Research Article

JMBE Journal of Microbiology & Biology Education

Here Is the Biology, Now What is the Mechanism? Investigating Biology Undergraduates' Mechanistic Reasoning within the Context of Biofilm Development

Sharleen Flowers,[a]* Kal H. Holder,[a] and Stephanie M. Gardner[a]
[a]*Department of Biological Sciences, Purdue University, West Lafayette, Indiana, USA*

Understanding molecular processes and coordinating the various activities across levels of organization in biological systems is a complicated task, yet many curricular guidelines indicate that undergraduate students should master it. Employing mechanistic reasoning can facilitate describing and investigating biological phenomena. Biofilms are an important system in microbiology and biology education. However, few empirical studies have been conducted on student learning of biofilms or how students utilize mechanistic reasoning related to systems thinking to explain biofilm formation. Using mechanistic reasoning and the theory of knowledge integration as conceptual and analytical frameworks, we examined the features of 9 undergraduate biology students' mechanistic models of a specific transition point in biofilm development. From these data, we constructed a model of knowledge integration in the context of biofilms, which categorizes students' knowledge based on features of their descriptions (e.g., entities, correct connections, and the nature of connections). We found that 4 of 9 students produced a fragmented model, 4 of 9 students produced a transitional model, and 1 student produced a connected model. Overall, students often did not discuss cell-cell communication mechanics in their mechanistic models and rarely included the role of gene regulation. Most connections were considered nonnormative and lacked important entities, leading to an abundance of unspecified causal connections. We recommend increasing instructional support of mechanistic reasoning within systems (e.g., identifying entities across levels of organization and their relevant activities) and creating opportunities for students to grapple with their understanding of various biological concepts and to explore how processes interact and connect in a complex system.

KEYWORDS biofilms, mechanistic reasoning, knowledge integration, undergraduate biology, gene regulation, cell-cell communication, phenotypic expression

INTRODUCTION

Mechanisms and mechanistic reasoning within systems

One omnipresent goal in the study of biology is to understand how the natural world works. For functional biologists, this means uncovering and elucidating the mechanisms that govern biological phenomena. Similarly, experts advocate for undergraduate students in biology to develop proficiency in constructing and interpreting explanations of biological phenomena (1). Tasking students to generate mechanisms addresses these calls, but building mechanistic explanations of biological phenomena can be complex. A mechanism describes how the things or entities engage in activities which produce change over time and space (2). In complex systems, the components of mechanisms interact across various levels of organization and different spatial locations, and multiple mechanisms can affect each other. Therefore, explaining biological phenomena involves the difficult task of incorporating mechanisms across levels of organization and localizations within a system and between systems (3, 4).

Breaking down phenomena according to the components of mechanistic reasoning can aid students in thinking about and building explanations of biological phenomena. Derived from Machamer and colleagues' characterization of how scientists explain scientific phenomena (2), researchers have identified several attributes of students' mechanistic reasoning, such as describing target phenomena as well as identifying entities and their properties, activities, and spatial organization (5). Studies have shown that students' mechanistic reasoning, generally and in the context of biological systems,

Editor Sarah Fankhauser, Emory University
Address correspondence to Department of Biological Sciences, Purdue University, West Lafayette, Indiana, USA. E-mail: sgardne@purdue.edu.
*Present address: Sharleen Flowers, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Boulder, Colorado, USA.
The authors declare no conflict of interest.
Received: 17 November 2022, Accepted: 3 May 2023,
Published: 18 May 2023

is not always well-developed (e.g., (6)) and students often exhibit various difficulties. Specifically, biological entities can be microscopic and unobservable, which makes conceptualizing the biological process problematic (7, 8). For example, entities such as proteins and genes and their associated activities may be misunderstood or forgotten when students reason with processes that have effects at the cellular level. Subsequently, coordinating the different parts of the mechanism across physical levels of organization has been demonstrated to be a challenge (3, 9–11). Thus, it is important for education researchers to understand how students use mechanistic reasoning and to help students overcome difficulties specifically associated with mechanistic reasoning within systems.

Biofilms as a relevant biological context to explore student thinking

Biofilms represent a complex system in which individual bacteria aggregate together on a surface and build a polymeric matrix of polysaccharides, proteins, and extracellular DNA (12). Bacterial biofilms have a significant impact in human society and the environment. For example, biofilms are exploited in bioremediation, biomining, and wastewater treatment. Furthermore, biofilms have been implicated in the pathogenesis and spread of disease and are harmful when they form on medical devices, pipes and machinery, household surfaces, etc. (13). As a principal topic of microbiological, environmental, and medical research, biofilms are an important concept in biology education and serve as a suitable context to explore student mechanistic reasoning within systems.

Despite the significance of biofilms in society and biology undergraduate education, there remains a considerable knowledge gap regarding students' understanding of biofilms. The American Society for Microbiology's Curriculum Guidelines for undergraduate microbiology recommend the instruction of bacterial biofilm formation as a component of two core competencies in microbiology (14). Yet, few empirical studies have been conducted around student learning of biofilms. A keyword search of biology education journals and medical education journals using the term "biofilm" failed to produce any results regarding how students understand biofilms. Much of the educational research regarding biofilms comprises descriptions of classroom activities to introduce basic concepts of biofilm development to young audiences and the public (15–17). There is a need to fill this gap in our knowledge as well as provide curriculum that enhances knowing and understanding of biofilms (18).

Theoretical frameworks guiding our work

We employed the mechanistic reasoning model and the theory of knowledge integration to elicit and characterize students' knowledge networks of biofilms. Drawing from the literature on mechanistic reasoning, we prompted mechanistic explanations by asking students to explain how a particular transition point in biofilm development occurs (2, 19, 20) and characterize explanations using important features of mechanisms. Specifically, we examined how students used and connected mechanistic ideas to build their explanation (e.g., entities or players, activities, and causality), the level of organization of the players, and where the processes are happening (3, 5, 21). Because building a mechanistic explanation for biological phenomena in complex systems such as biofilms requires an integrated understanding of biological concepts, we also leveraged the theory of knowledge integration in the analysis. As operationalized by Clark and Linn (22), knowledge integration describes the process by which students process new information by checking it against prior knowledge and ideas gained from formal and informal education as well as personal experiences. This framework has been used to guide curriculum, assessments, and education research to elicit and describe how students integrate new knowledge into their existing knowledge networks. Knowledge that does not conflict with information preexisting in their knowledge network becomes integrated, but knowledge that conflicts can become isolated from other ideas (22). Thus, it is important for these linkages to be supported with correct ideas to build a strong knowledge network that can be applied across a variety of contexts. Guided by these ideas, we used knowledge integration as a basis to build mechanistic model representations of the students' explanations. Additionally, we characterized the knowledge and relationships within the mechanistic models as well as the correctness of these elements. Finally, we incorporated all the characteristics described by mechanistic reasoning and knowledge integration to build a model of knowledge integration of students' knowledge networks in the context of biofilms.

Research aims

Despite the need for students to comprehend biofilm formation, there remains a gap in the literature describing how biology students use mechanistic reasoning within systems to understand and reason with biofilms. Addressing this critical need will provide the necessary foundations on which to build future research-informed teaching approaches for university educators. Therefore, we conducted a qualitative case study (23) to answer the question of what are the features of biology undergraduate students' explanations in the context of biofilm development?

