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Perspectives

The Boscombe Valley mystery: A lesson in the perils of dogmatism in science

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The central dogma enunciated by Francis Crick and the postulate that sequence defines protein structure and function put forth by Christian Anfinsen (also referred to as the Thermodynamics Hypothesis) are some of the most fundamental tenets of molecular biology that have had very profound influences and implications. They were formulated based on observations (evidence) that was obvious to the preceptors. However, as is well known, exception is the rule in biology and several works in the literature cite examples that appear to challenge these dogmas suggesting that being dogmatic can be perilous. In this perspective, using the *Boscombe Valley Mystery* from Sir Arthur Conan Doyle's fabled Sherlock Holmes stories as a paradigm, I ponder the necessity to revise the two dogmas in light of new evidence, especially concerning prions and intrinsically disordered proteins, much like the call for revising the Modern Synthesis to enunciate the Extended Evolutionary Synthesis.

Keywords. Anfinsen's dictum; central dogma; intrinsically disordered proteins; Lamarckism; natural selection; non-genetic mechanisms; prion

1. Introduction

In the Sherlock Holmes story, '*The Boscombe Valley Mystery'* by Arthur Conan Doyle (1891) a young man is accused with the murder of his father who was found dead with injuries to the head by a blunt object. All the evidence seems to point towards him since he was carrying a gun and had had a violent altercation with his father just before he was discovered with the body. 'Based on the prima facie evidence and first-hand reports, the police inspector, Lestrade, charges the young man with murder and takes him into custody. At the inquest, the suspect pleads he is innocent since just before the tragedy he had spent several days out of town and upon returning, had gone hunting to the lake where he heard a cry of 'Cooee' with which he and his father called each other. Thus, he had headed in the direction of the cry. However, the old man was surprised to see his son since he was under the impression that the son was out of town. The accused further states he then left the scene following the unpleasant encounter mentioned in Lestrade's report but soon after returned when he heard a scuffle only to find his father lying on the ground in a pool of blood trickling from the back of his head. Thus, he immediately ran to help the old man. When asked what his father's last words were, the son said the only thing that was quite distinct was the dying man's reference to 'a rat'. These two clues, the 'Cooee' cry from the father who had no knowledge of his son being the vicinity at the time, and his dying words, 'a rat', that seemed worthless to everyone else including the supposedly astute Lestrade, are the most valuable ones to Holmes. After a detailed investigation of the scene and consultation of the autopsy report in the coroner's notes, Holmes determines that in fact, it was not the son, but it was the dead man's old accomplice from Australia who had murdered him. Holmes has a confidential conversation with the latter who admits his guilt saying that the diseased was an evil character who was blackmailing him with

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knowledge of crimes they committed together when they were in Australia. In the end, Holmes's arguments and strong objections are sufficient to acquit the accused.

With this short story illustrating Sherlock Holmes's legendary analytical mind and deductive reasoning powers as a preamble, here I highlight two examples in biology, the central dogma by Francis Crick, and protein structure/function postulate by Christian Anfinsen (also referred to as Thermodynamic Hypothesis), where ostensible interpretations of the observations and biased viewpoints dissuaded, perhaps discouraged, others from recognizing, much less considering, alternate and plausible explanations. We shall consider prions and intrinsically disordered proteins (IDPs), that are thought to underscore the caveats of these two dogmas. While the prions *appear* to serve as evidence against the central tenet of molecular biology dogma, IDPs *appear* to challenge Anfinsen's dictum (Babu 2016; Das *et al*. 2018).

But as we shall see, neither prions nor IDPs invalidate the central dogma of molecular biology or Anfinsen's dictum (Vila 2020). However, acknowledging their unconventional features i.e., the ability to self-template and transfer inheritable information in a conformation-based manner (by Crick), and perform biological functions in the apparent absence of secondary structure as we know it (by Anfinsen), would have been less dogmatic and perhaps, more progressive. My intent is not to be invidious; on the contrary, my goal is to persuade the opinionated and encourage the noncommitted to adjudicate their prerogative so that the problem is approached with an unbiased mind.

2. Genetic material – The classical view

Classical work by Alfred Hershey and his graduate student Martha Chase in the early 1950s provided strong evidence that DNA is the genetic material that determines the inherited characteristics of a functional organism (Hershey and Chase 1952). At the time, the only exception were viruses such as the Tobacco Mosaic Virus that contain only RNA suggesting that RNA may also act as the genetic material. Subsequent work by Gierer and Schramm (1956) and Fraenkel-Conrat (1956) showed that tobacco plants could be infected by inoculation with the RNA alone, and that, that infective virus particles could be reconstituted by mixing together the protein and RNA.

