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Engineering Novel Proteins with Orthogonal tRNA: Artificial Causes that make a Difference

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§1. Introduction

Model organisms, the use of green fluorescent proteins, and orthogonal transfer RNA (tRNA) are examples of artificial causes being used in biology**.** Recent work characterizing the research interests of biologists in terms of a common set of values has ruled out artificial causes as biologically interesting. For instance, Kenneth Waters argues that biologists are primarily interested in causes that actually obtain. Similarly, Marcel Weber argues that biologists are primarily concerned with biologically normal interventions. Both views express a widely received attitude about the interests and goals of biologists as being primarily concerned with the contingent facts of our world. While I agree with this general attitude about the contingent nature of biology, I argue that neither view fully accounts for the diversity that distinguishes the discipline. Along with actual and biologically normal causes, biologists are also interested in artificial causes for technological and observational purposes. I maintain that research interest in artificial causes provides some pragmatic reasons for thinking that research programs in cellular biology aren't driven by a common core set of values.

§2. Causal Selection

Recent claims about the explanatory interests of biologists have emerged from the causal selection debate. Causal selection is the thesis that some causes from among the set of genuine

causes are ontologically significant. Philosophers have analyzed causal selection in terms of difference-making and INF-specificity. The gene serves as the paradigmatic cellular factor of causal selection in biology for both approaches.

Causal selection in philosophy of biology is the rejection of Millean parity. Millean parity grants that there is a way to distinguish between causes and non-causes, but denies that there is any principled way of singling out some causes as ontologically important to an effect. Distinguishing among causes may be justified owing to pragmatic values or interests, but there is no ontological basis that differentiates some causes from others. There is controversy over who actually holds Millean parity.[1] Nevertheless, an argument that is often read as a defense of Millean parity in biology invokes the complex and highly interdependent nature of developmental systems as evidence for the claim that any ontological distinction among causes is arbitrary and unjustified.[2]

Difference-making and INF-specificity analyses of causal selection hold that the singling out of some causes as ontologically significant is justified on ontological grounds. Proponents of causal selection argue that implicit in the experimental practices of cellular biologists are criteria for the singling out of some factors – most notably the gene – for the purposes of manipulating and controlling biological outcomes. These criteria are justified not because of pragmatic considerations, but rather because of the ontology of causal relationships.[3]

The difference-making criteria analyzes causal selection in terms of factors whose presence or absence makes a difference to an outcome.[4] Suppose that of the ten mouse traps set

[1] Griffiths and Stotz 2013; Griffiths forthcoming.
[2] Lewontin 1974; Griffiths and Gray 1994; Oyama, Griffiths and Gray 2001.
[3] To say that difference-makers or INF-specific causes are ontologically significant isn't to say that such causes are foundational, metaphysical entities. Difference-makers and INF-specific causes are ontologically significant because their criteria don't' reflect the interests and values of the speaker or observer. Satisfying the criteria of difference-maker or INF-specificity is to possess a set of causal properties that other relevant factors do not.
[4] Sterelny and Kitcher 1988; Waters 2007.

up in my home only one snaps. The traps are identical in all relevant ways. The amount of tension on the spring and the amount of bait resting on the catch are the same and so is the size, shape and weight of each. The cause whose presence explains the difference between snapped and unsnapped traps is the passing by of a mouse. In this scenario, the mouse is the important cause of the difference among traps; it is the difference maker.

Genes often function as difference makers in biological experiments. A common experimental method for testing the causal contribution of a cellular factor in developmental biology involves carefully controlled breeding regimens of model organisms. These breeding regiments generate populations of organisms that are genetically identical with the exception of one (or several) factor(s) that is allowed to vary in the population. Genes are often the factor that is allowed to vary against an otherwise identical genetic and environmental background.[5] On the assumption that the population is uniform in all relevant ways, researchers infer that differences in a phenotypic trait – say, eye color – are due to differences in a particular gene or set of genes.