METHODS

Student population

The data presented here were gathered as a case study to provide a rich characterization of student thinking at a single institution (24). During the Fall semester of 2019, we distributed an e-mail invitation for our study over the biology majors listserv at a large public midwestern university with very high research activity. We had an initial pool of 12 interested students; however, only 9 followed-up for interviews. The only

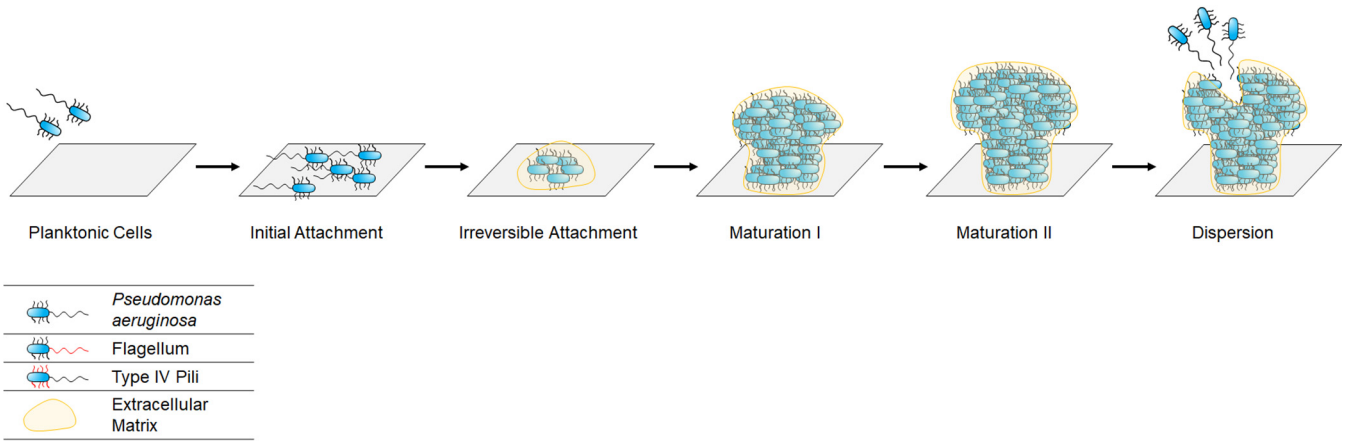

Figure 1. In a biofilm, individual bacterial cells attach to a surface and aggregate together to form a complex, slime-encased community. Secreted molecules such as polysaccharides, proteins, and extracellular DNA build a sticky polymeric matrix that help stabilize the biofilm to the surface. Bacteria living in these communities can exhibit different behaviors, which is facilitated by the sending and receiving of signals.

FIG 1. Model of biofilm development. Participants were provided with this figure, legend, and caption during the interview.

selection criteria was having declared a biology major. The class standing of students within our sample was three sophomores, three juniors, and three seniors. The biology majors of our nine students were the following: one Genetics; one Ecology, Evolution, and Environmental Biology; three Neurobiology and Physiology; and four General Biology. As biology majors, all participants had taken the same set of introductory biology courses, which covered topics relevant to our study. Additionally, in their second year, all biology majors take a cell biology and genetics course providing them with additional, more in-depth opportunities to learn topics relevant to this study. While three of our participants were enrolled in the cell biology course at the time of the interview and had not yet taken the genetics course, we did not observe a difference in their explanations and knowledge network features. None of the students had previously taken a microbiology class, but one student was currently enrolled in the institution's Introduction to Microbiology course. This student was three-quarters of the way through the semester; however, this did not give them an advantage in our study context. Due to the small sample size of this qualitative research study, we were not able to pursue analyses related to student demographics and performance on interview tasks.

This research study was approved under institutional review board 1806020745.

Data collection

We conducted semistructured, think-aloud interviews (25) in a small private office on the university campus. Sessions were audio-recorded and lasted between 30 and 90 min. All students were compensated with a $20.00 Amazon gift card for their time and travel. During the Spring and Fall semesters of 2018, we piloted the interview protocol with seven General Biology major students (five juniors and two seniors) to determine if the questions were eliciting responses that gave insights into their thinking. Responses were not notably different from those in the current study; thus, we feel confident that the students in the pilot provided us with valuable insights to refine our protocol. We also integrated feedback from four experts not directly involved in our research study (three science, technology, engineering, and math education researchers and one biology educator).

Data collected during the interviews were part of a larger study on students' knowledge integration and mechanistic reasoning within various biological contexts. At the beginning of the interview, students first defined what we are calling subsystems. We use the term subsystem, as it is descriptive of a component of a larger system (4). These subsystems were the following: gene regulation, cell-cell communication, and phenotypic expression. Students reviewed basic definitions of the subsystems and described relationships between the three subsystems in an open context. The purpose of these tasks was to activate and establish the students' baseline knowledge of the three subsystems and then to provide textbook definitions to help ensure students were not constrained by potential gaps in their knowledge, which may have limited their ability to engage in the rest of the interview. These specific subsystems were chosen since they are integral features of biofilm development but are also relevant and transferable across many biological contexts. A full description of the methods and data from the open context portion of the interview will be reported elsewhere (submitted for publication). Here, we report on student responses to questions posed in the context of biofilms (see Appendix S1 in the supplemental material).

First, we showed participants a model depicting biofilm development and an accompanying short, descriptive paragraph (Fig. 1). This model and figure caption were designed in 2017 based on the biofilm literature (12, 26–35) and reviewed by two

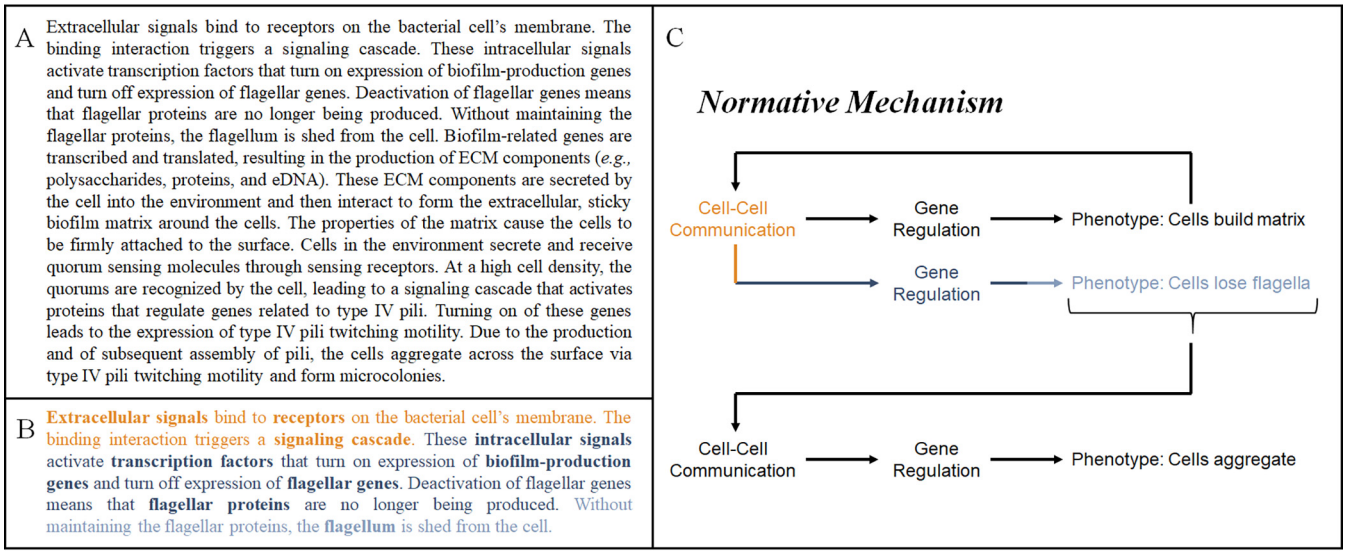

FIG 2. Abridged biofilm mechanism with example coding and mechanistic model. (A) Our simplified passage describing how bacterial cells transition from initial attachment to irreversible attachment during biofilm development. (B) An example of how we identified cell-cell communication mechanics (in yellow-orange), gene regulation mechanics (in dark blue), and bacterial phenotypes (in light blue) as well as entities (in bold). (C) A mechanistic model representing the entirety of the simplified passage in panel A and example coloration matching the coding in panel B.

