



How evolution dismantles and reassembles multienzyme complexes

Andrea Mattevi^{a,1}

The function and architecture of the pyruvate dehydrogenase (PDH) multienzyme complex is one of the most fascinating and fundamental topics covered by basic biochemistry courses (1–4). PDH converts the pyruvate generated by glycolysis to acetylCoA that can then be fueled into the Krebs cycle. Three enzymes—E1p, E2p, and E3—and five cofactors are involved in the overall PDH reaction. The E1p component uses thiamine phosphate to decarboxylate pyruvate and attaches the resulting acetyl group to an oxidized lipoamide carried by a E2p enzyme. The catalytic domain of E2p then transfers the acetyl group to CoA, generating the acetylCoA product. An E3 enzyme finally reoxidizes lipoamide to generate NADH. Multiple copies of the three enzymes form large megadalton complexes that share a similar design and mechanism of function beside variable compositions among species. In PNAS, Bruch et al. (5) show that the mycobacterial PDH features an unusual architecture that may allow it to function in combination with other α -ketoacid dehydrogenase complexes. This design may reflect specific metabolic and regulatory needs by *Mycobacteria* and related microorganisms.

With its flexible lipoyl arms and a rigid catalytic domain, the multidomain E2p forms the core of the PDH complexes that may comprise 24, or even 60, E2p chains (6). The E1p and E3 enzymes bind to the peripheral subunit-binding domain of E2p, creating a remarkable assembly whereby the peripheral subunits and domains can fluctuate while being anchored to the E2p catalytic domains. The lipoyl arms can thereby visit the active sites of E1p, E2p, and E3 to efficiently channel substrates and products across the enzymes (1). Channeling avoids the inappropriate diversion of reaction intermediates to other metabolic pathways. Likewise, the reductive power generated by the oxidative decarboxylation of pyruvate is wholly directed to NAD⁺ without any side reaction or reactive oxygen species generation.

E2p is part of a family of acyltransferases whose prototype is chloramphenicol acetyltransferase, an enzyme involved in antibiotic resistance (7). Members of this family generally feature a trimeric organization. In the case of E2p, the trimer is tightened through a strand exchange structural mechanism: The large β -sheet at the center of the E2p catalytic domain is extended by residues of a nearby subunit that form an additional “invading” β -strand (8). With its compact triangular shape, the highly conserved E2p trimer functions as a building block for the construction of the higher-order assemblies that form the PDH cores. In gram-negative bacteria, eight trimers form a 432-symmetric cube (7). Each trimer is located on a vertex of the cube and its orientation positions the flexible lipoyl arms and peripheral subunit-binding domains on the cube’s external surface. In gram-positive bacteria and eukaryotes, the E2p core is typically formed by 20 trimers that come together to form a 532-symmetric icosahedron with the trimers located at the triangular vertices of the icosahedron (Fig. 1A). How can virtually identical trimers equally form both cubes and icosahedra? The issue can be discussed with reference to quasi-equivalence (9). This concept was originally applied in biology to explain the similar, but not identical, subunit packing in the icosahedral viruses comprising more than 60 identical units (10). In the PDH cores, the interactions between trimers are mediated by a 3_{10} -helical segment formed by the E2p C-terminal residues. These short helices protrude from every E2p to reach out toward a small pocket on a subunit of an adjacent trimer (Fig. 1B). Though involving only a few amino acids, these interactions are sufficiently strong to tighten the multimeric PDH cores. Small variations in the protruding direction of the C-terminal 3_{10} -helices allow the trimers to change their orientations and assemble either as cubic or icosahedral cores whose intertrimer interactions can be described as “quasi-equivalent.” This concept found further confirmation from the analysis

^aDepartment of Biology and Biotechnology, University of Pavia, 27100 Pavia, Italy

Author contributions: A.M. wrote the paper.

The author declares no competing interest.

This open access article is distributed under [Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 \(CC BY-NC-ND\)](https://creativecommons.org/licenses/by-nc-nd/4.0/).

See companion article, “Actinobacteria challenge the paradigm: A unique protein architecture for a well-known, central metabolic complex,” [10.1073/pnas.2112107118](https://doi.org/10.1073/pnas.2112107118).

¹Email: andrea.mattevi@unipv.it.

Published December 22, 2021.

