MUTATION, DEVELOPMENT, AND EVOLUTION

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The basic source of all hereditable change or variation in living orgamisms has been generally accepted to be due to the process of mutation, and thusly, the major or paramount mechanism of evolution has come to be regarded as equiv: :alent to the process of mutation extended through time. Mutation involves an alteration or modification of the structural organization of the genetic material; and because of the nature of the DNA molecule, such material is capable of an infinite or nearly infinite number of variegated modifications.

Of the mutations due to the alteration of the genetic material, two classes may be delineated. One class is usually accompanied with observable chromosomal changes, whilst the other is not usually associated with such visible changes in the genetic material i.e. the chromosomes, but is submicroscopic, for in as much as it may involve only a few purine and pyrimidine bases. The former type of mutation occurs on a grosser level as it involves changes or re-arrangements in the specific juxipositional or spatial relationships of the different chromosomal segments, a particular segment being defined as a large number of DNA bases (or nucleotides) in a specific sequence.[1] These gross changes evince themselves as inversions, translocations, deficiencies, and as tandem duplications.

The answer as to whether or not a gene can be defined as being equivalent to one of these chromosomal segments may, I believe, become clearer towards the end of this paper. For the present moment, I shall refer to a gene as being synonomous with some small, but arbitrary, section of the chromosome, pointing out in so doing that this tentative definition adverts to only one of the meanings which can be applied to the concept, 'gene'. In point of fact, as this discussion develops/.....

1. Chromosomes in metazoa are also composed of proteins (histones) which appear to be attached lengthwise to the phosphate esters of the nucleotides, and not to the bases themselves, so as not to interfere with DNA replication and M-RNA synthesis.

develops it may well become apparent to the reader that the meaning of a gene is that it is a concept capable of many meanings, each of such equally as valid.

In the light of the tentative definition just posited, a gene mutation would or could be defined as a non-observable mutation occurring within a small chromosomal section, while the other type of mutation, involving the re-ordering of chromosomal segments, could be regarded as a chromosomal aberration. Yet,

> "this should not be taken to mean that gene mutations can be rigorously distinguished from chromosomal aberrations. Indeed, deficiencies and duplications that are too short to be seen under the microscope may be inherited as gene mutations ... Goldschmidt even contends that all mutations represent small chromosomal aberrations. Whether or not this is true, it is probable that what we call gene mutations are actually a residue left after the elimination of all changes for which a cytological visible basis can be found. Now, how small the smallest chromosomal alterations are which can be identified under a microscope depends upon the organism concerned (since some forms have large and others small chromosomes), as well as upon the powers of observation of the investigator. The term 'mutation' is used, then, in two senses. In the broader sense it covers any change in the genotype no matter how produced (that is by a gene change or by a chromosomal aberration). In the narrower sense, a mutation is a change in the properties of a single gene."[2]

> "The significance of this difficulty in establishing the structural basis of mutation becomes evident when it is recognized that our knowledge of genetics and our recognition of genes is based to a very large extent on the study of mutations. A gene is recognized [as the determinant of a certain trait] because it has an allelic form which gives a different phenotype. The action of the gene in determining the phenotype is ordinarily deduced [or inferred] from a comparison of contrasting phenotypes."[3]

In many cases contrasting phenotypes manifest contrasting genotypes whilst similar phenotypes do not necessarily imply similar genotypes.

With regard to genotypes, it is important to realize that a change in genotype can also be wrought by the formation of new gene combinations consequent upon/.....

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2. Dobzhansky, Principles of Genetics.

3. Wagner, Genetics and Metabolism.

upon segregation and the fusion of gametes. Since a specific gene combination or genotype cannot be maintained completely from generation to generation, but changes somewhat in the very process of its being transmitted, such a change in the whole genotype, even though slight is able only to last for the duration of the organism's life provided that no alteration in chromosomal structure occurs within that same period. Such a change of genotype as dependent on or conditional upon the mechanisms of segregation and sexual fusion can be regarded as a fluxial mutation, as opposed to a durable or inherited mutation. A "fluxial mutation" would be the broadest meaning which could be ascribed to the term, mutation. Ultimately, fluxial mutations rest on the foundation of inheritable mutations, as different gene combinations could not be existant without the presence of a large number of multifarious genes. Implicit in the notion of fluxial mutation is an additional meaning as to what a "gene" may be - that is, a gene in this case takes on the meaning of a specific genotype or a complex matrix of interacting units (chromosomal regions.)

