Viral-Induced Repair of Damaged Neurons with Preservation of Long-Term Information Content

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ABSTRACT

An organic cell, as an automaton, derives energy from the metabolism of its own constituents for self-maintenance under the guidance of its genetic control program, which will not function properly if damaged or if a certain level of environmental deprivation or interference is exceeded; if allowed to proceed, such conditions lead to complete degradation. Although they increment it, methods exist for virtually halting such deterioration by stopping all biochemical processes; other means will be required to restore or augment a control program, or enrich the environment to enhance repair ability. It has been proposed that appropriate genetic information be introduced by means of artificially constructed virus particles into a congenitally defective cell for remedy; similar means may be used for the repair of more general cell damage exceeding the functional limit. Progress is being made in relevant areas such as virus/cell specificity, temperature-sensitive viral mutants, identification of RNA and DNA codon sequences, *in vitro* DNA synthesis, viral disassembly and assembly, and metabolic pathway determination. Further work is needed also in isolation of viral capsid programs, specific cell function subprograms, metabolic repair pathways, identification of enriching nutrients, replication of repair virions, infection methods, and quality control. *In situ,* the repair program must use means such as protein synthesis and metabolic pathways to diagnose and repair any damage. Applied to brain neurons, this may destroy long-term information content, which appears to be stored ultimately in molecular form, often proposed to be in a feedback cycle involving mRNA and protein synthesis. This information can be preserved by specifying that the repair program incorporate appropriate RNA tapes into itself upon entry and release them on termination of repair. This method of cell repair is applicable to many forms of brain damage and may be used as a research tool in investigating metabolic processes as well as information content and storage.

VIRAL-INDUCED REPAIR OF DAMAGED NEURONS WITH PRESERVATION OF LONG-TERM INFORMATION CONTENT

By Jerome B. White

ABSTRACT

An organic cell is a self-repairing automaton, but if environmental interference exceeds a certain limit, damage will become total. Freezing can be used to halt progressive damage along with all metabolism, but means are required to restore or augment the cellular genetic control program, or enrich the environment to enhance repair ability. It has been proposed that appropriate genetic information be introduced by means of artificially constructed virus particles into a congenitally defective cell for remedy; similar means may be used for the more general case of repair. Progress has been made in many relevant areas. The repair program must use means such as protein synthesis and metabolic pathways to diagnose and repair any damage. Applied to brain neurons, this might destroy long-term information content, which appears to be stored in molecular form, often suggested to be in a feedback cycle involving mRNA and protein. This information can be preserved by specifying that the repair program incorporate appropriate RNA tapes into itself upon entry and release them on termination of repair.

CELLS AS AUTOMATA

In 1936, Alan Turing, British mathematician and one of the fathers of the modern electronic computer, wrote that it was possible to

> compare a man in the process of computing a real number to a machine which is only capable of a finite number of conditions q_1, q_2, …, q_R which will be called "*m*-configurations." The machine is supplied with a "tape" (the analogue of paper) running through it, and divided into sections (called "squares") each capable of bearing a "symbol." At any moment there is just one square, say the *r*-th, bearing the symbol S(*r*) which is "in the machine". We may call this square the "scanned square". The symbol on the scanned square may be called the "scanned symbol". The "scanned symbol" is the only one of which the machine is, so to speak, "directly aware." However, by altering its *m*-configuration the machine can effectively remember some of the symbols which it has "seen" (scanned) previously. The possible behaviour of the machine at any moment is determined by the *m*-configuration q_n and the scanned symbol S(*r*). This pair q_n, S(*r*) will be called the "configuration:" thus the

> configuration determines the possible behaviour of the machine. In some of the configurations in which the scanned square is blank (i.e. bears no symbol) the machine writes down a new symbol on the scanned square: in other configurations it erases the scanned symbol. The machine may also change the square which is being scanned, but only by shifting it one place to right or left. In addition to any of these operations the *m*-configuration may be changed. (17, 231)[1]

Each such machine will compute a mathematical function; its behavior may be specified by a standard description, or S.D. Turing further states that:

> It is possible to invent a single machine which can be used to compute any computable sequence. If this machine *U* is supplied with a tape on the beginning of which is written the S.D. of some computing machine *M*, then *U* will compute the same sequence as *M*. (17, 241f)

This universal computing machine, as he calls it, will thus imitate the action of any specific computing machine.

