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ORIGINAL PAPER

Constraint-based reasoning in cell biology: on the explanatory role of context

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Abstract

Cell biologists, including those seeking molecular mechanistic explanations of cellular phenomena, frequently rely on experimental strategies focused on identifying the cellular context relevant to their investigations. We suggest that such practices can be understood as a *guided decomposition* strategy, where molecular explanations of phenomena are defined in relation to natural contextual (cell) boundaries. This "top-down" strategy contrasts with "bottom-up" reductionist approaches where well-defined molecular structures and activities are orphaned by their displacement from actual biological functions. We focus on the central role of microscopic imaging in cell biology to uncover possible constraints on the system. We show how identified constraints are used heuristically to limit possible mechanistic explanations to those that are biologically meaningful. Historical examples of this process described here include discovery of the mechanism of oxidative phosphorylation in mitochondria, molecular explanation of the first steps in protein secretion, and identification of molecular motors. We suggest that these instances are examples of a form of downward causation or, more specifically, *constraining relations*, where higher-level structures and variables delimit and enable lower-level system states. The guided decomposition strategy in our historical cases illustrates the irreducibility of experimentally identified constraints in explaining biological activities of cells. Rather than viewing decomposition and recomposition as separate epistemic activities, we contend that they need to be iteratively integrated to account for the ontological complexity of multi-level systems.

Keywords Mechanistic heuristics · Guided decomposition · Recomposition · Cell biology · Constraints · Constraint-based reasoning · Protein secretion · Oxidative phosphorylation · Molecular motors

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1 Introduction

Philosophical and historical case analyses of experimentation and theorizing in biology provide insights into the reasoning strategies used to explain biological phenomena. In their influential book *Discovering Complexity*, William Bechtel and Robert Richardson (1993/2010) used such cases to articulate a set of heuristic strategies to achieve mechanistic explanations as a combination of *decomposition*, *localization*, and *recomposition*. A mechanism is in this context understood as "a structure performing a function in virtue of its component parts, component operations, and their organization" (Bechtel, 2006, p. 26). On this account, mechanistic heuristic strategies are depicted as both *reductionistic* and *holistic*. They are reductionistic because scientists must decompose biological systems into simpler units and localize parts on which to intervene. They are holistic because mechanistic explanations also rely on recomposition to situate mechanisms in the context of a larger biological system. It remains unclear, however, how recomposition can be accomplished such that there is confidence that the resulting explanation of a biological phenomenon accounts for the mechanism operating in the living system. The aim of this paper is to explore this philosophical question through historical case studies from cell biology.

From a perspective of "ruthless reductionism" (Bickle, 2003), contextual features may be seen as merely providing the background conditions for causal explanations. In Bickle's account from molecular neurobiology, the mechanism stays the same throughout the discovery process, and rather than a holistic strategy, recomposition amounts to simply adding back in the initial or background conditions, such as cellular parameters, after characterizing the main explanandum: the molecular mechanisms. Similarly, among some molecular biologists there is the assumption that close examination of individual molecules or collections of molecules is sufficient for achieving a mechanistic account of biological phenomena. As Alexander Powell and John Dupré note, molecular biology historically encouraged "the belief that a detailed understanding of individual molecular properties may be sufficient to account fully for cellular and organismic phenomena" (Powell & Dupré, 2009, p. 56). What this extreme reductionistic viewpoint ignores, however, is that the context provided by upper levels of organization are crucial elements of the molecular explanation.

In the revised introduction to *Discovering Complexity*, Bechtel and Richardson (1993/2010) stress that mechanistic heuristics are fallible or even "false" (cf. Wimsatt, 2007) in the sense that they imply a provisional assumption that biological systems can be decomposed into localizable operations, like the workings of a machine. Living systems, however, are not always neatly organized into functional modules, but are at best "near-decomposable" (Simon, 1962, 1977). As a result, mechanistic heuristics entail an inherent risk of neglecting aspects of biological systems that cannot be localized and decomposed into independent operations. According to Bechtel and Richardson, recomposition often fails, either because not all critical components are identified or because "the organization was not adequately recovered in the reconstituted system" (p. xxxviii). Hence, recomposition is not merely a simple process of mixing together the elements studied through reductionistic analysis but must also recover in the reconstituted system crucial aspects of the original system's organization.

This paper offers further support to the view that recomposition requires more than integration of knowledge gleaned from reductionist analysis. But we also take a further step in questioning whether recomposition and decomposition should be considered as separate activities. In our view, an analysis of biological systems requires what we refer to as *guided decomposition*, an epistemic strategy that continuously considers the contextual boundary conditions as constraints upon biological mechanisms (Matlin, 2022, p. 271). In essence, guided decomposition consists of a series of recursive steps of decomposition *guided* by a holistic view of the biological system that is referenced throughout the decomposition process (Matlin, 2022). How this works in practice is described in further detail in the following case studies.

As defined by Sara Green and Nicholaos Jones, "constraints are conditions—or representations of conditions—that both limit and afford a certain scope of possible structures and functions that can be instantiated in a system of a particular type" (Green & Jones, 2016, pp. 345–346). We suggest that guided decomposition is a type of *constraint-based reasoning*, where scientists identify how higher-level constraints simultaneously *limit* some possibilities of lower-level states but also *enable* functions that would be impossible in an unconstrained system (cf. Hooker, 2013). A simple example is how a rigid vertebrate skeleton is a structural constraint that simultaneously limits the flexibility of bodily movements but also enables an upright position that resists gravitational forces on land. Similarly, we argue, cellular and subcellular constraints are not merely background conditions for molecular explanations in cell biology, but central to how the mechanisms function. Drawing on this conceptual framework, we suggest that the epistemic strategy employed in historical cases from cell biology can be interpreted as attempts to understand biological mechanisms through the utilization of concrete cellular constraints, such as lipid membranes and cytoskeletal elements.

So far, constraint-based reasoning has mainly been discussed in the context of systems biology as a heuristic strategy to identify patterns of structural organization through abstraction from detail, typically involving mathematical modeling (Green & Jones, 2016). But constraints are also central for the disciplinary identity and methods of cell biology, as cell biologists aim to understand the distinctive ontological features of cells and cellular structures. Thus, compared to molecular biology, cell biology engages in investigations of macro-scale constraints on molecular processes. As highlighted by the *Stanford Encyclopedia of Philosophy* entry on Cell Biology: "Constraints […] account for why macro-scale objects exhibit different properties than their constituents. Constraints are not explained by laws but rather serve as boundary conditions that must be ascertained empirically. Accordingly, to the degree constraints explain biological activities of cells, these activities cannot be reduced, in the sense of being derived from the principles of chemistry or physics. Instead, researchers must, on the basis of empirical inquiry, identify the constraints actually realized in living cells" (Bechtel & Bollhagen, 2019, pp. 21–22). This quote highlights how the epistemic *and* ontological autonomy of higher-level structures are central to cell biology. Explaining the biological activities of cells thus hinges on experimental identification of constraints and their impact on the mechanism of interest. Bechtel and Bollhagen exemplify the importance of constraints through the example of feedback control in gene regulatory networks (see also Winning &

Bechtel, 2018). This example, however, does not completely capture constraints of specific focus to cell biology, compared to molecular or systems biology. We suggest that more attention should be given to the epistemic strategies of past and present practices of cell biology.