Not only is a durable mutation a result of a modification in the genetic material, but a durable mutation can be attributed to a change in the number of chromosomes, since such a change in number produces a new combination of genes which can endure in progeny. Polyploid and aneuploid plants are quite different in phenotype from diploid plants, and in a self-fertilizing plant, a hexaploid situation, for example, can be transmitted over a great many generations. A cross between two triploid individual plants may give rise to a large number of individuals which are triploid, while a minority of the progeny may be diploids. With respect to these latter progeny, the triploid state could not be regarded as a durable or permanent mutation, while with respect to the majority of triploid progeny, the triploid state, as produced in the parental generation, could be regarded as a heritable or durable mutation or modification. If tetraploid/.....

tetraploid individuals give rise to diploid progeny, and if the generations of progeny which have egressed from the original diploid parents are themselves diploid, then one may regard the mutation leading to the diploid state as being durable even though such a mutation may have taken place as an aspect of the very processes of segregation and sexual fusion.

It has been shown that the alteration of the genetic material, or the production of durable mutations, can be induced artificially by exposing the organism to certain agents or conditions. X-rays, radiation from radioactive materials, and U.V. have mutagenic effects. Also, extreme temperatures induce mutations. Many types of chemicals can as well act as mutagenic substances or mutagens. Certain organic and inorganic peroxides are known to act as mutagens. Mustard gas and related compounds have strong mutagenic effects. Certain types of natural purines and pyrimidines; such as caffeine, adenine, amino-purine, and 5-bromouracil; have been used with considerable success as mutagens. Also, alkylating agents, such as ethyl methane sulfonate, have been found to be mutagenic. Formaldehyde has also been discovered to be a mutagen, as well as nitrous acid and acridine.

It is through the application of such mutagens that the frequency of mutation is greatly increased. The natural mutation frequency or natural rate of mutation is that observed under ordinary or natural circumstances of observation, without the use of abnormal external agents or conditions, such as unnatural chemical conditions, extreme temperatures, and high levels of radiation. The cause or stimulus or agency of natural, or what is also referred to as spontaneous mutation, may in part be natural or background radiation. Even so, according to Dobzhansky,

> "a majority of [natural] mutations are produced by causes other than radiations. The origin of most spontaneous mutations remains an unsolved problem."[4]

4. Dobzhansky, Principles of Genetics

The problem seems to become even more magnified when one notes that not all genes in the species mutate spontaneously at the same rate, especially so when one recalls the fact that the overall average rate of natural mutation is different for different species, and may as well vary from sub-species to sub-species. In addition, Muller found evidence that the rate of sex-linked mutation is different in different stages of the germinal cycle. For example,

> "the sperm accumulated during the pre-imaginal life of a male [Drosophilia] show a two to three times greater mutation frequency than those produced six to nine days later... It is clear that at least in Drosophilia the time rate of mutation is not constant in all cells of the individual during its life span, and that it probably varies not only from one stage of development to the next but even within the different stages ..." 5

The above phenomena cannot really be explained by stipulating all natural mutations to be due to extrinsic factors alone. If one does not assume the cause of all natural mutations to be due to external agencies, the only alternative left is to assume that an organism has within itself the agencies or conditions which bring about mutations, or alterations in the organism's own genetic material. This would mean that the occurrence of natural mutations may not be accidental or random, but determined by factors naturally residing or created within the cell or body of the organism. What would be the nature of such factors or agencies? The answer may at first not be apparent, but when one recalls that most, if not all, of the chemical mutagens that have been used artificially by man to induce mutations are themselves the natural or organic products of specific reactions included in cellelar metabolism, it becomes not difficult to realize that an organism can produce its own chemical mutagens, and inflict them on itself, in a manner of speaking, thereby inducing its own mutations. Yet it is important to realize that genes determine the existence of the series of chemical reactions which result in the creations of these organic mutagens, and thus, it would appear/.....

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5. Wagner, Genetics and Metabolism.

appear that there are particular genes that are ultimately the agents or factors effecting the process of mutation on other genes.

These ultimate agents of natural mutation, which I shall refer to as muterons or mutator genes, may also induce the formation of organic mutagens other than those known to man, and it is quite feasible that specific ones of such may cause mutations within specific chromosomal regions (genes), while other variant ones may cause respectively different mutations within the same region (gene). Even some of the known organic mutagens appear to act in a fairly specific manner. Before pointing out some examples of the specificity of known mutagens, it would be best to give a brief description of how some of them cause mutations.

Ethyl methane sulfonate, a known mutagen, acts chemically on DNA to change given bases into others thereby alterating the DNA code. Ethyl methane sulfonate appears to cause methylation of guanine and adenine in DNA with the result that the methyl derivatives are then excised and eliminated from the DNA by enzymes and either replaced by different bases, the replacement being mediated by the action of still other types of enzymes, or not replaced to produce deletions. More shall be said later about the role some enzymes play in mutational activity.

