

## **The Case for Reverse Translation**

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The theoretical and experimental evidence for a “reverse translation” mechanism in animal cells is reviewed. Mekler’s (1967) theory is presented as the most likely means for reverse translation. This theory is shown to be consistent with the postulate of the “central dogma” that molecular information does not pass out of protein molecules once it has gotten in.

The importance of nucleic acid changes in: (1) the immune response, (2) evolution, (3) cancer, (4) cell differentiation, and (5) learning in animal brains is mentioned and each topic is related to the reverse translation hypothesis. Because of the intense research effort in immunology, the experimental data which indicate an active role for antigen in antibody formation and the data indicating the importance of RNA in the immune response are dealt with more thoroughly.

### **1. Introduction**

The principal molecules of the cell are the DNA, the RNA and the proteins. The lipids, fats, carbohydrates, amino acids and other biomolecules are either ingested directly from the environment or are made by the cell’s enzymatic machinery. Those molecules constructed in the cell are the end products in a chain of command from the DNA to the RNA to the proteins which bring about the actual synthesis of the molecule. Therefore, when talking about the dynamics of information exchange within the cell, we are concerned primarily with the nucleic acids and the proteins.

When the structure of DNA was solved by Watson & Crick in 1953, it was immediately apparent that DNA replication might occur by means of a template action of one DNA molecule onto another complementary one. By 1958 the mechanism by which the DNA might transmit its genetic information to the cell had been theoretically worked out by Crick and by 1964 this schema had been largely demonstrated experimentally (Hahn, 1973).

The three information exchanges involved (DNA to DNA, DNA to RNA, and RNA to protein) became widely known as the “central dogma” of

molecular biology. Schematically:



And from this basis all of the molecular aspects of genetics, immunology and evolutionary theory have been built.

## 2. Reverse Transcription

As early as 1964, however, a “reverse transcription” mechanism was predicted by Temin in order to account for certain virus replication problems, and in 1970, this information exchange was also demonstrated experimentally (Temin & Mizutani, 1970; Baltimore, 1970). As of 1976 the “central dogma” had been expanded to include some strange variations, mostly associated with viruses, e.g., reverse transcription and RNA replication (Spiegelman & Haruna, 1966). But generally speaking, the central dogma is considered by most biologists to be untouched by these viral oddities; the “normal” cell is believed to work along the lines of the central dogma of 1958.

Yet experimental data collected over the past five years together with old theoretical problems brings into doubt the completeness of the central dogma—even with regard to the normal cell. Importantly, the enzyme responsible for reverse transcription (the RNA-dependent DNA polymerase or “reverse transcriptase”) has been detected in normal animal cells (Scolnick, Aaronson, Todaro & Parks, 1971; Ward, Humphreys & Weinstein, 1972; Okabe, Gilden & Hatanaka, 1973; Hayward & Hanafusa, 1973). It is particularly abundant in thymus and spleen cells suggesting that it is involved in the immune response. Unless catching viral diseases has some evolutionary advantages (Anderson, 1970), it is likely that the gene for this particular enzyme has been selected to be a part of the normal cell *because it performs a useful function there*. The exact role of the reverse transcription process is an open question—but a question under open and serious debate (e.g., Temin, 1970; Hahn, 1973; Green & Gerard, 1974). Only those secure in their dogma can afford to overlook what is apparently the *fourth* major information exchange within the cell. Schematically, the information exchanges within the *normal* cell become:



The significance of reverse transcription is particularly critical in that five of the most important problems of molecular biology are concerned with the modification of the nucleic acids. They are:

- (1) the problem of neoplastic development (cancer) and the transformation of the normal cell;
- (2) the problem of genetic change (mutation) in evolution;
- (3) the problem of generating sufficient genetic diversity needed for antibody production (the immune response);
- (4) the problem of nucleic acid changes due to learning experiences in animal brains (memory);
- (5) the problem of gene expression and/or modification in the process of cell differentiation (ontogeny).

Current theory holds that "random mutation" (of one kind or another) is the likely mechanism of gene alteration in cancer, evolution and antibody production (while the nucleic acid changes in differentiation and learning remain in question), but the existence of the reverse transcription enzymes offers a possible cellular means by which genetic change may occur without resorting to the unprovable, statistically unlikely "random mutation" theories. Specifically, reverse transcription may be the means by which viral information is read into the normal cell's genome. This process has been worked out in considerable detail both theoretically and experimentally with regard to neoplastic development. The rival oncogene (Huebner & Todaro, 1972) and provirus (Temin, 1972) theories differ significantly only with regard to *when* the viral information is thought to be incorporated into the cell—evolutionarily long ago in the species' history (the oncogene hypothesis) or continually and at any time in the individual organism's life (the provirus hypothesis). For the purposes of this essay, the differences between these theories are unimportant; by either theory during the "misevolution" of neoplastic tissue, the insertion of viral material into the otherwise normal cell probably occurs by means of reverse transcription.