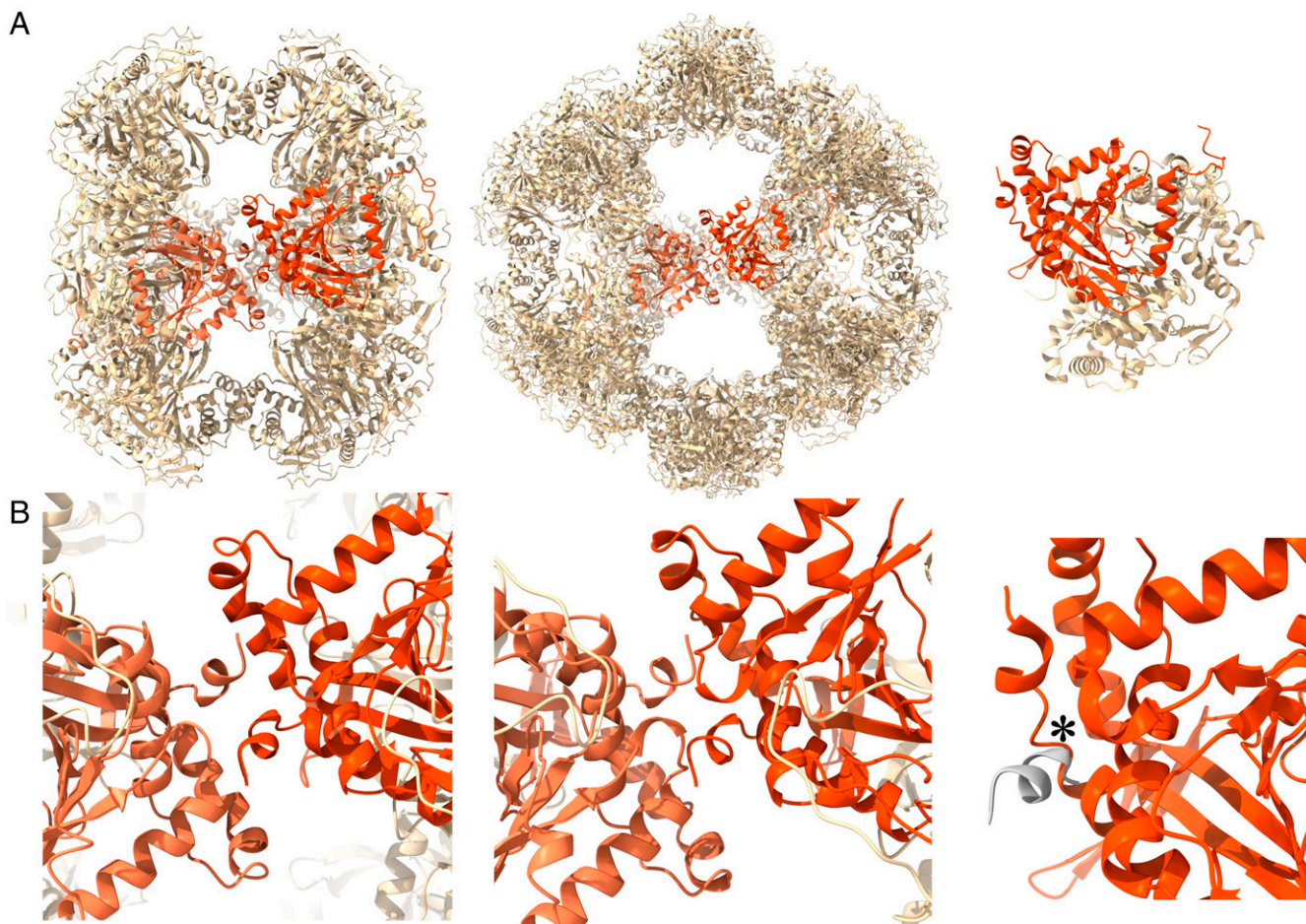


Fig. 1. Symmetry diversity among the PDH complexes. (A) The cubic core formed by *Azotobacter vinelandii* E2p (Protein Data Bank [PDB] ID code 1eae, *Left*), the icosahedral core formed by *Geobacillus stearothermophilus* E2p (PDB ID code 1B5S, *Middle*), and the trimeric *C. glutamicum* E2p (PDB ID code 6zzk, *Right*). Only the catalytic domains are shown. In A and B, the complexes are viewed along their two-fold axis and two subunits at the trimer-trimer interface are shown with two shades of red. (B) Zoomed-in views of the trimer-trimer interface in the cubic (*Left*) and icosahedral (*Middle*) cores. The equivalent residues of the trimeric *C. glutamicum* E2p are also shown (*Right*). In the trimeric E2p, the C-terminal 3_{10} -helix is preceded by a three-residue insertion (marked by an asterisk) and differently oriented compared to the equivalent residues of a cube-forming E2p (in light gray). The orientations are the same as in A.

of the PDH-related 2-oxoglutarate dehydrogenase (part of the Krebs cycle) and branched-chain α -ketoacid dehydrogenase (amino acid catabolism) complexes. The E2 components of these systems can assemble into cubic or icosahedral cores following the same structural principles governing E2p multimerization (11).