Organic peroxides react with DNA bases, apparently by attaching hydrogen and hydroxly radicals to the bases, and such attachment results in the conversion of one type of base into another. The net affect of this is the alteration of the DNA code.

Some mutagenic purines and pyrimidines are mutagenic by virtue of the fact that they are analogues to the pyrimidines and purines which go to make up DNA, and hence its code. 5-bromouracil is an analogue of thymine.

> "If a 5-bromouracil molecule replaces a thymine in a DNA chain, a BU-A nucleotide pair will be formed with the bases joined by hydrogen bonding (BU = 5-bromouracil.) This would mean that when the two chains of the double helix/.....

helix separate and duplicate ... one of the single chains will carry a BU base. It is conceivable that this chain might not function to produce a continuing strand, but on the other hand, the BU in it may form a hydrogen bond with another adenine, BU-A or with a guanine to give BU-G. If thymine and cytosine ... are made available, the BU-A combination would be expected to revert to the original T-A conditions and the BU-G combination might become C-G. This would cause a change in base sequence at this point from T-A to C-G, and presumably manifested as a mutation ... It should also be noted that the bromouracil may ... replace a cytosine to give a BU-G double nucleotide which by the same ... process might become BU-A, and then T-A resulting in ... C-G to T-A."[6]

Other base analogues, 2-Aminopurine for example, may probably cause such changes in sequence as C-G to G-C, or A-T to T-A, or G-C to T-A.

Nitrous acids react readily with amino groups converting them to hydroxyl groups. Only three DNA bases contain amino groups. The reactions of nitrous acid with these particular bases (adenine, guanine, and cytosine) produces hypoxanthine, xanthine, and uracil respectively.

> "These could presumably act as analogues and cause mutations in the same fashion as 5-bromouracil, provided that the analogues are formed in situ in the nucleic acids, for these compounds have not been shown to be mutagenic in the free state."[7]

One may even regard nitrous acid as being specific in its actions, as it only reacts with three out of the four normal DNA bases.

5-Bromouracil and 2-aminopurine appear to be specific in their respective mutational activities. For example,

> "Freese has found that mutations produced in the region (gene) of T-4 in the presence of 5-bromouracil ... generally located at the same sites, that is, they give the same spectrum. ... Mutations induced by the aminopurines tend to cluster at somewhat different sites."[8]

Ethyl/.....

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6. Ibid.

7. Ibid.

8. Ibid.

Ethyl methane sulfonate ...

> "shows considerable mutagenic specificity in phage, since it causes a reversion of those mutants induced by 2-aminopurine, but not those induced by 5-bromouracil."[9]

Various alkylating agents have different effects in producing reversions or back mutations. In adenine inosital double mutants of Neurospora Crassa Bromoethyl methane sulphonate caused per 10^6 Macroconida 152 reversion to ad+, while the same chemical only produced on the average .04 reversions to ins+. Ethyl methane sulphonate produced 17.4 ad+ revertants and 11.3 inos+revertants.

> "The Fahmys have done extensive analysis on D. melanogostu treated with a number of different types of nitrogen mustards and have concluded that the proportion of visible mutations to lethals obtained depends on the specific mutagen used" [10]

Finally, it is important to note that another mutagen, hydroxlyamine (NH_2OH), acts chemically only on cytosine, resulting eventually in all G-C syndromes (pairs) being eventually changed to A-T pairs.

If these organic mutagens are ultimately produced by genes, one would expect great amounts of mutagens to be existent in the cells of all species at all times, thereby bringing about the same type or degree of mutational activity at all times during the life spans of the divers organisms. However, it was pointed out before that mutations may occur more frequently at one stage of the life span than at other stages, or more often in some species as opposed to others, whilst some individuals of a species may have a higher frequency of mutations as compared to other individuals. Furthermore, it is to be noted that esters of sulfonic acids do not bring about mutations in the spermatogonia of Drosophilia, but nevertheless eventuate mutations in the spermatocytes, spermatids, and mature/.....

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9. Ibid.

10. Ibid.

mature sperm, the mature sperm being the most susceptible to the mutagenic action. In contradisinction to the activity of the methane sulfonic acids a cysteine derivative, S-2 chloroethylcysteine, only induces mutations in spermatogonia.