Reverse transcription as an important part of evolution and the immune response has not been given much consideration to date—primarily it would seem because even if reverse transcription were proven beyond a doubt to be involved in these processes, again a problem arises in accounting for the genetic variation in the (viral) RNA (which gives rise to variation in the cellular DNA, which in turn produces phenotypic changes in the organism). That is, even if the long-suspected hypothesis of random changes in the DNA giving rise to even more complex organization were circumvented by means of a reverse transcription mechanism, we are left with the question of the origin of the changes in the RNA molecules. We may again speculate about "random mutation"—this time of the RNA molecules, but we will have neither simplified the theory nor, from the point of view of the cell, will we have suggested a more orderly and dependable mechanism by which the cell

can improve and/or diversify its genetic information. Reverse transcription may indeed be an important part of the distribution of certain nucleic acid sequences (in the form of viruses or virions) during cell differentiation (Stavrianopoulos, Karkas & Chargaff, 1971), viral diseases (Temin & Baltimore, 1972), and in the cell cloning of the antibody response (Tremin, 1974). RNA or RNA-protein complexes are known to be transmitted between cells (in viral diseases) and theorized to be transmitted between cells (in cell differentiation and the immune response); they are known to be transmitted between organisms and species [in viral diseases and immunity transfer experiments (Paque & Dray, 1968)], and postulated to be transmitted between organisms and species in evolution (Anderson, 1970; Temin, 1974). So while we must not underplay the significance of reverse transcription in these various biological phenomena, it must be understood that *reverse transcription itself does not explain genetic change—only genetic distribution.*

### 3. Mekler's Reverse Translation Theory

In order to explain genetic change by means other than the theoretical cure-all, random mutation, an obvious place to look is at the possibility of "reverse translation". In brief, reverse translation would be an information exchange from protein molecules to nucleic acid (most likely RNA) sequences. *A priori*, there are no sound reasons to reject such a possibility. Reverse transcription went undiscovered for 6 years after the central dogma was pieced together, primarily because almost no one was looking for it. Furthermore, it has been calculated that for every one cellular protein that science has thus far been able to identify, there exist one thousand others which remain unknown (Hahn, 1974), which makes any out of hand closing of the search for reverse translation enzymes premature.

The chief biochemical argument against reverse translation is concerned with the unlikelihood of macromolecular information, once built into a protein molecule of ever "getting out" again. It is known that from the time of translation at the ribosomes, the polypeptide sequences assume their natural twisted configurations (Anfinsen, 1973; Whitney & Tanford, 1965) and that only under very harsh conditions can the proteins become denatured, i.e., straightened out (and therefore made available for a reverse translation process). Although experimentally such a denaturation process is possible, the chemical conditions necessary (high concentrations of urea or acid) are not conducive to cellular life. Under these severe artificial conditions, all proteins including the reverse translation enzymes along with the protein being reverse translated would be denatured. Clearly, this sort of process could not be a part of normal cellular existence.

As sound as that argument against a denaturing and literal decoding of a protein molecule is, it does not apply to a different reverse translation mechanism suggested by Mekler in 1967, which does *not* demand the (unlikely) unravelling of proteins to allow for reverse translation. He has noted that in all cells, amino acids are often associated with their respective tRNA molecules—as is necessary for normal protein synthesis. What this means for reverse translation is that any molecule which is foreign to the given cell (i.e., any antigen) will be surrounded not merely by polar amino acids, but by amino acids with tRNA moieties already attached. This situation is outlined in Fig. 1. The antigenic molecule is thereby surrounded

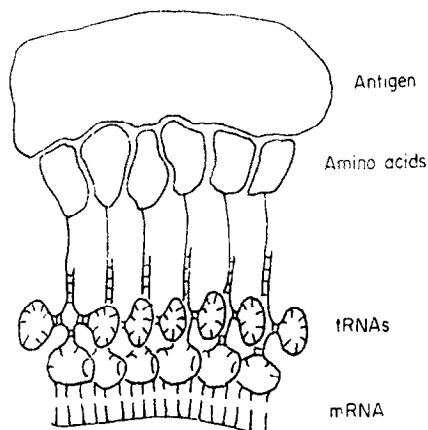


FIG. 1. Mekler's reverse translation hypothesis. The aggregation of polar amino acids which are linked to their appropriate transfer RNA molecules results in a tRNA anticodon sequence which may then be transcribed to an mRNA sequence.

by two layers of molecules: (1) the amino acids in direct contact with the antigen—arranged in conformity with the steric and electromagnetic surface of the antigen, and (2) the tRNAs covalently attached to the amino acids. To complete the reverse translation process, a means of decoding the tRNA anticodons into an mRNA-like sequence must be postulated. This is admittedly hypothetical at this time, but it is biochemically feasible and, in terms of template activities, it has known biological precedents. Mekler's theory of reverse translation does *not* entail the rightly criticized denaturing of proteins within the cytoplasmic milieu, nor any unknown variety of information transfer. It *does* entail an enzyme system which aligns the tRNA molecules next to one another (not unlike the alignment for translation) and which aligns and polymerizes the resultant mRNA sequence [not unlike transcription or the RNA polymerization process (Dravid, Pete & Mandel,

1971)]. The information transfer itself would be due to the well-known template action of nucleic acids on nucleic acids (aa-tRNA  $\rightarrow$  mRNA).

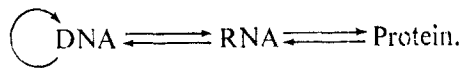
Mekler's reverse translation mechanism is, in essence, extremely simple and in fact does not violate (a liberal interpretation of) the "central dogma" as propounded by Crick (1958). That is, even assuming the validity of Mekler's reverse translation, molecular information (i.e., amino acid sequences) does not pass out of the given antigenic protein and into an mRNA sequence, but rather the antigen is "interpreted" in its natural configuration. Again, the surface structure of the molecule is "read" through the aa-tRNA to mRNA reverse translation process. The sequence of amino acids in the protein itself bears no linear relationship whatsoever to the sequence of amino acids gathered upon its surface. Consequently, the mRNA sequence which is complementary to the tRNA anti-codons also has no linear relationship to the amino acid sequence (and earlier mRNA and DNA sequences) of the antigenic protein. Strictly speaking, macromolecular information does *not* get out of a protein once it has gotten in.

