

## LETTER TO THE EDITOR

# Is the Concept of Regulation Overused in Molecular and Cellular Biology?

The essence of molecular and cellular biological research, as indeed of any other scientific field, is to understand the causes of observed effects and to derive rules that allow future predictions. Biological systems are invariably complex, and observed outcomes are the result of a series of events involving multiple factors. The synthesis of a single compound often requires the chemical transformation, and sometimes active transport, of several intermediates and multiple proteins. The synthesis of each protein is a complex process by itself, starting from gene transcription through RNA processing, translation, and posttranslational modifications. In turn, the transcription of each gene is also a complex process requiring many proteins and other molecular factors.

One way to figure out what goes on in the cell is to organize these steps in a hierarchical fashion, or, more sophisticatedly, as a network of interactions. We do that in our minds and then often on paper (or on screen). Such charts help us direct our research toward the cellular components that make all this happen.

Researchers have always wanted to know if certain cellular components are more important than others and should therefore be studied in more detail. The term “important” is difficult to define because obviously if any nonredundant component in a nonredundant pathway does not work, the final outcome will not be achieved. Nonetheless, one impetus for calling some components important may be the realization that changes in abundance or activity of such components could result in disproportionate, even drastic, effects on the outcome (i.e., they can act as virtual on/off switches). The terms “regulation” and “control” have thus been applied, for example, to certain aspects of gene transcription (e.g., in the *lac* operon in *Escherichia coli*) and to the inhibition of an enzyme that catalyzes the committed step

in a pathway by the final product of that pathway. The existence of such switches makes sense evolutionarily because it is likely that mechanisms have been selected that prevent the cell from devoting energy to producing the entire set of components (such as RNA, proteins, and metabolic intermediates) of a pathway when achieving the final effect is not beneficial to the cell at this point. The logical extension of this argument—selection against unnecessary expenditure of energy—is that in addition to on/off switches, living organisms have evolved mechanisms to quantitatively change the rate of cellular processes in response to internal and external cues. Indeed, the identification and dissection of such regulatory processes have come to occupy center stage in molecular and cellular biology.

However the concept of regulation in molecular biology might have been defined initially, nowadays one encounters the application of the terms “regulation” and “control” to almost any situation when cause and effect are discussed. This, in my opinion, has rendered the concept less meaningful because if every step is said to be regulated, then there is no qualitative distinction between a factor that regulates something and a factor that causes something. In addition, in many cases when the term “regulation” is applied, closer examination reveals that direct evidence for the presence of a regulatory mechanism is not presented. Since it is not the purpose of this letter to criticize specific articles, I will avoid citations and instead illustrate general examples of this trend, followed by discussion of the appropriateness of invoking the terms “regulation” and “control” in such situations.

(1) A gene is often said to regulate the production of the protein or RNA it encodes. Clearly, this sweeping use of the term “regulation” renders all genes regulatory genes and does not add to our understanding of the processes involved.

(2) Likewise, a protein often is said to regulate, or control, a biochemical pathway simply due to its enzymatic activity. It is well established that in many pathways, most, if not all, enzymes involved contribute to the observed flux, and increasing or decreasing the amount of any given enzyme in the pathway may lead to incremental changes in the flux. Is the observation that an incremental change in the activity levels of an enzyme causes a correspondingly incremental change in the flux by itself sufficient to conclude that this enzyme is the rate limiting one or that this enzyme, or its gene, regulates the pathway?

(3) Likewise, the observation that a change in the level of a given compound causes an observable change in biochemical, developmental, or morphological phenotype often leads to the conclusion that this compound regulates or controls the process responsible for the observed phenotype. Is such an observation, by itself, sufficient to draw conclusions about regulation?

