Chapter 2
Mechanisms Versus Causes in Biology and Medicine

Lindley Darden

Abstract Biologists use knowledge of mechanisms for explanation, prediction, and control. Philosophers of biology, working in the new mechanistic philosophy of science, have identified features of an adequate description of a biological mechanism. The very abstract schema term "cause" may refer to any of various components of a mechanism, or even conditions needed for it to operate. A case study of the disease cystic fibrosis illustrates the advantages (and complexities) of identifying the various stages of the relevant mechanisms. Such knowledge is more useful than merely claiming that a mutation in the CFTR gene causes the disease, given the goals of explanation, prediction, and control of disease symptoms. Knowledge of "mechanism produces phenomenon" is often much more useful for explanation, prediction, and control than "C causes E."

1 Introduction

Contemporary biologists often seek to discover mechanisms. Many such discoveries were major achievements in twentieth-century biology, such as the mechanism of Mendelian heredity (Morgan et al. 1915; Darden 1991), the numerous mechanisms of cellular metabolism (Bechtel 2006), mechanisms in neuroscience (Craver 2007), and the mechanisms of DNA replication, protein synthesis, and gene expression in molecular biology (Watson et al. 2007; Darden and Craver 2002). Philosophers of biology are now studying the nature of biological mechanisms in "the new mechanistic philosophy" (Skipper and Millstein 2005).

The team of Peter Machamer, Lindley Darden, and Carl Craver characterized mechanisms and applied that characterization to cases from molecular biology and

L. Darden (✉)
Department of Philosophy, University of Maryland, 1125A Skinner Building, College Park 20742, MD, USA
e-mail: darden@umd.edu

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neurobiology (Machamer et al. 2000; hereafter referred to as MDC). Others worked on mechanisms in such fields as biochemistry and cell biology (Bechtel and Richardson 1993, 2010; Bechtel 2006), evolutionary theory (Barros 2008), medicine (Thagard 1998; Moghaddam-Taaheri 2011), and the social sciences (e.g., Hedström 2005). Philosophers work to analyze the relation of this new work on mechanisms to traditional topics in philosophy of science, such as explanation (Bechtel and Abrahamsen 2005; Craver 2007) and causation, addressed in diverse ways by Jim Bogen (e.g., 2004, 2005, 2008), Bill Bechtel and Carl Craver (e.g., Craver and Bechtel 2007; Craver 2007, Ch. 3), Stuart Glennan (1996, 2002, 2010), and Jim Woodward (e.g., 2002).

Biologists seek mechanisms for three reasons: explanation, prediction, and control. In this chapter, I will argue that within the mechanistic sciences, such as molecular biology and molecular medicine, the claim "C causes E" is impoverished compared to the claim that "this mechanism produces this phenomenon." Knowledge of a mechanism in the biological sciences is usually more useful for explanation, prediction, and control than merely being able to label something as a cause. Furthermore, the new mechanists emphasize the importance of characterizing (and recharacterizing as work proceeds) the phenomenon that the mechanism produces. Such characterization is a rich description, providing guidance and constraints in the search for the mechanism.

I proceed as follows. In Sect. 2, I summarize one current view of biological mechanisms, the MDC characterization of biological mechanisms. In Sect. 3, I first summarize what we said in the MDC paper about the relation of the analysis of mechanism to an analysis of cause. Then, I expand it to conjecture what "C causes E" might refer to, from the perspective of biological mechanisms. In Sect. 4, I take up the extension of the MDC account to medicine and illustrate the power and complexities that the search for mechanisms plays in an example from medicine. Medical researchers seek mechanisms not just to give explanations for disease symptoms but also to predict the occurrence and severity of the disease and control the outcome for the patient's benefit. We might say: "A mutation in the CFTR gene causes cystic fibrosis." But that is much too simple. To illustrate the usefulness of knowledge of mechanisms, I trace the history of our understanding of the mechanisms that account for, and therapies to treat, the disease of cystic fibrosis. This example illustrates general features about the role of discovering mechanisms for explanation, prediction, and control in fields with practical aims, such as medical research.

