 RESEARCH ARTICLE

Crick's Adaptor Hypothesis and the Discovery of Transfer RNA: Experiment Surpassing Theoretical Prediction

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Historically, hypotheses failed in most cases to correctly forecast the workings of complex biological systems. Francis Crick's adaptor hypothesis, however, stands out as an exceptional case of a confirmed abstract prediction. This hypothesis presciently anticipated the existence of RNA adaptors that function as bridges between amino acids and the chemically different nucleic acid template for proteins. Crick conjectured that the adaptors are enzymatically charged with cognate amino acids, they bind to complementary protein-coding nucleic acid, and their liberated amino acids are incorporated into growing protein chains. Independently from and concomitantly with Crick's hypothesis, Mahlon Hoagland and Paul Zamecnik conducted experiments with no guiding theory that culminated in discoveries of the actual adaptors, transfer RNA (tRNA) molecules, and their amino acids charging enzymes. This paper traces the parallel histories of Crick's adaptor hypothesis and of the experimental discovery of tRNA and compares their relative impacts on the scientific community. Remarkably, despite the brilliance of Crick's confirmed prognostication, it had marginal impact on practicing scientists. Conversely, Hoagland and Zamecnik's experimental discoveries and their evidence-based model of protein synthesis had immediate and enduring impact. I discuss possible explanations for the different impacts of the theoretical prediction and experimental identifications of the same entities.

Keywords

protein synthesis • adaptor hypothesis • transfer RNA • Francis Crick • Mahlon Hoagland • Paul Zamecnik

1 Introduction

1.1 Predictive versus *Accommodation Hypotheses and Theories*

Philosophers of science distinguish between two types of hypotheses and theories. Hypotheses of one category *predict* novel entities or facts prior to their empirical discovery, whereas theories

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of a different type *accommodate* and *explain* previously gathered data. It is generally agreed that the capacity of *predictive hypotheses* to forecast novel facts makes them aesthetically more pleasing than *accommodation hypotheses* that explicate extant knowledge. However, thinkers disagree on the validity of a broader *predictivist thesis* (also dubbed *predictivism*; Maher 1988), which argues that prediction of novel facts provides greater *epistemic certitude* than does accommodation of known data. Whereas many thinkers have advanced various versions of the predictivist idea, a sizable school of thought has also rejected the notion that predictions have epistemic advantage over accommodation of data.

First raised by Renaissance astronomers, predictivist ideas were articulated in the seventeenth century by Descartes, Huygens, and Leibnitz (Giere 1983) and expanded and refined by thinkers of the nineteenth to twenty-first centuries (for selected writings in support of predictivism see: Achinstein 1990, 1994; Lipton 2004 2005; White 2003; Douglas and Magnus 2013; Kahn et al. 1992; Lange 2001; Worrall 2014; Maher 1993; Barnes 1996; McIntyre 2001; Hitchcock and Sober 2004; Gardner 1982; Whewell 1860; Popper 1963; Lakatos 1978; Hempel 1966; Duhem 1991). On the other hand, opposing scholars have raised substantial arguments against the predictivist thesis (see for instance: Collins 1994; Harker 2008; Howson 1988; Howson and Franklin 1991; Achinstein 1994; Scheffler 1957; Keynes 1921; Mill 1843).

1.2 Putting Predictivism to Test: Cases from the Histories of Physics and Chemistry

A historical approach to the philosophy of science tests the legitimacy of philosophical theses by examining their validity in actual cases from the history of science (Laudan et al. 1986; Brush 2015). Employing this method, historians of physics and chemistry put the predictivist thesis to test by comparing the relative impacts that novel predictions and accommodation hypotheses had on practicing scientists[1] (Barnes 2005; Brush 1989, 1990, 1993, 1994, 1996, 2015; Earman and Glymour 1978; Gardner 1982; Howson and Franklin 1991; Lipton 2004; Maher 1988; Scerri 2005; Scerri and Worrall 2001; Worrall 2005; Zahar 1973). In appraising most of these studies, Brush concluded that despite their intuitive appeal, predictions of novel facts did not impact physicists or chemists to greater extents than did explanatory theories:

> A successful prediction may yield much favorable publicity for a theory (including statements that call attention to the novelty of the phenomenon predicted) and thereby force other scientists to give it serious consideration. But subsequent evaluation of the theory in the technical literature do not seem to give greater weight to the prediction of novel facts than to persuasive deductions of known facts. (Brush 1989, 1127)

1.3 Complexity of Biological Systems Thwarted Predictions of Their Workings

Successful predictions in biology are much less frequent than in the physical sciences. Predictive hypotheses on the workings of complex biological systems have generally failed because of their inevitable blindness to yet unidentified constituents and effectors of the systems. Evolutionary biologists (Scriven 1959; Mayr 1985, 1988) pointed out that because biological systems are shaped by multiple and largely unknown causes and effects, their behavior is hard or impossible to predict. This point was cogently articulated by Ernst Mayr (1988):

[1] Among the better studied predictions in physics and chemistry were Einstein's prediction of gravitational light bending (Brush 1989); forecasts made by Alfvén's electromagnetic plasma theory (Brush 1990); predictions in big bang cosmology (Brush 1993); forecasts in quantum mechanics (Brush 1994); and Mendeleev's projection of existence of three elements in his incomplete periodic table (Barnes 2005; Brush 1994, 1996; Howson & Franklin 1991; Lipton 2004; Maher 1988; Scerri 2005; Scerri & Worrall 2001; Worrall 2005).

> A belief in universal, deterministic laws implies a belief in absolute prediction. The ability to predict was therefore the classical test of the goodness of explanation in physics. In biology, the pluralism of causations and solutions makes predictions probabilistic, if it is possible at all. Prediction in the vernacular sense, that is, the foretelling of future events, is as precarious in biology as it is in meteorology and other physical sciences dealing with complex systems. […] As Scriven (1959) pointed out, the ability to predict is not a requirement for the validity of a biological theory. (Mayr 1988, 19–20)

Historians of science have assessed the relative contribution of novel predictions in forming knowledge in selected areas of biology. One carefully studied case was Morgan's chromosome theory of heredity (CTH) (Morgan et al. 1915). Analysis showed that American and British geneticists adopted this theory mostly because of its ability to explain empirical data on Mendelian and sex-linked inheritance, on disjunction, and on linkage groups. These accommodations of empirical data impacted scientists to a greater extent than the confirmation of the CTH predictions (Brush 2002, 2015, 397–412).

Similar assessments revealed that it was empirical evidence and not predictions that convinced evolutionists in the 1950s that natural selection drives evolutionary change (Brush 2009, 2015, 442–464). Yet, because evolution and natural selection are multidimensional processes, they are unlikely to yield a definitive picture of the relative contributions of predictive versus accommodation hypotheses.

Population geneticists weighed the success of hypotheses that predicted fluxes in populations of *Drosophila*. It was initially claimed that measurements of past population dynamics of three species of *Drosophila* produced statistical estimators that predicted with high confidence future fluctuations in these populations (Poole 1976, 1978). It was pointed out, however, that such predictions can succeed only if past and future conditions remain the same. More importantly, even if statistical models could correctly predict future population fluxes, they would not provide any insight into the fundamental biological basis of either past or future population changes (Anderson 1978b, 1978a). A more recent attempt was made to forecast future morphological evolution of stick insects on the basis of long-term observations and experiments. Results indicated, however, that predicting future morphological changes on the basis of past data required a yet undiscovered full understanding of the biology of the organism (Nosil et al. 2018).

Because biological systems appeared to be less complex at the molecular level than at the organismal or population levels, it could be thought that their working would be easier to predict. Indeed, some major theories effectively delineated fundamental molecular-level principles of biology. Such was the Watson and Crick (1953) proposition that complementarity of replicated DNA strands was the key to faithful gene inheritance. Other grand theories were Crick's (1958) sequence hypothesis and his central dogma, and the operon model of regulation of gene expression (Jacob and Monod 1961). However, in contrast to these productive global theories, hypotheses that attempted to envisage physical and functional details of particular multicomponent bio-molecular systems were consistently unsuccessful. Thus, for instance, theories were wide off the mark in picturing the makeup and modes of function of the machineries of protein synthesis or of non-lysosomal degradation. Similarly, theories did not contribute to the elucidation of the biosynthesis of DNA or RNA or to the discovery and dissection of the RNA splicing apparatus. Failures of predictive conjectures in these and similar cases were primarily due to their inevitable ignorance of yet undiscovered constituents of the studied systems. It was ultimately left, therefore, for stepwise experimentation to identify components of such systems and to elucidate their workings. Thus, for instance, because messenger RNA was not envisaged and searched for in the 1950s, the dominant theory of the time erroneously maintained that

it was ribosomal RNA which mediated transmission of genetic information from DNA to the protein synthesis machinery (Fry 2016, 365–370). Similarly, theories made wrong predictions on the mode of non-lysosomal degradation of proteins (Fry 2018), the mechanism of translation of nucleic acid into protein, or the manner of antibody formation (Fry 2020). Ultimately, these and other complex bio-molecular systems were elucidated by experiment and organization of the gathered data into accommodation theories. In failing to provide effective forecasts of the workings of complex biological systems, predictive hypotheses therefore held no epistemic value.