In beginning to set forth an explanation to these aforementioned phenomena, one must first realize that in an organism not all genes are acting, or are active, at all times during the life period of the organism. It is highly conceivable, therefore, that regulatory or controlling (operator) genes of the Jacob-Monod system switch muterons on and off at specific times during the life span, with the result being that mutagens are only produced at specific times, and then, eventually converted to nonmutagenic substances by enzymes, the existence of these enzymes being wrought by still other specific genes. In fact, such genes should be considered as anti-muterons, for in as much as they negate, or eventually cancel out, the effects of mutagens produced by the muterons. It is quite possible that in many cases as soon as the mutagenic substances are produced many are converted, before they have a chance to act, to non mutagenic materials. Such a process would serve to control the degree or the number of mutations allowed to occur at a specific time, as only a few mutagens would not be converted by the enzymes. This would, in part, explain why different organisms, populations, and species manifest differential mutat-:ional rates. Also, the fact that different species have different genes may also be taken to mean that different species have respectively different types of muterons, one type producing more effective mutagens in one given species while the other type would be producing less effective mutagens in another given species. Different individuals may also differ in the types of muterons each has, and this would also serve to explain their differentiated effects in different organisms; such differentiated effects may, in addition, involve a

given individual having induced mutations within itself which differ, in most part, from those induced in other individuals.

The reason as to why different genes within the same species undergo different mutation rates may be explained by stipulating that in the given species certain muterons are present which produce only mutagens which display a higher frequency of specificity for one type of gene as opposed to another. Or, one may also think of the possibility of two different muterons within a species each determining the production of a specific mutagen, each type of mutagen being only specific for a given gene while one mutagen, X, is highly effectual in producing a high rate of mutation in gene X whereas mutagen Y is not effectual in producing a high rate of mutation in gene Y.

It is quite feasible that two mutagens may react with one another, producing thereby a mutagen which is more effective than either of the reactants. The process by which this may come about would as well be mediated by muterons. In this connection, it is possible that normal metabolites which are themselves non-mutagenic react with one another, producing highly mutagenic substances. Such a reaction would be effected by a particular enzyme which would or could be produced only by a particular muteron. In this respect, esters of methane sulfonic acid in themselves may not be mutagenic, but become converted to mutagens when they react with a specific metabolite produced only during the periods when spermatocytes, spermatids and mature sperm are brought into existance. S-2 - chloroethylcysteine may as well be non-mutagenic in itself, but may produce a mutagen through a reaction with a metabolite created only during the period of spermatogonia formation. After such a period the mutagenic products may be converted to non-mutagenic products by specific enzymes produced by anti-muterons, switched on by operator genes after the period of spermatogonia formation. This would/.....

would explain why S-2 chloroethylcysteine only manifests its mutagenic effects during the stage when spermatogonia are being developed. Whether or not the mutagen produced from S-2 chloroethylcysteine is specific for certain genes would remain to be settled, but it is quite possible that indeed it is one of the naturally produced mutagens, unknown to us, which is only specific for one gene and no others.

Yet, what factor would make a mutagen specific for only one type of gene? All genes have the same four purine and pyrimidine bases. Therefore, what would differentiate one gene from another, as far as the specificity of the mutagen is concerned, or in other words, why would a mutagen react with one gene and not another, when both genes had the same four types of bases? Even though all genes have the same four bases, different genes differ in the particular sequence of these bases as well as in the number of any one particular base. Are we able to say, therefore, that a mutagen, such as ethyl methane sulfonate is drawn to or has an affinity for a certain sequence of a small number of bases, such a specific sequence being a small section of the DNA code of a particular gene? I believe this is only part of the answer ... Chemical investigation has shown that each particular species of molecule; whether it be oxygen, water, methane, or adenine; has a particular shape which is due to the particular spatial arrangement or juxtaposition of the electron orbitals of the atoms making up the respective molecules. That is, when a specific molecule is formed from different atoms, the electron orbitals of the different atoms hybridize in a specific manner or pattern, and the result of this particular hybridization of electron orbitals is the formation of a molecule with a characteristic shape. By the same token, the particular shape of a complex molecule is, in effect, the result of the hybridization of the shapes of its component molecules, or if you will, the resultant of the fusion of a great many electron orbitals into a particular shape. The shape/.....

shape of a complex molecule is different from that of the shapes of its component molecules, when the latter are in the free state, even though all the component molecules may happen to be of the same type. This is another way of saying that the whole is different from its parts.

Adenine, a complex molecule, has a particular shape or spatial configuration of its electron orbitals. Thymine also has a particular shape different from that of adenine's shape. The other two bases also have characteristic shapes. A sub-sequence of four adenines synthesized together as part of the DNA code would be a molecule more complex than adenine, and ever more important, that particular sub-sequence would be a very complex molecule different in shape from that of adenine, in as much as the electron orbitals of the adenine molecules would fuse or hybridize to form a structure with a new shape. In this light, the sub-sequence ATAT would produce a resultant shape different from the shape of the sub-sequence AAAA, and sub-sequence TTTT would result still in a different shape. The resultant shape would be a function of the particular sub-sequence. If one would consider the entire sequence of bases making up a chain of DNA molecule, and not small sections of such a chain, as we have been doing, then this sequence (chain) of bases would manifest itself as a continuum of different shapes,[11] corresponding to different sub-sequences, graded into one another. Each arbitrary portion or section of the DNA code would correspond to a particular shape. In this light, a particular mutagen may only have an affinity for a particular sterochemical shape, which only exists at one small section of a specific gene. Because of this affinity for a particular shape on the part of the mutagen, the mutagen would or could only react with that particular gene and in that particular region (section) of the gene. Another type of mutagen may be drawn to or have an affinity for a different shape which may so happen/.....