2 The MDC Characterization of Mechanisms

A mechanism is sought to explain how a phenomenon is produced. Our team of Machamer, Darden, and Craver characterized mechanisms in the following way:

> Mechanisms are entities and activities organized such that they are productive of regular changes from start or set-up to finish or termination conditions. (MDC 2000, p. 3)

The MDC *characterization* of mechanisms is not a *definition* giving necessary and sufficient conditions for the term's usage in all cases. Instead it is a characterization to capture the way biologists use the term, as informed by our detailed examination of cases from molecular biology and neurobiology and also informed by philosophical reflection on requirements for *productive* changes.

An example of a biological mechanism is the mechanism of protein synthesis. From the beginning of the field of molecular biology in the 1950s, one of the phenomena puzzling biologists was how proteins are synthesized. By the 1970s, molecular biologists and biochemists had discovered the key details of the mechanism of protein synthesis (Darden and Craver 2002). The mechanism is often represented by the abstract schema, called the "central dogma" of molecular biology:

$$\text{DNA} \rightarrow \text{RNA} \rightarrow \text{Protein}$$

It may also be represented by much more detail as in Fig. 2.1, with structures of entities, the organization of the mechanism components within a cell, and the temporal stages and movements depicted by arrows. The mechanism begins in the nucleus with the unwinding of the DNA double helix and the synthesis of messenger RNA. The long ribbon of mRNA moves into the cytoplasm where it attaches to the cell organelle, the ribosome. The ribosome is the site where transfer RNAs, carrying their respective amino acids, attach to the messenger RNA (in a specific order, determined by the genetic code). The growing chain of amino acids will later leave the ribosome and fold into a three-dimensional protein (not shown in Fig. 2.1).

This example illustrates many of the general features of biological mechanisms. These are listed in Table 2.1. The first feature is "phenomenon" because the first step in the search for a mechanism is to identify and characterize a puzzling phenomenon of interest. Next are componency features. The mechanism is composed of entities and activities, sometimes further organized into functional modules. Functional modules are groups of entities and activities that play a given role in the mechanism and may recur in mechanisms of the same abstract type, e.g., the module for translation in the mechanism of protein synthesis (discussed below).

Note that the entities in the protein synthesis mechanism are not all at the same size level. Working entities of the protein synthesis mechanism range from small ions to larger macromolecules to cell organelles (composed of macromolecules). Size level and mechanism level need not, and often do not, correspond (Craver 2007, Ch. 5). Mechanisms have working components of a certain size, with structure and with other properties that enable them to engage in the activities that drive the mechanism.