1.4 The Exceptional Case of Crick's Prescient Adaptor Hypothesis

Francis Crick's so-called 'adaptor hypothesis' presents a rare case of a confirmed abstract conjecture in biology. This astute hypothesis stands in stark contrast to previously reviewed theories whose conceptions of the constitution and mode of operation of diverse complex bio-molecular systems were incorrect (Fry 2016, 365–370, 2018, 2020). Crick's hypothesis warrants the special analysis of this paper because it is singled out by its exceptional success from the habitual failures of other theoretical predictions about the working of other systems. In his adaptor hypothesis Crick prefigured the existence of amino acid-specific RNA adaptors and of specialized enzymes that charge them with particular amino acids. He also conjectured that the amino acid carrying adaptors bind to complementary protein-coding RNA template and their liberated amino acids are incorporated into a growing polypeptide chain (Crick 1955). When Crick introduced and disseminated his hypothesis in 1955–57, he was entirely unaware of concomitant, closely pertinent, experimental findings of the Harvard biochemists Mahlon Hoagland and Paul Zamecnik (Crick 1988, 94). These investigators discovered the actual adaptors—transfer RNA (tRNA) molecules—and identified enzymes that activate and charge the amino acids onto cognate tRNA. Based on these empirical data, they also formulated a skeleton theory of protein synthesis that shared many similarities with Crick's hypothesis. Curiously, while Crick was unaware of their findings, Hoagland and Zamecnik also had no knowledge of his adaptor hypothesis as they were carrying out their experiments (Hoagland 1959, 93–94; Zamecnik 1960).

The historically rare case of Crick's abstract hypothesis is especially instructive because of Hoagland and Zamecnik's unknowing, concomitant, affirming experiments. Intriguingly, historical evidence shows that despite its striking brilliance, Crick's hypothesis was mostly ignored or distrusted by contemporaneous researchers. By contrast, the experimental findings of Hoagland and Zamecnik had immediate and enduring impact on practicing scientists. It was, therefore, the empirical discoveries of Hoagland and Zamecnik and not Crick's celebrated hypothesis that exposed the scientific community to the mechanism of translation of protein-coding nucleic acid.

2 The Adaptor Hypothesis

2.1 Birth of the Hypothesis: Historical Background

Crick's interests in the mid-1950s encompassed protein structure (Crick and Rich 1955; Rich and Crick 1961, 1955) and the spatial organization of coat proteins of RNA viruses (Crick and Watson 1956; Crick and Watson 1957). A third, and arguably his most important, area of inquiry was the nature and properties of the nucleic acid genetic code and how it encodes proteins. Crick's earliest direct contribution to this field was his critical evaluation of an abstract model of the structure and translation of the code that had been proposed by the theoretical physicist George Gamow. Crick dismantled most of Gamow's propositions in an informal

paper that he circulated in 1955 among a few interested scientists. In its place he offered his so-called *adaptor hypothesis* that was independently confirmed by the parallel experimental work of Hoagland and Zamecnik. In hindsight, Gamow's incorrect model served, therefore, as a useful, disposed scaffolding for Crick's more accurate model.

The Gamow model. Starting in the 1940s, early speculations linked protein synthesis to nucleic acids (Fry 2016, 277–280). This insinuated linkage gained a sharper focus when Watson and Crick opined in 1953 that: "It seems likely that the precise sequences of bases [in DNA] carries the genetical information" (Watson and Crick 1953). This terse statement provoked George Gamow, a physicist with no direct experience in biology, to consider ways in which base sequences in nucleic acid encode ordered arrangements of amino acids in proteins. Starting a correspondence with Watson and Crick in the mid-1953, Gamow aired ideas on the possible nature of the code and its probable mode of translation into proteins.[2] The core question with which Gamow grappled in the first half of the 1950s was later enunciated by Crick and associates (Crick et al. 1957): "The problem of how, in protein synthesis, a sequence of four things (nucleotides) determines a sequence of many more things (amino acids) is known as the coding problem." In his first attempt to answer this question Gamow constructed a theoretical model of a code based on stereo-chemical fit between amino acids and inter-nucleotide spaces in a DNA template. He first described this model in an October 22, 1953 letter to Linus Pauling (Gamow 1953) and then delineated his ideas in two formal publications (Gamow 1954a, 1954b). In this model DNA served as direct protein-encoding template that accommodated stereochemically fitting amino acids in inter-nucleotide clefts. Specifically, Gamow postulated that amino acids inserted into hypothetical, diamond-shaped niches between two adjacent base pairs in the DNA double helix. Allegedly, specific combinations of three out of the four nucleotides in two adjacent base pairs dictated specific contours of each particular cavity.[3] Combinatorially, there were twenty possible different base triads and twenty different contours, a number that conveniently matched the twenty different amino acids. Gamow's model further purported that after becoming embedded within their specific cavities in the DNA, the amino acids bonded to one another to form a polypeptide chain. (For more detailed descriptions of the model, see Olby 2009, 223–228; Fry 2016, 280–284.)

Gamow's theory endowed the code with several attributes. First, because every base pair in the DNA was a part of two bordering cavities, it was also a part of two code words (later named codons). Each base pair in such an overlapping code dictated the identity of two adjacent amino acids. This feature of the code limited the number of possible permutations of two contiguous amino acids. Another feature of the Gamow code was its lack of polarity such that a string of code words would be translated at equal probability to the polypeptide chain A-B-C-D- and its reverse D-C-B-A-. Last, this model raised a prospect of a degenerate code in which several different code words encode the same amino acid.

Heeding some of Crick's criticisms of his model (section 2.2), Gamow later replaced DNA with RNA as the direct template, professing that amino acids lodged in presumed clefts in the secondary structure of single-stranded RNA. He also conducted computer-aided calculations in an attempt to define code words. Although his efforts to solve the code problem were unsuccessful, Gamow made a historically notable contribution by creating the so-called RNA Tie Club: a virtual think tank dedicated to elucidation of the genetic code. Twenty members of the Club—biochemists, physicists, and mathematicians—stood for the twenty amino acids, while

[2] Facsimiles of Gamow's letters are in an appendix to Watson (2001) and in the Francis Crick NIH 'Profiles in Science' archive: https://profiles.nlm.nih.gov/ps/retrieve/Narrative/SC/p-nid/153/p-docs/true.

[3] Default identity of the fourth base was supposedly determined by its complementary hydrogen bonding to the third nucleotide.

four 'honorary' members represented the 4 nucleotides. The habitually traveling Gamow kept the geographically scattered club members abreast of new, real or imagined, developments in the pursuit of the code. In addition, he encouraged members to circulate informal papers with ideas on the code. Crick first introduced his adaptor hypothesis in one such internal, never-published manuscript (Crick 1955), which is considered now to be the most famous unpublished paper in the annals of molecular biology.

2.2 Emergence of the Adaptor Hypothesis

In December of 1953 Gamow met for the first time with Crick who was then working at Brooklyn Polytechnic.[4] Their two-hour animated discussion revitalized Crick's enthusiasm for the code problem (Olby 2009, 221). Thinking about the code problem for the rest of his sojourn in the United States, Crick became increasingly critical of the idea of stereo-specific fit between amino acids and cavities in a DNA template. Instead, he came up with an alternative model of adaptor molecules that specifically bind amino acids and direct them to particular code words in the nucleic acid template. The adaptor hypothesis was hatched in August 1954 while Crick was still in the United States (Judson 1996, 284), and it was fully articulated in an informal manuscript "On Degenerate Templates and the Adaptor Hypothesis"[5] that Crick wrote after his return to Cambridge, in the winter of 1954–55. Circulated among members of the RNA Tie Club in early 1955,[6] the paper critiqued the Gamow model, raised ideas on some principal features of the code, and most notably introduced the adaptor hypothesis. A timeline of the launch and subsequent dissemination of the hypothesis is charted in figure 1 (top line).

Crick opened his RNA Tie Club communication with epigraph from *Qabus-Nama* ("A Mirror of Princes" in Persian): "Is there anyone as utterly lost as he that seeks a way where there is no way?" This quote from the rather obscure ruler of the Ziyarid dynasty and poet Kai Kāvus ibn Iskandar (1021–1099), seemed to reflect a pessimistic sense of dead end in the pursuit of the code. In point of fact, however, the paper presented ideas that significantly advanced the definition of the nucleic acid code and its mode of translation to proteins. The following sections describe Crick's critique of basic tenets of the Gamow model and his alternative adaptor hypothesis. Not covered are parts of the paper that dealt with overlap, degeneracy, and directionality of the code.

2.2.1 Crick's criticisms of the Gamow model

Crick contested central elements of the Gamow model. Yet, his evaluation was not entirely negative, as he praised Gamow for bringing up the prospect of degenerate and overlapping code. He also graciously acknowledged Gamow's role as leader of the quest for the code:

> Finally it is obvious to all of us that without our president [of the RNA Tie Club] the whole [code] problem would have been neglected and few of us would have tried to do anything about it. (Crick 1955, 6)

[4] Brooklyn Polytechnic is now the New York University Tandon School of Engineering.

[5] A facsimile of the handwritten manuscript is at the Wellcome Library Web site (https://wellcomecollection.org/works/j7qpsmdm), and its typewritten version is in the NIH 'Profiles in Science' online archive: https://profiles.nlm.nih.gov/spotlight/sc/catalog/nlm:nlmuid-101584582X73-doc.

[6] A hand-written inscription (presumably Crick's): "early 1955" appears on the title page of the typed manuscript. According to Olby (2009, 232), the paper was sent to members of the Tie Club in January 1955. Curiously, and most likely erroneously, Crick (1988, 95) himself later dated his return to England (and thus the writing of the paper) to the fall of 1956.