An automaton is, roughly, an entity with these properties:

a) it can take on any of a number of distinct internal configurations, or, states;

b) it can be affected by any of a number of stimuli, or, inputs;

c) it is affected by these inputs in that its internal state changes;

d) depending upon the internal state, the input, and the resulting internal state, it may engage in one of a number of actions, or outputs.

It should be clear that a Turing machine corresponds to this definition. So does a computer, and so do many familiar objects. Eventually, research turned to the theory of automata of generality greater than Turing machines. Investigations were made into automata which, apart from or in addition to printing or modifying tapes, would construct objects. In some cases, the object to be constructed would be an automaton. Specifically, the automaton to be constructed would be a replica of the constructor. Self-repairing and self-maintaining automata, as well as self-reproducing ones, have been investigated.

In 1948, John von Neumann lectured on self-reproducing automata. He showed that it was possible to describe an automaton on something resembling a tape, or one-dimensional chain:

> Given any automaton X, let $f(X)$ designate the chain which represents X. Once you have done this, you can design a universal machine tool A which, when furnished with such a chain $f(X)$, will take it and gradually consume it, at the same time building up the automaton X from the parts floating around freely in the surrounding milieu. All this design is laborious, but it is not difficult in principle, for it's a succession of steps in formal logics. It is not qualitatively different from the type of argumentation with which Turing constructed his universal automaton. (19, 84)

Von Neumann also showed that there is a fundamental reason for having an automaton construct another, whether the latter is different from the former or not, by using a description rather than taking an example apart or copying. However, von Neumann requires "… that there exists an automaton *B* which has this property: If you provide *B* with a description of anything, it consumes it and produces two copies of this description." (19, 84)

I will let von Neumann summarize the construction of a self-reproducing automaton in his own words:

The general constructive automaton A produces only X when a complete description of X is furnished it, and on any reasonable view of what constitutes complexity, this description of X is as complex as X itself. The general copying automaton *B* produces two copies of $f(X)$, but the juxtaposition of two copies of the same thing is in no sense of higher order than the thing itself. Furthermore, the extra unit *B* is required for this copying.

Now we can do the following thing. We can add a certain amount of control equipment C to the automaton $A + B$. The automaton C dominates both A and B, actuating them alternately according to the following pattern. The control C will first cause B to make two copies of $f(X)$. The control C will next cause A to construct X at the price of destroying one copy of f(X). Finally, the control C will tie X and the remaining copy of $f(X)$ together and cut them loose from the complex $(A + B + C)$. At the end the entity $X + f(X)$ has been produced.

Now choose the aggregate $(A + B + C)$ for X. The automaton $(A + B + C) + f(A + B + C)$ will produce $(A + B + C) + f(A + B + C)$. Hence auto-reproduction has taken place.

[The details are as follows. We are given the universal constructor $(A + B + C)$, to which is attached a description of itself, $f(A + B + C)$. Thus the process of self-reproduction starts with $(A + B + C) + f(A + B + C)$. Control C directs B to copy the description twice; the result is $(A + B + C) + f(A + B + C) + f(A + B + C)$. Finally, C ties the new automaton and its description together and cuts them loose. The final result consists of the two automata $(A$

1 The first number indicates a bibliography entry, the second the page(s) thereof

$+ B + C$) and ($A + B + C$) + f ($A + B + C$). If B were to copy the description thrice, the process would start with one copy of ($A + B + C$) + f ($A + B + C$) and terminate with two copies of this automaton. In this way, the universal constructor reproduces itself.]

This is not a vicious circle. It is quite true that I argued with a variable X first, describing what C is supposed to do, and then put something which involved C for X. But I defined A and B exactly, before I ever mentioned this particular X, and I defined C in terms which apply to any X. Therefore, in defining A, B, and C, I did not make use of what X is to be, and I am entitled later on to use an X which refers explicitly to A, B, and C. The process is not circular. (19, 85)

Consider a self-reproducing automaton in a changing milieu. Such an automaton must of necessity be highly structured. High degrees of structure are susceptible to degradation through entropy if the components making them up are required to engage in activity simply because complex structure is very improbable and any change will tend toward states of greater probability, which have less structure. This entropy can be counteracted if external energy is available and the automaton's control program can utilize it to maintain the structure. Self-reproduction is activity; therefore, a self-reproducing automaton will tend to be degraded. In addition, any action, such as self-reproduction, requires energy. A self-reproducing automaton must have energy sources if it is to act and remain in existence. Energy conversion itself requires action, and so tends to degrade an automaton. Thus, to prevent eventual, and possibly rapid total collapse, an automaton must of necessity possess self-repair and self-maintenance abilities.