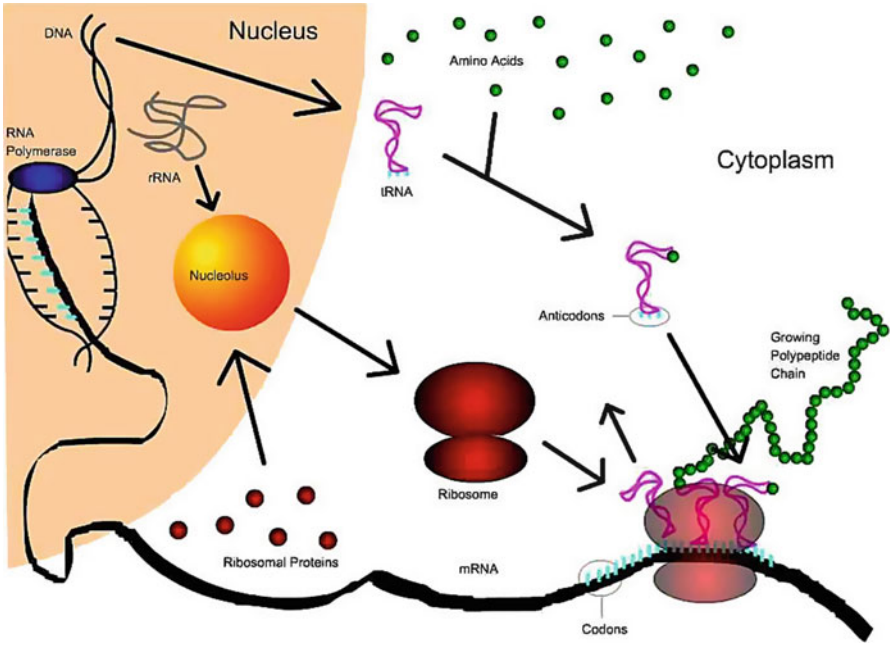

Fig. 2.1 Mechanism of protein synthesis

Table 2.1 Features of mechanisms

Phenomenon
Components
Entities and activities
Modules
Spatial arrangement of components
Localization
Structure
Orientation
Connectivity
Compartmentalization
Temporal aspects of components
Order
Rate
Duration
Frequency
Contextual locations
Location within a hierarchy
Location within a series

Modified from Darden (2006, Table 12.1) and Craver and Darden (2001, Table 2.1)

The mechanism's components have spatial and temporal organization. Spatial organization includes location, internal structure, orientation, and connectivity (both among component parts within the mechanism and to other mechanisms before the start condition and after the termination condition). Sometimes a molecular mechanism is compartmentalized, e.g., occurring in one part of the cell and surrounded by a membrane that protects its parts from dissipation and attack or from attacking other parts of the cell. (Lysosomes, e.g., contain caustic enzymes that break down waste materials; their enzymes are enclosed in that cell organelle and thus do not attack other cellular components.) Also, the stages of the mechanism occur in a particular order and they take certain amounts of time (duration). Some stages occur at a certain rate or repeat with a given frequency. In addition to the componency, spatial, and temporal features of a mechanism, the mechanism may be situated in wider contexts—in a hierarchy of mechanism levels (Craver 2007, Ch. 5) and in a temporal series of mechanisms (Darden 2005). These features of mechanisms can play roles in the search for mechanisms, and then they become parts of an adequate description of a mechanism. What counts as an adequate description (i.e., how much detail needs to be specified) depends on the context in which an explanation of the puzzling phenomenon is sought and the purposes for which the description is to be used.

One use is to make predictions. When the mechanism is in place and the start conditions obtain, then the orderly operation of each stage of the mechanism results in the production of the phenomenon. Hence one can predict what the outcome will be. However, if a portion of the mechanism is broken, then one can predict that the earlier stages operated and an intermediate product accumulates (or perhaps no product at all is produced). Knowing about the intermediate stages allows more fine-grained predictions about what is the output of each stage and what will happen when a stage breaks. A scientist may be able to run a mental simulation of the mechanism and thereby predict what phenomenon it will produce or to predict what will happen if a part of the mechanism is broken. (On mental simulations of mechanisms operating, see Bechtel and Abrahamsen 2005.) However, sometimes the complexity of the mechanism makes mental simulations difficult. Computational simulation models of the mechanism are more useful, especially for quantitative predictions about, e.g., concentrations of products (e.g., Eisenhaber 2006) or for predicting complex spatial interactions as in molecular dynamic simulations (e.g., Watanabe et al. 2010).

A *mechanism schema* is a truncated abstract description of a mechanism that we know how to fill with more specific descriptions of component entities and activities, such as the schema for the central dogma, discussed above. In contrast, a *mechanism sketch* cannot (yet) be instantiated. Components are (as yet) unknown. Sketches may have black boxes for missing components that are sought as the search for the mechanism proceeds. An adequate description of a mechanism (in the context of a given puzzling phenomenon) is an account with all the black boxes filled, with the overall organization specified (e.g., linear or cyclic), and with the features of Table 2.1 noted.

3 Mechanisms and Causes

In this section, I briefly discuss ways that talk of "cause" and "effect" may possibly be mapped to talk of "mechanism" and "phenomenon." This is not a thorough discussion of the many topics addressed by those analyzing causation. Rather it is just a brief foray, from the perspective of some of the recent work on biological mechanisms, to show how much more impoverished talk of "causes" is compared to talk of "mechanisms."

Possible referents of the term "cause" are many and varied from the mechanistic perspective. Something that is designated as a cause may refer to a piece of a mechanism. MDC analyzed mechanisms as composed of both entities (with their properties) and activities. Activities are producers of change; they are constitutive of the transformations that yield new states of affairs. As Machamer (2004) noted, activities are often referred to by verbs or verb forms (e.g., participles, gerunds). Molecules *bond*, helices *unwind*, ion channels *open*, and chromosomes *pair* and *separate*.