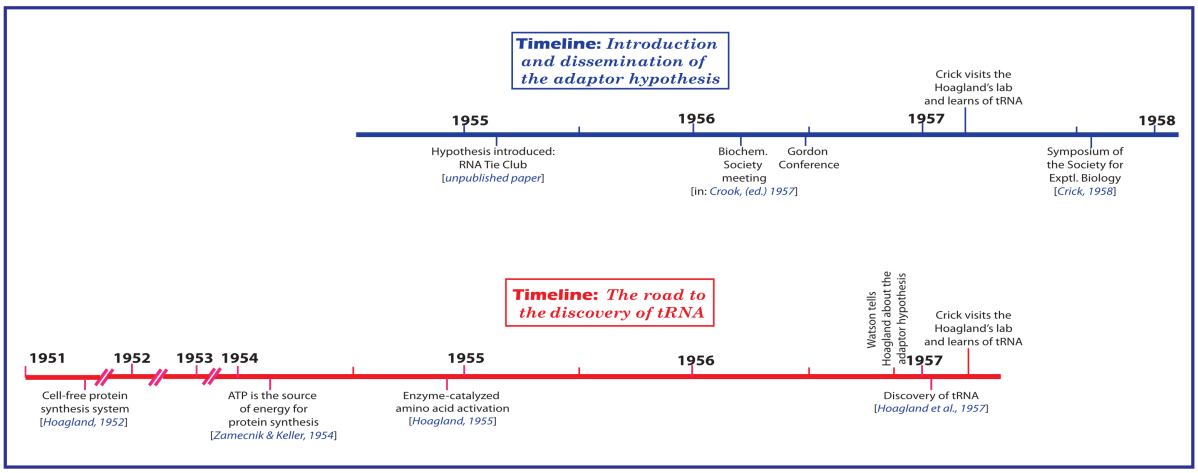

Figure 1: Timelines of the introduction and dissemination of the adaptor hypothesis (*upper line*) and the parallel development of a cell-free protein synthesis system, and discoveries of amino acid activation, activating and charging enzymes, and tRNA (*lower line*). Marked along the axes are dates of conference presentations, submission of key papers, and significant meetings. Precise dates of these events are detailed in the body of the text and in footnotes.

In the main, however, Crick considered principal assumptions of the Gamow model to be mistaken or unlikely. First, he narrowed Gamow's list of amino acids (Gamow 1954a) to just twenty unmodified building blocks of veritable proteins.[7] Second, Crick tested Gamow's tenet of an overlapping code by examining amino acid sequences of a few proteins that had just been determined by Fred Sanger. Based on these data he concluded that the code was likely non-overlapping. (For technical details, see Fry 2016b, 284–285). This conclusion was later validated by broader similar analysis (Brenner 1957) and in direct mutagenesis experiments with Tobacco Mosaic Virus (Tsugita and Fraenkel-Conrat 1960). Crick's third point was that the Gamow model allowed unlikely bidirectional reading of cavities in the DNA and thus an equal production of both A-B-C-D- and inverse D-C-B-A- polypeptides. Last, Crick's fourth critical comment was that the Gamow model involved unlikely investment of excessive free energy to maintain acceptable fidelity of protein synthesis (Crick 1955).

2.2.2 The adaptor hypothesis as an alternative to the Gamow model

Crick's fifth, and most central objection to the Gamow model concerned its underlying structural assumptions. Finding the idea of direct stereo-chemical fit between DNA template and amino acids to be groundless, he replaced it with a conjecture of adaptors.

Crick discounted Gamow's structural assumptions on two accounts. First, an amino acid in a polypeptide chain matches by size only a single base pair in the common B conformation of DNA and not two as Gamow posited. Crick's (1955) second and more important point was that there was no structural basis for the presumed stereo-chemical fit between amino acids and differently shaped inter-nucleotide cavities in the DNA template:

> I cannot conceive of *any* structure (for RNA or DNA) acting as a direct template for amino acids, or at least as a specific template. In other words, if one considers

[7] Crick excluded unusual amino acids that appear in small peptides such as diaminobutyric acid, oxytocin or vasopressin, and D amino acids that are present in polypeptides such as gramicidin.

> the physical-chemical nature of the amino acid side chains we do not find complimentary [*sic*] features on the nucleic acid. Where are the knobby hydrophobic surfaces to distinguish valine from leucine and isoleucine? Where are the charged groups, in specific positions, to go with the acidic and basic amino acids? (7)

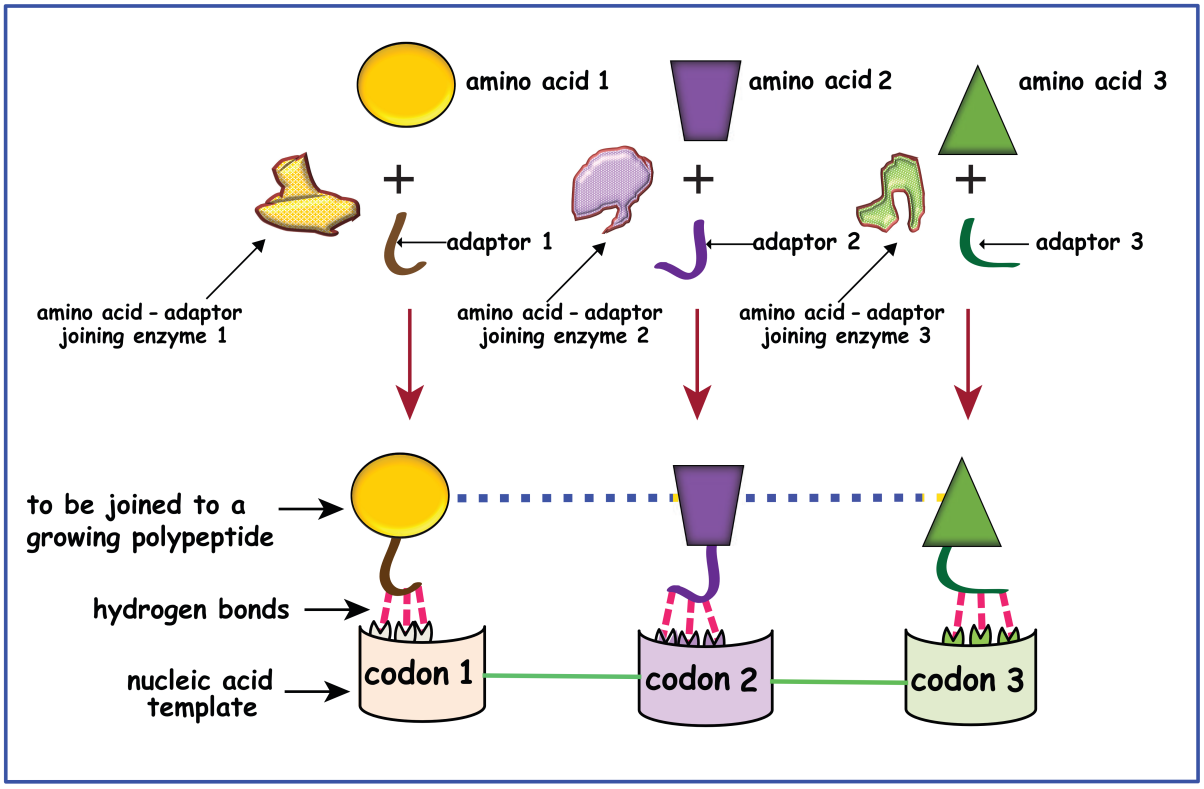

Figure 2: Illustration of Crick's original adaptor hypothesis (Crick 1955). Shown are three out of at least 20 different hypothetical RNA adaptors, each one comprising three and a few additional nucleotides. Specialized enzymes were proposed to 'chemically join' each amino acid onto its particular adaptor that then hydrogen bonded to complementary code word in an RNA template. Crick's original hypothesis did not specify how the amino acids detached from the adaptors and joined growing polypeptide chain.

Dismissing the idea of direct stereo-specific interaction of amino acids with a nucleic acid template, Crick presented instead an alternative *adaptor hypothesis*. Under this hypothesis adaptor molecules mediated *indirect* interaction between the amino acids and template (fig. 2). In essence, Crick proposed that specialized enzymes charge amino acids onto cognate adaptor molecules. The charged adaptors next form complementary hydrogen bonds with corresponding code words in the nucleic acid template and release their amino acids to be joined to a growing protein chain. Thus, Gamow's notion of specific direct spatial fit between amino acids and nucleic acid was replaced by an idea of specific hydrogen bonding of adaptor to template:

> What the DNA structure *does* show (and probably RNA will do the same) is a specific pattern of *hydrogen bonds*, and very little else. It seems to me, therefore, that we should widen our thinking to embrace this obvious fact. (Crick 1955, 7)

Expounding in full his adaptor idea, Crick further wrote:

> each amino acid would combine chemically, at a special enzyme, with a small molecule which, having a specific hydrogen-bonding surface, would combine specifically with the nucleic acid template. […] In its simplest form there would be 20

> different kinds of adaptor molecule, one for each amino acid, and 20 different enzymes to join the amino acid to their adaptors. Sydney Brenner, with whom I have discussed this idea, calls this the "adaptor hypothesis,"[8] since each amino acid is fitted with an adaptor to go on to the template. (1955, 8)

Interestingly, Crick did not stipulate in his 1955 hypothesis how the adaptors liberate their amino acids to be linked to growing polypeptide chain. However, he did insightfully speculate that the number of different adaptor molecules may be greater, or possibly lower, than twenty:

> It is also conceivable that there is more than one adaptor molecule for one amino acid, and the number 20 may be simply an accident (in any case we need a code for "end chain", so perhaps 21 would be more reasonable). Alternatively the same adaptor molecule might fit on in more than one way (related, say, by a rotation of Θ°). (1955, 9)

Countering potential criticism indicating absence of evidence for the existence of adaptors or their complexes with amino acids, Crick argued that such molecules were "in short supply" relative to a great excess of free amino acids and were thus hard to detect.

