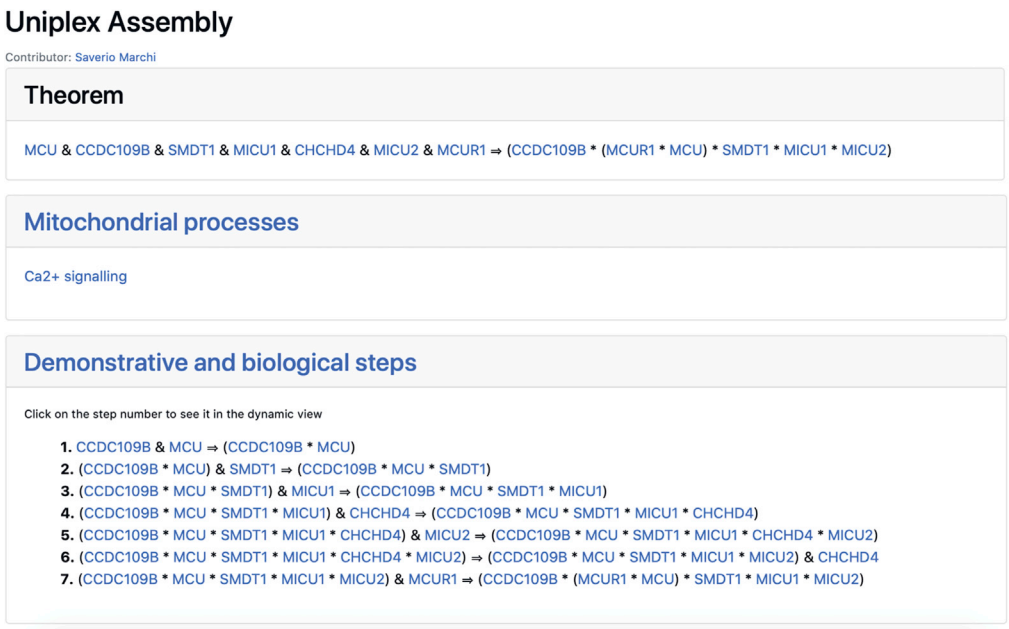

Figure 3. The "uniplex assembly" logical view

The overall pathway is presented as a theorem, whereas clicking on the box "Demonstrative and biological steps" allows the visualization of all the step-by-step reactions in Zsyntax language. Clicking on individual proteins pulls up additional information, including the link to Gene Ontology.

It belongs to the category of Ca^{2+} Signaling processes. It is defined as the following *Zsyntax* theorem (shown in Figure 3):

MCU & CCDC109B & SMDT1 & MICU1 & CHCHD4 & MICU2 & MICUR1 ⇒ (CCDC109B * (MCUR1 * MCU) * SMDT * MICU1 * MICU2).

The premise of the theorem, i.e. the left-hand side of the ⇒ symbol, lists all the proteins needed by the pathway, while the conclusion, i.e. the right-hand side, describes the final complex that is the Uniplex complex. Actually, in *Zsyntax* the conclusion of the theorem should contain also all the residual resources produced by the pathway, that is, the conclusion should be (CCDC109B* …) & CHCHD4 because the CHCHD4 protein is needed during the process but does not belong to the Uniplex complex, but we omit all the residuals for simplicity while keeping them in the graph visualization.

After presenting the overall pathway as a theorem, a click on the box "Demonstrative and biological steps" displays the sequence of logical steps (that is of EVFs) that prove the theorem and correspond to the actual sequence of biochemical reactions leading to the formation of the uniplex complex:

1. CCDC109B & MCU ⇒ (CCDC109B * MCU)
2. (CCDC109B * MCU) & SMDT1 ⇒ (CCDC109B * MCU * SMDT1)
3. (CCDC109B * MCU * SMDT1) & MICU1 ⇒ (CCDC109B * MCU * SMDT1 * MICU1)
4. (CCDC109B * MCU * SMDT1 * MICU1) & CHCHD4 ⇒ (CCDC109B * MCU * SMDT1 * MICU1 * CHCHD4)
5. (CCDC109B * MCU * SMDT1 * MICU1 * CHCHD4) & MICU2 ⇒ (CCDC109B * MCU * SMDT1 * MICU1 * CHCHD4 * MICU2)
6. (CCDC109B * MCU * SMDT1 * MICU1 * CHCHD4 * MICU2) ⇒ (CCDC109B * MCU * SMDT1 * MICU1 * MICU2) & CHCHD4
7. (CCDC109B * MCU * SMDT1 * MICU1 * MICU2) & MCUR1 ⇒ (CCDC109B * (MCUR1 * MCU) * SMDT1 * MICU1 * MICU2)