In MDC, we discussed the relation between cause and activity:

> Activities are types of causes. Terms like "cause" and "interact" are abstract terms that need to be specified with a type of activity and are often so specified in typical scientific discourse. (MDC 2000, p. 6)

We followed Elizabeth Anscombe (1971, p. l37), who noted that the word "cause" itself is highly general. It needs to be specified by other, more specific, causal verbs. Anscombe included the following in her list: scrape, push, dry, carry, eat, burn, and knock over.

Activities are one way to specify causes. An important feature of activities is that they come in types that have been discovered as science has changed. Over the centuries, scientists discovered new types of activities and their ways of operating. Once they are discovered and their modes of operating well understood, types of activities become part of the "store" or "library" of mechanism parts used to construct mechanistic hypotheses in a particular biological field (Darden 2001; Craver and Darden 2001). The kinds of activities most important in molecular biological mechanisms are, first, the push/pull geometrico-mechanical activities familiar since the beginning of the seventeenth-century mechanical worldview and, second, the many forms of chemical bonding discovered in the nineteenth and early twentieth centuries. Each field finds the activities that drive its mechanisms. A major advantage of the MDC view of causes as types of activities is that the vague term "cause" must be made more specific. The specific way that a specific change is brought about must be found in order to have an adequate description of a mechanism.

Methodologically, activities can sometimes be identified independently of the specific entities that engage in them. For example, the melting temperature of the DNA double helix indicated that it contained weak hydrogen bonds, even before the specific subcomponents (the DNA bases) exhibiting those bonds had been identified. More generally, activities may sometimes be investigated to find their

order, rate, duration, and sphere of influence more or less independently of the entities that engage in those activities.

A specific kind of activity produces a specific kind of change. Finding necessary and sufficient conditions to characterize the many diverse kinds of production is difficult and not required for their scientific discovery (Bogen 2008). Rather than seeking a general definition of production, it is more insightful to consider specific kinds of activities and the means for discovering them. As Machamer suggested in MDC, human beings directly experience many kinds of activities, such as collision, pushing, pulling, and rotating—the activities in mechanisms often discussed in the seventeenth century. Scientists have since discovered many kinds of activities not directly detectable by human senses, such as attraction and repulsion, electromagnetism, and movements across membranes to achieve equilibrium. Science students must be trained to understand how these activities work so that, with education, they can "see" (understand) how mechanisms employing them operate.

Moving beyond what MDC claimed about activities and causes, I note that relating "C causes E" to mechanisms may call attention to some piece of a mechanism other than an activity. As Stuart Glennan (1996) notes, analysis of "C causes E" may require an entire underlying mechanism to lay out all the stages between C and E. In such a case, C refers to the entire mechanism at a lower mechanism level. Alternatively, C may refer to an early stage of the mechanism (consisting of entities and their activities) with the other stages between C and E left unspecified. Hence, "cause" may refer to nearer or more distant stages in the mechanism, prior to the stage (E) of interest.

In addition to entities and activities and organization, MDC noted that mechanisms have "start or setup conditions." If a mechanism requires a signal or start condition (some don't, e.g., some biological mechanisms run continuously), then that may be called a "triggering cause" or a "sufficient cause." When the trigger is present (and the set conditions are available), the mechanism begins to operate. Something called a "necessary cause" might be any nonredundant part of the mechanism or, instead, part of the setup conditions for a mechanism to operate. Setup conditions for mechanisms are many and varied. Although some of the setup conditions are known and indicated (such as in the materials and methods sections of scientific papers), they cannot be fully specified, even in controlled laboratory conditions. (This issue is well known in discussions of ceteris paribus conditions.)

My goal in this section thus far has been to try to map the C of "C causes E" onto some piece of a mechanism or to its start or setup conditions. Now let's turn to E, the effect. Presumably that corresponds to the phenomenon of interest. An important starting point for finding a mechanism is to characterize the puzzling phenomenon that the mechanism produces (on recharacterizing the phenomenon as research on the mechanism proceeds, see Bechtel and Richardson 2010). Presumably, the characterization of the effect is similarly important in constraining adequate claims about its cause.