In this paper, we describe how constraint-based reasoning contributes to the discovery of molecular mechanisms that explain cellular phenomena. Both the history and current practice of cell biology are replete with relevant examples. Among these are the discovery of the mechanism of mitochondrial oxidative phosphorylation (1950–1970s), the study of protein targeting and transmembrane transport (translocation) in protein secretion (1950–1990s), and identification of motor proteins involved in neuronal vesicle transport (1980s). Here we focus on all of these. While aspects of the cases have been examined in detail in previous work (Weber, 1991, 2005; Rasmussen, 1995; Allchin, 1996, 1997; Prebble, 2001; Bechtel, 2006; Weber and Prebble, 2006; Prebble, 2010; Scholl & Nickelsen, 2015; Matlin, 2016, 2018, 2020, 2022), they have not been viewed through the conceptual framework of constraint-based reasoning. In the final sections, we discuss the general features of constraint-based guided decomposition that transcend individual cases and link the strategy of constraint-based reasoning to ontological implications that are currently discussed through the notion of downward causation in the philosophical literature.

2 Cell biology: a rich field for philosophical insights

The discipline of cell biology developed in the mid-twentieth century (Bechtel, 2006; Moberg, 2012; Matlin, 2016, 2022; Matlin et al., 2018). Like its nineteenth-century cousin cytology, cell biology relies on microscopy to identify and investigate cellular phenomena. It's original innovation, however, was the integration of microscopy with the controlled and systematic disruption of cells and isolation of cell parts, often followed by the biochemical analysis of those parts. In this manner, cell biology overcame the hesitancy of cytologists to physically disrupt cellular organization to get at the chemical mechanisms underlying many cellular phenomena (Matlin, 2022). In the twentieth century and beyond, this integrated strategy led to mechanistic explanations at the molecular level of many cellular processes. While utilizing biochemical techniques, cell biology was (and is) distinct from the discipline of biochemistry which, traditionally, relied upon the complete disruption of cells to isolate and purify individual enzymes and other substances prior to any investigation of their activities and functions (Matlin, 2016, 2022; Bechtel & Bollhagen, 2019). This sort of reductionistic bottom-up strategy was by the 1950s and 1960s mimicked epistemologically by a form of molecular biology that relied upon detailed structural analysis of biological macromolecules (Kendrew, 1967).

During the nineteenth century, cytologists' use of improved optical microscopes and innovative staining techniques revealed that eukaryotic cells in particular contain a variety of inclusions. With the exception of the nucleus, many of these were believed to be non-living and temporary *formed elements* in the cytoplasm. By the end of the first quarter of the twentieth century biologists accepted that cells are bounded by a plasma membrane and that at least some inclusions are also membrane-bounded

and are permanent features of all cells (Matlin, 2022). One prominent example is the mitochondrion that was visible in living cells stained with the vital dye Janus Green. This implied that mitochondria are associated with specific chemical activities and, by analogy with the physiology of more complex multicellular organisms, are an organ or *organelle* of the cell. By mid-century, use of the electron microscope, an instrument with vastly greater resolving power than the optical microscope, revealed that eukaryotic cells are filled with many more membrane-bounded organelles than previously recognized, suggesting a highly developed functional organization (Herbert Gasser, 1949 Rockefeller Institute report, cited in Moberg, 2012, p. 106).

A philosophically intriguing aspect of many historical cases in cell biology is the role of intracellular organizational entities such as membranes and cytoskeletal elements in the discovery and functioning of biological mechanisms. Viewed from a purely morphological perspective, it seems evident that the membranes that both encircle cells and divide cells into distinct compartments act as constraints on the functional activities of the cell. The cytoskeleton has a similar role. In the 1930s biologists began to refer to a cytoskeleton as a way of accounting for the anisotropy of cellular structure and motility, and by the 1950s identified a type of filament called microtubules in cells by electron microscopy (Zampieri et al., 2014; Worliczek, 2020). Later work led to the discovery of other kinds of filaments in nearly all cell types. By physically polarizing cells and giving directionality to cell motility, cytoskeletal elements, like cell membranes, also constrain cellular functions.

Investigations of cells that utilize constraints such as membranes and the cytoskeleton as an intrinsic part of a decomposition strategy may be a fruitful heuristic to get at mechanistic details of cellular functions while highlighting essential organizational features related to the original biological context that will aid in the recomposition process. Indeed, as we will describe, this is precisely what the integrated strategy of cell biology utilizing guided decomposition achieved. The importance of this epistemic strategy, however, also reveals more fundamental roles of constraints as constitutive of biological functions.

3 Cell biology case studies

3.1 Case 1: mitochondrial membranes and the mechanism of oxidative phosphorylation

As mentioned previously, mitochondria were identified as a ubiquitous organelle of eucaryotic cells at the end of the nineteenth century, in part through staining with the vital dye Janus Green. Because the staining reaction required oxidation of the dye, the very fact that mitochondria were stained suggested that the mitochondrial chemical environment was oxidizing. Oxidation, by removing an electron, converts the oxidized substance to a state of lower potential energy. Hence, early on, mitochondria

were suspected of having a role in energy production and were even referred to as the "power plant" of the cell (Claude, 1948).[1]

The chemical reactions that break down the complex molecules absorbed by living organisms through oxidation reactions were a major focus of the discipline of biochemistry as it coalesced at the beginning of the twentieth century. Over time, biochemists began to understand that such reactions are catalyzed by enzymes, and their strategy to study them consisted of extracting active enzymes from cells and then attempting to reconstruct the reactions in vitro. As part of this heuristic process, sometimes derisively referred to as *grind and find*, they frequently destroyed the structure of cells and tissues to get at the enzymes. Indeed, so long as the extracted material could catalyze reactions of interest, they considered the elimination of biological structure as irrelevant to the investigative process (Bechtel, 2006; Matlin, 2016; Bechtel & Bollhagen, 2019).

In experiments carried out primarily in the first half of the twentieth century this approach led to the discovery of metabolic pathways such as glycolysis. In this case, the strategy of extracting enzymes from cells and tissues without regard for cell structure was effective because the enzymes catalyzing glycolytic reactions are found in the soluble cytoplasm and are easily released. Glycolysis breaks down glucose through a series of reactions that yield the energetic molecules adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide (NADH). The mechanism for synthesizing ATP in glycolysis is called *substrate-level phosphorylation* because high-energy phosphorylated intermediates generated by the breakdown of glucose are used to phosphorylate adenosine diphosphate (ADP) to form molecules of ATP (Bechtel, 2006; Prebble, 2010; Matlin, 2016).

By the 1940s biochemists recognized that even more energy in the form of ATP was produced in conjunction with another metabolic pathway called the citric acid (or Krebs) cycle through a process known as *oxidative phosphorylation* that ultimately leads to the complete breakdown of glucose and related compounds to carbon dioxide. Although the reactions of the citric acid cycle were understood, biochemists did not know how ATP was generated. Based upon the reactions that produced ATP in glycolysis, they assumed that the mechanism was likely to be a form of substrate-level phosphorylation and began searching for high energy phosphorylated intermediates (Bechtel, 2006; Matlin, 2016).