Soon after (in 1957), Francis Crick put forth the central dogma which states that once 'information' has passed into protein it cannot get out again (figure 1). The transfer of information from nucleic acid to nucleic acid, or from nucleic acid to protein may be possible, but transfer from protein to protein, or from protein to nucleic acid is impossible. By 'information' Crick meant the precise determination of sequence, either of bases in the nucleic acid or of amino acid residues in the protein (Crick 1958). The only exception that Crick thought could happen was the reverse flow of information from RNA to DNA by reverse transcription in certain viruses. However, it is important to note that, per the central dogma, these landmark discoveries also made it very clear that proteins do not play any role in genetic inheritance.

The discovery of reverse transcriptase by David Baltimore (1970), and independently by Howard Temin and Satoshi Mizutani in (1970), provided evidence supporting the dogma; however, it was criticized as an oversimplification in some quarters. More specifically, it was stated, 'The central dogma, enunciated by Crick in 1958 and the keystone of molecular biology ever since, is likely to prove a considerable over-simplification.' Taking umbrage, Crick wrote in his 1970 *Nature* article, 'This (above) quotation is taken from the beginning of an unsigned headed 'Central dogma reversed', recounting the very important work of Dr. Howard Temin and others showing that an RNA tumour virus can use viral RNA as a template for DNA synthesis. This is not the first time that the idea of the central dogma has been misunderstood, in one way or another. *In this article I explain why the term was originally introduced, its true meaning, and state why I think that, properly understood, it is still an idea of fundamental importance*.' (The last sentence is italicized by me for emphasis).

In ending this celebrated paper, Crick wrote, 'Although the details of the classification proposed here are plausible, our knowledge of molecular biology, even in one cell – l*et al*one for all the organisms in nature – still far too incomplete to allow us to assert dogmatically that it is correct. There is, for example, the problem of the chemical nature of the agent, of the disease scrapie: see the articles by Gibbons and Hunter and by Griffith. Nevertheless, we know enough to say that a non-trivial example showing that the classification was wrong could be an important discovery. It would certainly be of great interest to find a cell (as opposed to a virus) which had RNA as its genetic material and no DNA, or a cell which used single-stranded DNA as messenger rather than RNA. Perhaps the so-called repetitive DNA is produced by an

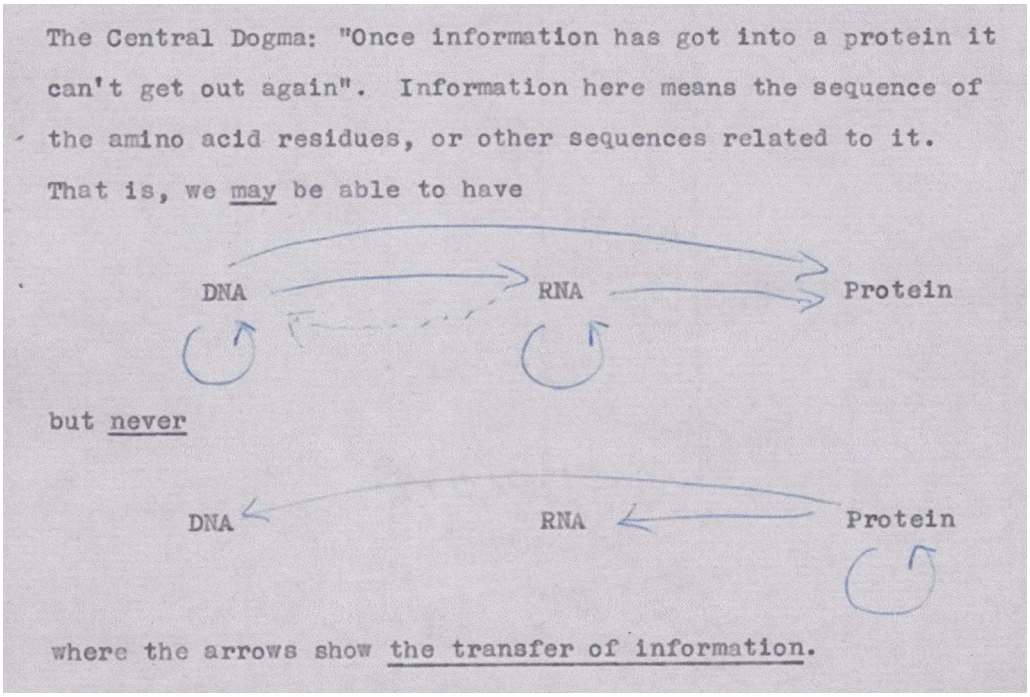

Figure 1. Crick's first outline of the central dogma, from an unpublished note made in 1956. Reproduced from Cobb M (2017) *PLoS Biol*. **15**(9): e2003243.