One of the aims in finding causes is to enable humans to exert control. As is sometimes said, a cause is a handle that can be turned to do something. What we wish to control is E, the outcome. The goal of control of the outcome is especially

important in medicine, so now we turn to an example from that field. The example shows that knowledge of the mechanisms operating or failing to operate provides a better handle than knowing that a single "X causes disease Y."

4 Control of the Outcome in the Disease of Cystic Fibrosis

One might say: "A mutation in the CFTR gene causes the disease cystic fibrosis." But this is an impoverished claim, compared to a description of the myriad mechanisms involved in the etiology of the disease.

In medical contexts, the puzzling phenomenon may be described and redescribed in various ways, as work proceeds to discover the mechanisms producing the disease. Also, the phenomenon of interest is different for those attending to different stages in the progress of the disease. Yet other characterizations of the phenomenon may be provided by those doing fundamental research versus those tasked with treating patients, so this case also illustrates different ways of characterizing the phenomenon within the contexts of pure versus applied research. Different characterizations of the phenomenon focus attention on different mechanisms or stages of a given mechanism involved in the disease etiology.

The phenomenon to be explained in what has come to be called the disease of "cystic fibrosis" changed over time, as groups of symptoms were clustered, the gene discovered, some of the activities of the malfunctioning protein found, and later stages of the disease dissected. One can tell a tidy story about the discovery of the normal mechanism, about the many ways it can break, and about how this knowledge has been and is being used in designing drug therapy. This perspective views disease as a broken-normal mechanism and therapy as aimed at restoring normal functioning (Moghaddam-Taaheri 2011).

However, one can view the medical mechanistic picture in a more complex way. One can ask: Is there some other mechanism that can restore chloride transport function rather than fixing the broken mechanism? Alternatively, as is common in medicine, one can just focus on mechanisms that will aid in alleviation of symptoms of those living with the disease. More specifically, one can seek drugs that will aid in preventing the lung infections that typically lead to death for cystic fibrosis patients. For some of these cases, the current understanding of the mechanisms provides powerful tools for medical researchers, but for other cases many black boxes remain.

In the early 1990s, it looked as if the story of conquering cystic fibrosis would be a simple one: gene discovered, mechanism and mutations understood, and guidance provided for therapies for intervention. However, the genotype-phenotype relations are more complex than anyone studying a disease (seemingly) produced by a single gene defect had reason to expect. Some aspects of the connections between the gene mutations and protein defects and the many phenotypic symptoms of the disease are still not well understood.

The following subsections proceed as follows. First, I recount the history of the discovery of the gene associated with cystic fibrosis. Then I describe the different mechanisms associated with the disease of cystic fibrosis, based on different ways of choosing the puzzling phenomenon of interest. The characterization of the phenomenon is a crucial step in delineating the mechanism of interest. The choice of the phenomenon (the effect?) and the goal of the research focus attention on different aspects of a single mechanism (the cause?) or on different mechanisms (different causes?) within the framework of a single disease. It is much too simple to say that a single mutation in a single gene causes the disease of cystic fibrosis.

4.1 History of Cystic Fibrosis Prior to the Discovery of the Gene

Work in the early to mid-twentieth century connected symptoms in the lungs, pancreas, and sweat glands. Medical researchers found that recurrent respiratory infections, raised levels of chloride in sweat, and insufficient pancreatic enzymes were all problems in the epithelial tissues in those organs and glands. The disease was named "cystic fibrosis," but the specific nature of the defect in epithelial tissues was unknown until the 1980s (Knol 1995).

4.2 Discovery of the CFTR *Gene*

Mitchell Drumm (2001), a graduate student and then postdoc who worked in Francis Collins's lab at the University of Michigan in the 1980s, wrote a lively first person account of the discovery of the gene involved in cystic fibrosis (CF). When these medical researchers started their investigation of CF, all the aspects of the molecular genetic mechanism were a black box. Population genetic studies of families with CF patients had shown that the disease is hereditary, not sex linked, and requires two copies of the mutant gene to produce the disease symptoms; carriers with one copy are not sick. In more technical genetic terms, it is an autosomal recessive disease. It is more prevalent in those with Caucasian European ancestry than among other groups in the USA. Before 1989, the gene was not known and the protein it produces was unidentified. However, earlier work on the symptom of salty sweat indicated that the protein was involved in the transport of chloride in and out of the cell (Quinton 1983; discussed in Pearson 2009).