To which nucleic acid template did the adaptors bond? At first Crick seemed to think of DNA as the direct template for protein synthesis:

> If we accept the idea that what matters in DNA are the hydrogen-bonding sites, it seems plausible to assume that each "site" will combine with one adaptor and one adaptor only. […] This requirement is not essential but it is likely if adjacent adaptors have to be combined with the DNA at the same time for polymerization to occur. (1955, 14)

Notably, already at that early time he had the insight to raise the possibility that adaptors hydrogen-bond to an RNA and not to a DNA template:

> I have tacitly dealt with DNA throughout, but the arguments would carry over to some types of RNA structure. If it turns out that DNA, in the double-helix form, does not act directly as a template for protein synthesis, but that RNA does, many more families of codes are of course possible. (Crick 1955, 16)

Yet, lacking at the time knowledge of the structure and properties of RNA, Crick could not ascertain that it was the actual adaptor-binding template:

> Incidentally the protein sequences we use to test out theories—insulin, for example—are probably RNA-made proteins. Perhaps a special class of DNA-made protein exists, almost in small quantities (and thus normally overlooked).[9] Except perhaps where there are giant chromosomes. In particular base pairing may be absent in RNA or take a radically different form, and there may be more than one base to the asymmetric unit. Without a structure for RNA one can only guess. (Crick 1955, 16–17)

[8] Crick and Brenner started to discuss the code in August 1954 when both attended a New Hampshire Gordon Conference on nucleic acids. Brenner suggested the term 'adaptor hypothesis' when he stayed with the Crick family during his visit to Cambridge in late 1954 (Judson 1996, 284). Crick eventually recruited Brenner to the Cambridge MRC Unit in 1956 and for the next twenty years the two shared an office and engaged in vigorous and fertile exchange of ideas.

[9] In mentioning DNA-directed protein synthesis, Crick likely referred to contemporaneous reports by the Rockefeller Institute biochemists Vincent Allfrey and Alfred Mirsky of protein synthesis in isolated nuclei (Allfrey 1954; Allfrey et al. 1955).

2.3 Known to Relatively Few, The Adaptor Hypothesis was Doubted or Dismissed

Because Crick's paper was circulated only among the few members of the RNA Tie Club, it had *minimal immediate* impact. One unconvinced reader was Jim Watson, then at California Institute of Technology. In a February 10, 1955 letter to Crick[10] he tersely remarked: "Your TIECLUB note arrived during visit.[11] Am not so pessimistic.[12] Dislike adapters."[13] Some of the leading students of the code and protein synthesis, including Gamow, Alexander Dounce, Paul Zamecnik, and Henry Borsook, met on April 4–6, 1955 at the Oak Ridge National Laboratories for a symposium on enzyme and protein structure. Discussing state of the art developments in protein biosynthesis, none of the speakers at the meeting mentioned Crick or his adaptor hypothesis (i.e., Zamecnik et al. 1956; Borsook 1956). Most pointedly, Dounce and Gamow, both members of the RNA Tie Club and likely readers of Crick's paper, evaluated code word composition, overlap, and degeneracy without mentioning Crick's hypothesis (Dounce et al. 1956).

Shortly after circulating his paper, Crick made two pubic presentations of his hypothesis. First, he spoke in February 18, 1956 in a London Biochemical Society symposium on the structure of nucleic acids and their role in protein synthesis.[14] As Brenner later reminisced the audience contested the hypothesis[15] (Brenner 2014; see also McElheny 2004, 89):

> So he [Crick] gave the lecture and biochemists stood up in the audience and said this is completely ridiculous, because. […][16] What people don't realise [*sic*] is that at the beginning, it was just a handful of people who saw the light, if there were twenty enzymes, we biochemists would have already discovered them. To them, the fact that they still hadn't, went to show that this was nonsense I can put it that way. So it was like belonging to an evangelical sect, because there were so few of us, and all the others sort of thought that there was something wrong with us.

Crick also spoke about the adaptor hypothesis in a June 25–29, 1956 Gordon Conference on proteins and nucleic acids. Among those attending were key players in the areas of the code and protein synthesis: Gamow, Alex Rich, Dounce, and Zamecnik.[17] As a rule, proceedings of Gordon conferences have no written records, and I could not find any account of Crick's talk at the 1956 conference. Interestingly, however, this event illustrates the disconnection that existed between Crick's theory and the contemporaneous experimental discovery of tRNA. The conference could have provided an opportunity for Crick and Zamecnik to compare notes on their respective hypothesis and its experimental corroboration. However, according to Hoagland, Zamecnik had missed Crick's presentation:

> In the summer of 1956 Francis gave an informal talk on ribosome structure and the adaptor idea at a Gordon conference in New Hampshire, not far from Boston,

[10] Facsimile of the letter in: https://profiles.nlm.nih.gov/ps/retrieve/ResourceMetadata/SCBBJL.

[11] Watson referred to Gamow's visit to Caltech.

[12] Watson was likely alluding to Crick's guarded position on the definition of the code.

[13] I failed to find written record of the basis to Watson's initial negative view of Crick's hypothesis or to the obvious indifference of other members of the Tie Club to this conjecture. Yet, by 1956 Watson had already become well versed in the hypothesis, such that it was he who introduced it to Hoagland (see section 4.1).

[14] A short version of this lecture was published a year later: (Crick 1957).

[15] The weight of Brenner's testimony should be carefully appraised considering that he had given it 58 years after the described event.

[16] At this point Brenner pointed out that 'scientists' (alluding to the Zamecnik and Hoagland group) were already at that very same time isolating the first aminoacyl-tRNA synthetase.

[17] Speakers at the conference and titles of their talks were advertised in *Science* 22 (2): 361 (1955).

> in the evening at the end of a five day meeting. Paul Zamecnik had attended the meeting but had left early and had not heard Francis's paper. (1990, 96)

Yet, Crick was present at the conference when Zamecnik gave a talk on its third day titled "Studies on the Role of RNA and Nucleotides in Protein Synthesis." By the time of that talk enzyme-catalyzed activation of amino acids has already been discovered (Hoagland et al. 1956) and five months later Hoagland and Zamecnik submitted for publication their first report of the discovery of tRNA (Hoagland et al. 1957). Considering this timeline and the fact that RNA was mentioned in the title of Zamecnik's talk, it is likely that it included first hints of tRNA. Because the historical record shows that Crick learned about the discovery of tRNA and of amino acid charging enzymes only in January 1957 (section 4.2), it must be deduced that he either missed the talk or that its implications escaped him.

Crick also presented his adaptor hypothesis in a Society for Experimental Biology symposium on biological replication of macromolecules that took place in London in September 19, 1957. In this talk titled "On Protein Synthesis," which was published one year later (Crick 1958), he addressed cardinal questions of the hour: the sequence hypothesis and the central dogma, protein synthesis, and the adaptor hypothesis.[18] At the time of this presentation Crick was already aware of the discovery of amino acid activating and charging enzymes and of tRNA (see section 3). Yet, because the empirically estimated size of tRNA was larger than the size that he imagined for his adaptor, Crick did not equate tRNA with the adaptor. The 1957 talk included, therefore, repeated exposition of the adaptor hypothesis, a mention of the experimental detection of tRNA and amino acid charging enzymes and some speculations on the relationships of the adaptor to tRNA. Crick's seminal talk is discussed in greater details in a following section (4.2.1).

3 Experimental Discoveries of Amino Acid Activating and Charging Enzymes and of tRNA and Their Incorporation Into Accommodation Model of Protein Synthesis

Prior to Crick's formulation and dissemination of the adaptor hypothesis, Paul Zamecnik and Mahlon Hoagland from the Huntington Memorial Hospital and Harvard University conducted a series of experiments that culminated in the discovery of amino acid activating and charging enzymes (heretofore termed aminoacyl-tRNA synthetases) and of transfer RNA (tRNA). Having been completely unaware of Crick's hypothesis, Hoagland and Zamecnik never intended to search for adaptor molecules. Rather, with no preconception of the mechanism of protein biosynthesis and thus without a guiding theory, they aimed to experimentally dissect and define the cellular protein synthesis machinery. A crucial starting point of their project was the establishment of a cell-free protein synthesis system. Next, the energy requirements of this *in vitro* system were defined, and its essential sub-cellular components were isolated. Subsequent experiments revealed that the first step in protein synthesis was an ATP-dependent activation of free amino acids by specialized enzymes: aminoacyl-tRNA synthetases. Next discovered were amino acid-specific tRNA species that the aminoacyl-tRNA synthetases charged with cognate amino acids. The amino acid carrying tRNA molecules were then shown to associate with RNA template on ribosomes and their liberated amino acids were incorporated into growing polypeptide chains. A timeline of these major discoveries is charted in figure 1 (bottom line).

[18] See Thieffry and Sarkar (1998) and Cobb (2017) for appraisals of this seminal talk and its historic importance on the occasions of its respective fortieth and sixtieth anniversaries.

The following subsections briefly delineate the main steps in the experimental discovery of aminoacyl-tRNA synthetases and of tRNA and outline their accommodation into an evidence-based model of protein synthesis. Many authors described these masterful discoveries in great detail (Zamecnik 1960, 1979, 1984; Siekevitz and Zamecnik 1981; Barciszewska et al. 2016; RajBhandary and Kohrer 2006; Spirin 1999; Rheinberger 2016; Giege 2006; Pederson 2005; Hoagland 1989) and (Morange 1998, 133–134; (Fry 2016, 313–356), while others explored historical and philosophical implications of these findings (Darden 2006, 65–97; Rheinberger 1992b, 1992a, 1993, 1995, 1997, 2006; Burian 1993; Darden and Craver 2002).