microbiology faculty members. The short figure description given to all participants also provided a foundation for student reasoning during the interview. After orienting students to the figure, the interviewer then asked general questions about what the participants saw in the figure and what entities or players they thought may be involved in the transition point between initial attachment and irreversible attachment (Fig. 1). This then led to the question, "Describe to me how the transition point from initial attachment to irreversible attachment occurs," which we intentionally framed using how language to prompt mechanistic explanations (2, 19, 20). It was the responses to this question that formed the data set reported here. If necessary, the interviewer also prompted the participant to think about the previous players they named and to phrase their answer as a sequence of events. At this point in the interview, it was expected that the students had sufficiently reflected on the subsystems and would be primed to incorporate the subsystems into their explanation of the transition point (36, 37).

Analysis

Audio recordings from the interviews were transcribed verbatim, and students' explanations of the transition point were analyzed using inductive and deductive coding (23), mechanistic reasoning (2), and the theory of knowledge integration (22). To aid in the analysis, we composed a normative mechanism of how *Pseudomonas aeruginosa* bacterial cells transition from initial attachment to irreversible attachment based on relevant and highly cited biofilm literature (12, 26–35). However, this mechanistic description was detailed beyond what would be expected of students (e.g., specific genes and protein names) (Appendix S2). Our expectation was for students to apply their knowledge from cell biology and genetics—which we cued earlier in the interview—to reason through the transition point. As we were not interested in revealing and analyzing specific microbiological knowledge, we generated a simplified version of the mechanism (Fig. 2A).

We first sought to create mechanistic models that represented the students' verbal descriptions of the transition point. Deductive coding of our normative mechanism revealed that cell-cell communication, gene regulation, and three different bacterial phenotypes (i.e., cell aggregation, flagellum loss, and production of matrix) were relevant to this specific transition point (Fig. 2B). Using these three subsystems as pillars, we then leveraged knowledge integration to detect linkages between the subsystems. We scanned the mechanism for descriptions or naming of the subsystems and the sequence of events connecting the subsystems. We then drew an arrow connection between subsystem names to represent these interactions that occur during the transition point (Fig. 2C). We repeated this coding process to generate mechanistic models of the student data.

Using knowledge integration and mechanistic reasoning as analytical frameworks, we examined students' mechanistic models for (i) correctness of connections, (ii) nature of connections, (iii) correctness of ideas, and (iv) nature of ideas. These dimensions were chosen because knowledge must be sorted and connected correctly to integrate into knowledge networks and have qualitative attributes of mechanistic reasoning to support application and transfer of knowledge networks to different contexts. Each dimension is described in detail below, and their codebooks can be found in Appendix S3.

To evaluate (i) correctness of connections, we compared the normative mechanistic model to the students' models and identified alignment of connection types. Each of the connections

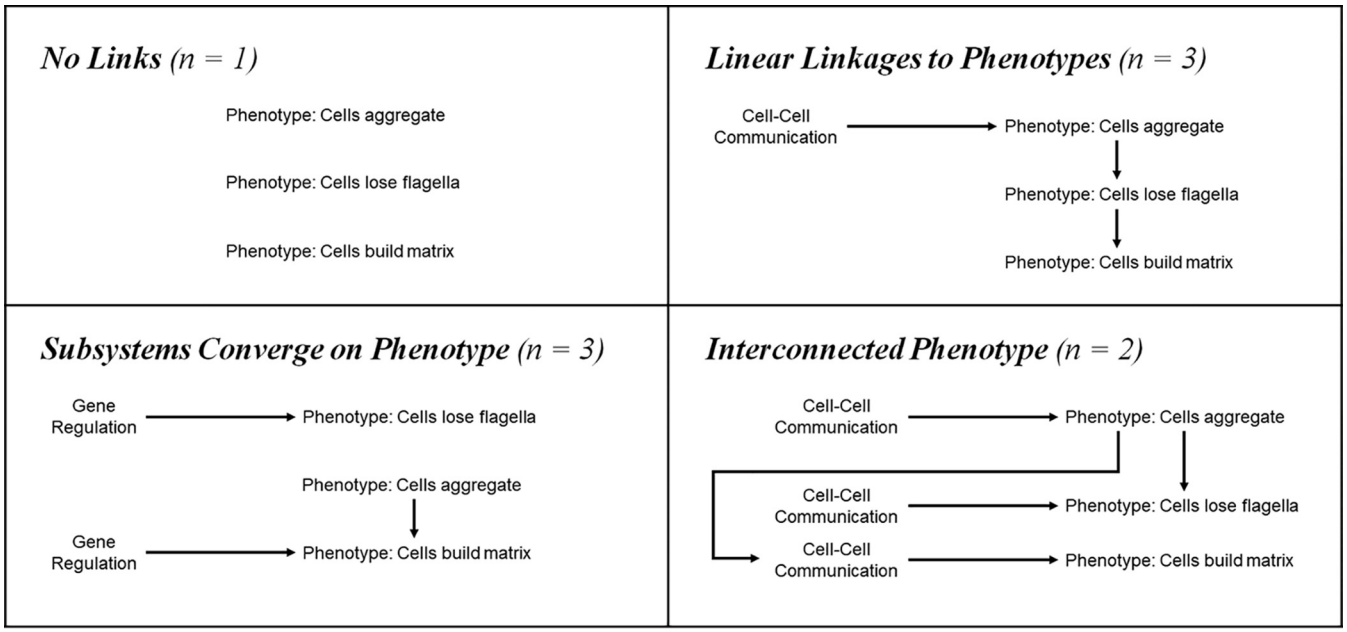

FIG 3. Students' mechanistic models of the initial attachment to irreversible attachment transition point in biofilm development. Exemplars of students' mechanistic models from each of the categories (except no links) are depicted. In parentheses are the number of students whose explanations fell into model structures in the particular category.

was also evaluated for (ii) nature of the connections by analyzing the ways in which the students described how the subsystems are linked, guided by the literature (2, 38, 39) and previous work (submitted).

For (iii) correctness of ideas, we examined the normative mechanism for knowledge elements relevant to the transition point and identified the following features: nine entities (see Appendix S3), three bacterial phenotypes, cell-cell communication mechanics, and gene regulation mechanics. We expected students to use these specific entities along with cell-cell communication and gene regulation mechanics to explain how the three visible phenotypes of cell aggregation, flagellum loss, and production of matrix occur. We decided that for a thing to count as an entity, it must meet one of the following criteria: (i) the entity engages in an activity (an action or a change occurring over space and time) (2); (ii) the entity is being acted upon by another entity during an interaction (3); or (iii) properties (such as structural attributes, spatial relations, orientations, or state) are described for an entity (2) (Fig. 2B).

To analyze (iv) nature of ideas, we evaluated descriptions of the level of organization of players (3) and the localization of processes (3, 5, 21) using a previously written codebook (submitted). During iterative refinement of the codebook against the data, we chose to characterize the "players" as opposed to "entities" to gain a holistic view of the level of organization in the students' explanations. Machamer and colleagues (2) defined entities as the things that engage in activities and/or have described properties. However, because this was not common in students' responses, we report on the overall players instead.