Some of the leading biochemists who worked independently to discover the mechanism of oxidative phosphorylation in the late 1940s and 1950s were Edwin Slater, Albert Lehninger, David Green, and Efraim Racker. Both Slater and Lehninger proposed versions of what became known as the *chemical hypothesis*, described years later by Racker: "[O]xidative phosphorylation proceeds analogously to the reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase [a glycolytic enzyme] via a high-energy intermediate A∼X which contains no phosphate and is a derivative of a respiratory catalyst. The chemical hypothesis included also the conversion of A∼X

[1] Edmund Cowdry, writing in 1924, noted that "although the view, that mitochondria take part in protoplasmic respiration, has been well received by cytologists and serves as a useful and convenient working hypothesis, it is still only a theory and must be regarded as such" (Cowdry, 1924, p. 325). Cowdry also listed a variety of other speculative functions of mitochondria.

to X∼Y, a high-energy intermediate that does not involve a member of the respiratory chain. A∼X or X∼Y are transformed by phosphorolysis to X∼P, from which the phosphate group can be transferred to ADP to form ATP" (Racker, 1976, p. 53).[2]

Initially, these groups carried out experiments to look for high-energy intermediates involved in oxidative phosphorylation using the same extractive strategy that was employed in the study of glycolysis. Beginning in 1946 results began appearing in the literature that helped to jump start the work. A few years earlier Albert Claude, a cancer investigator at the Rockefeller Institute for Medical Research in New York, began developing techniques of *cell fractionation* in which cells are gently broken open and the contents separated quantitatively by centrifugation. Through these efforts he and his colleagues reported the isolation of mitochondria from cells and the concentration of oxidative enzymes in the mitochondrial fraction, including an enzyme of the citric acid cycle. By 1947 the purification technique had been improved such that the isolated mitochondria both morphologically resembled mitochondria observed in whole cells by light microscopy and stained with Janus Green, suggesting that the mitochondria prepared in this fashion were functionally intact (Hogeboom et al., 1947). Within a short time, these results were confirmed by others and biochemists working on oxidative phosphorylation began using isolated mitochondria instead of whole tissues as a starting point for their experiments.

At the same time as he was developing cell fractionation, Claude, in collaboration with his colleague Keith Porter, also figured out how to use *electron microscopy* to examine eucaryotic cells, publishing the first results in 1945 (Porter et al., 1945). Within a few years, George Palade, a scientist in the Claude laboratory who had been involved in improving cell fractionation techniques for isolating mitochondria, began studying the morphology of mitochondria as seen in the electron microscope. In results published in 1952 and 1953 Palade described mitochondria as narrow elongated structures of somewhat variable sizes similar to those of bacteria that were enclosed by a double membrane. The inner membrane, Palade reported, was folded into a series of "ridges" that he named *cristae mitochondrialis* (Fig. 1) (Palade, 1952, 1953).[3]

While the biochemists working on oxidative phosphorylation were aware of Palade's morphological studies, his observations had few practical effects on their strategy for finding the high-energy intermediates postulated in the chemical hypothesis. Tissues rich in mitochondria and later isolated mitochondria were treated with a variety of detergents, extracted with organic solvents, and mechanically disrupted by grinding and by freezing and thawing in attempts to release the enzymes and intermediates participating in oxidative phosphorylation (Matlin, 2016). Although it was recognized that maximum phosphorylating activity was dependent on the intactness of mitochondria, mitochondrial structure was often viewed as an obstacle to enzyme isolation rather than a key to understanding the mechanism of oxidative phosphorylation. As Lehninger stated much later in 1965: "Actually, very few biochemists concerned themselves with the possible importance of the fact that respiratory enzymes

[2] The symbol "∼" or "squiggle" represents a high energy bond between two molecules.

[3] Palade debated the detailed structure of mitochondria with the electron microscopist Fritiof Sjöstrand (see description in Rasmussen, 1995; Matlin, 2016).

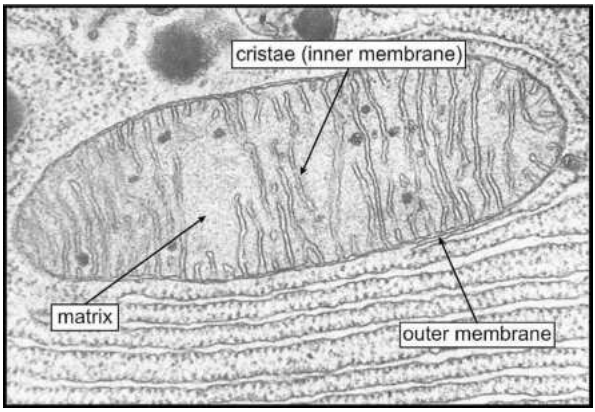

Fig. 1 Structural constraints in mitochondria. Electron microscopic image. Note the large amounts of rough endoplasmic reticulum (ER) in the cytoplasm below the mitochondrion (see Fig. 2 and text). Slightly modified and labeled from Fig. 218 in Don W. Fawcett 1981. *The Cell*, *Second Edition.* Philadelphia: W. B. Saunders Company. Used with permission of Elsevier.

were found to be associated with particulate matter of cells and tissues. It was part of the biochemical *Zeitgeist* that particles were a nuisance and stood in the way of purification of the respiratory enzymes" (Lehninger, 1965, p. 6). As the work of this set of biochemists proceeded, they managed to prepare mitochondrial extracts that were somewhat active in ATP production. However, the search for the postulated phosphorylated high-energy intermediate failed to progress.

In 1961 Peter Mitchell published what he referred to as the *chemiosmotic hypothesis* to explain oxidative phosphorylation in mitochondria (Mitchell, 1961). Mitchell was not unknown to the other biochemists working on oxidative phosphorylation, having studied biochemistry at Cambridge University while both Edwin Slater and David Green were also at the institution (Prebble, 2001; Matlin, 2016). At the time of his proposal, however, he was recognized mainly for his work on metabolite transport in bacteria. At Cambridge, Mitchell's initial thesis advisor was James Danielli, who, with his colleague Hugh Davson, had proposed a model of biological membrane structure in the 1930s that remained generally accepted well into the 1960s (Matlin, 2022). When Danielli left Cambridge, Mitchell was assigned a new supervisor Ernest Gale, which led to his work on bacteria. Mitchell was also mentored by David Keilin, the discoverer of cytochromes and an investigator of electron transport in cells. Given this background, Mitchell's knowledge of membranes and membrane transport was likely more nuanced than that of the others working on oxidative phosphorylation.

Mitchell's chemiosmotic hypothesis for oxidative phosphorylation was radically different from the chemical hypothesis favored by most others. He proposed that electron transport within the inner mitochondrial membrane generated a proton gradient across the membrane whose potential drives ATP production when protons flow back across the membrane through a membrane-associated ATPase.[4] No high-energy phosphorylated intermediate was needed. Furthermore, his hypothesis helped to explain why certain detergent extracts of mitochondria and other preparations made from sonically disrupted mitochondria were still able to carry out oxidative phos-

[4] Though referred to as an ATPase, it was understood that it was responsible for ATP synthesis and not degradation. It is now more properly called an ATP synthase.

phorylation; both consisted of closed membrane vesicles that were still able to generate a proton gradient.

One inspiration for the Mitchell hypothesis, as it became known, was certainly Mitchell's work on bacterial transport. Another key contributor was his focus on the particular form of the mitochondria as described by Palade (Matlin, 2016). Mitochondria were not only of the same approximate size as the bacteria that Mitchell studied, but also contained an intricate folded inner membrane, a feature that suggested an important relationship to mitochondrial function. Indeed, when Palade described the structure of mitochondria seen in the electron microscope, he correlated existing biochemical data with his morphological studies to suggest that the inner mitochondrial membrane might contribute to the functional organization of mitochondrial enzymes (Palade, 1952, 1953). Mitchell apparently believed that a proposed mechanism that took mitochondrial structure to heart was more plausible than one that viewed the structure as an obstacle to be overcome, as was the case with the chemical hypothesis.

Eventually, after years of debate and some modifications, the chemiosmotic hypothesis was accepted as correct in the 1970s. Efraim Racker, who had by then succeeded in isolating the ATPase from mitochondrial membranes, carried out a crucial supporting experiment (Racker & Stoeckenius, 1974; Allchin, 1996).[5] In collaboration with Walter Stoeckenius, Racker incorporated the ATPase in a lipid vesicle with bacteriorhodopsin purified from the purple bacterium *Halobacterium salinarium*. Bacteriorhodopsin is a membrane protein able to generate a proton gradient across the membrane in response to light, a property that is essential for purple bacteria to survive in environments with high salt concentrations. Racker and Stoeckenius demonstrated that when lipid vesicles reconstituted with the ATPase and bacteriorhodopsin were illuminated, the proton gradient led to the production of ATP.