Bruch et al. now report on another variation on the theme (5). They show that the PDH complexes from many *Actinobacteria*, including the *Mycobacteria*, simply consist of an E2p trimer. No high-order assemblies could be detected under any experimental conditions using small-angle X-ray scattering or other solution methods. The crystal structures of E2p from *Mycobacterium tuberculosis* and *Corynebacterium glutamicum* rationalized the minimal low-symmetry composition featured by the actinobacterial PDH. The catalytic domain of E2p forms a standard trimer as observed in the larger PDH complexes. Similarly, the 3_{10} -helical conformation of the C-terminal residues is conserved. However, there is a critical difference: The helix is oriented inward to interact with residues of its own protein chain (Fig. 1B). This conformation and associated intrasubunit interactions make the C-terminal residues unavailable for establishing

the intertrimer contacts underlying the cubic and icosahedral E2p cores. Structural comparisons further reveal that a three-residue insertion is located just before 3_{10} -helical residues. The inserted amino acids enable the C-terminal 3_{10} -helix to turn back toward its own subunit, determining the inward conformation of the *M. tuberculosis* and *C. glutamicum* structures (Fig. 1B). Bruch et al. (5) go further and explore the use of this three-residue insertion as a fingerprint motif to identify other E2p sequences potentially featuring a trimeric assembly and unable to form large multimers. Their analysis convincingly shows that most actinobacterial E2p sequences contain this insertion, which is instead not present in other phyla. Curiously, the E2 components of the branched-chain α -ketoacid dehydrogenase of the actinobacteria do not possess the fingerprint insertion and form classical cubes as demonstrated by cryogenic electron microscopy analysis. Clearly, evolution has developed a simple and yet effective strategy to dismantle the large multimeric E2p assemblies and form the less-conspicuous trimeric cores of the actinobacterial PDH complexes.

The presence of the three-residue insertion in E2p strictly correlates with the presence of a gene encoding for a protein known as

OdhA. As originally described by Usuda et al. (12), OdhA naturally fuses the catalytic domains of the decarboxylase and transferase components of the 2-oxoglutarate dehydrogenase that are merged into a single protein devoid of any lipoyl domain. The organisms featuring OdhA further lack the genes encoding for the standard E1 and E2 components of the 2-oxoglutarate dehydrogenase complex. Why does such a reshuffling of domains occur? It is speculated that OdhA marks the presence of hybrid supercomplexes. OdhA might be loosely associated or even form stable heterooligomers with the trimeric E2p core and/or E1p component of the low-symmetry actinobacterial PDH. This hypothesis implies that the lipoyl domains of the E2p components, as well as the E3 enzyme of the PDH, may serve the active sites of E1p and E2p as well as of OdhA. The electrons deriving from pyruvate and oxoglutarate decarboxylation would thereby reach the same NADH-reducing E3 enzymes. This hypothesis raises several interesting questions. How would such multicatalytic supercomplexes be assembled? How can the lipoyl domains cope with five different enzymatic reactions? Does the envisioned supercomplex feature any high-order symmetry that somehow resembles the classical PDH and related complexes? Addressing these issues through structural and enzymological studies will be the challenging next frontier for the field.

The low-symmetry and structural variations featured by the actinobacterial PDHs further pose a more general question: Why can the symmetry and composition of the PDH and similar complexes so largely vary across different phyla and along evolution? The tenet is that the characteristic combination of rigidity and flexibility together with the physical proximity of multiple active sites adds to the efficiency of the PDHs that can

operate through the controlled diffusion and channeling of the substrates, intermediates, and products (1, 12, 13). However, *Actinobacteria* demonstrate that the metabolic needs can also be met by lower-symmetry complexes that may even mix pyruvate and oxoglutarate decarboxylation activities, normally performed by distinct complexes. Thus, substrate channeling may only be one side of the coin. The PDHs and similar complexes can be viewed as nanocompartments with locally enhanced metabolite densities that can be especially sensitive to the metabolic state of the cell. Variations in the number and stoichiometry of the composing enzymes can largely alter the trafficking of metabolites (14, 15). In *Actinobacteria*, the postulated colocalization of pyruvate and oxoglutarate decarboxylations in the same assembly may enable the coregulation of two NADH-producing reactions. In eukaryotes, PDHs are regulated through phosphorylation by the PDH kinases, or PDKs. The large multimeric nature of the eukaryotic PDH is likely to amplify this signaling cue, gatekeeping the entry to the Krebs cycle (16). Enzymology and metabolism can now rely on powerful tools to study protein evolution (17) and metabolic fluxes (18). The results by Bruch et al. (5) highlight the PDH as an excellent system to investigate the interplay of enzyme and metabolism regulation with the evolution of protein oligomerization and assembly.