Nevertheless, the foreign protein (or any other antigenic molecule) may be read or interpreted by the amino acid-tRNA to mRNA alignment process so that the cell can obtain genetic information useful in dealing with the antigen (by producing antibody molecules complementary to it). Although there is no simple *sequential* relationship between the antigenic protein and that which is produced subsequent to reverse translation, the two protein molecules themselves have an important *functional* relationship in having complementary surfaces. Just as the DNA sequence related to an antigenic protein and the DNA sequence of its related antibody have an extremely distant relationship to one another, still the antibody-antigen relationship itself is very close. Particularly with regard to the immune response, the mutagenic aspects of reverse translation would therefore be quite specific—always in response to a specific antigen and therefore related to the construction of a complementary antibody molecule. Again, this does not mean that the new (reverse transcribed) DNA sequence has any direct relation to the DNA sequence of the antigenic protein, yet the genetic changes are specific for the given antigen—i.e., non-random.

Reverse translation in the sense of a denaturing and exact decoding of the amino acid sequence of a protein is apparently neither possible, nor would it necessarily be of any benefit to the cell. The theoretical possibility of an exact replication of a foreign protein by means of denaturation and literal reverse translation seems unnecessarily complicated when an exact copying process, if beneficial to the cell, could be accomplished more easily through the reverse transcription of a virus particle bearing the relevant gene. Such a process has been suggested as an important mechanism in evolution,

accounting for the remarkable solution of similar problems by similar methods in evolutionarily distant species (Anderson, 1970). In terms of conventional evolutionary theory, an *in toto* exchange of genes is heretical—although biochemically possible if not probable. Such a gene exchanging process, however, would have only limited possibilities for generating new gene sequences—mostly by means of recombination of viral genes with cellular genes. The question of generating substantial genetic diversity in evolution and the immune response remains unanswered.

On the other hand, some sort of diversity generating mechanism must be postulated at least with regard to the immune response, if not for other cellular processes as well. Reverse translation followed by reverse transcription, such as Mekler suggests, is—on theoretical grounds—a logical schema. It would provide a cellular means for creating diversity other than through random errors, and without demanding new template ideas. Schematically:



Of course, the validity of such a hypothesis, its theoretical attractiveness aside, lies in the experimental data.

#### 4. Immunology

There now exists more experimental evidence indicating a reverse translation process in the field of immunology than anywhere else. The reason for this is that, although genetic diversity in various other fields is relevant, and ultimately of course extremely important, the diversity of genes in the immune response has some unique properties. Significantly, tremendous genetic diversity is apparently generated within the lifetime of each individual organism. This may be the case in evolution and cancer as well, but by discussing large populations of organisms (i.e., the net “gene pool”) and the statistical probabilities of mutation, the time scale and frequency can be put into a theoretical context where change is random and slow. The immune response is different in that the time span is extremely short—genetic changes occurring in the first few months of development if not within a few days of exposure to antigen—and the “mutation” occurs regularly in response to specific antigens. So, although “random mutation” mechanisms have been postulated as producing immune diversity, they cannot be “normal” (i.e., evolutionary) mutation mechanisms, but instead they must be special, extremely rapid mutation processes which occur only on limited segments of the variable region of the antibody gene.

The three varieties of theory which are generally considered to be serious contenders for explaining antibody diversity are: (1) the somatic recombination theories; (2) the somatic mutation theories, and (3) the germ-line theories. All three have serious weaknesses; only the timidity to consider the anti-central dogma "reverse translation" concept has prevented their abandonment.

The somatic recombination theories (Gally & Edelman, 1970; 1972) have the distinct theoretical advantage of predicting a large variety of antibody molecules produced by a relatively small number of recombinable antibody genes. By postulating, say, 1000 antibody genes which can be broken up and recombined in four segments, ten million different combinations could arise—more than enough to account for the known antibody diversity. The discovery that antibodies have constant and variable regions limited the ways in which recombination could occur and made any proposed mechanism for such rather complex. But, since the sequencing of actual antibody molecules has been undertaken, the recombination theory has run into real problems. Specifically, there appear to be virtually no recombined segments found in two or more antibodies (Smith, Hood & Fitch, 1971). As the sequence data accumulates, more and more (smaller and smaller) recombining segments must be postulated in hope of experimental verification. The theoretical advantages of such a scheme, however, rapidly disappear as the recombination theory begins to resemble the germ-line theories in demanding very large numbers of recombinable germ-line segments. Notable also is the fact that as the number of segments increases, the recombination mechanism takes on new complexity. Instead of being a simple "crossover" involving three or four pieces, it becomes a massive reshuffling of numerous smaller gene segments, which of course is biochemically unprecedented.

The somatic mutation theories (Brenner & Milstein, 1966; Cohn, 1972) avoid the *ad hoc* recombining problem and the sequence data indicating no distinct recombining segments, but the mutation theories must rationalize their own *ad hoc* mechanism of mutation. Maintaining that the antibody gene undergoes "normal" mutation over its full length was possible 10 years ago, but the knowledge of constant, variable and hypervariable regions within the antibody genes makes any simple mutation mechanism impossible today. Not only mutation but "hypermutation" of a nucleotide sequence and strict non-mutation of nearby nucleotides must be postulated to fit with empirical findings. Such an *ad hoc* mutation hypothesis presents a problem in that again a biologically unprecedented mechanism must be asserted to maintain the theory. Furthermore, the types of mutations of the hypervariable region as indicated by comparative studies of immunoglobins show that mutations are, rather than random, typical of those found in other sets of evolutionarily related proteins (Smith, Hood & Fitch, 1971).