Based upon this brief historical review, it is possible to identify key reasoning strategies that contributed to discovery of the mechanism of oxidative phosphorylation. Certainly, one initial accelerant was the guided decomposition of cells to yield structurally intact and functional mitochondria. This innovation was of such strategic importance that it was adopted immediately by nearly all investigators working on the problem. After that, a crucial step was decomposition of mitochondria themselves. Most of the biochemists ignored the constraints on possible mechanisms posed by the complex membranous structure of the mitochondria described by Palade using the electron microscope. In addition to the integrity of the membrane, its asymmetric orientation determines both the direction of proton transport across the membrane and the location where metabolites that feed oxidative phosphorylation interact with the membrane. This constraint was suggested by morphological differences between the inner mitochondrial *matrix* and the cytoplasm surrounding mitochondria, as well as biochemical experiments on mitochondrial preparations (Fig. 1).[6] Rather than tak-

[5] Although an enzymologist and supporter of the chemical hypothesis of oxidative phosphorylation, Racker also exploited morphological techniques to prove that he had reconstituted the ATPase in the mitochondrial membrane in a spatially correct way (Kagawa & Racker, 1966). Eventually, this study may have affected his impression of Mitchell's hypothesis and influenced his decision to try the reconstitution strategy, although it is difficult to know for sure (Matlin, 2016).

[6] In publications in 1966, Mitchell emphasized the importance of this asymmetry for the interpretation of results with sonically disrupted (i.e., decomposed) mitochondria (Mitchell, 1966a, b).

ing such constraints into account, biochemists instead assumed that mitochondria were simply a bag of enzymes, an assumption that had proved successful when they deciphered the glycolytic pathway. Their decomposition of mitochondria was anything but guided, and this proved to be a major oversight. As Racker remarked in 1976: "It is indeed curious that for many years the biochemists working on oxidative phosphorylation completely ignored the fact that they were working with organized, vesicular structures" (Racker, 1976, p. 46). Mitchell, on the other hand, proposed a mechanism that took the constraints of mitochondrial structure seriously. Similarly, Racker and Stoeckenius, working with a minimal and heterologous reconstituted system, recognized the necessity of retaining a key constraint of mitochondria in the form of a closed and asymmetric membrane vesicle, and provided critical results supporting Mitchell. The decomposition of mitochondrial function proceeding from Mitchell's intact mitochondria to Racker's vesicles was carefully *guided*—the membranous constraints so characteristic of mitochondria were retained (Fig. 1), enabling the mechanism of oxidative phosphorylation *as it occurs in cells* to be deciphered.

3.2 Case 2: the "integrated strategy" to study the first step of protein secretion

The technical advances in cell fractionation and electron microscopy pioneered by Albert Claude and his colleagues in the 1940s and 1950s that underpinned the work on mitochondria were soon applied to the study of protein secretion. Protein secretion is the process by which certain proteins are transported from their site of synthesis in the cytoplasm out of the cell. While almost all cells do this, some cells, such as those of the exocrine pancreas, are specialized to secrete most of their protein synthetic output. Initiated primarily by George Palade, these studies were to span more than thirty years at the Rockefeller Institute (later Rockefeller University), leading to a detailed molecular explanation of the first step in secretion, the sequestration of newly synthesized secretory proteins in the endoplasmic reticulum (ER) (Matlin, 2022).

Claude and Porter first observed a "lace-like reticulum" in their initial examination of cells by electron microscopy in 1945 (Porter et al., 1945). Porter provided a more detailed description in the early 1950s, but Palade gradually took over the project, soon publishing two electron microscopic studies that established the near ubiquity of the ER in eucaryotic cells (Palade & Porter, 1954; Palade, 1955b).[7] These morphological observations demonstrated that the organelle consisted of an intricate series of membrane-bounded narrow anastomosing tubules running through the cytoplasm. Palade also discovered small particles in the cytoplasm, many of which appeared to be attached to the cytoplasmic surface of the ER membrane (Palade, 1955a). Independent work by others suggested that these particles, soon to be named ribosomes, were related to the process of protein synthesis. In pancreatic exocrine cells, Palade observed vast arrays of ER studded with ribosomes (which Palade called "endoplas-

[7] Based upon his publications in the 1950s, Porter may have been more interested in broadening the application of electron microscopy to various types of cells and tissues than pursuing a single biological problem in depth, as well as making technical improvements to electron microscopy. In contrast, Palade, who tried cell fractionation and histochemistry soon after arriving at Rockefeller, preferred to initiate a multi-year and multi-pronged study of protein secretion. Palade also recognized both the power and limitations of electron microscopy, as suggested by his work on the ultrastructure of mitochondria (Matlin, 2022).

mic reticulum of the rough-surfaced variety," or rough ER), as well as large numbers of cytoplasmic vesicles without ribosomes that appeared to contain aggregates of proteins. Based upon these morphological findings, Palade postulated that the rough ER and many of the cytoplasmic vesicles were involved in the synthesis, transport, and release of secretory proteins from the cell. At the same time, he realized that his electron microscopic images, as dramatic and clear as they were, were only suggestive and, by themselves, would not allow him to prove that the rough ER and vesicles participated in secretion or to establish molecular mechanisms for the process, his ultimate goal.

To overcome these limitations, Palade designed what he referred to as an "integrated strategy" that would combine morphological observations using the electron microscope with cell fractionation and biochemical analysis of cell fractions (Palade & Siekevitz, 1956a, b). To accomplish this, he recruited the biochemist Philip Siekevitz to the project, and they began by focusing on the rough ER. Significantly, their approach was not to use electron microscopy and cell fractionation as separate investigative techniques, but rather to use electron microscopy to *guide the decomposition* of cells as they were disrupted and the rough ER isolated for biochemical analysis. Thus, after breaking open cells by mechanical homogenization and separating membrane-bounded cell parts by centrifugation at various speeds (called differential centrifugation), they examined the isolated cell parts by electron microscopy to link their identity to intact structures in undisrupted whole cells. This procedure placed the isolated parts firmly in the context of the intact cell and increased confidence that the outcome of their biochemical analysis of parts would yield results that were biologically meaningful.

Palade and Siekevitz soon discovered that the tubular rough ER broke into small vesicles upon cell homogenization that they called rough microsomes because of the ribosomes attached to the outside of the microsomal vesicle membrane (Fig. 2) (Palade & Siekevitz, 1956a, b; Matlin, 2022). Despite these changes to the morphology of the rough ER caused by cell disruption and fragmentation of the tubular organelle, they realized that rough microsomes were a reasonable and legitimate surrogate for the rough ER and could, therefore, serve as the subject of further experiments to probe the function of the rough ER in cells. Stated another way, rough microsomes retained important *structural constraints* exhibited by the intact rough ER. Specifically, both the rough ER and rough microsomes were sealed membrane-bounded compartments, both were uniformly oriented such that ribosomes were bound to the outer surface (analogous to the cytoplasm facing surface in intact cells), and both contained electron-dense content presumed to be secretory proteins that had, by some means, been transported into their interiors (Fig. 2). These realizations now permitted Palade and Siekevitz and a new colleague, Günter Blobel, to begin focusing on the systematic decomposition of rough microsomes (Matlin, 2022).