By the 1980s, molecular biological techniques for finding a gene could proceed quickly if the protein and its accompanying messenger RNA could be identified. A complementary DNA, called "cDNA," could be constructed from the messenger and then used as a probe for finding the nuclear DNA and the location on the chromosome where the gene resided. But the search for the CF gene had to proceed without such technological reversal of those later stages of the mechanism. It was

the first gene to be discovered whose protein product was not known beforehand (Drumm 2001, p. 86).

Three groups in North America collaborated in the gene's discovery, bringing different techniques and areas of expertise. Lap-Chee Tsui at the Hospital for Sick Children in Toronto screened the chromosomes of families with CF children, locating the gene on chromosome 7, near certain known markers. Francis Collins's lab at the University of Michigan did the molecular analysis of the chromosome by a process that Collins had invented, called "chromosome jumping." The DNA of the chromosome was chopped up and circularized. Using this chromosome jumping technique, the Collins lab group found related markers more quickly than permitted by the slower technique called "chromosome walking," which required more laborious analysis of linear sequence overlaps. The third collaborator was John Riordan, also in Toronto in the 1980s, who constructed complementary DNA libraries, using messenger RNA from CF tissues. Putative stretches of DNA could be matched against the cDNAs to see if that gene was active in CF tissues.

A comparison between a putative normal gene and the same stretch of DNA from a CF patient found that three bases were missing in the disease gene. As Drumm remarked: "I think we were all expecting a more striking change in the gene if it were truly a mutation that caused CF" (Drumm 2001, p. 87). The gene was sequenced and various hypotheses proposed as to its functional role in cellular mechanisms. (On functions from a mechanistic perspective, see Craver 2001.) Given the similarity of some of its structural domains to other sequences whose function was known, the protein looked like it would reside in the cell membrane and conduct chloride ions across the membrane. Collins, Tsui, and Riordan named it the "cystic fibrosis transmembrane conductance regulator"—"CFTR" for short—in three papers published in *Science* in 1989 (Kerem et al. 1989; Riordan et al. 1989; Rommens et al. 1989).

The CFTR gene is large, with approximately 180,000 base pairs on the long arm of chromosome 7. It produces a large protein with 1,480 amino acids, organized into several different functional domains. Several classes of mutations produce the disease. Researchers have identified the specific locations of the mutations within the gene and traced the different ways each mutant breaks the mechanism. Some mutations are so severe that no protein is synthesized. However, the mutation that occurs in about 90 % of patients with cystic fibrosis in the USA (Rowe et al. 2005) is less severe. Three bases are deleted in the CFTR gene. During protein synthesis, this deletion results in one missing amino acid: phenylalanine at position 508 (of the 1,480 amino acids). Although missing only one amino acid, such Delta F 508 mutant proteins do not fold properly. The misfolded proteins do not implant into the cell membrane to properly transport chloride ions in and out of the cell (Kirk and Dawson 2003). Normally, the cellular machinery degrades misfolded proteins, but not all such mutant protein is degraded (important in potential drug therapy as we will discuss below). Details about the mechanism of degradation, or lack thereof, are black boxes (Bridges 2003).

4.3 Mechanisms Related to Cystic Fibrosis

So, there is a tidy story that we can now tell about the normal gene and the synthesis of the normal CFTR protein and about how different mutations produce different defects. Consider the mechanism for producing the protein with the Delta F 508 mutation. Each stage of the mechanism becomes a potential target for therapy. As Susan Lindee has discussed, the early hope was for gene therapy to replace the defective gene. The many problems with this approach include finding an appropriate vector for delivering the large gene, getting the gene into the appropriate cells (even in the lung cells which are more accessible than those in other organs), getting the gene to a safe location (either chromosomal or an extrachromosomal plasmid) so as not to disrupt other mechanisms, getting sufficient amounts of genes into the cells, preventing the immune system from rejecting any foreign matter used to take the gene into the cell, and getting the genes to respond to cellular regulatory signals to turn on the gene but not to overproduce the protein (Curlee and Sorscher 2003). These problems have yet to be solved; the prospects for successful gene therapy look dim in the case of CF (Lindee and Mueller 2011).