11. Such a continuum of graded shapes could be regarded as the secondary-primary structure of DNA in distinction to the primary and secondary structures also manifested by a DNA molecule.

happen to exist at another portion (region) of the same aforementioned gene. Of course it is possible that two genes may respectively have small sections which are identical in shape, and thusly, a mutagen having an affinity for such a shape would be specific for those two genes. It would in the light probably be more accurate to think of natural mutagens as being region or section specific rather than gene specific, each region of a gene corresponding to a given shape, and each such shape having only one specific mutagen capable of being drawn to it.[12] Once attached to a specific region, the mutagen would react only with the bases within that region, though it is likely that only one or two bases would be the objects of the reaction.

As one will recall the actions of the two base-mutagens, 5-bromouracil and 2-amino-purine, were respectively specific for two small but different regions within the rII gene (a larger region). Since these are analogues, such specificity in their case would mean that 5-bromouracil only replaces thymine within a certain region, while 2-aminopurine can only replace some other base in a different region. In order for a 5-bromouracil to be able to replace a thymine base in a DNA chain, the thymine would first have to be excised by an enzyme. Only after this process could an enzyme then insert the 5-bromouracil into the space once occupied by the thymine. One and the same enzyme could very well be doing both the excising and the inserting. In this situation, it would seem highly probable that the enzyme rather than the mutagen has a specific affinity for a specific DNA regional shape. The enzyme, in this light, would be seen as having two types of specificity, one for the 5-bromouracil and another for the particular shape of the specific DNA region.

Indeed, it is well known that enzymes have sites or stereochemical configurations/.....

12. It is important to note in this regard that antigen - antibody specificity is believed to be based upon the shapes of antigen and antibody molecules.

:ations making them able to attach to two or three types of substrates, and no more. By virtue of a particular enzyme having a particular site or sites, such an enzyme would only have an affinity for a substrate molecule having itself a particular shape. For this reason different enzymes would respectively have only affinities for different sets of substrates.

Keeping this in mind, then, a specific enzyme could very well be seen as picking up a specific base-analogue-mutagen, carrying the mutagen to a specific DNA region for which the enzyme has also an affinity, and inserting the mutagen into that region, upon removing one of the bases from that section. In this connection, two different enzymes, specific for two different base-analogue-mutagens, say 5-bromouracil and 2-aminopurine, would be specific for two different sections or regions within a gene, by virtue of these different regions having different shapes. It would appear, therefore, that base-analogue-mutagens may be only <u>indirectly</u> specific, in so far as their specificity is dependent on or mediated by the activity of an enzyme. And yet, it is the very enzyme which is the major agency about the mutation. If anything, both the base-analogue and the enzyme should be regarded as dual agencies in bringing about this type of mutation within a DNA sequence of bases.[13]

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13. In this connection it must also be noted that a DNA sequence of bases com-:poses only one chain of the molecule. The molecule also has another chain of bases which complement with those of the other chain. The two complementary sequences of bases in each respective chain corresponds to the same code and also would represent two complementary continuums of graded shapes. If a given non-base-analogue mut-:gen modifies only one of these continuums in a specific region the other chain will have remained unaltered and a moisaic mutation would ensue i.e. only one half of the daughter cells from the original mutant cell will have altered DNA. Since most mutations are not moisaic it is quite conceivable for a mutagen not only having the specificity for one region in one chain but an additional spec-:ificity for the corresponding region in the complementary chain, as well. Being so, two molecules of a given mutagen each with dual specificities for the respec-:tive shapes of the two complementary chains within a specific region could be seen as acting simultaneously, or nearly so, in the respective chains, within the specific region..... However, in view of what has just been said about the role of enzymes in mutation activity, it/.....

mutation.[13] In this respect, the enzyme becomes in itself a mutagen, but indeed a much more sophisticated one than the ordinary type of chemical mutagen we have so far been considering.

Since such mutagenic enzymes are produced by genes, one would have to regard these genes as being another particular class of muterons, which in bringing about a specific mutation work together with the class of muterons which produce or predestine the existence of the mutagenic base-analogues. Even the deletions or addition of bases through the activity of a non-base-analogue, acridine, involves the direct assistance of enzymes, and such enzymes may, therefore, be regarded as auxiliary mutagens.