New experiments established that the ribosomes bound to rough microsomes were synthesizing secretory proteins and "vectorially discharging" them into the microsomal lumen. These included both in vivo radioactive labeling of secretory proteins followed by cell fractionation and the in vitro demonstration that isolated rough microsomes could synthesize secretory proteins on their own. Furthermore, the ribosomes appeared to be tethered to the microsomal membrane by the proteins

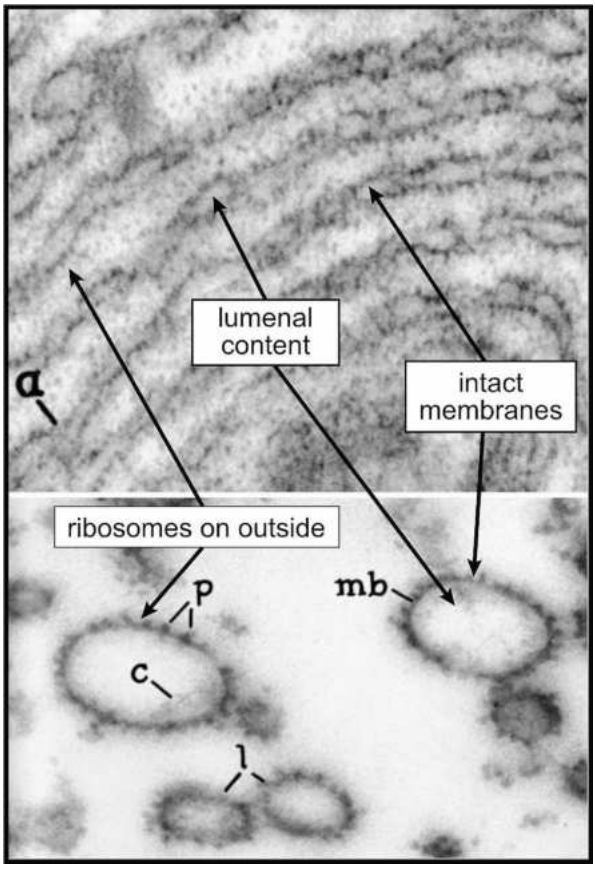

Fig. 2 A comparison of structural constraints in the pancreatic endoplasmic reticulum (upper panel) and isolated rough microsomes derived from the exocrine pancreas (lower panel). Electron microscopic images. Note that the magnification of the lower panel is higher than that of the upper panel. The lower panel consists of cross-sectioned vesicles. Modified and labeled from plates 172 (Fig. 3) and 175 (Fig. 9). ©1956 George E. Palade and Philip Siekevitz. Originally published in "Pancreatic Microsomes: An Integrated Morphological and Biochemical Study." Journal of Biophysical and Biochemical Morphology 2 (6):671–690. Used with permission of the Rockefeller University Press.

being synthesized (called the nascent chains), suggesting that passage of the proteins through the microsomal membrane occurred during protein synthesis. (Matlin, 2022).

At this point Blobel assumed leadership of the project. He began, with the initial collaboration of another colleague David Sabatini, by carefully decomposing rough microsomes into microsomal vesicles "stripped" of ribosomes, and then reconstituting them by adding back ribosomes, messenger RNA coding for specific proteins, and protein synthesis factors to yield rough microsomes again capable of protein synthesis and translocation in vitro (Matlin, 2022). At this point electron microscopy continued to be used to monitor certain aspects of the decomposition such as the removal of ribosomes, but biochemical measures designed to ensure that key constraints of the original rough microsomes remained intact throughout the decomposition procedure were also used. These included, for example, the addition of proteolytic enzymes to the outside of the vesicles to demonstrate the preservation of both their topographical orientation and integrity as a barrier. In this way, proteins translocated into the interior of microsomal vesicles were shown to be "protected" from proteolytic degradation. These sorts of experiments, combined with sequencing of the proteins synthesized in vitro by the reconstituted rough microsomes, demonstrated that secretory proteins are first synthesized as precursors with a short segment

of amino acids at the beginning of the nascent proteins that serves as a *signal* to not only direct the proteins and ribosomes to the surface of the microsomal membrane but also to facilitate translocation across that membrane (Blobel & Sabatini, 1971; Blobel & Dobberstein, 1975a, b).

Blobel, and soon other laboratories, then began to further decompose rough microsomes, eventually isolating proteins involved in directing or "targeting" ribosomes with their exposed nascent chain signals to the microsomal membranes and proteins making up the channel in the membrane through which the newly made secretory proteins are transported. With the characterization of these proteins, including determination of their three-dimensional molecular structures at high resolution, the molecular mechanisms (by now there were several variations) that carried out the first step in secretion, the synthesis and sequestration of secretory proteins in the ER, were firmly established (Matlin, 2022).

When the full sequence of investigations leading to these findings is reviewed, it is evident that the iterative or even recursive nature of the integrated strategy devised by Palade helped to increase confidence that the molecular mechanisms discovered actually reflect events in the living cell. Rough microsomes remained firmly linked to the rough ER in intact cells throughout the decomposition process, making recomposition of these events into biologically meaningful mechanisms straightforward. Certainly, other sorts of studies carried out contemporaneously, particularly the use of secretion deficient mutations in yeast (a eucaryotic microorganism capable of protein secretion) and bacteria, also supported the contention that what was found in vitro was what happened in living cells (Deshaies & Schekman, 1987).[8]

The use of isolated rough microsomes in in vitro or "cell-free" experiments, particularly by Blobel, illustrates the importance of constraints in epistemic strategies to investigate cellular mechanisms. The fundamental constraints exhibited by the ER in intact, living cells were retained throughout the series of decompositions carried out in the search for molecular mechanisms. The existence of an intact, properly oriented membrane barrier was crucial to establishing how secretory proteins were targeted to and translocated across the microsomal membrane. As in the previously described mitochondrial studies, crucial evidence for the functionality of proteins identified as key factors in ER targeting and translocation was eventually obtained by the reconstitution of these factors into lipid vesicles (Matlin, 2022). Without the constraints provided by the vesicle membrane, however, any activities of the isolated proteins were functionally meaningless. Their biological properties only emerged in reconstitution experiments because key constraints of the lipid vesicles matched those of the ER in living cells, and the use and maintenance of these constraints throughout the investigative process was what facilitated their discovery. In essence their epistemic strategy to preserve *in vivo constraints* in *in vitro experiments* provided a means to conduct molecular studies within the context of the whole cell. Reductionistic experiments were thus carried out within a semi-holistic environment.

[8] By their nature, experiments that use mutations to identify components and investigate mechanisms are intrinsically carried out in the context of the living cell (Beckwith, 2002, pp. 78–79). Further discussion of the contribution of genetic analysis to the epistemic strategy of cell biology is beyond the scope of this paper.

3.3 Case 3: fast axonal transport and the discovery of kinesin

Microscopy has also been important in the study of neural mechanisms, particularly the functions of neurons that send signals throughout the nervous system and other target tissues via the nerve cell processes known as axons and dendrites. Both axons and dendrites are long, thin membrane-bounded structures that extend from the cell bodies of neurons to transmit action potentials to synapses. Depending upon the organism and tissue, axons can extend for many centimeters or longer, complicating the cellular task of transporting materials needed for the maintenance and repair of neurons from the cell body to the nerve terminals and back. In studies of nerve injury after World War II, scientists observed that axons swelled to three times their normal diameter when constricted, suggesting that an enormous transport of fluid was occurring within the axon (Matlin, 2020). Additional work in the 1960s using radioactive tracers demonstrated that the movement of some materials in the axon, most likely vesicular components or proteins, occurred very quickly at rates of several hundred millimeters a day, termed *fast axonal transport*. An important question was what mechanism made this directed movement of vesicles possible.