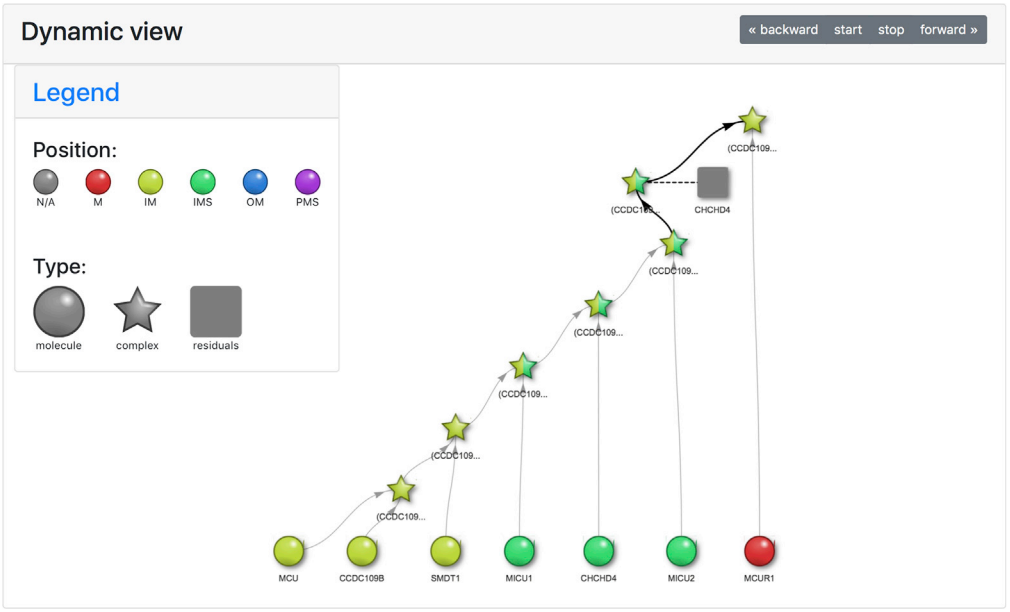

Figure 4. The "uniplex assembly" dynamic view

The final frame resumes all the different steps that lead to the formation of the uniporter complex. The multiple colors represent the different sub-mitochondrial localizations. Proteins are depicted as spheres, multi-protein complexes as stars. Residual proteins or molecules, which participate in the reaction but are not part of the final product, are depicted as squares. Clicking on the "backward" and "forward" buttons (top right corner), the user can dissect the entire pathway step-by-step or can navigate through an automatic slow animation ("start" button).

The first 2 steps describe the formation of the channel forming unit, composed by CCDC109B * MCU * SMDT1, whereas the passages from 3 to 6 report the assembly of the key regulators MICU1-2, which occurs in a CHCHD4-dependent fashion. Finally, the previous aggregate and MCUR1 react to producing the complex (CCDC109B * (MCUR1 * MCU) * SMDT1 * MICU1 * MICU2), which corresponds to the uniplex (Figures 3, 4, and 5). Each bond between the 6 proteins of the complex is represented by *, the brackets allow us to represent what is bond with what. In this case, MCUR1 interacts directly with MCU (Mallilankaraman et al., 2012; Tomar et al., 2016).

These seven steps are interactively illustrated by two different dynamic visualizations that can be navigated either through an automatic animation or by moving backward and forward by clicking on buttons. The graph-based visualization (Figure 4) illustrates which proteins in the initial aggregate bind to form an intermediate complex. In this view, the circle denotes a simple protein, the star denotes an intermediate or final complex, and the square denotes a residual protein. Colors denote the position of proteins and complex into the mitochondrion: red for the matrix, yellow for the internal membrane, green for the inter-membrane space, blue for the outer membrane, and violet for the peri-mitochondrial space. The gray color is reserved for unknown or irrelevant positions, while a star with multiple colors indicates a complex of proteins that occupy different sub-mitochondrial districts. For instance, the uppermost star in Figure 4 corresponds to the complex produced by the seventh step (its full *Zsyntax* name appears when pointing the mouse over the star, and simple graph rearrangements can be done through mouse dragging). The two edges entering that star show that this complex is the result of the interaction between the aggregate obtained in the previous step and the MCUR1 protein, residing on the mitochondrial matrix.