RNA→DNA transfer. Any of these would be of the greatest interest, but they could be accommodated into our thinking without undue strain. *On the other hand, the discovery of just one type of present day cell which could carry out any of the three unknown transfers would shake the whole intellectual basis of molecular biology, and it is for this reason that the central dogma is as important today as when it was first proposed*.' (The last sentence is italicized by me for emphasis). Thus, despite being aware (his reference to the prions in scrapie disease), and acknowledging the phenomenon as *plausible*, the omission of alternatives to the genetic material in his postulate, is rather puzzling.

The two articles Crick refers to above were the two back to back landmark articles published in *Nature* on the mechanisms by which a pathogenic protein could encipher its own replication blueprint without a genetic code. In the article by Gibbons and Hunter (1967), the authors write, 'There is evidence to suggest that the infectious disease scrapie is caused by an agent which does not depend on a nucleic acid for its ability to replicate. In the first of the two following articles it is suggested that scrapie can best be considered to arise from a replicable change in the structural pattern of a commonly occurring unit membrane. In the second article it is suggested that the agent is a protein and three possible mechanisms for its self-replication are proposed.'

The article by Griffith (1967) attempts to provide a theoretical framework and the author writes, 'It has been suggested that the agent responsible for scrapie, which affects sheep, has a very low molecular weight ($\sim 2 \times 10^5$) and is probably a protein without nucleic acid. It can infect goats, rats, mice or hamsters as well as sheep. This behaviour would not have been surprising had scrapie contained DNA or RNA because any cell contains the machinery to copy arbitrary sequences of nucleotides. It is not generally thought, however, that they can copy polypeptides and the idea that scrapie is a protein therefore presents difficulties. In this article I discuss the self-replication of proteins and argue that there are at least three distinct kinds of way in which it could occur. *This shows that there is no reason to fear that the existence of a protein agent would cause the whole theoretical structure of molecular biology to come tumbling down*'. (The last sentence is italicized by me for emphasis.)

3. Prions (proteins) as genetic material

Stanley Prusiner (1982) purified the infectious agent and because of its novel properties which distinguished it from viruses, plasmids, and viroids, he coined the term 'prion' to denote a small proteinaceous infectious particle which is resistant to inactivation by most

procedures that modify nucleic acids. It is now well recognized, largely through Prusiner's seminal work, that prions are a paradigm-shifting mechanism of inheritance in which phenotypes are encoded by self-templating protein conformations rather than nucleic acids.

This discovery provided the impetus and was followed by the realization that the unusual genetic behavior of yeast mutants, [*PSI*$^{+}$] and [*URE3*], could be explained by the prion-like behavior of two previously identified cellular proteins (Wickner 1994). [URE3] is an altered form of the URE2 protein whose normal function is to turn off utilization of poor nitrogen sources when a better source of nitrogen is available. [PSI] on the other hand, is the prion form of the yeast SUP35 protein involved in translation termination. This seminal finding provided a logical explanation of how stable phenotypic traits in yeast can be inherited in a non-Mendelian fashion in the absence of any nucleic acid determinant. By analogy with the mammalian prion protein PrP, the proposed protein-based non-Mendelian inheritance of [*PSI*$^{+}$] and [*URE3*] would arise as a consequence of a self-perpetuating change, most likely in conformation, of a cellular protein (Tuite 2000).

4. Prions are IDPs

Subsequently, work by Lindquist and her colleagues offered compelling evidence that several IDPs in yeast are functionally equivalent to prions. In a remarkable paper from Susan Lindquist's laboratory (Chakrabortee *et al*. 2016) that was a true tour de force, the authors showed that the transient overexpression of nearly 50 of these yeast proteins resulted in traits that remained heritable long after their expression returned to normal for scores of generations. These traits were beneficial, had prion-like patterns of inheritance, were common in wild yeasts, and could be transmitted to naive cells with protein alone. Remarkably, however, most inducing proteins were not known prions and did not form amyloid. Instead, they displayed characteristics of nucleic acid-binding proteins with large IDRs and are evolutionarily conserved. These data establish a common type of protein-based inheritance through which IDPs can drive the emergence of new traits and adaptive opportunities.