3.1 Establishment of A Cell-Free Protein Synthesis System

3.1.1 Early ideas on the mechanism of protein biosynthesis

A theory of protein synthesis that prevailed from the 1930s to the early 1950s alleged that polymerization of amino acids was catalyzed by reversely acting proteolytic enzymes. According to this model, proteases retained their specificities of hydrolysis of peptide bonds between two particular amino acids when they reversely acted under favorable conditions to catalyze α-peptide bond formation between corresponding amino acids. To polymerize different amino acids into polypeptide chains, proteases of different specificities were presumed to act in concert. First raised in the early 1930s (Wasteneys and Borsook 1930; Voegtlin et al. 1933), this idea was supposedly supported by the experimental work of Max Bergmann and his student Joseph Fruton at the Rockefeller Institute (Bergmann 1942; Bergmann and Fraenkel-Conrat 1937; Bergmann and Fruton 1938, 1941; Fruton 1941, 1950). Although the protease-catalysis theory of protein synthesis was contradicted by energetics calculations and other considerations (Borsook and Huffman 1938; Loftfield et al. 1953) it persisted until the early 1950s (Campbell and Work 1953; Linderstrøm-Lang 1952; Steinberg and Anfinsen 1952; Zamecnik 1950).

Independently from protease-catalyzed protein synthesis, autonomous lines of research exposed linkage between cytoplasmic RNA and protein biosynthesis. First, protein synthesis activity was found to be positively correlated to the RNA content of various cell types (Caspersson and Schultz 1938, 1939; Caspersson 1947). Second, fluctuations in protein synthesis corresponded to amounts of cytoplasmic RNA (Brachet 1941, 1947, 1950). Also, unperturbed maintenance of protein synthesis in the RNA-rich cytoplasm of enucleated *Amoeba proteus* cells indicated that the nucleus, and thus DNA, were dispensable for synthesis of protein (Brachet 1955). Separating tissue homogenates into sub-cellular fractions by centrifugation at different speeds, Albert Claude isolated nucleoprotein particles that contained all the then detectable cytoplasmic RNA. Initially termed 'small granules' (Claude 1938) and later 'microsomes' (Claude 1943), these nucleoprotein particles were vesicle-like particles made up of fragments of the endoplasmic reticulum and of ribosome particles of ~40% RNA and ~60% protein.[19] Because of their low sensitivity, analytical methods of the time detected only microsomal RNA while minor RNA species that comprised up to ~20% of the total cytoplasmic RNA, remained unseen. As a result, Thus, microsomal RNA was thought for a relatively long time the only type of RNA in the cytoplasm.

3.1.2 Early attempts to synthesize proteins in the test tube

Soon after the release of radioactive isotopes to the research community by the end of World War II (Creager 2013) chemists synthesized ^{14}C- and ^{35}S-labeled amino acids (Loftfield 1947; Melchior and Tarver 1947a, 1947b). Measurement of incorporation of such radiolabeled amino

[19] For detailed history of the discovery of ribosomes see (Rheinberger 1995, 2016).

acids into acid-insoluble macromolecular polypeptides promptly became an accepted method for monitoring protein synthesis *in vivo* and *in vitro* (Fry 2016, 319–320). First attempts to conduct and gauge *in vitro* synthesis of protein were made with tissue slices. These experiments revealed that synthesis of protein was an energy (ATP) consuming reaction (Frantz et al. 1947; Zamecnik et al. 1948; Winnick et al. 1947). Also, synthesis was higher in slices of rapidly proliferating tissues than in slices of resting tissues (Winnick et al. 1947; Greenberg et al. 1948; Borsook et al. 1950b). However, since tissue slices could not be dissected into sub-cellular fractions, they did not afford insight into the composition of the protein biosynthesis machinery.

3.1.3 Construction of cell-free protein synthesis system

Zamecnik's earliest interest was in the examination of possible differences in the protein biosynthesis capacities of cancer and normal cells. Using isotopically labeled amino acids, he initially monitored their incorporation into proteins in slices of normal and cancerous tissues (Zamecnik et al. 1948). Although he found that incorporation was energy (ATP) dependent, he adhered at that early stage to the then consensual idea that peptide bonds were formed by reversely acting proteolytic enzymes (Zamecnik 1950). Because of the limited experimental utility of tissue slices, his laboratory took a next theory-free exploratory step of establishing a more manipulatable cell-free protein synthesis system. Philip Siekevitz, who was working in the Zamecnik laboratory in 1951–52, undertook to develop such a system (Siekevitz and Zamecnik 1951; Siekevitz 1952).[20] Using rat liver homogenates, he monitored incorporation of ^{14}C-labeled alanine into acid insoluble macromolecular material that was later authenticated as a true measure of protein synthesis (Zamecnik and Keller 1954). As in tissue slices, protein synthesis in the cell homogenate was energy (ATP) dependent (Siekevitz 1952; Zamecnik and Keller 1954).

Applying differential centrifugation and precipitation of microsomes at low pH, Siekevitz separated the liver cell homogenates into sub-fractions of nuclei, mitochondria, microsomes, and post-mitochondrial supernatant (Siekevitz 1952). As previously seen in live animals (Keller 1951; Hultin 1950; Borsook et al. 1950a), most of the incorporated radiolabeled amino acid was localized in the microsomal sub-fraction which thus appeared to be the likely site of protein synthesis (Siekevitz 1952). Whereas each isolated sub-fraction was incapable of incorporating amino acids by itself, recombining the microsomal and the post-mitochondrial supernatant fractions reinstituted synthesis to the level of unfractionated homogenate This observation first hinted that the supernatant fraction contained element(s) essential for protein synthesis.

3.2 Discoveries of Amino Acid Activation, Activating and Charging Enzymes, and tRNA

Using the cell-free protein synthesis system, Hoagland, Zamecnik, and associates initially discovered that prior to being incorporated into proteins, amino acids were activated by specialized activating enzymes. Subsequent experiments identified transfer RNA (tRNA)[21] and showed that it was charged with cognate amino acids by the activating enzymes, currently named aminoacyl-tRNA synthetases. Based on these landmark discoveries Zamecnik and Hoagland proffered a tentative evidence-based skeleton scheme of protein synthesis (Hoagland et al. 1957).

[20] Siekevitz' seminal 1952 paper was received for publication in the *Journal of Biological Chemistry* on July 11, 1951 and appeared in print on April 1, 1952. Reception dates of this and subsequent key publications from the Hoagland and Zamecnik laboratory are marked in the timeline in figure 1.

[21] These amino acid binding, low molecular size RNA molecules were initially called small RNA (S-RNA), then renamed soluble RNA (sRNA), and finally received their current designation of transfer RNA (tRNA).

3.2.1 Enzyme-catalyzed amino acids activation

In early 1955 Hoagland reported that amino acids were enzymatically activated in an ATP-dependent reaction (Hoagland 1955). This short communication and a subsequent more expansive paper (Hoagland et al. 1956) reported the detection of enzymes in liver cell homogenate[22] that catalyzed production of activated AMP∼amino acids (aa: amino acid; PP_i: pyrophosphate):

$$\text{aa} + \text{ATP} \longrightarrow \text{aa}{\sim}\text{AMP} + PP_i$$

Hoagland and associates obtained initial evidence that different amino acids were activated by different enzymes (Hoagland et al. 1956). Soon other investigators corroborated this preliminary report by isolating distinct amino acid-specific activating enzymes (Berg 1956; Davie et al. 1956; Demoss and Novelli 1955, retrospectively reviewed by Berg 2003; Giege 2006; Raj-Bhandary and Kohrer 2006; Spirin 1999).

3.2.2 Discovery of amino acid carrying tRNA

Hoagland, Zamecnik and Stephenson announced in early 1957 that prior to their ultimate incorporation into proteins, activated amino acids were transferred to and bonded with low-size RNA (Hoagland et al. 1957). In a subsequent paper they described in greater detail the transfer reaction and the amino acid carrying RNA molecules (Hoagland et al. 1958).

Transfer of activated amino acids to low-size RNA: Hoagland et al. reported in an initial account that incubation of a post-mitochondrial cytoplasmic sub-fraction that contained activating enzymes and ~5% RNA, with ATP and radiolabeled leucine resulted in transfer and covalent bonding of the radioactive leucine to low-sized RNA species that was later defined as tRNA (Hoagland et al. 1957). Subsequent experiments established that the covalent bonding of amino acids to cognate tRNA molecules was catalyzed by their specific activating enzymes (aminoacyl tRNA synthetases) (Hoagland et al. 1958).

$$\text{aa-AMP} + \text{tRNA} \longrightarrow \text{aa-tRNA} + \text{AMP}$$

The Zamecnik team and others next found that amino acids bonded to the 3′-termini of their corresponding tRNA (Hecht et al. 1958a, 1959; Hecht et al. 1958b; Zachau et al. 1958).

Transfer of amino acids from their tRNA carriers to nascent polypeptide chains: Zamecnik and Hoagland's next seminal discovery was the transfer of amino acids in the presence of microsomes, ATP, and GTP from their carrier tRNA to growing polypeptide chains (Keller and Zamecnik 1956; Hoagland et al. 1957). This observation showed that after their activation and bonding to cytoplasmic tRNA, amino acids were discharged from the tRNA and incorporated into growing polypeptide chains (Hoagland et al. 1958; Hoagland and Comly 1960). This scheme was soon substantiated and expanded by many research groups (section 3.3). Years later Hoagland vividly described the revelatory instance of his pioneering historical discovery:

> In the most suspenseful and exciting few hours of my professional life, I did an experiment that came out essentially as reported in the cited paper: having labeled the indigenous RNA of the soluble fraction with amino acids in the presence of ATP, reisolated the fraction's protein and RNA free of amino acids and ATP by reprecipitation at pH 5, I then incubated it with microsomes. To my delight, most of the counts on RNA were rapidly transferred to protein, in a reaction that specifically

[22] Almost at the same time another group reported detection of analogous enzymes in a bacterial cell extract (Demoss and Novelli 1955).