In the 1980s a preferred experimental system to study fast axonal transport was the squid giant axon (Matlin, 2020). While axons in most organisms are very thin, the squid giant axon is 300–700 micrometers in diameter and several centimeters long. At this size it is visible with the naked eye and can be easily dissected from the squid body and studied in isolation. Such work was carried out at the Marine Biological Laboratory (MBL) in Woods Hole, Massachusetts, where squid were readily available in the summer. In the isolated axons, scientists suspected that transport continued to occur. The material being transported could not, however, be easily seen with even the best light microscopes. Robert Allen and his collaborator Nina Allen from Dartmouth University, who spent summers at the MBL, discovered that a combination of a specialized light microscope and newly available video camera enabled them to detect objects in cells below the resolution of the typical light microscope (Allen et al., 1981). In collaboration with Scott Brady from Case Western Reserve University, who was studying fast axonal transport in the squid giant axon at the MBL, they were able to observe and record movement within the axon of what appeared to be vesicles on linear tracks, presumably some kind of filament (Allen et al., 1982).

With these observations, certain constraints characteristic of fast axonal transport became clear (Fig. 3). Transport occurred within the *axoplasm*, as the axonal cytoplasm was called. It was linear and aligned with the long axis of the axon. Transport of particles, presumed to be some kind of vesicles, appeared to occur on long, filament-like tracks and was bidirectional, that is, particles moved both away from and towards the location where the cell body existed prior to dissection of the axon. The rate of transport, as calculated from the video record, was consistent with the known rate of fast axonal transport. Because this was a dynamic system, a biochemical constraint, the requirement for some sort of energy source to power the movement, was presumed.

Brady soon decomposed the isolated axon to create a cell-free system to study transport (Brady et al., 1982). Treating the isolated axon like a tube of toothpaste, he squeezed the axoplasm out of the axonal plasma membrane. The extruded axoplasm

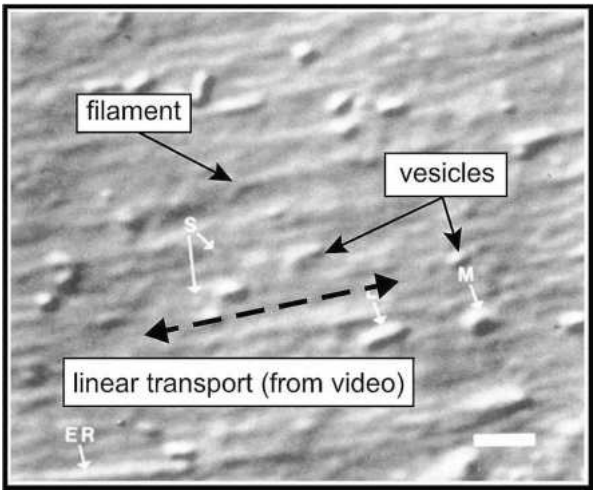

Fig. 3 Constraints visible in the squid axoplasm by video microscopy using the AVEC-DIC method to enhance contrast of structures below the resolution of light microscopy.

retained its shape and, when provided with the right buffered medium, continued to transport material in a manner identical to that of the intact axon when viewed in the video microscope. That is, the constraints identified in the intact axon were preserved in the isolated axoplasm. He then continued to decompose the system by physically teasing some of the axoplasm away from the edges of the preparation. Now, using the Allens' microscope, he could observe what appeared to be individual filaments that he believed to be microtubules with vesicles continuing to move along them. Keeping the energy requirement in mind, Brady treated his system with an ATP analogue and observed that the vesicles stopped moving on the filaments and "froze" in the attached state (Lasek & Brady, 1985).

This is a still image from a videotape record. Note the density of material in the axoplasm. Many of the imaged vesicles and other oblong or round objects are not transported along filaments in the video from which this image is taken; others move in two opposite directions along the filaments (as depicted by the double-headed arrow). The apparent sizes of the different objects and filaments are not accurate because the AVEC-DIC microscope renders objects below the resolution limit of the light microscope as similarly-sized structures. Labels added to Fig. 1; originally published in Robert Day Allen, Janis Metuzals, Ichiji Tasaki, Scott T. Brady, and Susan P. Gilbert. 1982. "Fast Axonal Transport in Squid Giant Axon." Science 218 (4577): 1127–1129. Used with permission of the American Association for the Advancement of Science.

At this point a competing research group that included an electron microscopist Bruce Schnapp from the National Institutes of Health and a biochemically-oriented graduate student Ron Vale from Stanford University entered the picture at the MBL. Using a similar video microscope combined with electron microscopy they demonstrated that vesicles moved on individual microtubules (Schnapp et al., 1985). Vale then used Brady's inhibitor trick to detect and purify a molecular motor protein that

he named kinesin capable of moving vesicles in one direction on microtubules (Vale et al., 1985a; Matlin, 2020).[9]

As in the mitochondria and protein secretion cases, the scientists working on fast axonal transport used microscopy to identify constraints on the system. They continued to use microscopy to guide decomposition of the axon to create a cell free system that preserved those constraints. Transport occurred in the axoplasm and was linear and aligned with the axon's long axis. Later, transport was linked to vesicles and was constrained by filaments identified by electron microscopy as single microtubules. The rate of transport observed microscopically in vitro was similar to fast transport in intact axons. Additional cycles of decomposition followed until molecular components of the mechanism were identified. As in the other cases, the preservation of in vivo constraints through successive cycles of decomposition enabled these investigators to conduct in vitro molecular experiments within the context of the intact axon.

4 Discussion

We have analyzed three case studies from three different eras of cell biology to illustrate an epistemic strategy based upon *guided decomposition* of complex living systems as a way of explaining biological phenomena at a molecular mechanistic level. In all three cases microscopy was used to guide decomposition as cells were broken into parts. An essential aspect of guided decomposition is the identification of key constraints in the intact living system and preservation of these constraints when experimenting on isolated parts. This recapitulation of in vivo constraints in reduced in vitro systems enables the investigation of phenomena, ultimately at the molecular level. It also facilitates recomposition of mechanisms into the intact whole because the parts remain linked to the whole throughout decomposition. That is, mechanisms discovered through reductive analysis can be reintegrated into the intact living system with some confidence that such mechanisms reflect what is actually happening in vivo.

In the discussed cases, the first step in guided decomposition was the identification of structural constraints in cells using morphological analysis. The process by which biologists decide what is significant when they observe cells and other biological materials in the microscope is complex and somewhat mysterious. Although typically only a few micrographs appear in published accounts of research, biologists often observe hundreds to thousands of images before choosing those they consider characteristic examples.[10] It is likely that this selection process requires a certain aesthetic judgement (Matlin, 2022). Recognition of aesthetic qualities in biological specimens allows patterns of regularities to be seen and appreciated, even if biologists are hesitant to admit an iota of subjectivity in their work. In his book *Visual*

[9] Shortly thereafter, Vale linked another known motor protein, dynein, to movement of vesicles in the opposite direction (Vale et al., 1985a, b).

[10] The current wide use of digital video imaging of live cells and the ability to post such material as supplements to online publications permits readers to recapitulate some of the observation process used by the scientists conducting the study. These posted movies, although extensive, are still highly selected and edited.

Cultures in Science and Technology, Klaus Hentschel states that, among scientists, "remarks about 'beauty' are tucked away as irrelevant sidelines, as ancillary feelings, as slightly embarrassing subjective deviations from the 'purely objective' main line of research" (Hentschel, 2014, p. 362). More concretely though, these visual practices permit biologists to identify structural constraints in cells. Palade's observations of mitochondria and the ER in the electron microscope are good examples. To determine mitochondrial structure Palade searched through images derived from multiple tissues from several mammalian species, protozoa, and even some plants, finally concluding that "A characteristic pattern of organization was found with the help of the electron microscope […] irrespective of the species providing the specimen and the cell type examined" (Palade, 1953, p. 209). Similarly, Palade looked at forty different mammalian and avian cell types before concluding that the ER was a network of membrane bounded cavities (Palade, 1955b). In both cases, the constraints identified as characteristic and significant structural features then guided decomposition of these organelles in the quest for molecular mechanisms.