The third type of theory, the germ-line theory (Smith *et al.* 1971; Hood & Talmage, 1970), maintains that the full array of antibody genes is inherited in the germ-line of the animal. Serious questions as to the likelihood of such a huge number of genes (larger than 1,000,000?) being devoted entirely to the immune system can be raised.† But more serious than the problem of number (and percentage of the entire genome) is the problem of justifying how so many genes could withstand the trials of natural selection to become a part of the genome when in fact only a small percentage of this mammoth repertoire would be utilized in the lifetime of any single organism. By postulating that so many genes exist, and have existed for generations, one is implicitly stating that they have a role in the organism's genetic make-up. Particularly with regard to the antibodies which are produced in response to man-made antigens (never before experienced in the entire history of the species), difficult questions arise as to how and why these genes could have become a part of the genome. In other words, if there is any selection pressure whatsoever determining which genes will and which genes will not be a part of the organism, the germ-line theory's huge antibody gene repertoire seems anomalous.

The difficulties of these conventional theories are well-known—the proponents of each skilfully elucidating the weaknesses of rival theories—but the evidence for a reverse translation mechanism is not widely known. Reverse translation as a part of the immune response would be the process by which the hypervariable region, generally only a dozen or so amino acids long, would arise. This segment would then be joined with the variable and constant regions by means of mechanisms which, although uncertain in this theory, are implicit in any but the germ-line hypotheses. The reverse translation theory, however, is to be preferred to germ-line theories on two counts: (1) it does not imply the phenomenal numbers of germ-line genes that the germ-line theories must postulate, and (2) it does not need an additional hypothesis concerning the “selective expression” of antibody genes. The second point is important. A reverse translation theory of antibody diversity means that the antigen itself has an “instructive” role in providing a template for the eventual mRNA sequence. The mechanism by which communication takes place from antigen to DNA is a normal biological template action (reverse translation and reverse transcription). As shown in Fig. 2, this process avoids the postulation of an “activation” or “selective derepression” mechanism which all three of the (non-instructionist) conventional theories must assert. The usual circumlocution of this problem is

† Jerne (1972) states, “It should be noted that more realistic models assuming the presence of a variety of antibody molecules of different affinity towards any idiotypic determinant, all lead to estimates of a repertoire higher than 5,000,000 in man.”

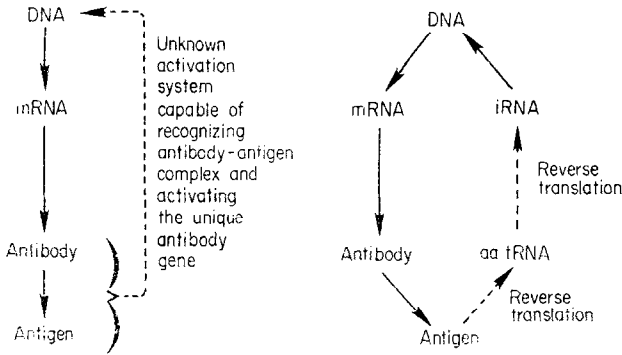


FIG. 2. Conventional theories (left) must postulate an "activation" system which uniquely derepresses the appropriate antibody gene. This hypothesis is in addition to any diversity generating hypothesis. An instructionist hypothesis, such as reverse translation (right), encompasses both processes in a single step.

to state that immunological activation is like all other gene activation processes (e.g., as in differentiation) and not very well understood. That is probably the case, but such words do not in any way simplify the matter. The fact is that "selective derepression" entails the recognition of antigen by antibody, and the recognition of antibody complexed with antigen by a protein (?) which would then recognize and derepress the antibody gene. In other words, the activation process itself approaches the complexity of reverse translation. Furthermore, the mechanism of activation from antigen to antibody gene is completely unknown. On the other hand, reverse translation accounts for antibody diversity and antibody gene activation in a single step. Through reverse translation and reverse transcription the relevant antibody gene (or hypervariable region of same) would be constructed *de novo*; it would be *the* active antibody gene in the given antibody producing cell. Its construction through reverse translation, replication (Jachertz, 1974a), and distribution to other potential antibody producing cells (i.e., cloning) would comprise both steps of (1) generating genetic diversity, and (2) activating (infecting) the cells to the production of specific antibody.

Cell cloning, a known feature of the immune response, would not require additional hypotheses, but would become an integral part of the reverse translation process. As Mekler has emphasized, the complex of antigen, amino acids, tRNAs and mRNA is virus-like. Its formation has been discussed; its distribution, i.e., the cloning of cells to produce identical antibody, would be analogous to viral infection. That is, the mRNA sequence would enter the B-cells of the lymph system as virions and be reverse transcribed by the RNA-dependent DNA polymerase. From that point antibody production could take place through normal transcription and translation of the

new DNA sequence. A noteworthy feature is that just as tissue is only very rarely invaded by more than a single virus at one time, similarly any given antibody producing cell can normally produce only one variety of antibody, i.e., it is "infected" by only one type of mRNA. The actual mechanism of infection is obscure, but possibly very similar in viral disease and cell cloning.

On the positive side (apart from the manifest difficulties of conventional antibody theories), there is significant evidence that antigen plays an active role in the immune response—again, an implicit part of reverse translation but anomalous in terms of non-instructionist theories.

Cunningham (1974) has reviewed the evidence which indicates the likelihood of antigen generating antibody diversity. He has stated that, although the older instructionist theories (e.g., that of Pauling) have been abandoned for good reason, there are nonetheless four major criteria upon which an "antigen generating diversity" theory is favored over the conventional scheme which holds that antigen merely comes into contact with *performed* antibody producing cells and somehow activates antibody production.

The four criteria (which Cunningham does not relate to reverse translation itself) are:

(1) *Number of B-cells.* If the genetic diversity needed for the various antibodies produced to any given antigen exists prior to the arrival of the antigen itself, then a large number of (uni-potent) B-cells would have to exist at all times to assure that any antigen will be found by its related antibody producing cell. If, on the other hand, the antigen is instrumental in generating antibody diversity, then relatively small numbers of B-cells would be sufficient. From that small array of germ-line B-cells, the full spectrum of antibody diversity could be generated due to contact with antigen. Experimentally it is found that only a small number of B-cells is normally present (Osoba, 1969; Lefkovits, 1972).