The internal structure of the complexes involved in the pathway is better visible in the second dynamic visualization provided by MitopatHs, the so-called dynamic mito-location. This view depicts *spatial* information about the pathway and clearly shows the location of the proteins and their aggregates inside the mitochondrion during the sequence of biochemical reactions. In Figure 5, we show the visualization of the sixth and seventh steps, to appreciate the final steps that conduct the structural association of the whole complex. Thus, the uniplex assembly pathway, as virtually all the processes managed by MitopatHs, can be inspected

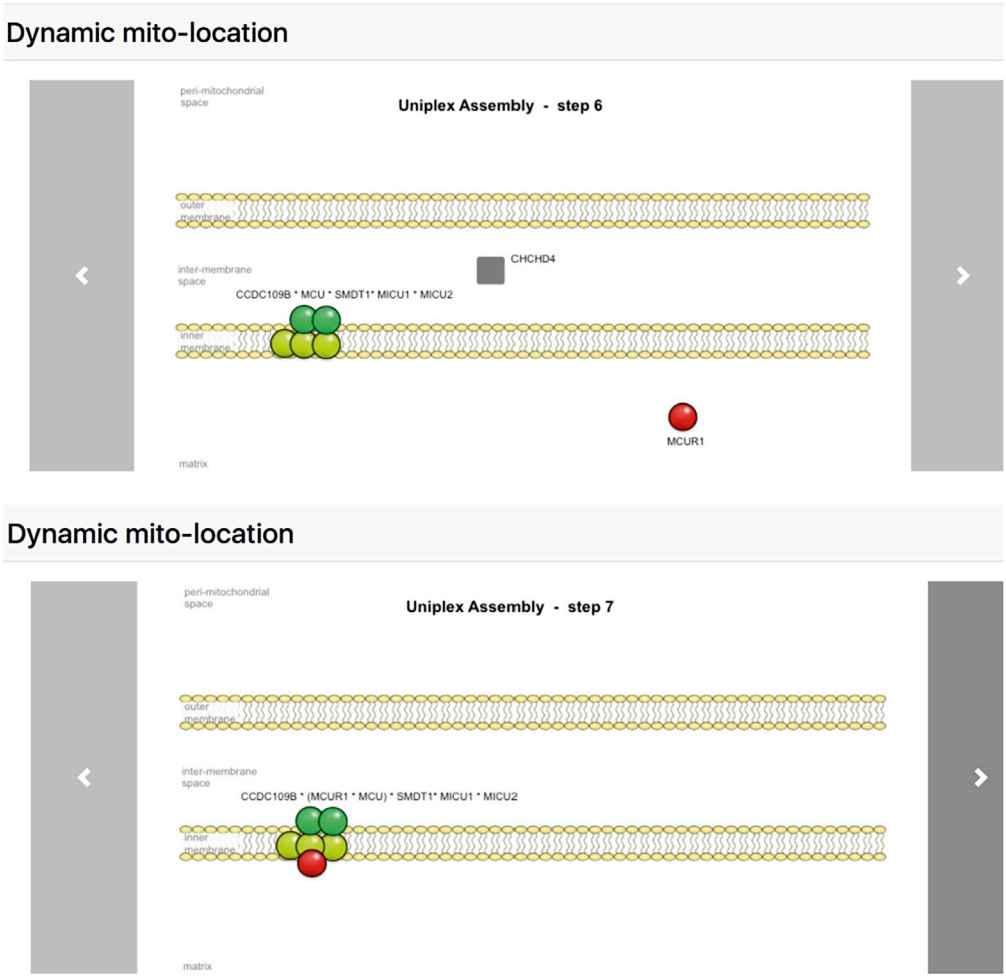

Figure 5. The "uniplex assembly" dynamic mito-location

In the last two steps, it is evident as CHCHD4, depicted as *residual* (gray square), is crucial for the proper uniplex assembly, but does not belong to the multi-protein complex (step 6). Conversely, MCUR1 effectively completes the channel by interacting with MCU (step 7).

in three different ways: the logical view (Figure 3), the graph-based visualization (Figure 4), and the spatial visualization (Figure 5).