More recently it was reported that at least one of these prion-like proteins drives self-assembly into gel-like condensates (Chakravarty *et al*. 2020). Of note, these proteinaceous particles are infectious despite not being composed of amyloid qualifying them as protein-based epigenetic elements. These proteins downregulate a coherent network of mRNAs resulting in improved growth of yeast cells under nutrient limitation. Thus, this unique non-amyloid self-assembly of RNA-binding proteins appears to drive a form of epigenetics beyond the chromosome, instilling adaptive gene expression programs that are heritable over long biological timescales at least in the yeast. Extending these observations in yeast, an IDP (PGL-1) was shown to form aggregate-like structures in germ cells of *C. elegans* (Kennedy 2020). Indeed, the PGL-1 aggregates were inherited for multiple generations after these animals no longer possess the mutation that originally triggered their formation. Taken together, these remarkable findings render the hypothesis that IDPs can also form self-propagating aggregates in animals and thereby mediate transgenerational inheritance highly tenable.

5. Structure defines function

In the late 1950s and 1960s, Christian Anfinsen's work on the enzyme ribonuclease revealed the relation between the amino acid sequence of a protein and its conformation. In a series of elegant publications, Anfinsen and colleagues proposed that the sequence of a protein contains the information required to adopt a defined structure and, hence, function. This led to what is now called as Anfinsen's postulate or the thermodynamic hypothesis, which states that 'the three-dimensional structure of the native protein in its normal physiological milieu is the one in which the Gibbs-free energy of the whole system is the lowest; that is, that the native conformation is determined by the totality of the interatomic interactions and hence by the amino acid sequence, in a given environment' (Anfinsen 1973).

Anfinsen's postulate served us well as it also provided a natural extension of the central dogma in that there is a direct relation between the information from DNA to protein is transferred via RNA. However, the discovery of the intrinsically disordered proteins (IDPs) or intrinsically disordered regions (IDRs) within ordered proteins in the late 1990s by Vladimir Uversky (Uversky and Dunker 2010), eclipsed the structure/function relation postulated by Anfinsen. IDPs are polypeptides or segments that do not contain sufficient hydrophobic amino acids to mediate co-operative folding' (Babu 2016). Such proteins contradict the classic 'lock and key' hypothesis of Fischer, and challenge the prevalent notion that under physiological conditions, proteins fold into a single 3D shape

determined by their amino acid sequence and having the minimum Gibbs free energy.

6. IDPs and evolution

IDPs are prevalent in all three kingdoms of life (Peng *et al*. 2015). Despite lack of structure, they play critical roles in numerous important biological processes (reviewed, Kulkarni and Uversky 2018; Uversky 2019). More importantly, IDPs have also been implicated in the origin of prebiotic life (Kulkarni and Uversky 2019; Matveev 2019) and the evolution of the first independent primordial living unit akin to Tibor Gánti's Chemoton (2003), which preceded the Last Universal Common Ancestor (LUCA) that is believed to give rise to the first unicellular form of life (see Kauffman 2011). Secondly, IDPs play a critical role in phenotypic switching. Therefore, they are also thought to be critical in multicellularity and hence, in major evolutionary transitions during evolution (Szathmáry and Smith 1995; West *et al*. 2015). Thirdly, as we discussed above, IDPs also facilitate the emergence of new traits and adaptive opportunities via non-genetic, protein-based mechanisms (Chakrabortee *et al*. 2016). Finally, IDPs are constituents of proteinaceous membrane-less organelles (PMLOs), where they often serve as drivers and controllers of biological liquid-liquid phase transitions responsible for the PMLO biogenesis (Darling *et al*. 2018). Consistent with this argument, it has been hypothesized that a potential site for the origin of life could be spaces between mica sheets that have multiple advantages and could serve as specific shelters for the primordial PMLOs (Hansma 2017). While the IDPs, and certain highly ordered proteins such as the P-gp that was shown to assume different folds due to a single nucleotide polymorphism that was synonymous with the most common, or wild-type, allelic sequence of *MDR*1 exhibited reduced transport functionality of P-gp (Kimchi-Sarfaty *et al*. 2007), are thought to challenge Anfinsen's rule (and even the central dogma, see Newman and Bhat 2007), I would argue they actually don't. Perhaps, they abide by a 'weak' Anfinsen's dictum analogous to the weak Pinsker conjecture since IDPs are not random coils (Kulkarni 2020).