(2) *Dose of antigen.* If antibody diversity exists in full at the time of contact with antigen, then even small doses of antigen should stimulate the appropriate high-affinity antibody cells. If, however, antigen generates diversity, then the greater the dose of antigen, the greater the chances of producing high-affinity antibody. Experimentally, it is found that larger doses of antigen produce higher affinity antibody (Siskind & Benacerraf, 1969).

(3) *Heterogeneity.* The heterogeneity of antibody production should decrease with time if certain subpopulations of B-cells are being selected from a much larger number of available antibody producers. On the contrary, the heterogeneity of the antibody response should increase with time if the antigen generates the genetic diversity. In line with Mekler's theory, numerous possibilities of varying affinity due to reverse translation from antigen to mRNA sequences should be possible; as long as antigen is present, new

mRNA sequences should arise. Experimentally, antibody heterogeneity is known to increase with time during the response (Miller & Segre, 1972; Kreth & Williamson, 1973).

(4) *Affinity*. If diverse preformed B-cells exist in the lymph system prior to the arrival of antigen, then the smaller the dose of antigen, the higher the affinity of the antibody should be. In other words, a limited number of antigen should activate only a limited number of high-affinity B-cells. If, on the other hand, antigen generates diversity, then the amount which generates maximum antibody response should also have maximum affinity, as is commonly found.

Furthermore, Cunningham & Pilarski (1974*a,b,c*, 1975) have demonstrated experimentally that: (1) entirely new antibody specificities arise during the course of an immune response, (2) variant antibody specificities are produced within a clone of antibody forming cells, and (3) clonal variation operates at a high frequency *in vivo* and that the rate of variation is influenced by the amount of antigen.

The above-mentioned evidence for a role of antigen in antibody diversity is largely indirect and, admittedly, related without many of the confusing subtleties involved in this work. In fact, there appears to be no experiment thus far which rules definitively for or against this proposition, but it is important to note that the antigen-generated diversity hypothesis has greater theoretical simplicity than the antigen-less diversity theories. Not only must *ad hoc* explanations be created for each of the four arguments of Cunningham, but the question of how antibody diversity itself is generated needs further hypotheses. The somatic recombination theories need *ad hoc* recombination mechanisms; the somatic mutation theories need *ad hoc* mutation mechanisms; and the germ-line theories must explain how antibody genes for synthetic antigens could have become a part of the genome before the antigen had been experienced in the history of the species, as well as explain the apparent differences in DNA content of cells exposed to antigen and those not exposed (Little & Donahue, 1970). Because of what is no more than conformity to prevailing views, the various instructionist aspects of the immune response have been overlooked or rejected by most biologists, but clearly a wide variety of evidence does exist that "instruction" is an important part of immunity. [Others who have noted the importance of the role of antigen in immunity are Makela & Cross (1970), Gershon & Paul (1971) and Gershon & Kondo (1972).]

Somewhat more direct evidence demonstrating reverse translation comes from the experimental work on RNA and the immune response (see the excellent review by Gottlieb, 1973). As far back as 1960 it was found that injections of RNase administered with antigen was effective in suppressing

immunity (Berenbaum, 1960), implying that RNA is the key factor in developing immunity. Furthermore, RNase administered with iRNA destroys immunity (Vyas, Ibrahim, Rao & Likhite, 1974). The fact that transcription inhibitors do not inhibit RNA production early in the immune response may indicate that the RNA is the product of a reverse translation process rather than transcription.

Jerne (1972) has noted that "the greatest support for the instructive theories come from the recent discoveries that macrophage RNA induces IgM synthesis and RNA-antigen complexes induce IgG synthesis. These RNAs undoubtedly function as messengers or instructors to the antibody forming cell." The central question of course is where does the RNA originate? If not from transcription from DNA, then it must arise either from RNA replication or reverse translation. Mitsuhashi, Saito & Kurashige (1974*a,b*) after extensive investigation into the role of RNA in immunity offer the following interpretation of the immune response: "(1) iRNA is produced by antigenic stimulation even though the mechanism is not known, (2) iRNA is increased by RNA replicase and transmitted by cell-to-cell contact, (3) new DNA is produced by an iRNA-dependent DNA polymerase activity and is the specific gene's coding for immunoglobulin peptide, and (4) antigenic stimulus causes the cells carrying this new DNA to become antibody forming cells." This train of events accounts not only for the continuous synthesis of RNA [as indicated by tritiated uridine incorporation (Mitchell & Nossal, 1963)], it clarifies the role of reverse transcription enzymes in the immune response, and indicates why no DNA synthesis occurs within the first 24 h of the immune response, but does occur after 24 h (Ortiz-Ortiz & Jaroslow, 1969). The known association of antigen with RNA (Campbell & Garvey, 1963) is of course reasonable within the framework of a reverse translation theory, but presents problems within the framework of a DNA activation model. The important point here is that antigen appears to activate or produce antigen-specific RNA molecules without transcription. This RNA, free of antigen, can in turn stimulate non-immunized cells to produce antibody specific for the antigen (Tanaka & Mitsuhashi, 1963; Mitsuhashi, Kurashige, Kawakami & Nojima, 1968; Johnson & Johnson, 1971).

The many experiments demonstrating the transfer of immunocompetence by the transfer of RNA and RNA-antigen complexes are also easily interpreted in terms of reverse translation. The RNA extracted from macrophages would, according to this theory, be either iRNA transcribed from tRNA anticodons via reverse translation, or amino acyl-tRNA-iRNA associated with antigen. The RNA-antigen complex would be a more potent stimulator of immunity than antigen alone in that only replication or reverse

transcription of the RNA would be needed to begin antibody production (Gottlieb, 1973).