As decomposition proceeded, microscopy and biochemical procedures, such as Blobel's use of proteolytic enzymes to demonstrate rough microsome integrity, ensured that the original in vivo constraints remained intact in in vitro systems. This validated the use of these in vitro systems as surrogates for the intact living system. Application of constraint-based reasoning in this manner to both the intact system and its parts extends the idea of constraints beyond features that reflect the organization of living systems to a need for concrete epistemological tools that assist both the decomposition and recomposition processes while also facilitating mechanistic explanations of biological phenomena.

Guided decomposition, illustrated in our cases as the use of microscopy and other experimental techniques to first identify constraints in intact living systems, and then to make sure that these constraints are preserved as cells and organelles are taken apart, means that the biological phenomena of interest are investigated in the context of the intact living system. Under these circumstances, recomposition of mechanisms is not separate from decomposition. Rather, recomposition is a continuous outcome of a decomposition process that constantly references constraints present in the context of the living cell. In our first case, Mitchell and others demonstrated that an intact mitochondrial membrane in isolated mitochondria or a closed membrane vesicle in decomposed mitochondria are required for ATP synthesis. Because the intricate structure and integrity of mitochondrial membranes in situ were recognized as critical in vivo structural constraints, it was straightforward to recompose Mitchell's proposed mechanism back into the living cell, that is, to make a convincing case that chemiosmosis was the way that cells actually carried out oxidative phosphorylation. The intact membranes present in isolated mitochondria and decomposed mitochondria capable of ATP synthesis resembled those seen in situ, and there was no reason to believe that they functioned differently from those of mitochondria in cells.

Similarly, in our second case, Palade, Blobel, and colleagues showed that isolated rough microsomes retained the constraints of the intact rough ER, including membrane topography and integrity, and were still able to vectorially translocate newly made proteins. This left little doubt that the recomposed molecular mechanisms they uncovered in the decomposed system were identical to those operating in the intact

cell. Finally, in our third case, Allen and Brady identified constraints on fast axonal transport in intact axons consisting of linear transport of vesicles on filaments. The preservation of these constraints through further rounds of decomposition under the guidance of microscopy increased confidence that the mechanism recomposed from these molecular parts was likely responsible for fast axonal transport in actual neurons.

In addition to structural constraints, guided decomposition of living systems requires attention to biochemical constraints. Since life exists in what is thermodynamically a far-from-equilibrium state, living systems require external energy inputs (Bertalanffy, 1950). Energy requirements are also constraints whose preservation is as important as that of structural constraints as the system is decomposed. Scott Brady's "freezing" of vesicular transport when he applied an ATP analogue to isolated axonal filaments meant that the hydrolysis of ATP was a constraint on further analysis. When kinesin was identified as the motor protein powering vesicle transport, its requirement for ATP satisfied that constraint and increased confidence that it was part of the mechanism of fast axonal transport. Bechtel and colleagues have similarly stressed that an adequate understanding of what they term "production mechanisms," that is, the parts and operations performing specific molecular interactions, requires an extended focus on external "control mechanisms" that constrain and direct flows of free energy (Winning & Bechtel, 2018; Bechtel & Bollhagen, 2019; Bechtel, 2022; Bich & Bechtel, 2022). Their analyses also support the view that biological mechanisms often are not completely decomposable but are embedded in context of complex networks of structural and energetic constraints. As a consequence, mechanistic analysis requires attention to both the structural (or organizational) context and the energetic requirements of the molecular components and processes of interest.

It is important to emphasize that the identification of constraints is a heuristic process in the sense that there is no certainty that the constraints chosen for epistemic purposes are those relevant to the biological phenomenon under investigation (Wimsatt, 2007). Even with a presumption of objectivity, biologists must select some features and ignore others, often based upon ancillary information provided by other independent investigations that inform a particular mechanistic hypothesis. Palade, for example, was familiar with nineteenth-century morphological studies of secretion that implicated cellular vesicles visible in the light microscope, convincing him that secretion might proceed by a vesicular process. He also correlated the abundance of rough ER in cells with their known secretory function through his observations with the electron microscope, suggesting that a focus on the rough ER was a reasonable choice (Matlin, 2022). At this point, preservation of rough ER constraints during decomposition of cells became of paramount importance to his studies while structural constraints on other parts of the cell were less relevant.

Another example illustrates how the selection of relevant constraints is conditioned by the phenomenon under investigation. In the 1970s biologists interested in studying the organization of photosynthetic reaction centers in chloroplasts chose as an experimental system a green alga with abundant thylakoid membranes (Chua et al., 1975). Chloroplasts, like mitochondria, have a double outer membrane called the envelope and internal stacks of thylakoid membranes where photosynthesis is localized. To study photosynthesis, then, the structural and biochemical constraints

of importance were the integrity of the thylakoid membranes and their ability to carry out photosynthetic reactions. Due to the organization of the alga used in these studies, isolation of intact thylakoid membranes was possible, but the isolation procedure completely disrupted the chloroplast envelope membrane. Breakage of the envelope membrane, a structural constraint, was however irrelevant to the study of photosynthesis, and there was no reason for it to be preserved. Later, the same research group began investigating the transport of cytoplasmically-synthesized proteins through the chloroplast envelope into the interior of the chloroplast (Chua & Schmidt, 1978). Now the structural constraint of the envelope and its preservation during decomposition of the cell and isolation of chloroplasts was relevant. This forced the group to switch from the alga to spinach as a source of chloroplasts since chloroplasts with intact envelopes could be isolated from spinach.

How far do the philosophical implications of constraint-based guided decomposition go? Is guided decomposition merely a convenient heuristic strategy, or does the complexity of (many) living systems require accounting for constraining relations? On the one hand, the use of cell-free or simplified in vitro systems as described here is an essential and powerful strategy to investigate molecular mechanisms in cells (Matlin, 2022). Because this strategy is inherently reductive relative to the level of the intact cell, some might argue that it is no different from other reductive strategies that use isolated molecules to investigate molecular biological phenomena. We believe, however, that there are crucial differences. In vitro systems can only legitimately be used to find out how cells work if it can be demonstrated that activities and processes uncovered in vitro obey important constraints present in vivo. The use of constraint-based reasoning distinguishes the strategy we describe in the case studies from those that take isolated molecular parts and attempt to reassemble biologically meaningful mechanisms from those parts. Constraints identified in intact cells *must be retained* in the cellular parts identified and isolated during decomposition. If not, the danger is that uninterpretable or even artifactual results will be generated. The biochemists searching for a high energy chemical intermediate in oxidative phosphorylation in extracts prepared by destroying mitochondrial structure fell into this trap. More recently, concerns have been raised that "omic" strategies in systems biology that rely on results from synthetic interactomes as the only constraints on functional relationships between proteins risk the generation of biologically irrelevant results (Green, 2017).