The enzymes thus far considered as mutagens appear to act only in conjunction with certain other mutagenic agents. However, in recent years, a class of enzymes have been discovered which act directly on the genetic material without the presence of other mutagens, such as 5-bromouracil and 2-aminopurine. These enzymes, though, have never really been regarded in the sense of mutagens, mainly because they negate or cancel out DNA alterations or damage wrought by such external agents as U.V. and X-rays. They act by restoring the original structure of the DNA (and hence the original code.) Such a process of restoration is in itself a mutational activity, and hence such enzymes, also referred to as repair enzymes, should be regarded as mutagens, or more precisely, as anti-mutagens, produced by still a different class of anti-muterons, other than that hitherto considered. The process whereby these anti-mutagenic enzymes act consists in excising the small section of altered DNA and replacing it with a particular sequence/.....

13. (contd.) it would seem more likely that once one chain, A, is altered within a certain section, X_A, an enzyme would then excise the corresponding section, X_B, from the complementary chain, B, and in place of the excised section would insert nucleotides which now would complement with those making up the substending section, X_A. The result would be that the base sequence of chain B would have been altered within the section, X_B. The nature of this alteration would hence correspond in code to the alteration effected within X_A. Therefore, in this situation an enzyme would also be acting as an auxialiary contributor to mutagenesis.

sequence of bases which corresponds to the original DNA code in that region. Some of these enzymes need light in order to carry this out and for this reason have been referred to as photorepair systems ~~by Witkin~~. Others do not require light and thereby have been referred to as the dark repair systems ~~by Whitehouse~~. These enzymes only ~~excise~~ sections which have been altered, and are, therefore, specific in their mutagenic (anti-mutagenic) activity. This would not be surprising if such enzymes have affinities only for shapes along the DNA molecule which can only come into being as a result of the realteration of DNA through mutation, as an alteration of DNA at a particular region would cause the creation of a new pattern of hybridization of electron orbitals at that section. The efficiency of a living organism would not only have such repair enzymes act on mutations wrought by U.V. and X-rays, but it would seem quite probable that an organism would have such repair enzymes efface mutations effected by the organism's own muterons.

If there be a class of enzymes which negate mutations, would it not seem possible that there also exists a class of anti-repair enzymes which cause mutations by not acting in conjunction with any other type of mutagen. Such independent mutagenic enzymes like anti-mutagenic enzymes (repair-enzymes), would excise sections of DNA, but unlike the altered portions chain excised by the repair enzymes, these sections excised by the mutagenic enzymes would be normal DNA sections, and, moreover, would involve both chains, not just one. The mutagenic enzymes would then replace such sections with different base pairs, or with the same base pairs but in different sequences, or may not replace the excised piece at all, and thereby produce a mutation in this fashion, as well. As one will recall, such a process whereby an enzyme or enzymes excised a section of one DNA chain and then replaced it with an abnormal sequence of bases was made in reference to a discussion pertaining to the action of a chemical mutagen, ethyl ethane/.....

ethane sulfate. However, in this case, such an enzyme only carries out this activity in conjunction with another type of mutagen and can therefore only be regarded as an auxiliary or associated mutagen. The independent enzymatic mutagens, on the other hand, would and could carry out this same process without the aid or stimulus of any other type of mutagen. Not only could such enzymes cause excisions, but they could also effect the inversion of small portions of DNA involving only three or four base pairs. Moreover, they could effect the exchange of different small portions of DNA from one region to another region, such portions capable of being as small as one base pair.[14] This would entail the DNA molecule taking on the status of a substrate on which different enzymes are able to act. It is well known that certain enzymes break down DNA. More important, it is known that certain enzymes act on DNA by attaching glucose and cellobiose to its OH groups. This occurs in the T-even series of bactereophages, and demonstrates that DNA is capable of acting as a substrate in enzyme - catal-:yzed reactions other than degradation. In this connection, other enzyme-catalyzed reactions could involve the conversion of one specific base into another, thereby serving to alter the code.

One can think of these independent mutagenic enzymes as being specific, as mutagens, in the same way as the ordinary chemical mutagens previously discussed. A given independent enzyme would only have specific affinities for two or three different shapes which only exist at specific regions along the DNA-continuum of graded shapes, and would therefore only be able to effect exchanges between these particular regions. Such exchanges could conceivably occur as well, between specific regions of different and non-homologous chromosomes. Conceivably, one of the enzymes could also excise a portion of a chromosome, then translocate such a/.....

14. The exchange or reshuffling of nucleotide pairs can be referred to as transnucleotidation.

a section to the homologue (or non-homologue), and in turn attach it to the homologue, thereby creating a duplication of certain genes or base sequences in the homologue. An exchange in such a case would not be reciprocal.