Protein degradation

The SMDT1 degradation pathway, directly accessible at https://web.math.unipd.it/mitopaths/pathway/SMDT1+degradation, belongs to the categories of Ca^{2+} signaling and Protein turnover. The mAAA proteases AFG3L2 and SPG7 degrade the SMDT1 (also known as EMRE) forms that are not incorporated in the uniporter complex, to ensure the proper assembly and function of the uniplex (Konig et al., 2016; Tsai et al., 2017).

As illustrated by the Zsyntax mode, the SMDT1 degradation takes place in four steps:

1. AFG3L2 & SPG7 ⇒ (AFG3L2 * SPG7).
2. (AFG3L2 * SPG7) & SMDT1 ⇒ (AFG3L2 * SPG7 * SMDT1).
3. (AFG3L2 * SPG7 * SMDT1) & ATP ⇒ (AFG3L2 * SPG7 * SMDT1 * ATP).
4. (AFG3L2 * SPG7 * SMDT1 * ATP) ⇒ (AFG3L2 * SPG7) & ADP.

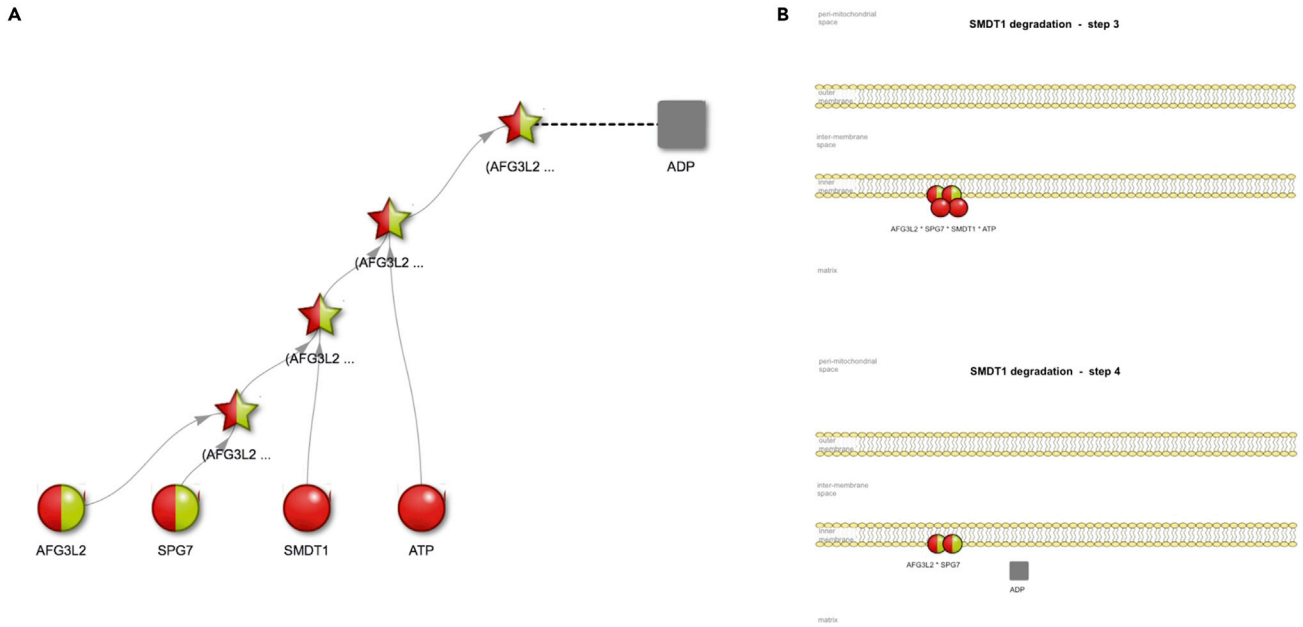

Figure 6. The "SMDT1 degradation" pathway

The dynamic view (A) and mito-location (B), showed as SMDT1 is degraded (identified as disappearance) by the AFG3L2-SPG7 complex, located at the IMM, facing the mitochondrial matrix, through the energy of ATP hydrolysis (identified as *residual* ADP).