Lastly, we performed a knowledge integration analysis in which we categorized students' explanations on a continuum of fragmented to integrated biological ideas, drawing on Southard and colleagues' theoretical model (39). We holistically used all of the previous analyses (weighed equally) to evaluate the alignment of students' explanations to the normative mechanism and subsequently characterize the integration of students' knowledge.

Trustworthiness

The first author of this study (S.F.) independently coded all transcripts and developed a working set of codes for the mechanistic model structures and their features. The last author (S.M.G.) analyzed a subset of the student data using the preliminary codes and met to compare coding and structures of mechanistic models. Once initial codes and structures were agreed upon, S.F. iteratively revised the codebooks for mechanistic models and knowledge integration analysis with the second author (K.H.H.), whom S.F. had previously trained in qualitative coding. During this process, the readers independently coded the data using the codebooks, met to discuss coding, and then refined the codebooks. Once the authors settled on finalized codebooks, S.F. and K.H.H. performed one final round of coding for all the data. Prior to discussion, both authors had over 80% agreement. All analyses were discussed to complete agreement.

RESULTS

Characterizing structures of mechanistic models

Four types of mechanistic models emerged from the data set from student explanations (Fig. 3; Table 1). Models

TABLE 1
Key features of mechanistic models describing biofilm transition point

Mechanistic model type	Key features
No links	• No linkages present; only lists phenotypes as occurring
	• Does not incorporate cell-cell communication or gene regulation subsystems
Linear linkages to phenotypes	• A sequential, linear explanation of one subsystem leading to the next subsystem
	• Includes phenotype leading to the next phenotype
Subsystems converge on phenotype	• A largely linear explanation with some nonlinear features (e.g., convergence)
	• At least one instance where a phenotype is influenced by two independent subsystems
Interconnected phenotype	• A largely linear explanation with some nonlinear features (e.g., convergence)
	• Phenotype is a causal subsystem that affects a cell-cell communication or gene regulation subsystem

varied by presence of explicit connections and how the subsystems are connected (e.g., linear or nonlinear). In our sample, almost all mechanistic models (8/9) had at least 2 subsystems causally linked, and the majority of mechanistic models (5/9) had at least 3 subsystems causally linked in one chain of connected ideas (e.g., the linear linkages to phenotypes exemplar in Fig. 3). The most complex and interrelated mechanistic models were seen with students we identified as interconnected phenotype. Overall, the majority of mechanistic models were linear and contained at least one description in which two subsystems converged on a phenotype subsystem.

Characterizing correctness of connections

Nearly all students (8/9) provided at least one explicit connection between subsystems in their explanations. From these connections, we found a diverse set of connections in the students' mechanistic models that were not represented in the normative mechanism (Fig. 2). Connections considered correct were descriptions of cell-cell communication to gene regulation (CCC → GR), gene regulation to phenotypic change (GR → P), and phenotypic change to cell-cell communication (P → CCC) (Fig. 2), and we labeled all other connections as incorrect. Out of the 8 students who described connections, only 1 included CCC → GR, some students (3/8) included GR → P, and few students (2/8) included P → CCC. All 8 students described incorrect connections of cell-cell communication to phenotypic change or phenotypic change leading to another phenotypic change. Of the 26 total connections, 30% were correct connections, and incorrect connections accounted for the remaining 70%. We also found that only one student described a connection using incorrect knowledge when they described how gene regulation changes the DNA sequence depending on the cell's needs. Otherwise, all other connections contained correct information.

Characterizing the nature of connections

The students' mechanistic models in our data set included three different nature of connection categories: mechanistic, specified causal, and unspecified causal (see Appendix S3 for definitions and more examples). These primarily differed in specificity and usage of mechanistic features (e.g., entities with causal activities). A mechanistic connection includes a causal sequence of events with many entities and activities to explain the phenomenon, such as in this quote: "[the bacteria] start signaling once they're around each other. . . like a certain signal secretes or compounds, and. . . they hit the receptors of other ones and they know to come close together." A specified causal connection contains a causal factor but does not provide a mechanistic sequence of events. For example, "Perhaps like [the bacteria] receive from their environment that they need something from and they would benefit from aggregating together. So something in their environment signals them to form [a community or group]." In contrast, an unspecified causal connection lacks any causal factors and merely states a temporal sequence, such as this description: "Somehow when [the bacteria] kind of combine together. . . they secrete molecules." Across the 8 students who described at least one connection, we found 4 mechanistic, 8 specified causal, and 14 unspecified causal connections. All students who described at least one connection in their mechanistic models also included at least one unspecified causal connection (8/8). The majority of students (5/8) described at least one specified causal connection, and a few students (3/8) provided at least one mechanistic connection.

Characterizing correctness of ideas

No student included all nine expected entities (Fig. 4). The highest number of correct entities a student included in their explanation was 7. The most commonly missed entities (≤3 students included) were extracellular signals, receptors/signaling cascade, transcription factors/proteins, and genes. No student included type IV pili as an entity in their explanations (this was in the figure they were provided). The most discussed entities (≥7 students included) were bacterial cells, flagella, extracellular matrix molecules, and the extracellular matrix. Many students (7/9) discussed other entities that were not in our list of appropriate entities but were not necessarily incorrect to include in their explanations (e.g., fats, RNA, and amino acids). In addition, the majority of students (7/9) described inappropriate entities in

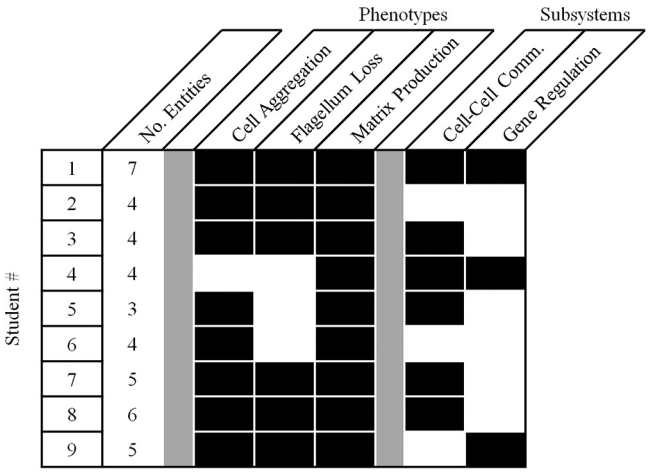

FIG 4. Evaluation of correctness of ideas in student explanations. The first column in the table lists the number of appropriate entities the student included in their explanation. The three columns under Phenotypes indicate if a student did or did not mention the phenotype. The two columns under Subsystems indicates if a student did or did not describe mechanics relating to the subsystem in their answer. A blackened box in the table represents at least one instance that the phenotype or subsystem was discussed.

their explanations that were ill-defined or irrelevant to bacteria (e.g., "nucleus," "something," and "stuff").

Two-thirds of students (6/9) discussed all three phenotypes in their explanations (Fig. 4). All students mentioned cells forming the extracellular matrix, almost all (8/9) discussed cell aggregation, and only 6/9 students included cells losing their flagella.

Very few students (2/9) integrated both cell-cell communication and gene regulation into their explanations (Fig. 4). We found that 6/9 students mentioned cell-cell communication at least once, and only 3/9 students mentioned gene regulation mechanics at least once.