Acknowledgments

I express my profound gratitude to my PhD mentor, Prof. Wim Hol, who supervised my research on PDH. I thank Andrea Gottinger for his help in preparing Fig. 1. The laboratory is supported by the Association Italiana Ricerca sul Cancro, Fondazione Cariplo, Italian Ministry for University and Research, and EU-H2020.

- 1 R. N. Perham, Swinging arms and swinging domains in multifunctional enzymes: Catalytic machines for multistep reactions. *Annu. Rev. Biochem.* **69**, 961–1004 (2000).
- 2 J. R. Guest, S. J. Angier, G. C. Russell, Structure, expression, and protein engineering of the pyruvate dehydrogenase complex of *Escherichia coli*. *Ann. N. Y. Acad. Sci.* **573**, 76–99 (1989).
- 3 L. J. Reed, From lipoic acid to multi-enzyme complexes. *Protein Sci.* **7**, 220–224 (1998).
- 4 M. Smolle, J. G. Lindsay, Molecular architecture of the pyruvate dehydrogenase complex: Bridging the gap. *Biochem. Soc. Trans.* **34**, 815–818 (2006).
- 5 E. M. Bruch et al., *Actinobacteria* challenge the paradigm: A unique protein architecture for a well-known, central metabolic complex. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2112107118 (2021).
- 6 L. J. Reed, M. L. Hackert, Structure-function relationships in dihydroliipoamide acyltransferases. *J. Biol. Chem.* **265**, 8971–8974 (1990).
- 7 W. V. Shaw, A. G. Leslie, Chloramphenicol acetyltransferase. *Annu. Rev. Biophys. Chem.* **20**, 363–386 (1991).
- 8 A. Mattevi et al., Atomic structure of the cubic core of the pyruvate dehydrogenase multienzyme complex. *Science* **255**, 1544–1550 (1992).
- 9 T. Izard et al., Principles of quasi-equivalence and Euclidean geometry govern the assembly of cubic and dodecahedral cores of pyruvate dehydrogenase complexes. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 1240–1245 (1999).
- 10 D. L. Caspar, A. Klug, Physical principles in the construction of regular viruses. *Cold Spring Harb. Symp. Quant. Biol.* **27**, 1–24 (1962).
- 11 A. Aevansson, K. Seger, S. Turley, J. R. Sokatch, W. G. Hol, Crystal structure of 2-oxoisovalerate and dehydrogenase and the architecture of 2-oxo acid dehydrogenase multienzyme complexes. *Nat. Struct. Biol.* **6**, 785–792 (1999).
- 12 Y. Usuda et al., Molecular cloning of the *Corynebacterium glutamicum* ('*Brevibacterium lactofermentum*' AJ12036) *odhA* gene encoding a novel type of 2-oxoglutarate dehydrogenase. *Microbiology (Reading)* **142**, 3347–3354 (1996).
- 13 S. Prajapati et al., Structural and functional analyses of the human PDH complex suggest a "division-of-labor" mechanism by local E1 and E3 clusters. *Structure* **27**, 1124–1136.e4 (2019).
- 14 S. Hezaveh, A. P. Zeng, U. Jandt, Full enzyme complex simulation: Interactions in human pyruvate dehydrogenase complex. *J. Chem. Inf. Model.* **58**, 362–369 (2018).
- 15 J. Yi et al., Effect of substitutions in the thiamin diphosphate-magnesium fold on the activation of the pyruvate dehydrogenase complex from *Escherichia coli* by cofactors and substrate. *J. Biol. Chem.* **271**, 33192–33200 (1996).
- 16 S. L. Zhang, X. Hu, W. Zhang, H. Yao, K. Y. Tam, Development of pyruvate dehydrogenase kinase inhibitors in medicinal chemistry with particular emphasis as anticancer agents. *Drug Discov. Today* **20**, 1112–1119 (2015).
- 17 G. K. A. Hochberg et al., A hydrophobic ratchet entrenches molecular complexes. *Nature* **588**, 503–508 (2020).
- 18 V. Pareek, H. Tian, N. Winograd, S. J. Benkovic, Metabolomics and mass spectrometry imaging reveal channeled de novo purine synthesis in cells. *Science* **368**, 283–290 (2020).