Biological cells, at various stages of development, are examples of self-reproducing and self-maintaining automata. The genes, or, the genome as they are collectively called, are a cell's control program. They constitute a description of the cell's structure and action. Of course the genome itself possesses complex structure, the first determination of which was made in 1953 by James D. Watson and Francis Crick, who later received Nobel prizes for their contributions. It consists of deoxyribonucleic acid, DNA, made up of two interwoven chains of smaller molecules. The analogy to the tape of an automaton is obvious. When a cell reproduces, the two chains of the genome separate; a complementary other half is then synthesized for each. Thus, the original control program is destroyed and two others are constructed. This is precisely analogous to von Neumann's formulation, which was made more than four years before the structure of DNA was determined.

The analogy is highlighted by Watson's statement about the situation in 1951: "… I had worried about the possibility that the gene might be fantastically irregular. Now, however, I know that genes could crystallize; hence they must have a regular structure that could be solved in a straightforward fashion." (21, 28)

Von Neumann's model eventually diverges from self-reproduction as it occurs in cells, but the similarities are striking.

Some authors, like Carl R. Woese, emphasize the automaton-theoretic aspects of cell development:

> In the present instance we are primarily concerned with only two classes of molecules in the cell: first, molecules whose primary structure has a high information content and whose secondary structures, and so forth, are in one sense of no real interest—that is, DNA, a large class of RNA, and most of the cell's protein; and, second, molecules that bring about the transfer of information from one kind of the *informational molecules* to another. The first class, we think of as tapes, and the second, because of the mode of action, as *tape readers*. To us, then, the cell becomes a conglomerate of tapes and tape readers.
>
> In cellular tape-reading processes, an input tape feeds linearly through the tape reader; the reading in all cases consists of producing an output tape whose monomer units and mapping rules are characteristic of the tape reader but whose information content, of course, reflects exactly that of the input tape. The cell contains three basic kinds of tapes and three kinds of tape readers. The primary (ultimate reference) tape is DNA, which undergoes two kinds of tape-reading operations. One, called replication, results in an output of two tapes each identical to the input tape. The other, called transcription, results in an output tape that is an RNA copy of a particular strand of the input tape (DNA being double-stranded) in addition, of course, to the original input tape itself. Under normal conditions, all RNA of the cell appears to be produced by the process of transcription. A special subclass of RNA, appropriately called message RNA, becomes an input tape for the translation tape reader, whose output is the polypeptide tapes. The latter class of tapes is, of course, never subject to a tape-reading operation itself. … It appears that the two remaining classes of RNA tapes, called ribosomal RNA and transfer RNA, are not themselves used as input tapes … but are incorporated as parts of the translation tape-reader system. (22, 5f)

Proteins, more generally polypeptides, are *translated* from RNA tapes through a complex process involving the association of an amino acid with three consecutive RNA bases. The resulting polypeptide chain is folded into a characteristic biologically active form. (20, 298ff)

Prominent among the proteins produced by a cell are enzymes, which are biological catalysts. Their synthesis

> … follows a double genetic control. The so-called structural genes determine the molecular organization of the proteins. Other, functionally specialized, genetic determinants, called regulator and operator genes, control the rate of protein synthesis through the intermediacy of cytoplasmic components or repressors. The repressors can be either inactivated (induction) or activated (repression) by certain specific metabolites. This system of regulation appears to operate directly at the level of the synthesis by the gene of a short-lived intermediate, or messenger, which becomes associated with the ribosomes where protein synthesis takes place. (9, 318)

DAMAGE AND GENETIC REPAIR

The proper function of a cell is dependent upon maintaining the normalcy of these factors:

1. physical environment; i.e., factors such as temperature, pressure, radiation, membrane integrity and so on;
2. chemical environment; i.e., the presence within certain limits of certain biologically active chemicals, ion concentrations, and so on, both within and without the cell membrane;
3. integrity of the control program.