So, consider the next module of the mechanism, the one after the gene itself, as a target: the messenger RNA. The CFTR gene contains not only the coding sequences that eventually direct the ordering of amino acids during protein synthesis but also spacer segments, called introns. A cell organelle, called a "spliceosome," processes the pre-mRNA to produce the mRNA; the spliceosome accomplishes this by snipping out the introns and binding the remaining coding segments together into the final messenger RNA. Researches have succeeded in inserting a minigene into the DNA of human lung tissue grafted onto a mouse. The minigene has the correct coding segment rather than the Delta F 508 three-base mutation. The gene is expressed at the same time as the CFTR gene, thereby overcoming one of the barriers to gene therapy. Then the splicing machinery is induced to put the correct segment into the processed messenger RNA rather than the mutant segment. Some success in the mouse system makes this look promising. However, it is still a long way from human clinical trials (Liu et al. 2002, 2005; discussed in Thomson 2002).

Currently, a primary area for targeted drug therapies is the next stage of the mechanism: the synthesis of the misfolded protein. For the Delta F 508 mutant, the three missing bases in the gene result in one amino acid missing from the protein, which then misfolds. Although some of the protein degrades, some of the misfolded protein remains in the cells. Therapy can be directed to finding drugs that aid in rescuing the undegraded misfolded protein so that it refolds and inserts into the cell membrane and functions (albeit at a reduced level) to transport chloride ions. A robotic process has screened millions of compounds for their effects on the misfolded protein and some promising drug candidates have been found. One is curcumin, a major constituent of the spice turmeric, which has shown promising effects in vitro and in mice models (Rowe et al. 2005, p. 1999).

In contrast to this random screening, rational drug therapy is also being explored. Medical researchers are using a more detailed understanding of the role of

additional molecules to try to correct the defect. These additional molecules, called chaperones, aid the CFTR protein to fold properly (Wang et al. 2006). The discovery of the role played by such additional molecules that interact with CFTR (produced by additional genes, called "modifier" genes) may explain a puzzling phenomenon about the relation between genotype and phenotype. It is puzzling why patients with the same two Delta F508 mutations can still vary in the severity of symptoms of the disease. One hypothesis is that this difference is due to different modifier genes in their DNA. Although cystic fibrosis seemed to be an ideal case of a disease caused by a mutation in a single gene, we can no longer hold such an overly simple view. The mechanism by which modifier genes work becomes important also.

Thus, we see the importance of the way the puzzling phenomenon is characterized in order to focus attention on the relevant aspect of the mechanism. When the puzzling phenomenon is the synthesis of the normal CFTR protein, that mechanism is fairly well understood. But when the puzzling phenomenon is why the Delta F 508 mutant protein fails to function properly, aspects of the mechanism by which the mutant form of the protein is synthesized and misfolded and degraded still have black boxes. Nonetheless, enough is known about that module of the mechanism to guide drug discovery efforts to find drugs to aid with refolding the misfolded protein.

However, when the puzzling phenomenon is a broader one, namely, how the mutant in the CFTR gene produces the symptoms of cystic fibrosis disease in the myriad organs that it affects, many of the details of these mechanisms are unknown. When what is taken to be puzzling is much later in the progress of the disease, even more black boxes remain. What are the stages of the mechanism leading to the thick airway mucus in the lungs that result in the fact that, as cystic fibrosis patients age, they become more susceptible to particular strains of bacteria that are more resistant to treatment? Various hypotheses as to how to fill this black box abound. As a recent review article said: "So far, a unifying mechanism responsible for the vast clinical expression of the disease in the CF airway has not been identified" (Chmiel and Davis 2003, p. 173).