Characterizing the nature of ideas: level of organization and localization

All students included players at cellular and macromolecular levels of organization, two-thirds of students (6/9) described players at undefined and environmental levels of organization, and about half of students (5/9) included molecular players in their descriptions (Fig. 5). Per our normative mechanism, a student's explanation should have included all four of the specified levels of organization categories and excluded the undefined level of organization. Only 1 student in our sample described an explanation in this manner, and 3 students included all five leveld of organization categories.

All students described processes occurring at an unspecified localization, almost all students discussed processes happening outside a cell (8/9) and at the cellular membrane (7/9), and few students (3/9) explicitly described processes occurring inside a cell (Fig. 5). Per our normative mechanism, a student's explanation should have included all three of the specified localization categories and excluded the unspecified localization, but we did not identify any student in our data set that described such an explanation. However, we did find 3 students who included all four localization categories in their description.

DISCUSSION

Synthesis of data: model of knowledge integration within a biofilm context

Informed by a theoretical model of knowledge integration in undergraduate molecular and cellular biology (39), we evaluated the ways in which students integrated knowledge into their mechanistic models. Using all the previously described characteristics from our analysis at equal weight, we identified mechanistic models lying on a spectrum of knowledge with defined categories of fragmented, transitional, and connected (Fig. 6). Key characteristics for each of the categories are provided in Table 2 (see Appendix S4 for a full description).

From our data set, we found that 4/9 students produced a fragmented model, 4/9 students produced a transitional model, and 1/9 students produced a connected model. These results demonstrate that many undergraduate biology students in our sample may not have possessed an integrated perspective of biological systems. While our study was not examining systems thinking specifically, in the context of reasoning within the biofilm system, students were often engaged in lower-level tiers of systems thinking, such as identifying relevant structures of the system, and were not reasoning about the relationships (4). Most students' ideas were isolated with few specified connections, if any connections, between various biological concepts. The majority of students' mechanistic models lacked the appropriate entities and subsystems needed to fully explain the transition point. This is consistent with the theory of knowledge integration: to have an integrated understanding of biology, students must sort appropriate entities into their respective processes (22, 39). Conflation of entities or ideas about various processes leads to loss of connectivity in mechanistic models (22). This is well-reflected in our data, since sparse and less-specified connections and few appropriate entities all contributed to fragmented models. Building a fully specified mechanistic explanation requires understanding of not only what relevant subsystems are related to each other but also what entities and activities support the linkage. In particular, things need to be described as entities and not as players to fit the definition of mechanisms (2). Improvement in this area may help transition students away from the fragmented end of knowledge integration and more toward a connected understanding of biological phenomena.

Reflecting on student models of biofilms

From these data, we conclude that students' mechanistic reasoning within the biofilm system was primarily limited by transfer of knowledge to an unfamiliar context and alternative conceptions or a lack of knowledge related to gene regulation.

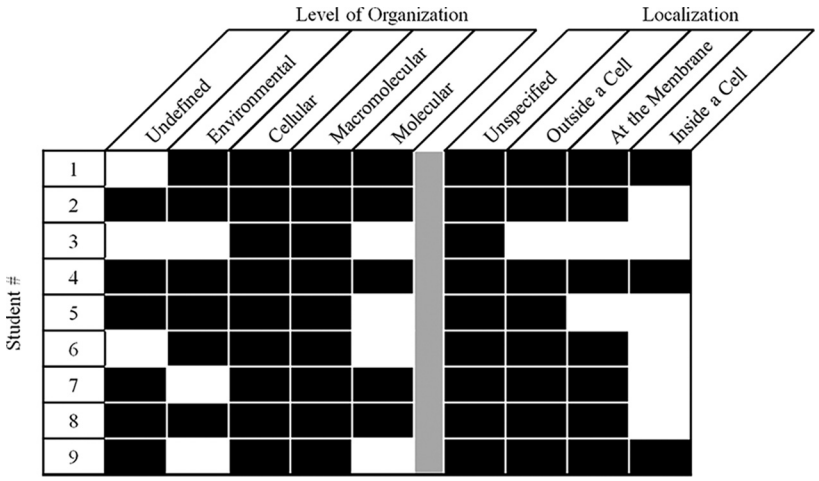

FIG 5. Level of organization of players and localization of processes coding. The five identified levels of organization are arranged on the left side of the table and the four identified localizations are arranged on the right side of the table. A blackened box in the table represents at least one instance where the student described a player at that level of organization or a process occurring at that localization.

We performed this study under the supported assumption that priming students earlier in the interview and providing a figure of biofilm development with a detailed caption would be sufficient for students to reason about the previous concepts in the new context (36). However, this knowledge transfer may have been too far (40, 41). The cell-cell communication and gene regulation subsystems and various connections between subsystems that were discussed earlier in the interview (submitted) were often not applied to the biofilm context. Additionally, processing scientific figures can be cognitively demanding (42–44). Interpreting and understanding external representations such as diagrams and illustrations requires skills in visual literacy and model-based reasoning (45, 46). Simply comprehending external representations is a challenge (47, 48), let alone comprehending a new figure situated in an unfamiliar microbiological context while in an interview setting. Students may have been given the necessary information in the form of a pictorial figure (e.g., the three phenotypes) and a figure caption (e.g., signals as an entity), but identifying this information and realizing how it is connected to the previous priming was potentially too cognitively difficult. For example, despite being labeled in the figure legend and noticed by almost all students (8/9) earlier in the interview (data not shown), no student utilized type IV pili as an entity in their explanations, even though they are necessary for the cell aggregation phenotype in biofilm development.

Students' understanding of gene regulation seems to be a barrier for correct description of this transition point in biofilm development. Transcription factors and genes were often missing from student explanations, which is consistent with previous work documenting students' uncertainty on the role of proteins and genes in the cell (7, 49). Additionally, studies have shown that students have varied ideas about gene expression and difficulties understanding the central dogma (49–52). Data from earlier in the interview indicated that the majority of our participants did not know the functional definition of gene regulation (submitted). Although students were cued to think about and review the definition of gene regulation, it is likely that they did not have the content knowledge or recognize its relevance to the biofilm context. Curriculum guidelines from the American Society for Microbiology emphasize that students should understand that gene regulation is influenced by external and internal stimuli (14). Additionally, understanding gene expression and how it influences the behavior of organisms is a core concept in undergraduate biology education (1). Put together, this forms a sequential connection of cell-cell communication affecting gene regulation which affects phenotype, which is the same framework we identified in the normative mechanism. However, our results showed that the least common connection was cell-cell communication to gene regulation and the most common connection was cell-cell communication to phenotype. By skipping the gene regulation step, the students posited that changes in cell-cell communication directly translated to changes in phenotype. This connection occurs in many biological contexts (e.g., long-term potentiation), but in the context of biofilm development, gene regulation is involved in each phenotypic change in the transition point between initial and irreversible attachment. The omission of gene regulation, and by extension that of proteins (7), prevents students from fully describing the transition point and signals a need to review the role of genes and proteins as well as the function of gene regulation in the cell.