> required GTP and proceeded in the presence of a large excess of free unlabelled amino acid. (Hoagland 1989, 104)

3.3 Based on Their Experimental Results, Hoagland and Zamecnik Framed a Skeleton Model of Protein Biosynthesis

Based on their discoveries, Hoagland et al. offered in 1957 a tentative explanatory model of the passage from free to activated amino acids, their bonding to tRNA, and finally incorporation into proteins:

> It is now further postulated that this initial activation of amino acids is followed by transfer of activated amino acids to S-RNA. This latter reaction is ribonuclease sensitive, while the former is not. GTP mediates the transfer of this activated amino acid to peptide linkage via the microsomes by a mechanism as yet unknown. (Hoagland et al. 1957, 216)

A year later they added more details to their model:

> We have suggested elsewhere [Hoagland, Zamecnik, and Stephenson 1957] a hypothetical reaction sequence for protein synthesis which accounts for the findings presented in this paper. Its central idea is that pH 5 RNA molecules, [meaning tRNA] each charged with amino acids in characteristic sequence, polymerize in microsomes (in specific order determined by the complementary structure of microsomal RNA[23] to higher molecular weight units with resultant configurational changes which permit peptide condensation between contiguous amino acids. (Hoagland et al. 1958, 256)

The model was graphically presented in Zamecnik's 1958–1959 Harvey Lecture (Zamecnik 1960; figure 3).

4 Experiment and Theory Converged: Hoagland Learned of the Adaptor Hypothesis and Crick Was Informed of tRNA

Crick on one hand and Hoagland and Zamecnik on the other hand were entirely unaware of each other's respective theory and experimental findings. It was only in 1956/7 that they learned on two separate occasions about their parallel feats.

4.1 Hoagland was alerted to the Adaptor Hypothesis

Hoagland first heard about Crick's adaptor hypothesis in late 1956. Decades later he described the circumstances of this revelation:

> At the end of 1956 I had my first visit from a card-carrying molecular biologist. Jim Watson had just become a professor of biology at Harvard and was probing the structure of the ribosome. He had heard rumors of my discovery of transfer RNA, and I eagerly told him of our findings. He was restlessly attentive and when I had finished, told me that Francis Crick had forecast the existence of transfer RNA-like molecules a year or so earlier. He wondered whether I had heard of

[23] At that time messenger RNA was as yet undiscovered and ribosomal RNA was believed to be the carrier of genetic information (the 'one gene-one ribosome-one protein' theory; Fry 2018).

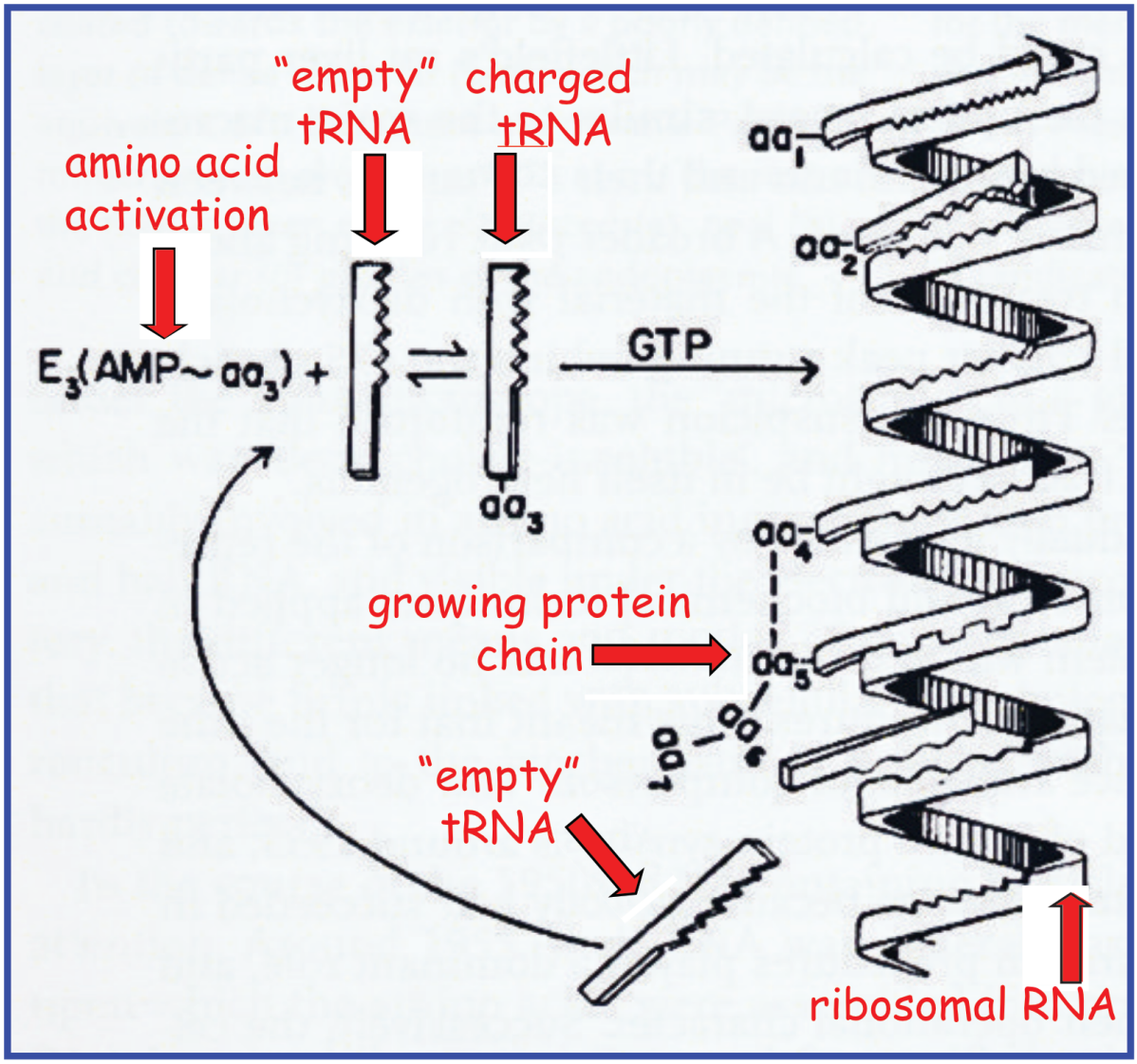

Figure 3: Zamecnik's evidence-based model of the path from free amino acids to their polymerization into protein. Original scheme in Zamecnik (1960) modified by Fry (2016, 346). Note that this model adhered to the time's idea that ribosomal RNA, and not the yet undiscovered mRNA, was the template for proteins.

> the adaptor hypothesis. I was astonished and a bit miffed that I had not! […] I was bowled over by the ingenuity and beauty of Francis's idea and sensed that it had to be the explanation of our experimental findings.[24] An image arose in my mind: we biochemical explorers were hacking our way through a dense jungle to discover a beautiful long-lost temple, while Francis Crick flying gracefully overhead on gossamer wings of theory waited for us to see the goals he clearly was gazing upon. (Hoagland 1990, 93; also see Hoagland 1989, 2004, 2006)

In an undated conversation with Judson, Hoagland recalled his somewhat more aggrieved reaction to the news of the adaptor hypothesis:

[24]Hoagland's reaction illustrates the epistemic power and the psychological impact of a successful prediction. This is akin to T. H. Huxley's reaction upon learning of Darwin's theory: "My reflection, when I first made myself master of the central idea of the 'Origin' was 'How extremely stupid not to have thought of that.'" (Huxley 1988).

> In fact, I can remember vividly leaning over a centrifuge in the particular laboratory and talking with Jim about it, and his saying, 'This is the interpretation of your results', Hoagland said. 'And, I can *sense, palpably,* my feeling of resentment, at the time, that Jim would be telling me how to interpret my result—but also the feeling that, God damn it, he is right. You know. It was just—it was just right.' (Judson 1996, 324)

Despite their different tenor, both versions similarly show that Hoagland subscribed almost instantly to the idea that tRNA and Crick's adaptor were synonymous. Importantly, however, Hoagland and Zamecnik did not need the adaptor hypothesis to explain their results since by that time they had already defined tRNA as "a link in the chain of intermediate reactions in protein synthesis" (Rheinberger 1997, 157 and sections 3.2.2 and 3.3). Yet, whereas Crick explicitly pointed to the crucial role of hydrogen bonding of his hypothetical adaptor and to the RNA template, Hoagland and Zamecnik more vaguely invoked complementarity between tRNA and an RNA template.

4.2 Learning of tRNA, Crick Posited That It Was Precursor to Smaller Adaptors

Crick first learned about tRNA and activating enzymes during his visit to the laboratory of Hoagland and Zamecnik in early 1957.[25] According to Hoagland (1990) he accepted at once that tRNA represented his putative adaptor:

> In the spring of 1957, Francis Crick came to visit us. He was elated. The discovery of an RNA species that apparently performed the task of his postulated adapters prompted him to enter the experimental fray.[26] (97)

The historical record contradicts, however, this description of Crick's prompt adoption of tRNA as the adaptor. In point of fact, Crick thought that tRNA molecules were *precursors* to much smaller adaptors. This idea grew from the contrast between Crick's original notion of an adaptor of just three or very few nucleotides and an empirically measured, larger size of tRNA. In 1956, Crick, John Griffith, and Leslie Orgel circulated among members of the RNA Tie Club an informal communication on postulated properties of the genetic code.[27] This and a subsequent formal paper (Crick et al. 1957), contended that the code consisted of non-overlapping trinucleotide code words that were complemented by amino acid carrying adaptors of three or very few nucleotides. Zamecnik's concomitant unpublished experimental measurements indicated, however, that tRNA was about sixty bases long (Rheinberger 1997, 159). Confronted with these results, Crick argued that tRNA molecules could not serve as adaptors because their large size would critically slow their diffusion and impede their entree into the microsomal site of protein synthesis (Olby 2009, 265). Writing to Hoagland in January 1957, Crick opined that tRNA was too large to go "intact into the microsomal particle." Instead he conjectured that

[25] Crick visited Hoagland's laboratory on January 17, 1957.