Reverse translation along with "processing" or "degrading" of the antigen would occur in the macrophages. That is, antibody diversity would have its true origin in the macrophages. The macrophages, as is experimentally known, would be non-specific, indiscriminate processors of antigen. There the iRNA sequence produced through reverse translation would originate in virion form, but would not be replicated or reverse transcribed. In the form of antigen-amino acyl-tRNA-mRNA complex or as an iRNA (mRNA) molecule alone, the relevant RNA would be transferred to lymph B-cells where replication (Mitsuhashi *et al.*, 1974), further B-cell "infection" (Jachertz, 1974b), and subsequently reverse transcription would occur, thereby making this new nucleic acid information a permanent part of the animal's immune system.

The existence of an RNA-dependent DNA polymerase which is specific for the "informational" RNA found in lymph cells (Jachertz, 1974a) and the finding that RNA-DNA complexes are more abundant in antibody producing cells than in non-producing cells (Koros, Koster & Mowery, 1971) is ample indication that reverse transcription is an important part of antibody production.

The infection of lymphocytes by macrophages by means of RNA or RNA-antigen complexes is indicated by a wide range of data, direct cytoplasmic connection between macrophages and lymphocytes (Schoenberg, Miuwaw, Moore & Weisberger, 1964), the clustering of antibody producing cells around macrophages (Miller & Avrameas, 1971), and the distribution of radioactively-labelled RNA between macrophages and lymphocytes (Fishman, Hammerstrom & Bond, 1963; Bona, Robineaux, Antennis & Chauvet, 1969). These aspects of the immune response are summarized in Fig. 3.

## 5. Evolution

Unfortunately, the argument for reverse translation with regard to the problems of evolution is largely of a negative character; as Dobzhansky (1967) has stated "As yet there is no satisfactory theory of mutation." Reverse translation may therefore fill the apparent gap, providing a theory of genetic change which is both biochemically sound and conceptually "satisfactory".

The discovery of Muller (1927) that genetic changes can be induced by strong doses of ionizing radiation provided the genetic basis for the "neo-Darwinian" or "synthetic" theory of evolution. In line with Muller's work,

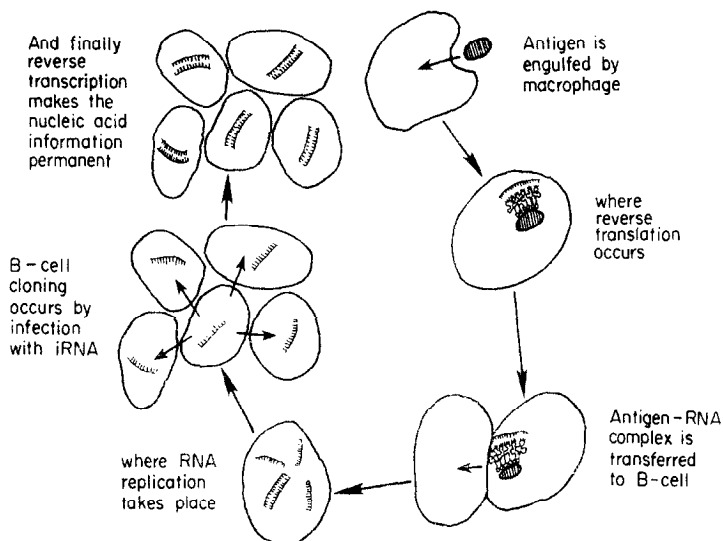


FIG. 3. The immune response.

random changes are theorized to occur at a frequency high enough in healthy organisms that given so many base pairs per cell mutating at a rate of so many per generation, etc., then of the relatively large number of chance alterations a few over a long period of time would be beneficial. The undeniable strength of this theory is that it cannot be disproven. Given enough random changes over a long enough period of time, the chimpanzee will type Hamlet and the cell will mistakenly invent endonucleases which cleave ultra-violet light-damaged DNA at the site of dimerization. In the world of sufficient randomization anything is possible, so attempting any definitive disproof of such theories must be abandoned. Nevertheless, it is relevant to point out the statistical odds which such a theory must overcome. To construct *de novo* a single protein of 100 amino acids (each uniquely required at its position) the odds would be a mere  $20^{100}$  to one—one chance in twenty per amino acid (Moorhead & Kaplan, 1967). As common sense tells us, random guessing is no way to go about constructing an intricate molecule. . .

The “neo-Darwinian” theory, which Darwin himself would have opposed in this regard, and the diatribes against Lamarck and Lysenko are familiar to all students of biology, but the mathematical and biochemical uncertainty of the neo-Darwinian hypothesis as it now stands is not widely acknowledged. Not only is the theory statistically unlikely (although not disprovable) and conceptually difficult if not empty (von Bertalanffy, 1967), the random mutation foundation is not as strong as it once was. That mutations (that

is, changes, random or otherwise) occur is disputed by virtually no one within the scientific world, but that these changes are "random" is doubtful. (In the sense that we cannot accurately predict what changes will occur when a cell or organism is exposed to near lethal doses of radiation or mutagenic chemicals, we might call the mutations "random", but this trivial and anthropocentric usage of the word should not be confused with the biochemical reality within the cell. Quite aside from our inability to predict specific mutations, the event itself may be an orderly cellular process. As with any other insufficiently investigated occurrence, that which appears at first to be random often entails precise causal mechanisms which can be discerned if we do not dogmatically close investigations by asserting their inherent randomness.)