There are even competing hypotheses, which may not be mutually exclusive, about why the airway mucus is thick and particularly susceptible to bacterial infections. Several hypotheses depend on the effects of malfunctioning chloride transport, leading to an imbalance of salt homeostasis or abnormal water absorption producing thicker mucus (Widdicombe 2003). However, new evidence points to a malfunctioning immune response. Neutrophils, which are a type of white blood cell, are recruited to fight bacteria. CF patients also have defective regulation of neutrophils, leading to an overabundance of them. The mechanism for this malfunctioning regulation of neutrophils is not well understood, although some of the entities and their activities have been identified (Gu et al. 2009). As neutrophils break down, the debris, especially their DNA, accumulates in thick mucus that is a site for colonization preferred by certain forms of bacteria. So, if the phenomenon that the physician wishes to alter is the overgrowth of specific strains of bacteria, then the therapeutic effects may be directed to neutrophil regulation, a much later

stage in the disease with different targets than the CFTR protein biosynthesis mechanism (Chmiel and Davis 2003).

This hypothesized mechanism of overexpression of the immune response to inflammation led to the unexpected prediction that anti-inflammatory drugs would be beneficial to CF patients. Without this hypothesis, one would have expected that anti-inflammatory drugs, such as ibuprofen, would have deleterious effects for lungs susceptible to infections. The normal inflammatory response, which recruits neutrophils to the site of an infection, is beneficial in the fight against bacteria. However, because the hypothesized mechanism suggested overexpression of this response, the drug therapy to reduce the response was subjected to a clinical trial, with some success (Konstan et al. 1995).

So, this case shows the many different ways the puzzling phenomenon can be identified and consequently the many different mechanisms that provide candidate "causes" for that chosen phenomenon. If the phenomenon to be explained is the synthesis of the normal CFTR protein, then the mechanism for that is well understood. If the question is the following—"what is the nature of the failure in that gene that leads to cystic fibrosis disease?"—then the answer is that there are many mutations that disrupt that mechanism in different ways (as we discussed, different classes of mutants disrupt the normal mechanism at different stages). If we focus on the most common mutant found in those with cystic fibrosis in the USA, the Delta F 508 mutation, then the puzzling phenomenon is how does the CFTR protein misfold and get degraded (or not). Although some of the details of the degradation mechanism are still black boxes, nonetheless, we know that the outcome is that some misfolded proteins are found in the cells. Hence, enough of the mechanism is understood to direct empirical or rational drug discovery efforts, which may find a way to correct the misfolding and transport the protein to the cell membrane. The goal is to elicit sufficient amounts of chloride ion transport to restore some of the normal function and alleviate some of the disease symptoms.

However, if we want to know the mechanism by which this mutant leads to lung disease and death in CF patients, then there are still many black boxes to be filled. Competing hypotheses have to be evaluated about crucial stages of different mechanisms. To decrease death due to bacterial infections, it may be possible to direct therapeutic effects to the regulation of overexpression of neutrophils rather than correcting the CFTR gene itself. A different mechanism, coming later in the progression of the disease, becomes the target mechanism for controlling one disease symptom.

This case shows that the mechanistic perspective adds much more detail than a simple claim that a mutated gene causes the disease cystic fibrosis. That vague claim has been eliminated in favor of a rich description of the many mechanisms involved. One would have thought that for a disease due primarily to a single gene defect, we could say that the mutation in the gene causes the disease and the way to fix it is to apply gene therapy to deliver a functional, non-mutated gene. Sadly such a simple fix did not work. This case shows the importance of knowing the different stages of the normal mechanism and the specific ways in which it breaks and even identifying different mechanisms that come into play as the disease progresses. All these aid drug discovery.

5 Conclusion

In our "Thinking about Mechanisms" paper (MDC 2000), we discussed the simple relation between one puzzling phenomenon and one mechanism. One might have thought one could easily identify the phenomenon as the effect (E) and the entire mechanism (or some piece of it) that produces that phenomenon as the cause (C). However, there are many candidates for what is to be designated as the cause and what is to be called the effect, once specific features of a mechanism and its setup and start conditions are identified. The cystic fibrosis case illustrates advantages and complexities gained by discovering the relevant mechanisms, given the goals of explanation, prediction, and control over disease in medicine.

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