[26] Hoagland referred here to his and Crick's later (failed) attempt to decipher the genetic code by finding anticodon triplets in isolated amino acid-specific adaptor molecules. Inviting Hoagland to Cambridge in the summer of 1957 as a collaborator, Crick's aim was "to isolate individual tRNA (S-RNA) species and identify the sequence responsible for binding a particular amino acid" (Hoagland 1990, 103). In hindsight, Crick and Hoagland's planned project was doomed to fail because nobody had at that time the necessary technical tools. In one sense, however, this historical episode was notable because it was one of very few instances of Crick's taking to the bench to perform experimental work (Hoagland 1990, 105; Olby 2009, 265–267).

[27] Archived in the NIH Profiles in Science (Orgel et al. 1956).

the activating and charging enzymes first acted as nucleases that cleaved tRNA precursors into trinucleotide adaptors that were then charged with the activated cognate amino acids[28]:

> How can we fit your results into this scheme? The idea is that your 'activating enzymes' cut up this new RNA into trinucleotides at the same time attaching an amino acid to each one.

The charged trinucleotide adaptors were next speculated to diffuse to the microsomes where they base-paired with an RNA template, liberated their carried amino acids which were then incorporated into growing polypeptide chains. Whereas Hoagland was open to the idea that tRNA molecules were precursors to smaller adaptors, Zamecnik was more cautious (Rheinberger 1997, 160). Thus, while Hoagland joined Crick in Cambridge to experimentally pursue this notion[24], Zamecnik published in 1958 and 1959 schemes of intermediate reactions in protein biosynthesis in which intact tRNA was depicted as the very carrier of amino acids with no mention of cleaved smaller adaptors (Zamecnik et al. 1958; Zamecnik 1960; fig. 3). Zamecnik also reasoned in his 1958–1959 Harvey Lecture that in order to be recognized by specific activating enzymes, adaptor molecules had to be bulkier than mere trinucleotides (Zamecnik 1960).

4.2.1 Crick reflected on the discovery of tRNA in a seminal 1957 lecture

As told, Crick gave in September 19, 1957 a celebrated, programmatic, public talk titled "On Protein Synthesis"[29] that appeared in print a year later (Crick 1958).[30] Discussion here is limited to only those parts of the talk that touched on the adaptor hypothesis, tRNA, and activating enzymes. In his remarks on the Hoagland and Zamecnik's findings, Crick succinctly cited their discovery of tRNA:

> Very recently an intermediate reaction has been suggested by the work of Hoagland, Zamecnik & Stephenson (1957),[31] who have discovered that in the first step the 'soluble' RNA contained in the 'pH 5' fraction became labelled with the radioactive amino acid. The bond between the amino acid and the RNA appears to be a covalent one. This labelled RNA can be extracted, purified, and then added to the microsomal fraction. In the presence of GTP the labelled amino acid is transferred from the soluble RNA to microsomal protein. This very exciting lead is being actively pursued. (1958, 150)

Although he used the adjective 'exciting' to describe the discovery of amino acid binding RNA (i.e., tRNA), Crick refrained from suggesting that it was a likely or even a potential candidate adaptor. In a separate section of the talk Crick described again his adaptor hypothesis. After briefly presenting the rational of the hypothesis, he speculated that the adaptor molecules were chemically nucleic acid:

> What sort of molecules such adaptors might be is anybody's guess. They might, for example, be proteins, as suggested by Dounce (1952)[32] and by the Hokins (1954)[33]

[28] The letter, cited in part in Rheinberger (1997, 159–160), also included hand-drawn scheme of the imagined breakdown of large tRNA into small amino acid-carrying and code-recognizing adaptors.

[29] Crick spoke at a Society for Experimental Biology symposium on biological replication of macromolecules that was held at University College London.

[30] In recognition of its importance, the talk and its historical context were weighed by several authors (see for instance Cobb 2015, 130–140, 2017; Thieffry and Sarkar 1998).

[31] Crick was referring here to Hoagland et al. (1957).

[32] Crick was referring here to Dounce (1952).

[33] Crick was referring here to Hokin and Hokin (1954).

> though personally I think that proteins, being rather large molecules, would take up too much space. They might be quite unsuspected molecules, such as amino sugars. But there is one possibility which seems inherently more likely than any other-that they might contain nucleotides. This would enable them to join on to the RNA template by the same 'pairing' of bases as is found in DNA, or in polynucleotides. (Crick 1958, 155)

Crick also maintained that the Hoagland and Zamecnik's activating enzymes catalyze the charging of amino acids onto cognate adaptors. However, he speculated that these enzymes also cleave precursor tRNA molecules to shorter amino acid carrying adaptors:

> If trinucleotides, say, do in fact play the role suggested here their synthesis presents a puzzle, since one would not wish to invoke too many enzymes to do the job. It seems to me plausible, therefore, that the twenty different adaptors may be synthesized by the break of RNA, probably the 'soluble' RNA. Whether this is in fact the same action which the 'activating enzymes' carry out (presumably using GTP in the process) remains to be seen. From this point of view the RNA with amino acids attached reported recently by Hoagland, Zamecnik & Stephenson (1957), would be a halfway step in this process of breaking the RNA down to trinucleotides and joining on the amino acids. (Crick 1958, 156)

4.3 tRNA Was Finally Identified as the Functioning Adaptor

It is hard to pinpoint the exact time at which Crick accepted that intact tRNA was indeed his adaptor. Decades later he recalled his evolving perception of tRNA:

> As every molecular biologist now knows, the job is done by a family of molecules now called transfer RNA. Ironically, I did not immediately recognize that these transfer RNA molecules were the predicted adaptor because they were bigger than I had expected, but I soon saw that there were no grounds for my objection. A little later Mahlon [Hoagland] came to Cambridge …. (Crick 1988, 96)

According to this rendering Crick abandoned the idea of tRNA as precursor to smaller adaptor molecules before the summer of 1957, the time of Hoagland's arrival in England. This is unlikely, since in his September 19, 1957 talk Crick was still promoting the concept of tRNA as precursor (Crick 1958). Also, under the spell of Crick's scientific and personal charisma, Zamecnik and Hoagland continued to pursue the idea of tRNA as precursor to shorter adaptors. This is indicated by what Zamecnik said in his 1959 Harvey Lecture (emphasis mine): "According to Crick's hypothesis, *and to our views*, the soluble RNA molecule appears to be too long and complex to serve in its entirety as a suitable transfer agent" (Zamecnik 1960). Thus, from 1957 till 1959 the Boston laboratory conducted futile search for small adaptor products of tRNA cleavage (Rheinberger 1997, 192). It was only in late 1960 that Hoagland and Lucy Comly announced their failure to find evidence for breakup of tRNA into "smaller utilizable pieces" (Hoagland and Comly 1960). It thus appears that by the end of 1960 the weight of accumulated experimental data convinced Crick, Hoagland, Zamecnik, and the broader community of biochemists, that Crick's adaptor and intact tRNA were synonymous.

4.4 Why Were Crick and Hoagland & Zamecnik Unaware of Each Other's Hypothesis and Experimental Discoveries?

What could be the reasons for the disconnection between the hypothesis and the independent experimental discovery of the same entities? In his comprehensive analysis Rheinberger con-

vincingly argued that a decisive factor was the cultural and epistemic divide that separated the nascent group of molecular biologists of the 1950s and the established larger community of biochemists. Whereas molecular biologists were interested in biological code and information, biochemists were engaged in isolation and characterization of new molecules and their interactions (Rheinberger 1997, 156–164). Another possible factor was the slow and limited scientific communication of the 1950s (no e-mails, video calls, or even inexpensive, transatlantic telephone calls). This likely minimized potential connection between Crick at Cambridge and the Harvard laboratory. Lastly, there was also the unfortunate, missed opportunity of interaction between Crick and Zamecnik when both attended the June 1956 Gordon conference.

5 The Experimental Discovery of tRNA Had Greater Impact Than the Theoretical Prediction of the Adaptor

The independence of the adaptor hypothesis from the experimental discoveries of tRNA and aminoacyl tRNA synthetases provides a rare opportunity to compare impacts of a stand-alone theoretical prediction and empirical discovery of the same biological entities.

Table 1: Number of mentions of the term "Adaptor Hypothesis" in articles published in leading professional journals between January 1, 1955 and February 10, 2020.

Journal	Number of articles mentioning "Adaptor Hypothesis"*	Earliest mentioning article†	Cited source‡
Science	4	1969	Crick (1955) [1] Crick (1958) [3]
Nature	--	--	--
Journal of Molecular Biology	11	1959	Crick (1957) [1] Crick (1958) [10]
Journal of Biological Chemistry	3	1970	Crick (1958) [2]
Biochimica et Biophysica Acta	1	1962	Crick (1958) [1]
Proceedings of the National Academy of Sciences, USA	15	1960	Crick (1955) [2] Crick (1958) [8] Crick (1963) [2] No source [3]

Contents of each of the listed journals were searched for the term "Adaptor Hypothesis." Mentions of this term in irrelevant contexts (epitope recognition, proteasome, etc.) were screened out, and only articles that quoted Crick's hypothesis are listed.