The most important experimental data which brings the concept of "randomness" into question are those which indicate that the establishment of permanent changes in the DNA is dependent upon both RNA and protein synthesis (Jensen & Haas, 1963). If mutation were as simple as Muller had imagined, the electromagnetic wave would react directly with the intact DNA producing a physical change therein. Subsequent transcription and replication would therefore be altered . . . the first of a long string of appropriate accidents necessary to produce a new protein in the cell. RNA and protein and other extragenomic molecules and events would be irrelevant to the mutation event. To the contrary, mutation frequency declines "when post-irradiation nutrient conditions are unfavorable to RNA and protein synthesis. Conversely, post-irradiation conditions conducive to synthesis of RNA and protein encourage genomic establishment of the mutations" (Jensen & Haas, 1963). Von Borstel, Cain & Steinberg (1971) report, "It has become increasingly clear in recent years that spontaneous mutations are due principally to factors intrinsic to an organism rather than to external agents such as background radiation. . . . That is, mutability is itself a phenotypic character subject to genetic control." Reverse translation and reverse transcription may be these biochemical "factors intrinsic to an organism" which bring about genetic changes. That the organism's biochemical response to external stimulants may not always be a constructive one leading to adaptability does not mean that the mechanism of mutation is "random". Rather, it can as cogently be argued that the mutation is a cellular attempt at alleviating "stress" at the genetic level. Yet, the idea of "random mutation" (with the many qualifications put on this term) remains a theoretical cure-all for the difficult problem of genetic diversity. If, however, changes in the genetic material are indeed mediated by other cellular molecules, then the idea of "randomness" lacks all but the most trivial descriptive meaning, referring only to *our knowledge* of the mutation event.



In a review of the mechanisms of mutagenesis, Bridges (1969) notes that the essential question is whether mutation is biological or chemical in essence—i.e., principally due to the interaction of DNA with enzymes or with electromagnetism and inorganic molecules. Physical chemistry since the time of Muller has shown that chemical changes, particularly *in vitro*, are in fact real and therefore are a possible means of change in evolution. But as molecular biology has developed it has become known that biological intervention through enzymes and enzyme systems is the principal mechanism of *in vivo* mutation. Statistically, far more mutations are brought about through cellular enzymes than by direct spontaneous (chemical) means. On theoretical grounds, mutations which are controlled by enzymes are far more likely to be the rare beneficial mutations of evolution than are the free-for-all accidents of chemical mutation. Bridges states, "The most weighty evidence against direct chemical change being important in mutagenesis, at least in bacteria, is the observation that the presence of a particular repair system . . . is necessary for nearly all of the mutagenic effect of ultra-violet and around 90% of that of ionizing radiation." He concludes, "One fact that has emerged quite clearly is that expressible mutations do not arise to a significant extent as a direct action of radiation upon genetic material. If such an action exists it has yet to be adequately demonstrated in a living system" (Bridges, 1969).

The details of the mechanisms of mutagenesis are complex and often not well established, but as a generalization, it is probably not safe to conclude with the neo-Darwinians that simply environmentally-induced errors account for more than a small fraction of the genetic changes which have occurred in evolution.

The argument against "evolution by random mutation" is an old one, argued variously by creationists, neo-Lamarckians and anti-Darwinists. Reverse translation as the mechanism of mutation does not bolster any of the above three philosophies. It is concerned exclusively with the biochemical event of "mutation". The mutation, once established through reverse translation and reverse transcription, then becomes a part of the organism's (or species') genotype which, in Darwinian fashion, is subjected to the trials of natural selection. Reverse translation does not imply that natural selection does not occur—on the contrary, because the reverse translation enzymes made those organisms which contain it, more fit to deal with environmental stimuli, they have survived in natural selection.† And of course reverse translation provides no obvious link between consciousness (or will) and genetic structure, as neo-Lamarckians might hold.

† If the reverse translation enzymes are considered "Lamarckian", then it can be said that through Darwinian natural selection, Lamarckian cellular mechanisms have evolved!

Nevertheless, the proven existence of reverse translation would mean philosophically a major change in the popular conception of evolution. In its simplest form, the development of cellular complexity may be due to cellular functions, not due to fortuitous cellular mistakes. Rather than mutations being essentially random in nature, mutations, whether beneficial or not, would be direct responses to foreign environmental molecules. This is not to say that mutation is orchestrated by external or metaphysical agencies, but merely that the cell has a biochemical means for responding to stimulation. Self-alteration, to the point of genetic change, as a means of adaptation would be an intrinsic aspect of cellular existence. The fact that such change may often be ineffectual or even malignant to the organism does not imply that the changes are blind mutations, from the viewpoint of the cell.

In this respect, the concept of reverse translation is more closely aligned with the various anti-neo-Darwinian theories (although, again, not anti-Darwinian!) in demanding mechanisms of generating complexity other than randomness (e.g., Whyte, 1965). The next link to a metaphysical Creator or, more likely, to consciousness and the mental state of the organism is as yet purely hypothetical.

## 6. Cancer

Cancer has been termed "misevolution" and as inevitable and of the same general character as evolution (Foulds, 1969). It involves the creation and development of genetic material which bears new molecular information . . . to the detriment of the normal tissue in which it grows. Although a wide variety of stimulants can induce neoplastic development, it is nevertheless widely held that viral genetic material is the key to transformation of the normal cell, the carcinogens or radiation functioning only as a trigger to the mutation or release of the viral message (Locke, 1974).