7. Why extended hypotheses

Crick only focused on living systems following the emergence of life on earth as we know it but not on prebiotic life akin to Tibor Gánti's Chemoton (Gánti 2003). Neither did he pay attention to the prions. It is true that, as far as we are aware, there is no extant form of life without DNA or RNA as the genetic material. But it can't be ruled out that proteins, particularly IDPs, could have acted as carriers of information, especially in life forms before DNA served as the genetic material, and in passing on the information transgenerationally as demonstrated by the prions and prion-like proteins. From this perspective, at least a dotted arrow going from protein to DNA (perhaps with a question mark) implying information transfer from phenotype to genotype and fill the gap in our knowledge on how acquired characteristics can get genetically assimilated and canalized (as proposed by Waddington 1942) may have rendered the central dogma more grandiose and served Crick better, a sentiment echoed by others as well. Thus, Eugene Koonin in his thought-provoking article (Koonin 2012) said, 'The prion-mediated heredity that violates the Central Dogma appears to be a specific, most radical manifestation of the widespread assimilation of protein (epigenetic) variation into genetic variation. The epigenetic variation precedes and facilitates genetic adaptation through a general 'look-ahead effect' of phenotypic mutations. This direction of the information flow is likely to be one of the important routes of environment-genome interaction and could substantially contribute to the evolution of complex adaptive traits.' The central dogma as Crick envisaged it, is absolutely correct; but lacks any evolutionary significance. And to quote Theodosius Dobzhansky (1973), 'Nothing in biology makes sense except in light of evolution. Otherwise, like Denis Noble (2018) says in his essay entitled, Central Dogma or Central Debate', 'The unidirectionality of sequence information transfer from DNA to proteins no more determines life than the QWERTY keyboard determines what I wrote in this article.'

Thus, the central dogma of molecular biology perhaps may not be valid as an 'absolute' principle; transfer of 'information' from proteins to the genome appears plausible. It is important to note that here, information *does not* imply the precise determination of sequence, either of bases in the nucleic acid or of amino acid residues in the protein as defined by Crick. However, information may be transferred from phenotype to genotype by IDPs (Mahmoudabadi *et al*. 2013; Kulkarni *et al*. 2013; Sonnenschein *et al*. 2014) as envisaged by the MRK hypothesis (Kulkarni and Kulkarni 2019). Furthermore, compelling evidence is now accumulating which indicates that information can also be transferred from phenotype to genotype via epigenetic changes (Jablonka and Lamb 1989; Jablonka and Lamb 2015; PMID: 25917417; Jablonka and Lamb 1995), piRNA-mediated

transgenerational inheritance of an acquired trait (Grentzinger *et al*. 2012; Duempelmann *et al*. 2020), non-genetic changes orchestrated by altered RNA and protein molecules (Samhita 2020), and other mechanisms (Smythies *et al*. 2014; Sciamanna *et al*. 2019; see Levine *et al* 2020 and cross-references therein). Indeed, such information can be genetically assimilated and transmitted through successive generations (Waddington 1953).

8. Learning and evolution

Although relatively unexplored and underappreciated, another implication of the phenomenon of information transfer from the phenotype to the genotype is the 'Baldwin effect'. It is quite logical to assume that in response to changes in its environment, an organism learns - defined as the heuristic of adjusting its response to the same inputs over time based on the outcomes of previous outputs - to acquire useful adaptations during its lifetime which can have a significant influence on evolution. Therefore, it would seem rather wasteful if the organism forfeited the advantage of the exploration performed to facilitate the evolutionary search for increased fitness. Thus, the fastest and most efficient way this goal maybe achieved would be to transfer information about the acquired (learned) characteristics that resulted in new phenotypes back to the genotype where it is gradually assimilated into its developmental genetic or epigenetic repertoire.