A variant type of independent enzyme - mutagen could be seen as only being able to bring about an inversion involving only a specific region, in as much as it has an affinity only for that region, whilst another type of enzyme could only excise a small section of DNA from a specific region, and in turn replace the excised portion, which is disregarded, with an abnormal sequence of bases, obtained from the purine and pyrimidine pool of metabolites. The nucleotides may be inserted individually and therefore randomly; or even as likely the nucleotides may at first, be coupled together randomly into groups, or sets of three or four by one enzyme,[15] and then inserted as groups or sets by a differ-:ent enzyme. More specifically, different sets of nucleotidal structures would be synthesized and a pool of them eventually formed. The inserting enzyme would be specific for only one set of a specific sequence, as well as a set which is complimentary to the first, and thus would only couple itself to these two com-:plimentary sequences. The enzyme would then join the sequences into a duplex, wherein their respective bases compliment with one another. Upon the completion of this, the enzyme would then insert the duplex-sets into the place from which the original excision was made, thereby producing an alteration of the DNA code at that particular region.

In fact, there does exist in cellelar systems, twenty or more variant enzymes, of which each is specific for a particular sequence of ribonucleotides. These different sets of joined ribonucleotides are known collectively as t-RNA. The enzymes/.....

15. Indeed, Ochoa has discovered an enzyme which couples nucleotides together randomly without regard for a template.

enzymes are specific in that each of the twenty attaches respectively a specific amino acid to a specific t-RNA molecule. The importance of such a system from the standpoint of this discussion is that a variation of such a system could be extended quite readily to include different enzymes being specific respectively for different sets of joined nucleotides, while at the same time having respective specificities for different regions of the DNA molecule.

In looking back over the independent enzyme-mutagens discussed, one may put them into different classes. One class would only bring about inversions; another would only eventuate exchanges; still another would only modify the bases; while yet another would only mediate the insertion of specific base-sequences, upon excision. Each member of each class would act only on a specific region (or regions) of the DNA molecule. The possible specificity of independent enzyme mutagens would be quite in fitting with the general character of the enzyme, in so far as the importance of an enzyme, in part derives from that chemical fact that a given enzyme can only be specific for a few substrates. Considering a substrate as large and as complex as DNA, one is able to think of different enzymes, each of which acting specifically on different regions of the molecule.

These independent-acting-mutagenic-enzymes would each, as the non-independent-acting-enzymatic mutagens, be produced by a specific muteron, but it is also possible that a non-mutagenic enzyme could be converted into an independent or non-independent enzyme-mutagen by its being acted upon by yet another enzyme. In this situation, one enzyme would be serving as a complex substrate for yet another enzyme to act upon, with the result being that the substrate is converted into a different molecule. The enzyme effecting the conversion or modification of the other enzyme could in a sense be regarded as an indirect-enzymatic mutagen, in as much as it is effecting the creation of a mutagenic enzyme. The phenomenon/.....

phenomenon of one enzyme serving as the substrate for as yet another enzyme does exist and an example of this phenomenon shall be given later, as well as the general implications of such a phenomenon as they relate to mutational activity.

Up to now no direct evidence has been cited in this paper which shows that mutational activity of some or many genes is under the control of, or is determined by mutator genes (or muterons). However, there appears to be some evidence for their existence in lower organisms. Yanofsky, in 1965, discovered that a certain gene in E. Coli and Salmonella induces transversions in a particular region (gene). He infers that it acts through or via the production of some mutagenic substance, because it also induces mutations inphages residing in the bacteria. It is quite possible that such a mutagen is an enzyme. In T-4 phage, ~~according to Auerbach~~, one of the genes which controls DNA replication by producing a DNA polymerease can undergo a mutation with the result that it proceeds to produce an abnormal or altered DNA polymerease, which, in turn, causes mutations in other parts of the phage DNA during replication, by inserting incorrect bases. This would be a good example of a mutator gene. Also, it shows that a non-mutator gene can be converted into a mutator gene through a mutation in the former. Furthermore, this would be a clear example or support for the existence of mutagenic enzymes, and more important, a beautiful example of a DNA molecule causating a mutation within itself. In this connection, it would seem quite feasible that a specific gene (or a section of DNA) could bring about a mutation within itself by producing a mutagenic enzyme which only acts under certain temperature conditions, while it is inactive under others. This may, in part, explain why changes in temperature bring about mutations. When and if a gene does bring about its own mutation, it might, as a result, produce another type of mutagenic enzyme which is capable of bring-ing about another mutation in a different portion of the gene. Again the result may be the production of still another type of mutagenic enzyme which in turn/.....

turn would modify or alter the gene in still another way; and the latest altered form would produce still another type of mutagenic enzyme ... and so on. Such a cycle whereby a gene self-induces mutations within its own structure could go on for a great length of time until a non-mutagenic enzyme is produced. And if such a non-mutagenic enzyme proves highly beneficial for the survival of the organism, the gene in its latest form or structure would be selected for and hence be maintained in the genome over many generations. A gene, capable of effecting mutations within itself, could be regarded as a self-mutating muteron, and the genome may be made up of a fair number of these self-mutating muterons.