Notably, the fourth step points out as SMDT1 is degraded (identified as disappearance) through the energy of ATP hydrolysis (identified as *residual* ADP). Figure 6 shows the dynamic visualization of this pathway, where the proteins AFG3L2 and SPG7 have a double color, since they are located at the inner membrane facing the mitochondrial matrix.

Distinctive features of the MitopatHs tool

The uniqueness of MitopatHs resides in its logical structure, which does not result only in presenting a series of reactions as a logical theorem, but allows for precisely digging deep into a specific molecular pathway, going beyond what is available elsewhere, such as KEGG, Reactome, or HumanCyc.

Looking at MitopatHs visualization of the uniplex assembly, it is easy to comprehend many molecular details of the pathway, including the interactions between the different subunits, their intra-mitochondrial localization, and the role of other factors, such as CHCHD4, (also known as Mia40), which mediates the link between MICU1 and its paralog MICU2 in a heterodimer (Petrungaro et al., 2015) without being an integral member of the Uniporter complex. Indeed, in step 6, CHCHD4 appears as a *residual* protein ((CCDC109B * MCU * SMDT1 * MICU1 * MICU2) & CHCHD4), indicating its role as an accessory factor for the correct formation of the Uniplex (Figures 3, 4, and 5). All this information cannot be extracted by visiting the corresponding pathway in Reactome (https://reactome.org/content/detail/R-HSA-8949151 and https://reactome.org/content/detail/R-HSA-8953461), KEGG (https://www.kegg.jp/kegg-bin/highlight_pathway?scale=1.0&map=map04020&keyword=mcu), or HumanCyc (https://humancyc.org/gene?orgid=HUMAN&id=G66-37888#). Similar considerations can be drawn from the comparison between the "SMDT1 degradation pathway" and its counterpart in Reactome (https://reactome.org/content/detail/R-HSA-8949659), or by analyzing the PARK6 (PINK1) degradation pathway in Mitopaths (https://web.math.unipd.it/mitopaths/pathway/PARK6%20(PINK1)%20degradation), which contains more molecular details than 3 different pathways available at Reactome (https://reactome.org/content/detail/R-HSA-5205681, https://reactome.org/content/detail/R-HSA-5205661 and https://reactome.org/content/detail/R-HSA-5205672). Of note, these processes are not described in KEGG and HumanCyc. Thus, although generic databases comprise pathways of all different organelles and are extremely useful to place the mitochondrial compartment in the whole cellular scenario, MitopatHs provides a mitochondrial-focused tool for the study of reactions and biological functions.

DISCUSSION

The web interface of MitopatHs has been designed to promote a data visualization style that is simple and uniform, to immediately understand the meaning of mitochondrial pathways, and connect them with other related biochemical processes. Therefore, MitopatHs is remarkably useful for those researchers that have limited experience in mitochondrial biology, which may come across the mitochondrial compartment during their research, such as by pinpointing a mitochondrial protein as a promised candidate during a genetic screen or identifying the deregulation of a mitochondrial process as a key pathogenic event of a certain disease. By entering the name of the protein in MitopatHs homepage, they can immediately have an idea of its role in mitochondrial activities, recognizing the binding partners and following the step-by-step reactions that characterize a specific pathway.

MitopatHs currently hosts 22 pathways, divided into 12 categories, and involving 69 proteins. This represents roughly 6% of the mitochondrial proteome (Rath et al., 2021) and 10% of the mito-proteins with known functions. However, the tool is conceived to foster a collaborative effort to collect and systematize the mitochondrial information, thereby promoting open science and the construction of shared knowledgeable information. Thus, with the launch of MitopatHs, we hope to rapidly increase the number of mitochondrial pathways (including further mutated pathways associated with diseases), by incorporating the suggestions from any researcher through the link "Contribute", located in the upper left corner of the site (Figure 1). Over the next 3 years from the launch of MitopatHs, we aim to curate all the mitochondrial proteins and describe approximately 1000 different molecular pathways.