Building on these reflections, we take one further step and suggest that the need for constraint-based reasoning is conditioned by ontological features of biological systems that has been described as *downward causation*. The notion of downward causation we find relevant for this purpose is what Claus Emmeche, Simo Køppe, and Frederik Stjernfelt call "medium downward causation" (Emmeche et al., 2000, p. 25). The term captures how higher-level entities are *constraining conditions* for the activity of lower levels (see also Woodward, 2021), and this notion is in alignment with how theoretically inclined systems biologists use the notion of downward causation in the context of multi-scale models of living systems (e.g., Ellis et al., 2012; Noble, 2012; Wolkenhauer & Green, 2013). Downward causation is here, like in Emmeche et al. (2000), defined as constraining relations where higher-level structures (or variables) define boundary conditions for lower-level processes. Denis

Noble emphasizes that modeling of the heart rhythm is not mathematically possible without accounting for how ionic currents are influenced by the constraining relations of the cell membrane (the cell voltage) (Noble, 2012, 2017). In his example, the oscillation of ionic currents is an emergent phenomenon that cannot be understood by attending only to the molecular scale. In this sense, constraints such as cellular and intracellular membranes not only limit the scope of biological possibilities or mechanisms but also *enable* biological functions that would be impossible to reach in an unconstrained system (see also Patte, 1971, 1973; Hooker, 2013; Green, 2018).[11] Similarly, our case studies articulate the impact of the biological boundaries formed by cellular and intracellular membranes and the cytoskeleton on what mechanisms can be realized in vivo.

While our description of what we call guided decomposition is based on historical case studies in cell biology, it is not specific to cell biology. Rather, it can be understood as a more general epistemic strategy to identify how the states or processes at a specific scale are impacted by higher-level constraints. A bottom-up strategy that attempts to model a living system from detailed studies of component parts would often fail, because the scope of possibilities would be practically intractable and because the system organization and other higher-level constraints are central to the functioning of multi-scale systems in general (Noble, 2012; Green & Batterman, 2017). In both physics and biology, scientists often adopt a "middle-out" strategy attending to how a phenomenon of interest requires identification of both material (lower-level) constituents and higher-level variables that define the boundaries for lower-level processes (for a more detailed discussion, see Batterman & Green, 2021). The notion of "higher-level constraints" does not refer to parameters at an absolute scale or level (such as the cell) but is defined by the system or processes of analysis. A higher-level variable in one analysis can be a lower-level variable in another. What is important, however, is that understanding living systems often requires an iterative process of going back and forth between reductionistic and more holistic approaches to situate mechanisms in their broader contexts and to identify what mechanisms are biologically meaningful.

Noble's work, as well as other examples described by philosophers of biology and physics, further illustrate how constraints are not only ontological features of complex systems but also have essential explanatory roles when modeling both biological and physical systems mathematically (Batterman, 2001, 2012; Mossio et al., 2013; Green, 2018; Bursten, 2021). It is often the case that differential equations used to describe certain phenomena at the molecular level can only be solved when boundary conditions (i.e., constraints) identified at higher levels are included in the analysis. As Noble states regarding modeling of the cardiac rhythm:

> One might think that, provided all the relevant protein mechanisms have been included in the model and if the experimental data are reliable, and the equa-

[11] Downward causation has been contested in the philosophical literature and associated with problems such as causal overdetermination. We do not have space to unpack the concerns. For further discussion of downward causation and responses to these objections, see Green (2018), Green and Batterman (2021) and Woodward (2021).

> tions fit the data well, cardiac rhythm would automatically 'emerge' from those characteristics. It does not. The reason is very simple and fundamental to any differential equation model. In addition to the differential equations you need the initial and boundary conditions. Those values are just as much a 'cause' of the solution (cardiac rhythm) as are the differential equations. In this case, the boundary conditions include the cell structure, particularly those of its membranes and compartments. (Noble, 2012, p. 58)

Similarly, Julia Bursten describes the complexities of modeling both sound transmission of a violin and the violin strings' harmonics. She notes that to explain the harmonics it is necessary to fix the endpoint of the string, which amounts to setting a boundary or constraint at the bridge end of the violin. This is equivalent to enabling explanations of biological phenomena by identifying essential constraints on the system, whether those explanations are achieved through molecular analysis or mathematical modeling à la Noble. She concludes that "the specification of a boundary, and the encoding of that boundary into a boundary condition on a mathematical model, are nearly universal preconditions for the generation of explanations in the physical sciences" (Bursten, 2021, p. 253). Thus, also in the physical sciences, boundary conditions cannot be reduced to mere background conditions but are often co-constitutive of the phenomena that scientists are interested in explaining.

What we are advocating is thus not a top-down approach where higher-level constraints always determine lower-level mechanisms. In systems biology, top-down approaches have been problematized for ignoring how structure not always determines function, for example, in debates about whether the robustness of gene regulatory networks can be explained by the topological features of the network alone, irrespective of the lower-level details (DiFrisco & Jaeger, 2019, 2020; Green & Batterman, 2021). Similarly, it must be recognized that the cell membrane is not always important as a constraint for all intracellular processes. In the case of fast axonal transport described previously, the microtubule cytoskeleton was a more important constraint than the axonal plasma membrane. Fast axonal transport proceeded normally in the extruded axoplasm in the absence of the axonal membrane. Thus, constraint-based reasoning is not merely a matter of identifying all possible constraints via microscopy, imaging, or topological network analysis. Rather, it is an iterative activity exploring how specific constraints may enable specific biological functions and guide the evaluation of whether proposed mechanisms are biologically meaningful.

5 Concluding remarks

In this paper, we have revisited the characteristics of mechanistic heuristics through the historical lens of case studies from cell biology. Our analysis emphasizes that recomposition, which aims to explain how biological mechanisms operate in living systems, goes beyond the integration of knowledge obtained through reductionistic analysis. We suggest that decomposition and recomposition should not be treated as separate activities. Rather, we advocate a guided decomposition approach that con-

tinuously—and iteratively—considers contextual boundary conditions as constraints on biological mechanisms.

We have documented this philosophical point through historical case studies in cell biology demonstrating how constraint-based reasoning contributes to the discovery of molecular mechanisms. Our examples include the discovery of mitochondrial oxidative phosphorylation, protein targeting and translocation in protein secretion, and the mechanisms behind neuronal vesicle transport. While these cases have all received attention from philosophers and historians of science before us, they have not previously been interpreted as instances of constraint-based reasoning. Yet, we contend that the role of cellular and subcellular constraints, such as lipid membranes and cytoskeletal elements, are central to the epistemic strategy of cell biology. What we call guided decomposition is also distinctive of the development of cell biology, emphasizing the integration of microscopy-based morphological investigations with the controlled disruption of cells and isolation of cell parts for biochemical analysis.

Moreover, we believe that the importance of membranes and the cytoskeleton as structural constraints on cellular functions, that is, as ontological features of biological mechanisms, has not received sufficient attention in the mechanistic literature. We have here suggested that the need for a guided decomposition is due to the importance of downward causation, understood as constraining relations where higher-level structures (or variables) define boundary conditions for lower-level processes. Hence, certain aspects of multi-scale systems only become "visible" or possible to study when looking "above" the specific level of analysis. William Wimsatt puts this beautifully when stating that "we must work back and forth between ontologies of different levels to check that features crucial to upper level phenomena are not simplified out of existence when modeling at the lower level (Wimsatt, 1997, S374). The alternative, what he calls a "perceptual focus" on a particular level, can be an obstacle even if the aim is to achieve "reductionistic" molecular explanations.

We hope that our historical case analysis can provide further support to the view that the cellular context must not be forgotten, or "simplified out of existence," when seeking molecular explanations of cellular phenomena. Guided decomposition is an attempt to avoid this pitfall by explicitly preserving constraints from the intact living system when experimenting on isolated parts. This strategy allows for the investigation of cellular phenomena at the molecular level and facilitates the reintegration of mechanisms into the intact living system with some confidence that they reflect what occurs in vivo. While we have described the characteristics of guided decomposition in the context of cell biology, we suspect that this is a common epistemic strategy in other areas of biology, and perhaps any empirical science dealing with multi-scale systems. We encourage further exploration and discussion of this question.

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Declarations

Conflicts of interest The authors declare no conflicts of interest.

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