Instructional implications

To cultivate the next generation of scientists, educators should scaffold mechanistic reasoning into the classroom when exploring how processes in biology function and integrate with other processes in the wider system. We recommend implementing tools such as the MACH model (53) or the MAtCH model (54), a guiding framework derived from how scientists describe mechanisms incorporating methods, analogies, theory, context, and the "how" of the mechanism, to help students

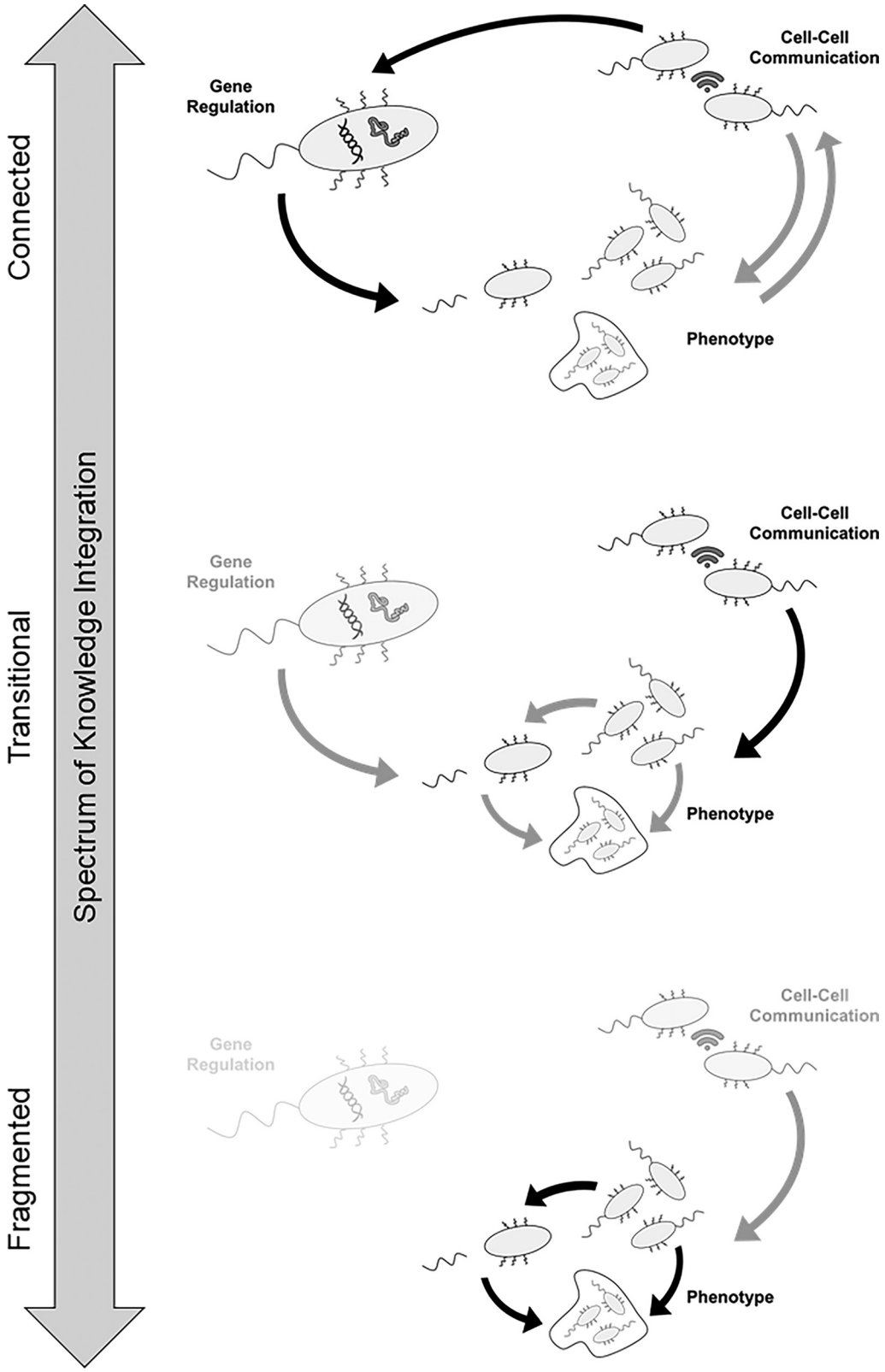

FIG 6. Model of knowledge integration in the context of biofilm development. Text and arrows in black represent subsystems and connections that are common characteristics of the model. Text and arrows in dark gray represent subsystems and connections that are less frequent. Text in light gray or lacking arrows represent subsystems and connections that are not a characteristic of the model. Opaque portions of the images next to subsystem names are similarly aligned: the most opaque images are common, slightly transparent images are less frequent, and very transparent images are not a characteristic.

TABLE 2
Key features of the three categories of knowledge integration in a biofilm context

Knowledge integration category	Key features
Fragmented	• Incorporates the fewest connections, mostly between phenotypes
	• Some include a connection between cell-cell communication and a bacterial phenotype, but none of them involve the gene regulation subsystem
	• Least specified and least correct
Transitional	• Incorporates more connections that include the cell-cell communication and gene regulation subsystems
	• The content and nature of information are more specified and correct
	• Missing some important characteristics, such as connections between cell-cell communication and gene regulation
Connected	• Highest number of connections that are more specified and correct
	• All three bacterial phenotypes are explained using the subsystems of cell-cell communication and/or gene regulation
	• The correctness and nature of the content are the most correct

identify the necessary components for building a complete mechanistic explanation. Additionally, instructors need to acknowledge the situational nature of knowledge and examine the boundaries of knowledge transfer. Instructors may assume that content learned in a previous lecture or course is readily available knowledge that can be used in any context (55). However, knowledge is tied to the specific contexts (e.g., environment, activity, and cultural) in which it is learned and used (56). In addition, different areas of science develop discipline-specific epistemic norms, values, beliefs, and heuristics (57). Thus, applying knowledge learned in one context to another requires disentangling contextual features and discipline-specific ideas from the concept and transferring only the relevant knowledge. Studies have shown that knowledge transfer is hard (40, 41, 58–60) and can be impacted by the extent by which transfer is "near" or "far" (40, 41, 61). Instructors should critically consider the contexts they ask students to transfer knowledge to and incorporate opportunities for practice to help students achieve knowledge transfer. We assumed that discussion of gene regulation, cell-cell communication, and phenotypic expression earlier in the interview would be sufficient cuing for students to incorporate those subsystems into their biofilm explanations. However, only two-thirds of students (6/9) and one-third of students (3/9) discussed cell-cell communication and gene regulation, respectively. Studies have shown that spoken instructor cues can impact student reasoning (62, 63) and explicit instructions to address specific criteria affects students' answers (64). Thus, we suggest inserting opportunities for students to activate prior knowledge and priming them to think about and use specific ideas at the time of teaching or assessment.

Limitations and future directions

In this study, we captured 9 students' mechanistic models of a transition point in biofilm development and sorted these models into three distinct knowledge integration categories based on previous work (39). Only one student fit into the connected category, and we saw no evidence of a nuanced category as theorized by Southard and colleagues (39). Expanding the study to other student populations in other life science majors and at additional institutions may reveal more students with a connected understanding of biology and may also yield interesting new insights. Increased probing and interview scaffolding may also prompt students to demonstrate a connected understanding of biology. In addition, our data collection may have been limited by asking students to explain biological phenomena from an unfamiliar context of biofilms. To reduce the cognitive load of the task and difficulty of transfer, we plan to ask participants in future interviews to provide their own context in which to embed the concepts of gene regulation, cell-cell communication, and phenotypic expression. One key characteristic of mechanistic reasoning is chaining forward and backward, which involves reasoning through previous interactions and predicting future events and vice versa, based on the known entities and activities of a mechanism (5). Future studies should investigate students' abilities to use chaining when reasoning about their mechanistic models. We propose using a "perturbation" (65) as a problem-solving task in which a particular characteristic of the system is changed and then the student uses their mechanistic model to reason through what would happen. Exploring the boundaries of students' mechanistic reasoning within systems will expand our understanding of how students reason about biofilms and other complex systems within biology. From these data, important curriculum and assessment tools can be created to enhance students' education of biofilms and of biological systems in general.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

ACKNOWLEDGMENTS

We extend gratitude to Eryn Sale for her assistance piloting the interview, Anupriya Karippadath for her valuable comments, and Gabby Rump for her help analyzing other data related to this study. We thank Nancy Pelaez, Ala Samarapungavan, and Thomas Walter for their review of the study during its early stages.