Limitations of the study

The current version of MitopatHs displays some important limitations. First, it does not provide information on the protein structure or the nature of the molecular interactions between different protein partners. Second, it does not take into account well-known cytosolic (non-mitochondrial) factors that have been reported to act also inside the organelle, such as AKT or TP53 (Marchi et al., 2019; Vaseva et al., 2012). Third, in some cases (as for the "complex assembly" pathways), we present a sequence of events/reactions, expressed through *Zsyntax* steps, although some of them might occur simultaneously. Nonetheless, for large multi-protein complexes, the correct stoichiometry between the different subunits may not be reported. For example, in the "uniplex assembly" pathway, we proposed that MCU firstly reacts with CCDC109B and SMDT1/EMRE to generate the core component of the complex (first and second reactions of the pathway), based on the logical deduction that the regulatory elements could assemble after the constitution of the pore. Moreover, the MCU assembles as a tetramer (Fan et al., 2020), together with multiple MICU or SMDT1/EMRE subunits (Payne et al., 2020; Wang et al., 2019). Since many aspects are not yet fully elucidated, largely due to the "young age" of the uniplex (the first component has been discovered in 2011 (Baughman et al., 2011; De Stefani et al., 2011)), we decided not to include these elements in the overall visualization, although we report such information in the main description of the pathway. However, any comment or issue about the pathways formalization can be submitted through the "Contribute" link and, after validation, can be added to the textual descriptions.

Last, since the *Z-interaction* operator * is binary, it cannot immediately express a direct bind event of more than two proteins without resorting to coupling them into sub-complexes. Moreover, the molecule bindings expressed in MitopatHs overcome the commutativity and associativity properties of the logical operator *, thus it is not possible to rely here on the automated theorem prover available for *Zsyntax* (Boniolo et al., 2015) to automatically infer whether a given complex is reachable from a given set of proteins through a (chain of) mitochondrial pathway.

Future perspectives

Future development of the software will focus on adding a static image that represents all the multiple biological steps of a specific pathway in the spatiality of the mitochondrial compartment, as well as a 3D animation for reproducing the entire process. Moreover, we aim to provide the option to export every single reaction (showed in the "Dynamic mito-location") as a PowerPoint slide (https://www.microsoft.com/it-it/microsoft-365/powerpoint). The software is built in a modular way, so that every operation on the knowledge base is performed through an API system based on JSON message passing, including pathway information retrieval or insertion of new data. This will simplify future integrations with other systems or enhanced graphical interfaces, that can be independent of the internal implementation details.

As mentioned above, a limitation of the tool is to not cover the activities of those molecular factors that translocate to mitochondria only upon certain conditions, or act in the surrounding area of the organelle. Our mid-term plan is to implement the MitopatHs resource with this information, with particular focus on the proteins located at the endoplasmic reticulum (ER)-mitochondria contact sites, also known as ER-mitochondria-associated membranes (Marchi et al., 2014; Perrone et al., 2020; Wu et al., 2018). In this manner, we will report and describe the functions of those proteins that (1) display additional intracellular localization, (2) transiently reside inside mitochondria (during specific physio-pathological scenarios), or (3) act onto mitochondria, to expand the wide range of biological pathways in which mitochondria are involved.

Resource availability

Lead contact

S. Marchi is the lead contact for this study.

Materials availability

There are no physical materials associated with this study.

Data and code availability

The tool generated during this study is available at https://web.math.unipd.it/mitopaths/

The source code of the web service is freely available at https://github.com/mitopaths. Data about proteins and pathways are available for download through MitopatHs' search API system, which is documented at https://web.math.unipd.it/mitopaths/documentation/api. Such a system, which is the same internally used by the web service, allows retrieving every piece of information stored by MitopatHs in JSON format, which is suitable for later storage, processing, or display.

METHODS

All methods can be found in the accompanying Transparent methods supplemental file.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.102324.

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The Graphical Abstract has been created with BioRender.com.

AUTHOR CONTRIBUTIONS

S.M., P.P., S.C., and G.B. conceived the project. M.Z. constructed the web server. S.M,. M.Z., and S.C. curated the formalization of Zsyntax theorems and graphical visualization. S.M., S.C., and G.B. wrote the manuscript. All authors read and approved the final version of the manuscript.

DECLARATION OF INTERESTS

The authors declare that they have no competing interests.

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