There is an upper limit of interference from the environment such that if this limit is exceeded, the control program will not be able to continue repair or maintenance. This limit may be lowered if the control program is itself damaged or diminished, and it is reasonable to assume that it may be raised if the control program is augmented and/or the constituent milieu of the environment is enriched. If the upper limit of interference is either exceeded or is lowered until normal functions cannot proceed, damage occurs, which if not slowed, stopped, or reversed, leads to complete degradation. Ultimately, any form of damage is manifested on the molecular level, and we may

> … outline the possible modes of molecular recovery in general terms. Three possible modes for dealing with damaged molecules in the cell might be listed as follows:
>
> I. The damaged molecule or part of a molecule may be restored to its functional state *in situ*. This may be accomplished by the activity of some enzymatic mechanism or it may simply result from the "decay" of the damage to an innocuous form.
>
> II. The damaged unit may be removed from the molecule or system which contains it and then be replaced with an undamaged unit to restore normal function.
>
> III. The damage may remain unrepaired in the system, but for one reason or another the system may be able to bypass or ignore the damage.
>
> All of these general modes have now been well documented … (8, 2f)

It is one of the theses of the cryonic movement that a method of slowing and virtually halting progressive cell damage exists, though it contributes an increment to it. This method is controlled freezing and storage. Concrete proposals for carrying out repair on the molecular level are required. Such methods for eventually effecting repair, or inducing regenerative processes to begin and continue at a rate greater than those of degradation are needed. Since a cell is formed and maintained under genetic control, it is reasonable to suggest that genetic control also be used to carry out degrees of repair greater than those the cell in its damaged condition could by itself provide. For each degree of damage greater than the normal regenerative abilities of a cell, a suitable enriched environment and augmented control program should be provided. The control program should be augmented as such, in the form of additional genetic information which will enable the cell to carry out emergency repairs, such as of a damaged membrane, gather nutrients from the environment, and restore normal functioning according to the standard control program.

All this will require that the cell be provided with a specifically enriched environment, and that supplementary genetic information be introduced into the cell. The latter occurs in the phenomenon known as transformation, defined as "the integration with the genome of a recipient cell of a small piece of exogenous genetic material, extracted from a donor cell and introduced into the reception as part of a free DNA particle." (12, 231)

It is of course not necessary that DNA used for repair purposes come from a donor cell; DNA has been synthesized (11, 78ff) (7, 2321ff), and synthetic DNA could be used for this purpose. Transformation has been studied in bacteria and higher organisms as well:

> In transformation certain bacteria change their hereditary makeup by absorbing DNA molecules from their environment. The ability to do this is induced by a giant-molecule factor synthesized by the cell. … The phenomenon of transformation in bacteria was first observed in 1928, led to the identification of DNA as the genetic material in 1944 and has since been recognized as a significant form of genetic intervention: a means whereby bacterial cells can acquire new genes (and thus new traits) with a frequency many order of magnitude higher than if such changes occurred only through random mutation. … In bacterial transformation a bit of DNA penetrates the boundary of a bacterial cell and becomes incorporated into the cell's genetic apparatus. (16, 38)

The situation is more complicated in higher organisms:

> … increasing numbers of workers have studied

> the possibility of genetic transformation mediated by nucleic acid in cells of higher organisms. *In vivo,* the difficulties encountered are numerous. In such systems, the injected DNA must be carried by the blood stream or by the plant sap to sites distant from the point of injection and the DNA molecules must pass several cell membranes before reaching their final site. (12, 231f)

Commenting on transformation, Tomasz states: "The importance of learning more about the mechanism is obvious, since the invasion of cells by extraneous genetic material is not restricted to the world of bacteria. Such events are the essence of all viral infections and may be responsible for the induction of some forms of cancer." (16, 38)

He also states:

> Work is now in progress … aimed at learning more about the mechanisms that somehow open and close the gates of cells to the entry of foreign genetic material. This work could eventually lead to better understanding of viral infection and could even contribute to the possibility of deliberate genetic intervention in higher organisms. (16, 44)

VIRUSES

Artificial viruses offer a possibility of transporting supplementary genetic information into a cell for repair purposes. Luria and Darnell define viruses as "entities whose genome is an element of nucleic acid, either DNA or RNA, which reproduces inside living cells and uses their synthetic machinery to direct the synthesis of specialized particles, the virions, which contain the viral genome and transfer it to other cells." (13, 3)

Expanding on this definition, they

> … attempt to convey the two qualities of a virus: first, the possession of a genetic material of its own which, inside a host cell, behaves as part of the cell; second, the possession of an extra-cellular infective state, represented by specialized objects, the virions, which are produced in the cell under the genetic control of the virus itself and serve as vehicles for introducing the viral genome into other cells. (13, 3)