The INF-specificity criteria is a refinement of the difference-making criteria. It analyzes causal selection in terms of a system of counterfactual dependence according to which a range of possible input states map onto a range of output states with paradigmatic cases marked by a one-to-one relationship between inputs and outputs.[6] INF-specific relationships are like a refrigerator thermostat with a range of different settings each of which corresponds to one and only one temperature. Setting the dial to 2 activates the cooling mechanism to bring the refrigerator to 2.5 degrees centigrade, while setting the dial to 3 raises the temperature to 5 degrees, and so on.[7]

[5] Waters' actual difference-making account derives heavily from the experimental methods of Bridges and Morgan (1919) whose work exclusively used genes as actual difference makers.
[6] Waters 2007; Woodward 2010; Weber forthcoming.
[7] INF-specific relationships admit of degrees. INF-specific relationships can vary in the degree to which they approximate a perfect one-to-one mapping. An INF-specific relationship can also have a greater degree of specificity in comparison to another INF-specific relationship insofar as the codomain of the latter is a proper subset of the codomain of the former.

Protein coding genes relate to the amino acid sequence of proteins in an INF-specific way. DNA consists of four types of nucleotide bases – adenine, thymine, guanine, and cytosine. Genes specify proteins in units of three nucleotides (or codons), which map onto one and only one of 20 canonical amino acids (with some redundancy). For instance, the codon with three cytosine nucleotides (CCC) always codes for the amino acid proline and nothing else.[8] The same goes for all other amino-acid coding codons. Setting aside the issue of redundancy, each variation in the codons of a gene maps uniquely onto one and only one protein. Thus, a range of gene variants systematically correlate with a range of proteins in much the same way as the settings of the thermostat in the previous example map onto a range of temperatures.[9]

Difference-making and INF-specificity are criteria for singling out some causes as ontologically significant to an effect. On both analyses, the gene serves as the paradigmatic cellular factor of causal selection in biology. Representatives from both approaches have offered justifications for why other cellular factors – most notably transfer RNA – aren't singled out as ontologically significant causes of proteins, which they take to be indicative of the explanatory values distinctive of biologists.

§3. tRNA: A Finicky Lot

Biologists rarely pick out transfer RNA (tRNA) as ontologically significant causes of proteins. Representatives from both the difference-making and INF-specificity analyses have argued that the practice of picking out genes rather than tRNA tells us something about the explanatory values of biologists. Weber argues that biologists are primarily interested in

[8] There is some redundancy in the genetic code in that more than one codon can specify the same amino acid. For instance CCA can also code for proline. This is how the relationship between genes and proteins have a degree of INF-specificity in the sense mentioned in the previous footnote.

[9] This is a rather idealized example. First, we are not considering whether all variations in a gene can produce viable proteins. Second, most (perhaps all) cases of protein synthesis (especially in eukaryotic cells) undergo various complex editing and splicing processes at the stages of gene expression leading up to translation, which can change the number of proteins a gene can specify.

biological normality while Waters argues that biologists are primarily concerned with actual difference makers. Both proposals are an attempt to identify a core set of investigative values that guide research in cellular biology.

tRNA are complex molecules found in most cells whose function it is to deliver the precise amino acid to the correct codon of a genetic message during protein synthesis. tRNA are a finicky lot. Their job requires it of them. Each tRNA has two important points of contact: the anticodon and the acceptor stem.[10] The anticodon recognizes only one type of codon and the acceptor stem recognizes only one type of amino acid.[11] This ensures that the correct amino acid associates with the correct codon. For instance, a tRNA whose anticodon only recognizes the cytosine nucleotide triplet (CCC) will only carry proline on its acceptor stem. A widespread caricature of protein synthesis says that codon specificity of tRNA is universal. From bacterium to elephant, each codon associates with its favorite amino acid and nothing else. According to this caricature, any variation in codon specificity of tRNA is likely to be fatal for the cell or organism.

Were the codon specificity of tRNA to vary, tRNA could satisfy the criteria for causal selection in much the same way as the gene. Against a genetic and environmentally uniform population, tRNA could serve as difference makers. For differences in the amino acid associated with particular type of tRNA could account for differences in protein. Similarly, a range of variation in the anticodons of tRNA could systematically correlate with a range of different proteins in an INF-specific way.

[10] See images 1 and 2 in appendix.
[11] tRNA recognize the correct amino acid with the help of another important enzyme called the aminoacyl-tRNA synthetase. This enzyme interacts with both the anticodon and acceptor stem to charge the tRNA with the appropriate amino acid.

Representatives from both the difference-making and INF-specificity approaches to causal selection assume that the lack of interest in varying the codon specificity of tRNA tells us something about the core investigative values of biologists. Marcel Weber has argued that the hypothetical INF-specificity of tRNA is irrelevant to the explanatory interests of biologists. For cellular biologists are primarily interested in biologically normal interventions or interventions that

1. may also be due to natural processes and
2. are compatible with the continued persistence of the biological entity that is being considered.[12]

Weber assumes that variation in tRNA codon specificity will inevitably fail to meet both criteria for biological normality. For not only does codon specificity not vary in the natural world, but its variation is often fatal for the biological entity in question. Hence, concludes Weber, biologists aren't interested in the hypothetical INF-specificity of tRNA.