As noted earlier, the central question concerning cancer is, "Where does the viral material come from?" The provirus theory maintains that viruses are constructed *de novo* in cells, but does not explain the precise mechanism of synthesis. Gross (1974) has likened this theory to a return to spontaneous generation, noting that not long ago viruses and bacteria were all thought to arise spontaneously, whereas today most of these cases of "spontaneous generation" are easily explained as infection from outside sources. The oncogene theory, which Gross would support, postulates that all viral genes exist somewhere within the gene pool of all species . . . thereby avoiding the spontaneous generation charge, but also not explaining where such genes originally came from! To postulate that it has long since been in

the species' gene pool necessitates further hypotheses to explain its original appearance. On the other hand, unexplained *de novo* virus construction is probably justifiably labelled as modern "spontaneous generation", but *de novo* virus production through reverse translation is a biochemical schema which deserves investigation. Cancer, in such a model, would be a disease of reaction to foreign antigens—foreign either to the organism as a whole or foreign to the tissue in which the antigen is found. The virion produced in reaction to the antigen would produce DNA through reverse transcription, which in turn would induce the production of RNA and protein in amounts appropriate to the degree of invasion by antigen. Cancer would be "mis-evolution" in the sense that it is a genetic response to external stimuli—but a response which, unlike the immune response or an evolutionary response, is not beneficial to the tissue or organism.

Temin (1974) has stated his view on cancer and reverse transcription thus: "The provirus hypothesis states that RNA-directed DNA polymerase activity exists in normal cells, that it plays a role in normal cellular processes like differentiation, that RNA tumour viruses (ribodeoxyviruses) evolved from this activity, and that cancer arises from variational events in the functioning of this activity." Although, to his credit, Temin has undertaken the difficult task of paradigm enlarging in introducing reverse transcription as a normal cellular process, his provirus theory suffers from the same problem as previous theories of viral disease and evolution in postulating (carcinogen induced) random changes in the viral RNA (Temin, 1970) when something more precise—for evolution or misevolution—is probably needed. Reverse translation would be a process related to reverse transcription in producing the original RNA fragments which are subsequently distributed and transcribed; reverse translation would be the "variational events" which produce the malignant genetic material.

If both reverse translation and reverse transcription are important aspects of cancer, then a case of cancer, and similarly a "case" of evolution, arises not by random chance, for it is regulated by cellular enzymes.

## 7. Cell Differentiation

The nature of the control of genetic material in bacteria is only imperfectly understood, so the control systems in many-celled organisms are even less clearly delineated. As in the study of cancer and the immune response, it is unlikely that DNA itself is a messenger molecule, travelling outside of both the nucleus and the cell membrane. RNA, however, seems a more likely means of cellular communication, and has been shown, first in 1958 by Niu, to travel between cells during cell differentiation. Whether or not this RNA

has informational content is a question of current debate (e.g., Hamburg, 1972). It seems unlikely that this problem will be resolved before definite answers are found concerning the role of RNA in cancer and the immune response.

Temin (1970) has speculated that reverse transcription plays a key role in differentiation by making RNA messages from neighboring cells permanent in the form of DNA. Reverse translation would supplement this theory by being the means by which relevant RNA messages are originally formed in response to *any* chemical communication from neighboring cells.

### 8. Learning

Learning and biochemical changes is a broad topic, again with a variety of evidence suggesting the importance of RNA and its specific alteration as being central to the physiological aspect of memory. In fact, each of the three classes of informational molecules, DNA, RNA and protein, have been implicated in memory, and each class of theory has a certain degree of experimental support (e.g., Mitchell, Beaton & Bradley, 1975). On theoretical grounds, it would be most likely that all three of these classes of molecules are involved, involved to a degree dependent upon the intensity of the learning experience and the resultant consolidation of the memory. Various reductionist theories have been constructed which would require a specific molecule within each nerve cell to "store" the memory. But in light of the anti-central dogma reverse transcription and reverse translation mechanisms, it would be foolish to expect storage in one class of molecules without communication with other classes. Specifically, if protein changes are implicitly a part of learning—currently a popular theory—then, whether these protein changes are merely steric or more profound changes of amino acid sequences, reverse translation of the new "antigenic" surfaces would be expected. In other words, RNA changes would be the *second* effect upon the cell, corresponding to relatively intense learning, i.e., relatively well-consolidated memory. Reverse transcription to DNA molecules would be the next step, a still deeper and rarer memory consolidation.

The role of reverse transcription with regard to memory has not yet been investigated, the only evidence of interest in this possibility being a brief comment by Temin (1970). The quantitative and qualitative changes in nerve cell RNA, first detected by Hyden in 1963, is certainly evidence of some sort of reverse translation-like process. The nature and extent of these RNA changes has been challenged and debated, but the central question is no longer, "Do RNA changes occur?" but rather, "What kinds of changes occur, how and why?" (see Gaito, 1972). If new species of RNA indeed

arise, then a reverse translation or similar mechanism is necessary, for certainly in the case of learning in the brain, random mutation hypotheses are utterly inappropriate.

### 9. Conclusion

The cell is a highly complex yet highly-ordered system of biomolecular information. The specificity and variety of its enzymes is truly remarkable, allowing for the storage of information in DNA molecules and its expression to protein through RNA molecules. Some 18 years since the promulgation of the central dogma, it appears that there are some additional information exchanges, i.e., additional enzyme systems, which allow for a more complete, more cybernetically efficient (Cook, 1976) system of internal communications. Reverse transcription was first predicted by Temin in 1964, and first put within its proper theoretical context by Mekler in 1967. Experimentally it was not demonstrated until 1970. Reverse translation was first predicted by Mekler in 1967 and, despite an abundance of indirect evidence, it has not yet been demonstrated experimentally.

If, instead of reverse translation, other mechanisms for generating genetic diversity are to be postulated, the five areas discussed briefly above each need their own separate mechanisms and, implicitly, their own separate enzyme systems. Mechanisms of mutation for evolution and cancer, mechanisms of hypermutation or recombination for the immune response, mechanisms of communication in cell differentiation and mechanisms of non-random DNA or RNA mutation in learning in the brain, all must be postulated in place of reverse translation.

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