Virus particles or virions consist basically of a control program in the form of a DNA or RNA tape, and an enclosing capsid which protects the program in the extra-host-cellular environment and administers the program to an appropriate host cell. The program, either in conjunction or not with the cell's control program, may use the intracellular environment to replicate additional virions. Some viruses do not so replicate: "Viruses are obligate parasites and in nature many of them give rise to latent infections causing little inconvenience to the host cell; only occasionally do they break out to produce symptoms of disease." (14, 15)

Watson expands on some such viruses:

> Some bacterial viruses … do not always multiply upon entering a host cell. Instead their chromosome sometimes becomes inserted into a specific section of a host chromosome. Then the viral chromosome is, for all practical purposes, an integral part of its host chromosome and is duplicated, like the bacterial chromosome, just once every cell generation. The virus chromosome when it is integrated into a host chromosome is called the *prophage*; those bacteria containing prophages are called *lysogenic bacteria;* and those types of virus whose chromosomes can become prophage are known as lysogenic viruses. In contrast, those viruses … that always multiply when they enter a host cell are called *lytic viruses*. (20, 204f)

Besides lysogenic viruses, there also exist defective ones, which by themselves are unable to reproduce when infecting a host, but which can grow in the presence of another, helper virus, which is able to supply one or more of the functions that a defective virus lacks, thus enabling the latter to multiply. (20, 474ff)

These characteristic properties of lysogenic or defective viruses are of course determined by specificities in the control programs, and the genes determining them would be relevant in the synthesis of artificial repair viruses.

NEURAL INFORMATION CONTENT

Every cell in a higher organism, including man, contains the genetic specifications for the entire organism, so only a percentage is used to maintain a particular cell. In some cases of damage, then, it might be more feasible to replace a damaged portion with an artificial component or grow a new one from another cell. This alternative is not attractive in the case of the central nervous system, especially the brain, since essential information content regarding memory and personality is stored there. As Ungar and Irwin state:

> The essential function of the nervous system is to receive, store, and retrieve information so as to modify behavior according to the changing conditions of the environment and past experience. Part of the information is built into the organism and stored in the genetic material: It determines the structure and organization of the system and directs the innate responses to stimuli. These responses vary in complexity from simple reflexes to the elaborate instinctive patterns of behavior observed in some species.
>
> However, in almost all animals behavior is modified by acquired information. Operationally, memory and learning comprise everything that enables organisms to change their behavior as a result of experience. (18, 144)

Repair, rather than replacement, is thus most crucial in the case of damaged neurons. In addition, information content in neurons, assuming it has not been already damaged, is likely to be destroyed during any large-scale repair process unless specific steps are taken to preserve it.

One favorable factor in brain information storage is that there appears to be considerable redundancy:

> It is well known that functionally identical information may be stored at multiple sites in the nervous system so that the system can function extremely well despite extensive ablation. This property of the nervous system is referred to as "redundancy." (2, 164)

Even if information is destroyed in one part of the brain, redundancy may ensure that it will nonetheless not be lost; as Flexner and Flexner report from an experiment: "This result was interpreted to mean that large areas of the brain participate in longer-term memory but that a relatively small area is sufficient to sustain it." (4, 1653)

Pribram theorizes on how redundancy may be achieved: "Experiments with monkeys have identified the brain areas involved in the recall of various learned tasks. Memory may take the form of interference patterns that resemble laser-produced holograms." (15, 73)

He expands on this hologram-like storage:

> … I believe there is now available a hypothesis about the nature of the memory trace that satisfies the known physiological requirements and that can be tested by experiment. It is perhaps not surprising that the brain may exploit, among other things, the most sophisticated principle of information storage yet known: the principle of the hologram. In a hologram the information in a scene is recorded on a photographic plate in the form of a complex interference, or diffraction, pattern that appears meaningless. When the pattern is illuminated by coherent light, however, the original image is reconstructed. What makes the hologram unique as a storage device is that every element in the original image is distributed over the entire photographic plate. The hypothesis is attractive because remembering or recollecting literally implies a reconstructive process—the assembly of dismembered mnemic events. (15, 73)

Flexner, Flexner, and Roberts discuss the nature of instinct, memory, and learning:

> Memory is thought to consist of overlapping stages. In the first stage the essential process is believed to be the electrical activity of those nerve cells which participate in a learning procedure. In this stage memory can be destroyed by electroconvulsive shock which disrupts this selective electrical activity. The period when memory is vulnerable to electroconvulsive shock in the mammal varies greatly, with a minimal value of less than 1 minute.
>
> The learning process also leads to changes of a permanent kind so that in man, for example, memory of an event in childhood may persist for life. Thus long-term memory appears to be a relatively stable condition reached as the outcome of events occurring in a period of consolidation. In this period electrical activity is transformed into a more permanent record. … Further clues to the nature of the learning process and memory can be obtained by considering instinctive or inherited behavior. Such behavior must be attributed to certain stable patterns of gene expression which become established during the development of the individual. These patterns of gene expression are dictated by the sequence of nucleotides in the DNA and are manifested during the complicated and mysterious process known as differentiation.
>
> Behavioral patterns acquired by learning or training are so similar to instinctive ones that they are often difficult to distinguish. Accordingly it is reasonable to assume that well consolidated, long-term memory has the same fundamental basis as instinctive behavior, that is, it is the manifestation of a stable pattern of gene expression. Nature frequently uses the same mechanism for a variety of purposes. (6, 1377)

They go on to propose a theory of how information is stored in neurons:

> We assume that an established memory of long duration depends, not on the continued presence of any protein or nucleic acid molecules, but on the establishment of a self-sustaining system for their synthesis. Such a system can occur whenever some of the products of a gene's expression act as inducers (or derepressors) of that gene. If the gene is repressed, inducers are not synthesized and the gene stays repressed. On the other hand, if the gene is induced for a sufficient time, inducers will accumulate above a critical level and the gene will stay induced. If, however, the synthetic processes are inhibited for a sufficient time, the level of inducers will fall below the critical level and the gene will revert to its repressed state.
>
> The processes involved in the establishment of a long-term memory can be described in terms of the self-inducing system. We assume that the initial

> learning experience triggers the synthesis of one or more species of mRNA. This mRNA alters the synthetic rate of one or more proteins which are essential for the expression of memory. These proteins are thought to modify the characteristics of synapses concerned in a learning process so that the passage of impulses between nerve cells is facilitated. In turn, the proteins or their products act as inducers of their related mRNA; in this way the concentration of the inducer proteins is maintained. In this view, expression of memory depends upon changes in proteins, changes which are initiated and sustained by qualitative and quantitative changes in mRNA produced by a learning experience. Loss of this mRNA would lead to loss of essential protein with consequent permanent loss of memory. In the presence of an inhibitor of protein synthesis, the concentration of essential protein could fall to levels too low for expression of memory, but loss of memory would be temporary if mRNA were conserved to direct the synthesis of protein when the inhibitor had disappeared. (6, 1381f)

To be sure, no certainty can be accorded the theory:

> Clearly only a beginning has been made in testing the hypothesis based on a self-sustaining system. The hypothesis is consistent with the results of Hydén and collaborators … who demonstrated an increase in nuclear RNA following training. It is also consistent with the recent finding by Zemp *et al* … that rate of synthesis of nuclear RNA is increased in a learning situation. There is, however, as yet no completely convincing demonstration that changes in RNA and protein are fundamental to memory. (6, 1382)

And evaluating the results of another experiment, Flexner and Flexner conclude that it "raises the possibility that the basic memory trace of maze learning may depend upon a long-lasting normal peptide(s) and so may make unnecessary the postulation of a self-sustaining system requiring messenger RNA." (5, 927)

Certainly there are many theories on how information is stored in neurons; but it seems clear that in some form or other, long-term information content, the only type likely to be present in a cryonically suspended patient, must have a molecular basis. In what follows I will nonetheless assume the self-sustaining RNA theory, being confident that any molecular-based information storage will be amenable to preservation by an appropriately devised repair control program.

PROBLEMS IN VIRUS PRODUCTION

Nobel laureate Kornberg speculates on future areas for research in biology:

> One is the exploration of the physical and chemical nature of DNA polymerase in order to understand exactly how it performs its error-free replication of DNA. Without this knowledge of the structure of the enzyme and how it operates under defined conditions in the test tube, our understanding of the intracellular behavior of the enzyme will be incomplete.
>
> A second direction is to clarify the control of DNA replication in the cell and in the animal. Why is DNA synthesis arrested in a mature liver cell and what sets it in motion 24 hours after part of the liver is removed surgically? What determines the slow rate of DNA replication in adult cells compared with the rate in embryonic or cancer cells? The time is ripe for exploration of the factors that govern the initiation and rate of DNA synthesis in the intact cell and animal. Finally, there are now prospects of applying our knowledge of DNA structure and synthesis directly to human welfare. (11, 78)

Of course, using artificial viruses for repair purposes necessitates being able to produce them, and much has already been done in the disassembly and reassembly of viruses, for instance:

> If, on the other hand, the components of the virus that causes the mosaic disease of tobacco are gently dissociated and then brought together under the proper conditions, they do reassociate, forming complete, infectious virus particles. The tobacco mosaic virus consists of a single strand of ribonucleic acid with several thousand identical protein subunits assembled around it in a tubular casing. (25, 61)

Aside from tobacco mosaic virus, other viruses have been synthesized, such as ϕX174. (7, 2321ff)

Wood and Edgar state some of the relationships involving protein synthesis in general and the process of assembling a virus:

> Molecular biologists have now provided a fairly complete picture of how genes carry out their primary function: the specification of protein structure. The segment of nucleic acid (DNA or RNA) that constitutes a single gene specifies the chain of amino acids that comprises a protein molecule. Interactions among the amino acids cause the chain to fold into a unique configuration appropriate to the enzymatic or structural role for which it is destined. In this way the information in one gene determines the three-dimensional structure of a single protein molecule.

> Where does the information come from to direct the next step: the assembly of many kinds of protein molecules into more complex structures? To build the relatively simple tobacco mosaic virus no further information is required; the inherent properties of the strand of RNA and the protein subunits cause them to interact in a unique way that results in the formation of virus particles. (25, 61)

They go on to conclude:

> The problem has now reached a tantalizing stage. A partial sequence of gene-controlled assembly steps can be written, but the manner in which the corresponding gene products contribute to the process remains unclear … Continued investigation … can be expected to provide further insight into how genes control the building of biological structures. (25, 74)

Regarding viral assembly and its research possibilities, Kornberg states:

> An obvious area for investigation would be the synthesis of the polyoma virus, a virus known to induce a variety of malignant tumors in several species of rodents. Polyoma virus in its infective form is made up of duplex circular DNA and presumably replicates in this form on entering the cell. On the basis of our experience it would appear quite feasible to synthesize polyoma virus DNA. If this synthesis is accomplished, there would seem to be many opportunities for modifying the virus DNA and thus determining where in the chromosome its tumor-producing capacity lies. With this knowledge it might prove possible to modify the virus in order to control its tumor-producing potential. (11, 78)

I am surely not the first to propose the use of artificial viruses for one purpose or another; Kornberg suggests:

> Our speculations can extend even to large DNA molecules. For example, if a failure in the production of insulin were to be traced to a genetic deficit, then administration of the appropriate synthetic DNA might conceivably provide a cure for diabetes. Of course, a system for delivering the corrective DNA to the cells must be devised. Even this does not seem inconceivable. The extremely interesting work of Stanfield Rogers at the Oak Ridge National Laboratory suggests a possibility. Rogers has shown that the Shope papilloma virus, which is not pathogenic in man, is capable of inducing production of the enzyme arginase in rabbits at the same time that it induces tumors. Rogers found that in the blood of laboratory investigators working with the virus there is a significant reduction of the amino acid arginine, which is destroyed by arginase. This is apparently an expression of enhanced arginase activity. Might it not be possible, then, to use similar nonpathogenic viruses to carry into man pieces of DNA capable of replacing or repairing defective genes? (11, 78)

The proposal set forth in this paper extends the use of artificial viruses to the more general case of repair of cell, especially neuron, damage. This will require artificial viruses which will infect neurons: "Viruses are selective in the cells they attack, the evidence indicating that this depends on whether the cells have available the particular receptors to which the viruses may attach themselves." (23, 7) It is known that there are many viruses capable of causing infection of the central nervous system (23, 9), so appropriate portions of the control programs of such viruses should be included in the production of repair virions. In addition, it needs to be assured that the viruses are able to reach the cells in question, either along natural routes of infection (24, 11), since the ability to make effective contact with a host cell is essential to infection (24, 59), or through micro-surgical injection techniques. In some cases it might be feasible to inject naked genetic material directly into a cell rather than administer it from without by a virus.

Viral mutants which operate at unusual or restricted temperatures exist; the control program characteristics specifying this might be useful if it is determined that repair should proceed at other than normal biological temperature (24, 26); but the important factor is that repair proceed faster than deterioration, whatever the temperature.