Ken Waters argues that the potential difference-making abilities of tRNA are also uninteresting to biologists insofar as biologists are primarily concerned with actual difference makers. Actual difference makers are difference makers that actually vary; whereas, potential difference makers are factors that could account for differences in an outcome were they to vary but happen to not do so. Biologists, argues Waters, are primarily concerned with actual, rather than potential, difference makers. Because codon specificity never actually varies, tRNA are merely potential difference makers whose varying "does not matter to biologists unless different tRNA has actually existed or is likely to exist actually in the future."[13]

[12] Weber forthcoming, 27.
[13] Waters 2007, 576.

In saying that biologists are primarily interested in studying biological normality or actual difference makers, Weber and Waters are identifying a core set of explanatory values that guide research in cellular biology. Biological normality and actual difference makers are concrete proposals of a more general attitude that says biology studies the contingent facts about the world. On this picture, biology is the science of a particular range of possibilities that is narrower than the range of possibilities that, say, physics studies. Biological generalizations don't describe laws of nature,[14] they contain many heterogeneous exceptions, and only hold in a restricted range of contexts.[15] In pointing to the finicky nature of tRNA, Weber and Waters are identifying a range of contexts in which biological generalizations do not hold and, thus, do not receive research attention.

Weber and Waters assume that the lack of interest in varying codon specificity of tRNA tells us something about the core investigative values of biologists. Variation in codon specificity of tRNA is neither biologically normal nor actual; hence, Weber and Waters conclude that biologists aren't interested in picking it out as ontologically significant. In pointing to a range of contexts in which biological generalizations do not hold, Weber and Waters are carrying on a general attitude that says biology studies contingent features of the world. While I agree with the general thesis about the contingent nature of biology, I consider whether these authors have identified the principle explanatory values of biologists in the next section.

§4. Artificial Causes

In discussing the achievement of variation in codon specificity of tRNA and other examples of artificial causes, I argue that biological normality and actual difference making do not account for the diversity of interests among cellular biologists. Artificial causes can be of

[14] Smart 1959; Beatty 1995.
[15] Mitchell 1997; Woodward 2000.

significant interest to biologists as forms of technology. Yet they can fail to be biologically normal in Weber's sense insofar as they are wholly unnatural. They also challenge Water's account of actual difference-making insofar as prior to being made actual causes, biologists must spend significant time thinking about the non-actual.

The development of site-specific incorporation of unnatural amino acids using orthogonal tRNA in living cells was first achieved by Peter Schultz and associates in 2000.[16] It involves the addition of a non-standard amino acid – an amino acid that doesn't belong to the 20 canonical ones – to a "stop codon" or the nucleic acid triplet that tells the protein synthesis machinery to halt protein production. Since every tRNA associates with one and only one amino acid, the incorporation of a non-canonical amino acid requires the engineering of an orthogonal tRNA; a tRNA that isn't native to the cell.[17] Orthogonal tRNA are produced in one organism (often bacteria) but are engineered to operate in the cells of another organism (bacteria, yeast, mice, roundworm, and human).[18] Orthogonal tRNA undergo random genetic modification and several rounds of trial and error (a process called directed evolution) to achieve variation in the anticodon and acceptor stem that function to associate the unnatural amino acid with the desired stop codon.

Orthogonal tRNA and other artificial causes are useful for observational purposes and for engineering solutions to real world problems. As an observational tool, artificial causes can allow researchers to gather data about the activities of the components of a causal system that are otherwise difficult to observe and measure. Orthogonal tRNA have been used to incorporate

[16] Wang et al. 2000.
[17] As mentioned in footnote 11, tRNA are charged with the appropriate amino acid with the help of an aminoacyl-tRNA synthetase. Site specific incorporation of unnatural amino acids, thus, requires the engineering of an orthogonal aminoacyl-tRNA synthetase enzyme as well as an orthogonal tRNA.
[18] Liu and Schultz 2010.

fluorescent markers at specific sites in a protein, making the conformation structure more easily visible.[19] Another powerful technology that has become standard in much of cellular biology has been green fluorescent protein (GFP) as a genetic marker. The GFP gene normally occurs in various marine organisms. By introducing the GFP gene into the genome of another organism, embryo development is made easily observable as fluorescent proteins are produced alongside a gene of interest.[20] Even the selective breeding of model organisms is a technology that enables researchers to observe the effects of difference makers in lab-raised populations.