(4) More subtly, elements that may be required for regulatory circuits to operate but that are not directly involved in the nonlinear modulation of such regulatory processes are nonetheless often called regulatory. For example, this label is sometimes applied to nucleotide sequences that are recognized by the general transcription machinery or those recognized by the protein translation machinery, when mutations in such sequences cause altered levels of protein to be made. Similarly, elements involved in general and invariable protein modification and degradation pathways are also often labeled as regulatory, based on similar evidence with mutants exhibiting altered protein levels or activity. Even when a specific regulatory circuit is clearly identified, it may involve many components. Should all of these components be called regulatory even when

## LETTER TO THE EDITOR

some are not directly involved in signal modulation? Should enzymes that catalyze the formation of a hormone be designated regulatory proteins? With present usage, will genetic analysis identify them as such?

(5) In addition, one often reads that any observed levels of a transcript, or an enzyme, or a metabolite, are the results of “finely,” “highly,” “precisely,” “strictly,” or “tightly” regulated processes. The basis for these claims appears to be the often implicit assumption that in a given set of environmental conditions the plant finds itself in, the observed levels of said transcript or protein are obtained through a mechanism calibrated to lead to a phenotype that is optimal for the fitness of the plant. However, this hypothesis is seldom tested experimentally.

The responses to the questions and comments made above will of course depend on one's definition of regulation and control. It is indisputable that living cells constitute a system that displays both homeostasis as well as the ability to change. The application of the terms “regulation” and “control” in their colloquial sense to describe mechanisms of change and homeostasis processes seems reasonable enough. However, to be useful, a scientific term needs to be both clearly defined and to make distinctions, and it appears to me that in the field of molecular biology, including plants, these terms are used by the majority of the practitioners in their colloquial and perhaps even anthropomorphic sense without the benefit of widely known and accepted scientific definitions.

Furthermore, the current use of common terminology by researchers in the different areas of molecular and cellular biology masks major differences in the meaning

of these terms to different users employing different concepts. For example, flux control theory assigns a fractional flux control coefficient to each component in a pathway, to be determined experimentally, as an estimate of its controlling effect on the flux. This approach, which in principle can be applied to most and perhaps even all processes in the cell, explicitly posits that each component may exert some, but not necessarily equal, control on the flux. Thus, it specifically recognizes the proportional, sometimes linear, effects that a change in a given component may have on the flux. While this concept invokes control, a qualitative distinction between regulatory and nonregulatory elements may not be meaningful under it. Molecular biologists who study gene function, on the other hand, routinely categorize genes and the proteins or RNA they encode into regulatory versus nonregulatory ones. Further down the spectrum, some geneticists conclude that a gene “controls” or “regulates” a process simply when an inactivating mutation in it causes a phenotype.

I believe it is time we engage in a public discussion of what we perceive the meaning and utility of the terms “regulation” and “control” to be, and, once such terms are clearly defined, what the standards for experimentally demonstrating regulation should be. A restrictive definition that includes only on/off mechanisms, such as the allosteric interactions that cause the drastic change in the ability of the *lac* repressor to bind to the *lac* operator or similar interactions that cause some feedback inhibition of enzymes, may be highly specific but, it can be argued, will miss out on many other important mechanisms that have more quantitative effects. However,

given that so much of what goes on in the cell is changeable (or stable in the face of changing environment), an expansive definition of regulation will likely result in the majority, or perhaps even all, of the cell's components and processes being implicated, thus lacking specificity.

In summary, given the present lack of clarity as to what we mean by regulation, I believe the incantatory invocation of this and similar terms and the reflexive affixation of the adjective “regulatory” to any cellular component whose modification leads to a changed outcome provide us with only the illusion of deeper mechanistic understanding. We need to clarify the concepts, and we need specific and well-circumscribed definitions. Here, I have deliberately chosen not to provide my own definitions because I wish to draw attention to the problem first. However, it is not my intention to sound negative. I do believe that well-defined concepts of regulation and control can be extremely useful in making sense of how plant cells work, and I hope that we can arrive at such definitions with input from our scientific community.

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