REFERENCES

1. American Association for the Advancement of Science. 2011. Vision and change in undergraduate biology education: a call to action. AAAS, Washington, DC.
2. Machamer P, Darden L, Craver CF. 2000. Thinking about mechanisms. Philos Sci 67:1–25. https://doi.org/10.1086/392759.
3. van Mil MHW, Boerwinkel D, Waarlo A. 2013. Modelling molecular mechanisms: a framework of scientific reasoning to construct molecular-level explanations for cellular behaviour. Sci Educ 22:93–118. https://doi.org/10.1007/s11191-011-9379-7.
4. Momsen J, Speth EB, Wyse S, Long T. 2022. Using systems and systems thinking to unify biology education. CBE Life Sci Educ 21:es3. https://doi.org/10.1187/cbe.21-05-0118.
5. Russ RS, Scherr RE, Hammer D, Mikeska J. 2008. Recognizing mechanistic reasoning in student scientific inquiry: a framework for discourse analysis developed from philosophy of science. Sci Educ 92:499–525. https://doi.org/10.1002/sce.20264.
6. Southard KM, Espindola MR, Zaepfel SD, Bolger MS. 2017. Generative mechanistic explanation building in undergraduate molecular and cellular biology. Int J Sci Educ 39:1795–1829. https://doi.org/10.1080/09500693.2017.1353713.
7. Duncan RG, Reiser BJ. 2007. Reasoning across ontologically distinct levels: students' understandings of molecular genetics. J Res Sci Teach 44:938–959. https://doi.org/10.1002/tea.20186.
8. Gilbert JK, Osborne RJ, Fensham PJ. 1982. Children's science and its consequences for teaching. Sci Educ 66:623–633. https://doi.org/10.1002/sce.3730660412.
9. Duncan RG. 2007. The role of domain-specific knowledge in generative reasoning about complicated multileveled phenomena. Cogn Instruct 25:271–336. https://doi.org/10.1080/07370000701632355.
10. Duncan RG, Tseng KA. 2011. Designing project-based instruction to foster generative and mechanistic understandings in genetics. Sci Educ 95:21–56. https://doi.org/10.1002/sce.20407.
11. Marbach-Ad G, Stavy R. 2000. Students' cellular and molecular explanations of genetic phenomena. J Biol Educ 34:200–205. https://doi.org/10.1080/00219266.2000.9655718.
12. Steinberg N, Kolodkin-Gal I. 2015. The matrix reloaded: how sensing the extracellular matrix synchronizes bacterial communities. J Bacteriol 197:2092–2103. https://doi.org/10.1128/JB.02516-14.
13. Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM. 1995. Microbial biofilms. Annu Rev Microbiol 49:711–745. https://doi.org/10.1146/annurev.mi.49.100195.003431.
14. Merkel S, ASM Task Force on Curriculum Guidelines for Undergraduate Microbiology. 2012. The development of curricular guidelines for introductory microbiology that focus on understanding. J Microbiol Biol Educ 13:32–38. https://doi.org/10.1128/jmbe.v13i1.363.
15. Couto JM. 2017. Biofilms for babies: introducing microbes and biofilms to preschool-aged children. J Microbiol Biol Educ 18:18.1.96. https://doi.org/10.1128/jmbe.v18i1.1273.
16. Marlow VL, Maclean T, Brown H, Kiley TB, Stanley-Wall NR. 2013. Blast a biofilm: a hands-on activity for school children and members of the public. J Microbiol Biol Educ 14:252–254. https://doi.org/10.1128/jmbe.v14i2.563.
17. McOwat K, Stanley-Wall NR. 2018. Biofilm building: a simple board game to reinforce knowledge of biofilm formation. J Microbiol Biol Educ 19:19.1.70. https://doi.org/10.1128/jmbe.v19i1.1355.
18. Woo KY. 2016. Physicians' knowledge and attitudes in the management of wound infection. Int Wound J 13:600–604. https://doi.org/10.1111/iwj.12290.
19. Abrams E, Southerland S. 2001. The how's and why's of biological change: how learners neglect physical mechanisms in their search for meaning. Int J Sci Educ 23:1271–1281. https://doi.org/10.1080/09500690110038558.
20. Mayr E. 1961. Cause and effect in biology. Science 134:1501–1506. https://doi.org/10.1126/science.134.3489.1501.
21. Lira M, Gardner SM. 2020. Leveraging multiple analytic frameworks to assess the stability of students' knowledge in physiology. CBE Life Sci Educ 19:ar3. https://doi.org/10.1187/cbe.18-08-0160.
22. Clark D, Linn MC. 2003. Designing for knowledge integration: the impact of instructional time. J Learn Sci 12:451–493. https://doi.org/10.1207/S15327809JLS1204_1.
23. Creswell JW, Poth CN. 2018. Qualitative inquiry & research design: choosing among five approaches, 4th ed. SAGE Publications, Thousand Oaks, CA.
24. Merriam SB, Tisdell EJ. 2015. Qualitative research: a guide to design and implementation, 4th ed. Jossey-Bass, San Francisco, CA.
25. Ericsson KA, Simon HA. 1980. Verbal reports as data. Psychol Rev 87:215–251. https://doi.org/10.1037/0033-295X.87.3.215.
26. Davies DG, Marques CNH. 2009. A fatty acid messenger is responsible for inducing dispersion in microbial biofilms. J Bacteriol 191:1393–1403. https://doi.org/10.1128/JB.01214-08.
27. Glessner A, Smith RS, Iglewski BH, Robinson JB. 1999. Roles of Pseudomonas aeruginosa las and rhl quorum-sensing systems in control of twitching motility. J Bacteriol 181:1623–1629. https://doi.org/10.1128/JB.181.5.1623-1629.1999.
28. Guttenplan SB, Kearns DB. 2013. Regulation of flagellar motility during biofilm formation. FEMS Microbiol Rev 37:849–871. https://doi.org/10.1111/1574-6976.12018.
29. Hengge R. 2009. Principles of c-di-GMP signalling in bacteria. Nat Rev Microbiol 7:263–273. https://doi.org/10.1038/nrmicro2109.
30. Hinsa SM, Espinosa-Urgel M, Ramos JL, O' Toole GA. 2003. Transition from reversible to irreversible attachment during biofilm formation by Pseudomonas fluorescens WCS365 requires an ABC transporter and a large secreted protein. Mol Microbiol 49:905–918. https://doi.org/10.1046/j.1365-2958.2003.03615.x.