* Only original research papers were included and mentions of "Adaptor Hypothesis" in review articles, book reviews, obituaries, and meeting reports were excluded.

† Year of publication of the article that was the first to mention "Adaptor Hypothesis."

‡ Numbers in square brackets are of articles that cited a listed paper as source of the term "Adaptor Hypothesis."

5.1 Impacts of Crick's Hypothesis and of the Experimental Discovery of tRNA: Historical Record

One way to assess the influences of Crick's hypothesis and of the experimental discovery of tRNA is by looking at the reactions of contemporaneous scientists to these autonomous developments. As detailed in section 2.2.1, the original text of the adaptor hypothesis (Crick 1955) and its subsequent dissemination in scientific meetings (fig. 1) were met with indifference or distrust. This is also illustrated by the fact that Crick's 1955 manuscript was cited for the first time only in 1960 (table 1) when the adaptor has already been recognized to be synonymous with tRNA (section 4.3). Most significantly, there is no detectable record of attempt to put the hypothesis to experimental test. Because Crick's adaptor hypothesis did not motivate experimentalists to test its veracity, it remained an infertile theoretical model.

In contrast to the unresponsive reception of Crick's hypothesis, the work of Hoagland and Zamecnik gained immediate and substantial recognition. Biochemists cited, replicated, and extended their findings on cell-free protein synthesis, enzyme-catalyzed amino acids activation and bonding to tRNA, and on discharge of the amino acids from tRNA and their incorporation into growing polypeptide chains. Promptly citing Hoagland and Zamecnik, numerous laboratories confirmed and expanded their findings (see for instance: Berg 1956; Davie et al. 1956; Demoss and Novelli 1955; Lipmann et al. 1959; Zachau et al. 1958).

5.2 Contrasting Impacts of Crick's Hypothesis and of the Experimental Discovery of tRNA: Bibliometric Evidence

Bibliometric analysis provides another way to assess the relative impacts of Crick's hypothesis and of the experimental discovery of tRNA. Low number of citations of Crick's relevant key papers (tables 1 and 2) indicates that his hypothesis elicited weak reaction, both when it was still a stand-alone theory (1955–1957) and after the adaptor had already been equated with tRNA. By contrast, high numbers of citations of relevant key reports indicate that the discoveries of aminoacyl tRNA synthetases, tRNA and their modes of operation had considerable impact.

Table 1 shows how many times the term 'Adaptor Hypothesis' was mentioned between 1955 and early 2020 in articles in leading professional journals. Quite surprisingly, this phrase appeared only sparingly in the listed journals. Moreover, 'Adaptor Hypothesis' was mentioned for the first time (by Crick himself), only in 1958, nearly two years after the adaptor has already been associated with the empirically discovered tRNA. As to the sources of the term, 70% of the articles (24 out of 34), cited Crick's 1957 lecture "On Protein Synthesis" (Crick 1958) which already spoke of the relationship between his adaptor and the concomitantly discovered tRNA (section 4.2.1). Only three articles cited Crick's original 1955 RNA Tie Club communication (Crick 1955), two papers referred to his 1963 retrospective review in *Progress in Nucleic Acid Research and Molecular Biology* (Crick 1963), a single article cited his 1956 talk at the Biochemical Society Symposium (Crick 1957), and three papers mentioned the hypothesis without ascribed reference.

Table 2 shows results of scanning of the 1955 to 2020 body of the scientific literature for citations of relevant key publications by Crick and by Hoagland and Zamecnik. As seen, Crick's papers on the adaptor hypothesis were poorly cited. His original RNA Tie Club manuscript (Crick 1955) was cited only 54 times in 65 years and most of the citations appeared in retrospective review articles rather than in reports of new research. Crick's (1958) seminal lecture "On Protein Synthesis," which already acknowledged the discoveries of tRNA and activating and charging enzymes, was cited more the 2600 times. However, only 24 of the citing articles mentioned the term 'adaptor hypothesis' (table 1), while the remaining >2600 citing papers addressed other cardinal elements in the lecture such as the sequence hypothesis, the central

dogma, etc. Importantly, the bibliometric data indicate that the impact of the adaptor hypothesis remained low even after it became well-known.

Table 2: Number of citations of Crick's three major presentations of his adaptor hypothesis and of key reports by Hoagland, Zamecnik, and associates on amino acid activation, activating enzymes, and tRNA (Source: Google Scholar, February 10, 2020).

Crick's Seminal Contributions		Hoagland et al., Seminal Contributions	
Publication	**Number of citations**	**Publication**	**Number of citations**
Crick (1955)	54	Hoagland (1955)	414
Crick (1957)[34]	?	Hoagland et al. (1956)	614
Crick (1958)	2644	Hoagland et al. (1957)	443
		Hoagland et al. (1958)	849

Whereas Crick's hypothesis prompted belated and weak reaction, the discoveries of Hoagland and Zamecnik had substantial impact as attested by the hundreds of citations that each of their key papers earned (table 2). Of equal importance, the reaction to their discoveries was immediate. For instance, Hoagland's report on amino acid activation (Hoagland 1955) was cited six times already in the year of its publication and 23 times in the subsequent year. Remarkably, most of the citing articles substantiated and expanded the findings of Hoagland and Zamecnik by reporting isolation of new activating enzymes and/or tRNA species.[35] Thus, whereas Crick's hypothesis was never put to experimental test, the work of Hoagland and Zamecnik inspired robust empirical confirmation and expansion.

5.3 Why Did Crick's Prescient Hypothesis Fail to Elicit Befitting Response?

It is rather surprising that despite its outstanding brilliance, Crick's adaptor hypothesis had only minimal impact on practicing scientists. What could explain this incongruity?

Some may argue that the two years that elapsed between the introduction of Crick's hypothesis in 1955 and its experimental corroboration in 1957–1958 were too short a time for the hypothesis to gain recognition. However, history shows that some hypotheses did gain due recognition, although they were confirmed soon after their introduction. Stephen Brush tallied time intervals that separated introduction of predictive theories in physics, chemistry, and biology from their subsequent corroborations. According to his account the natural selection theory of Mendelian inheritance has been accepted just three years after it was presented and the quantum mechanics theory was adopted one year after its introduction (Brush 2015, 488). Another potential explanation to the low effect of Crick's hypothesis is its communication to only the small audience of the RNA Tie Club (Crick 1955). This explanation is countered, however, by Crick's presentations of his hypothesis to hundreds of scientists in two meetings in 1956 (fig. 1). However, as witnessed by Brenner, audiences resisted or were indifferent to the hypothesis (Brenner 2014). Last, the possibility that the adaptor hypothesis was recognized only belatedly is countered by evidence that in the sixty-five years that elapsed since its intro-

[35] As far as I could determine, Hoagland and Zamecnik's work had been initially cited by practicing biochemists but not by members of the RNA Tie Club—even those who were experimental biochemists (i.e., Alex Rich, Martynas Ycas, Erwin Chargaff). One exception was Fritz Lipmann, an honorary member of the Club, who not only cited their findings but also became an active investigator of tRNA.

duction it had much smaller impact than the empirical work of Hoagland and Zamecnik (tables 1 and 2).

We can only speculate on deeper reasons for the surprisingly low relative impact of Crick's adaptor hypothesis. One likely element is the resistance of experimental biologists to theorizing. Because complex bio-molecular systems were assembled under indeterminate historical circumstances of selection and evolution, abstract theories usually fail to effectively prognosticate their compositions and modes of operation. Thus, in contrast to its dominant role in physics, theory in the life sciences is only secondary to observation and experiment. Crick's bent on theorizing in the tradition of physics made him, therefore, an odd man out in the overwhelmingly experimental community of biochemists. It should also be remembered that when he introduced his hypothesis in the mid-1950s, Crick had not reached yet his later prominence. His status at that time of an ex-physicist 'outsider' may also have contributed to the indifferent reaction of biochemists to his highly speculative abstract conjecture. By contrast, these scientists readily grasped and promptly followed the findings of Zamecnik and Hoagland which spoke the familiar language of experimental biochemistry. Another possible sociological/cultural reason for the dismissal of the adaptor hypothesis by traditional biochemists could be their alienation from the newly emerging group of molecular biologists who claimed to have a superior, emphatically theoretical, approach to fundamental problems in biology.

All told, evidence presented in this paper indicates that the adaptor hypothesis case did not conform with the predictivist claim of epistemic superiority of predictive theories over theories that accommodate known data. Although the adaptor hypothesis presciently predicted unknowns, its epistemic weight was lower than the Hoagland and Zamecnik's evidence-based model of protein synthesis (fig. 3). The higher place of experiment over prediction in this particular case was well articulated by Hoagland himself. Decades after Crick introduced his hypothesis and after the discovery of tRNA, he appraised the contributions of theory *versus* experiment. His conclusion, which this paper espouses, was that despite the brilliance of Crick's hypothesis, it was the more pedestrian experimental work that ultimately had the greater impact:

> Crick's adaptor idea, while one of the more brilliant insights in molecular biology, stands more as a monument to man's imagination than as the springboard to a major discovery. In this case, we biochemists could assert with pride that by dint of smaller imaginative jumps, hard labor and considerable luck we had unearthed tRNA and appreciated its significance for determining order without the aid of the hypothesis—even if its promulgation did precede our discovery. *In this instance, a grand theory neither substituted for nor guided the successful analytical dissection of the machinery of protein synthesis.* (Hoagland 1990, 96; emphasis mine)

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