Artificial causes can also help solve real world problems. As a tool for generating novel effects, artificial causes exploit the behavior of a causal system. The use of orthogonal tRNA to engineer antibodies that bind with cancer-fighting drugs (antibody-drug conjugates) is a new way to treat cancer while minimizing unwanted side effects.[21] Artificial causes may not always improve the observational abilities of researchers but can be a way of putting a causal system to a new purpose.

Research interest in artificial causes like orthogonal tRNA defies the explanatory values that Weber attributes to biologists. Artificial causes don't satisfy Weber's first criterion of biological normality insofar as they are wholly unnatural causes. Orthogonal tRNA are a technology only achievable in the lab. Hence, according to Weber, orthogonal tRNA and other artificial causes that fail to meet his first criteria of biological normality shouldn't be of interest to cellular biologists.

Waters' emphasis on the actual also has trouble accommodating artificial causes. Waters asserts that biologists are primarily interested in actual difference makers. As potential difference

[19] Wang et al. 2006.
[20] Chalfie et al. 1994.
[21] Axup et al. 2012.

makers until their creation in the lab, orthogonal tRNA should not have been of interest to biologists prior to 2000. But the development of orthogonal tRNA – and other types of artificial causes – requires that biologists imagine and hypothesize the non-actual in order to be made actual difference makers. Artificial causes are less of a serious challenge to Waters than to Weber. For once artificial causes are made actual, Waters' actual difference-making account can explain the research attention they receive.

While the development of artificial causes is a significant area of research in biology, the domain of biological inquiry also includes biological normality and actual difference makers. As Waters has pointed out, the use of actual difference makers has a long history in biology and it continues to be a powerful experimental method today. [22] Hence, Waters' focus on the actual may be over-stated rather than misguided. For orthogonal tRNA had to be made actual eventually lest a loss in research interest might have occurred. Similarly, much research involving the experimental variation of genes is research into biological normality. Again, Weber's emphasis on biological normality isn't misguided, but is rather over-stated. It is noteworthy that the orthogonal tRNA research program satisfies Weber's second criteria of biological normality. Part of Peter Shultz' success in developing orthogonal tRNA was that he was the first to achieve site specific incorporation of unnatural amino acids in living cells. Were the use of orthogonal tRNA to regularly result in the death of the biological entity, it is possible that research interest would have waned.

Not only are the explanatory values of cellular biologists diverse, but they are likely to change as research programs develop. While researchers have known for some time that the stop codon of some types of mRNA can be reassigned an amino acid, research on the frequency with

[22] For a recent use of actual difference makers that employ an epigenetic factor as an actual difference maker see Dolinoy et al. 2006.

which this occurs in single-cell populations hadn't been studied until recently. (Ivanova et al. 2014) A caricature of protein translation assumes that mRNA codon specificity to be universal across species. Yet, recent research shows that stop codon reassignment is fairly extensive in single-celled organisms. This new development challenges the caricature of protein translation that has been widely disseminated since the formulation of Crick-information and is likely to push research interests in new, unexpected directions. It may even push research interest in the direction of varying tRNA codon specificity – as the orthogonal tRNA research program has done.

While the explanatory values of cellular biologists are diverse, the immense rate at which research programs develop and mature into distinct sub-disciplines sheds doubt on whether biological inquiry is guided by a common set of core values. The values and interests of cellular biology grow and develop along with the maturation of distinct sub-disciplines and research programs; hence, it is unlikely that a core set of values will be shared among researchers. Any characterization of the common core values shared among cellular biologists is likely to turn out false shortly after it is announced.

§5. Conclusion

In discussing artificial causes, I show that focus on biological normality and actual difference makers doesn't account for the diverse explanatory values of cellular biologists. The interests and values of cellular biologists are diverse – including research programs that investigate artificial causes as well as biological normality and actual difference makers. Not only is cellular biology driven by a diversity of values, but such values are likely to change as research programs develop. The rapid rate at which research programs mature, thus, makes it

unlikely for cellular biologists to all share a common core set of values. For the values of biologists change alongside the research programs they motivate.

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