31. O'Toole G, Kaplan HB, Kolter R. 2000. Biofilm formation as microbial development. Annu Rev Microbiol 54:49–79. https://doi.org/10.1146/annurev.micro.54.1.49.
32. O'Toole GA, Kolter R. 1998. Flagellar and twitching motility are necessary for Pseudomonas aeruginosa biofilm development. Mol Microbiol 30:295–304. https://doi.org/10.1046/j.1365-2958.1998.01062.x.
33. Sauer K, Camper AK, Ehrlich GD, Costerton JW, Davies DG. 2002. Pseudomonas aeruginosa displays multiple phenotypes during development as a biofilm. J Bacteriol 184:1140–1154. https://doi.org/10.1128/jb.184.4.1140-1154.2002.
34. Stoodley P, Sauer K, Davies DG, Costerton JW. 2002. Biofilms as complex differentiated communities. Annu Rev Microbiol 56:187–209. https://doi.org/10.1146/annurev.micro.56.012302.160705.
35. Willey JM, Sherwood L, Woolverton CJ, Prescott LM. 2008. Prescott, Harley, and Klein's microbiology, 7th ed. McGraw-Hill Higher Education, New York, NY.
36. Lavrakas PJ. 2008. Encyclopedia of survey research methods. Sage Publications, Thousand Oaks, CA.
37. Hammer D, Elby A, Scherr RE, Redish EF. 2005. Resources, framing, and transfer, p 89–119. *In* Mestre JP (ed), Transfer of learning from a modern multidisciplinary perspective. Information Age Publishing, Greenwich, CT.
38. Becker N, Noyes K, Cooper M. 2016. Characterizing students' mechanistic reasoning about London dispersion forces. J Chem Educ 93:1713–1724. https://doi.org/10.1021/acs.jchemed.6b00298.
39. Southard K, Wince T, Meddleton S, Bolger MS. 2016. Features of knowledge building in biology: understanding undergraduate students' ideas about molecular mechanisms. CBE Life Sci Educ 15:ar7. https://doi.org/10.1187/cbe.15-05-0114.
40. Barnett SM, Ceci SJ. 2002. When and where do we apply what we learn?: a taxonomy for far transfer. Psychol Bull 128:612–637. https://doi.org/10.1037/0033-2909.128.4.612.
41. Kaminske AN, Kuepper-Tetzel CE, Nebel CL, Sumeracki MA, Ryan SP. 2020. Transfer: a review for biology and the life sciences. CBE Life Sci Educ 19:es9. https://doi.org/10.1187/cbe.19-11-0227.
42. Angra A, Gardner SM. 2018. The graph rubric: development of a teaching, learning, and research tool. CBE Life Sci Educ 17:ar65. https://doi.org/10.1187/cbe.18-01-0007.
43. Round JE, Campbell AM. 2013. Figure facts: encouraging undergraduates to take a data-centered approach to reading primary literature. CBE Life Sci Educ 12:39–46. https://doi.org/10.1187/cbe.11-07-0057.
44. Wright LK, Cardenas JJ, Liang P, Newman DL. 2018. Arrows in biology: lack of clarity and consistency points to confusion for learners. CBE Life Sci Educ 17:ar6. https://doi.org/10.1187/cbe.17-04-0069.
45. Jonassen D, Strobel J, Gottdenker J. 2005. Model building for conceptual change. Interact Learn Environ 13:15–37. https://doi.org/10.1080/10494820500173292.
46. Schönborn KJ, Anderson TR. 2010. Bridging the educational research-teaching practice gap: foundations for assessing and developing biochemistry students' visual literacy. Biochem Mol Biol Educ 38:347–354. https://doi.org/10.1002/bmb.20436.
47. Cook M, Wiebe EN, Carter G. 2008. The influence of prior knowledge on viewing and interpreting graphics with macroscopic and molecular representations. Sci Educ 92:848–867. https://doi.org/10.1002/sce.20262.
48. Schnotz W. 2002. Commentary: towards an Integrated View of Learning from Text and Visual Displays. Educational Psychology Rev 14:101–120. https://doi.org/10.1023/A:1013136727916.
49. Marbach-Ad G. 2001. Attempting to break the code in student comprehension of genetic concepts. J Biological Education 35:183–189. https://doi.org/10.1080/00219266.2001.9655775.
50. Lewis J, Wood-Robinson C. 2000. Genes, chromosomes, cell division and inheritance - do students see any relationship? Int J Science Education 22:177–195. https://doi.org/10.1080/095006900289949.
51. Newman DL, Coakley A, Link A, Mills K, Wright LK. 2021. Punnett squares or protein production? The expert-novice divide for conceptions of genes and gene expression. CBE Life Sci Educ 20:ar53. https://doi.org/10.1187/cbe.21-01-0004.
52. Wright LK, Newman DL. 2013. Using PCR to target misconceptions about gene expression. J Microbiol Biol Educ 14:93–100. https://doi.org/10.1128/jmbe.v14i1.539.
53. Trujillo CM, Anderson TR, Pelaez NJ. 2015. A model of how different biology experts explain molecular and cellular mechanisms. CBE Life Sci Educ 14:ar20. https://doi.org/10.1187/cbe.14-12-0229.
54. Jeffery KA, Pelaez N, Anderson TR. 2018. How four scientists integrate thermodynamic and kinetic theory, context, analogies, and methods in protein-folding and dynamics research: implications for biochemistry instruction. CBE Life Sci Educ 17:ar13. https://doi.org/10.1187/cbe.17-02-0030.
55. Sato BK, Lee AK, Alam U, Dang JV, Dacanay SJ, Morgado P, Pirino G, Brunner JE, Castillo LA, Chan VW, Sandholtz JH. 2017. What's in a prerequisite? A mixed-methods approach to identifying the impact of a prerequisite course. CBE Life Sci Educ 16:ar16. https://doi.org/10.1187/cbe.16-08-0260.
56. Brown JS, Collins A, Duguid P. 1989. Situated cognition and the culture of learning. Educ Res 18:32–42. https://doi.org/10.3102/0013189X018001032.
57. Samarapungavan A, Westby EL, Bodner GM. 2006. Contextual epistemic development in science: a comparison of chemistry students and research chemists. Sci Educ 90:468–495. https://doi.org/10.1002/sce.20111.
58. Butler AC. 2010. Repeated testing produces superior transfer of learning relative to repeated studying. J Exp Psychol Learn Mem Cogn 36:1118–1133. https://doi.org/10.1037/a0019902.
59. Sumeracki MA, Weinstein-Jones Y, Nebel CL, Schmidt SJ. 2019. Encouraging knowledge transfer in food science and nutrition education: suggestions from cognitive research. J Food Sci Educ 18:59–66. https://doi.org/10.1111/1541-4329.12158.
60. Wooldridge CL, Bugg JM, McDaniel MA, Liu Y. 2014. The testing effect with authentic educational materials: a cautionary note. J Applied Res Memory Cogn 3:214–221. https://doi.org/10.1016/j.jarmac.2014.07.001.
61. Perkins DN, Salomon G. 1992. Transfer of learning, p 1–13. *In* Husén T, Postlethwaite TN (ed), International encyclopedia of education, 2nd ed. Pergamon Press, Oxford, England.

62. Knight JK, Wise SB, Rentsch J, Furtak EM. 2015. Cues matter: learning assistants influence introductory biology student interactions during clicker-question discussions. CBE Life Sci Educ 14:ar41. https://doi.org/10.1187/cbe.15-04-0093.
63. Knight JK, Wise SB, Southard KM. 2013. Understanding clicker discussions: student reasoning and the impact of instructional cues. CBE Life Sci Educ 12:645–654. https://doi.org/10.1187/cbe.13-05-0090.
64. Schen M. 2012. Assessment of argumentation skills through individual written instruments and lab reports in introductory biology. Annual Meeting of the National Association for Research in Science Teaching, Indianapolis, IN.
65. Hmelo-Silver CE, Marathe S, Liu L. 2007. Fish swim, rocks sit, and lungs breathe: expert-novice understanding of complex systems. J Learn Sci 16:307–331. https://doi.org/10.1080/10508400701413401.