Indeed, this idea that learning can guide evolution was independently proposed in the late 19th century by Baldwin (1869), Osborn (1896) and Morgan (1896) although it is singularly referred to as the 'Baldwin effect'. Although empirical evidence may be lacking, theoretical studies by Hinton and Nowlan (1987) demonstrated that learning *can* be very effective in guiding the evolutionary search. These computer simulations revealed that learning alters the shape of the search space in which evolution operates. Thus, from an evolutionary perspective, the heuristics approach is much faster and far less energy-intensive than that required for the production of a whole organism by random mutations followed by natural selection (Hinton and Nowlan 1987). Subsequent studies by Behera and Nanjundiah (1995,1996,2004) demonstrated that although the relationship may not be as straightforward as was assumed by Hinton and Nowlan, phenotypic plasticity can potentiate evolution even when more realistic fitness schemes are simulated. Thus, it seems to me that invoking information transfer from protein (conformation) to DNA in a revised dogma of molecular biology would be more fulfilling and would account for how a trait is genetically assimilated, canalized and buffered against fluctuations as envisaged by Waddington (1957).

9. The order/disorder paradox

With the discovery of IDPs, it is now evident that ordered and disordered proteins lie at the extremes of a continuum. However, at the boundary, are proteins referred to as metamorphic proteins (Murzin 2008). Metamorphic proteins are proteins that are on the brink of thermodynamic stability. They are either weakly stable ordered systems or disordered but on the verge of being stable. In such marginal states, even relatively minor changes can significantly alter the energy landscape, leading to large-scale conformational remodeling and enabling them to switch folds (Bryan and Orban 2010). The structural transitions in metamorphic fold switches and polymorphic IDPs possess a number of common features including low or diminished stability, large-scale conformational changes, critical disordered regions, latent or attenuated binding sites, and expansion of function. Therefore, because these transitions are conceptually and mechanistically analogous, they represent adjacent regions in the continuum of order/disorder transitions (Kulkarni *et al*. 2018).

Anfinsen was aware that the basic facts needed to state the folding problem were already in place before his work which he acknowledged in his Nobel lecture (Anfinsen 1973) (for an interesting historical perspective, see Baldwin 2005). Thus, it is possible that he may have failed to realize that folding intermediates which are also present in nascent polypeptides that are yet to assume their final secondary structure can be functional as pre-molten and molten globules (Zambelli *et al*. 2012; Vamvaca *et al*. 2004; Uversky *et al*. 1996) or non-native partially folded state (Bemporad *et al*. 2008), or acknowledge the uniqueness of the prion proteins reported in the 1960s. Furthermore, proteins from the thermophilic organisms presented a paradox to Anfinsen's structure-function paradigm but were not acknowledged either.

Nonetheless, while Anfinsen's dogma may not be invalid, it may make more sense to enunciate an extended hypothesis to include the prions and IDPs some of which appear to function even in the absence of any discernable 3D structure – the so-called 'fuzzy logic' (Arbesú *et al*. 2018; Fuxreiter 2018) or assume rigid structure upon specific post-translation modification (Bah *et al*. 2015) or interaction with a partner (Sugase *et al*. 2007), metamorphic proteins that can switch folds, and proteins such as the P-gp that can fold

differently to assume different functions despite having an identical sequence (Kimchi-Sarfaty *et al*. 2007). Indeed, it has recently been demonstrated that IDPs can form tight complexes with disassociation constants in the picomolar range in the absence of any ordered structure (Borgia *et al*. 2018) underscoring the need to revisit Anfinsen's postulate.

10. Conclusions

Had it not been for Sherlock Holmes's uncanny abilities to discern the facts by considering the not so obvious alternatives, an innocent man in the *Boscombe Valley Mystery* story was destined to be punished at the assize because the 'evidence' albeit circumstantial, was interpreted in only one, rather biased way by those seeking a motive behind the crime. I implore the reader to only regard the Sherlock Holmes story as an analogy to an overlooked fact, and as the moral of the story indicates, one should consider all possibilities as remote as they may seem before adjudicating a postulate. Like I stated at the outset, my intent here is not to criticize these two postulates or their celebrated preceptors. Instead, based on the arguments presented, especially with regard to the IDPs, I think that both postulates while certainly NOT wrong, could benefit from an extended synthesis much like contemporary evolutionary biologists advocate the need to revise the Modern Synthesis and formulate the 'Extended Evolutionary Synthesis' (Pigliucci 2007; Laland *et al*. 2015; Müller 2017). The IDPs, although misinterpreted to have challenged not only Anfinsen's dogma but also central dogma, provide a basis for extended versions of the two dogmas.

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