PROBLEMS IN VIRAL-INDUCED REPAIR

The following is a summary, claiming no finality or completeness, of the problems on whose solution a technology of cell repair using artificial viruses depends:

Capsid programs need to be isolated or created to ensure that a particular artificial virus will be specific to the type of cell it is supposed to repair. Those portions of the cell genome dealing specifically with the cell's own function must be isolated and perhaps incorporated into the virus in case the cell's own program is damaged. The metabolic pathways necessary to effect repair of a certain degree of damage must be determined and coded into the repair program. Supplementary nutrients to assist repair of the same degree of damage must be determined, prepared, and suitably administered. A preparatory program designed to replicate repair virions in large numbers must be devised. Natural and mechanical methods of infecting all the cells in a damaged or possibly damaged area must be developed. Quality control of many batches of virions must be maintained. Non-replication within the cell must be assured. It must be ascertained whether or not repair should be carried out

by one virus, or by successive infection of several, each acting alone or in conjunction with earlier ones. Once *in situ*, the repair program should determine, by preliminary synthesis, or immediate reaction to specific abnormal products, the degree and kind of damage, if any. According to the diagnosis it should then shift to the appropriate subprogram and carry out the repair, organizing the resources available in the cell and in the enriched extracellular environment. It should determine when repair is complete, and provide for its own disposition. This method may be used to repair any type of cell, but I am interested especially in repair of neurons, where preservation of information content is critical.

PRESERVATION OF LONG-TERM INFORMATION CONTENT

Assuming the self-sustaining RNA theory, how will the control program preserve information content while repair is proceeding? This information can be preserved by requiring the repair program to incorporate the appropriate RNA tapes before large-scale repair is carried out, and, after it is complete, release them to function as before. A possibility for a technique of accomplishing this exists in the phenomenon known as hybridization. Watson discusses an experiment:

> If a heated DNA solution is slowly cooled, a single strand can often meet its complementary strand and reform a regular double-helical molecule. This ability to renature DNA molecules permits us to show that artificial hybrid DNA molecules can be formed by slowly cooling mixtures of denatured DNA from two different species. For example, hybrid molecules can be formed containing one strand from a man and one from a mouse. (20, 266)

Of course, preservation of the information content here involves RNA, and DNA-RNA hybridization occurs; Watson mentions a special case in which "… the RNA product remains attached to its DNA template, allowing the isolation of a hybrid DNA-RNA double helix." (20, 307) Such hybrids are often used in experiments, for instance "… to show the complementarity in nucleotide sequences between an RNA molecule and one of the two strands of its DNA template …" (20, 310) In fact, Adair, Wilson, and Glassman discuss hybridization in conjunction with brain information content:

> There remains the question of the function of RNA that shows the response to training. Since it has been shown that preribosomal RNA attaches to polysomes, the increase in radioactivity in polysomes reported here may reflect increased labeling of either messenger or ribosomal RNA. Regardless of whether mRNA, rRNA, or both are involved, however, the increase in radioactivity would seem to signal the beginning of the synthesis of either a new protein (or proteins) or of an increased rate of synthesis of proteins that are being made continuously. Tests to distinguish between these alternatives by using DNA-RNA hybridization techniques are underway. (1, 921f)

Kates and McAuslan discuss it in connection with viruses: "Viral messenger RNA was assayed by specific hybridization with RP [rabbit poxvirus] - DNA." (10, 316)

Since RNA molecules used for information storage are doubtless genetically specified, a repair control program might carry out DNA-RNA hybridization of all such molecules at the appropriate time after entry into a neuron, and then incorporate the resulting hybrids into its main program in much the same manner as prophage is incorporated into the genome of a lysogenic cell. When repair is complete, the hybrids would be detached, the RNA molecules released, and allowed to function as before.

CONCLUSION

The general method outlined here has its obvious use in the repair of nervous tissues especially human. Repair of all types of damage—caused by factors mechanical, chemical, pathological, aging, freezing, thawing, and so on—is intended. The method may be partly realized by using its theoretical aspects as a research tool to investigate metabolic pathways in organisms such as bacteria as well as information content and storage in human and other neurons. As Darnell states:

> … the most effective means to study animal cell biology is to choose an appropriate virus to introduce a controlled set of genes which perform or cause to be performed the set of events one wishes to analyse. The obvious limitation to this approach is that we do not know a virus which will cause any and every event of interest in animal cell biology. It is nevertheless true that a vast array of interesting problems can be attacked, through the analysis of virus functions at the level of molecular interactions. One possible example of such a problem is the study of the synthesis and entry into organized structures of membrane proteins derived from viral genes. (3, 160f)

I hope that the method cursorily outlined here is still concrete enough to encourage those who are concerned with problems of repair of brain damage, whatever its origin. ■

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