It has been known for some time that some parts of a genome are not necessarily chromosomal in nature but represent motile bodies containing genetic material residing in the cytoplasm and transmitted outside the nucleus to the progeny, by means of the egg cytoplasm in higher organisms. Such bodies have all the properties of chromosomal genes in that they are a stable system of self-perpetuating bodies with clear effects on the phenotype. Moreover, such bodies or episomal genes are capable of mutation. Chloroplasts the effectors of photosynthesis are one good example of such bodies ...

> "In maize, Rhoades has made a study of the relation between a plastid abnormality [a mutated episomal gene] and a regularly transmitted [chromosomal gene]. He found that maize plants homozygons for a recessive allele ij (iojap) in chromosome VII may be variegated, with yellow or white ... striping, or are all white in which case they die as seedlings, owing to the presence of defective [mutated] colorless plastids [which are unable to bring about photosynthesis.] Reciprocal crosses of ij/ij striped plants with normal green (Ij/Ij) ones give different progenies ... When normal green plants (Ij/Ij) are used as females and pollinated by pollen from ij/ij (striped) plants, the F_1 plants, Ij/ij are whole green (normal); but when ij/ij /striped plants) are pollinated by Ij/Ij pollen for F_1 plants (Ij/ij) are of three different kinds; normal green, striped and all white (non viable) seedlings. Whether or not the F_1 plants, all of which have the same [chromosomal] genotype, Ij/ij develop varigation obviously depends on the ovules from which they arose, that is/.....

is on the source of the egg cytoplasm ... When the striped F_1 plants, ... as females, are test-crossed with normal Ij/Ij, as males, they produce in some cases only normal green, in some cases striped, and in others only white seedlings. Some of the latter are Ij/Ij, but even when they lack the ij allele, they inherit the abnormal [mutated] plastids. Rhoades has given additional evidence for the conclusion that under the influence of ij/ij [chromosomal] genotype, plastids [episomal genes] may become permanently changed to the colorless state and that this change [or mutation] is perpetuated when the allele responsible for the change [mutation] is replaced by its normal allele."[16]

Hence the preceding would be a good example of a gene (ij) in a higher organism causing or inducing a mutation in another gene even though the latter happens to be a motile episomal gene transmitted via the cytoplasm.

Even more striking would be the induction of a mutation or mutations in chromosomal genes by episomal genes (episomes). In fact, there is good evidence for the existance of such a phenomenon.

"Dawson and et al have described a leucineless strain of Salmonella typhimurium infected with an episome which caused a high frequency of mutation to prototrophy ... An episome influencing the mutation rate for streptomycin resistance in E. coli has also been described [by Gunderson in 1963] ... Ginozoa and Painter described ... a resistance-transfer-factor [a resistance transfer episomal gene] which carries information for moderate drug resistance to chloramphenicol and dihydrostreptomycin, and mutability to high-level resistance. After elimination of the episome from high level resistant strains, a low, non-transferable resistance was generally retained. It was deduced that high level resistance was obtained by coping the information for resis-:tance carried by the episomal particle [gene] in the chrom-:osome."[17]

These episomal motile mutator genes or muterons would represent still a different class of muterons from the ones previously considered. The latter represent non-motile mutator genes which make up permanent sections of the chromosomes./.....

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16. Dobzhansky, Principles of Genetics.

17. D. Sompolinsky et al, "Transferable resistance factors with mutator effect," "Mutation Research", March/April 1967.

chromosomes. Moreover, they only appear to be capable of producing mutations in other genes through the production of some type of mutagen e.g. mutagenic enzyme, and therefore their mutator effects are not brought about by the muterons making direct contact with the gene that is to be mutated. However, as we have just seen, in the cases of some episomal muterons, the mutator effect only takes place through the direct physical contact of the episomal muteron with the gene to be mutated.

In maize, McClintock has found strong evidence for the existence of episomal muterons or what she refers to as "controlling elements". They reside in the nucleus and effect mutations by making direct physical contact with the region of the chromosome to be mutated. These episomal muterons of which some may be composed of RNA, a slightly variant form of the genetic material, are capable of moving from one part to another of the same chromosome, or from one chromosome to another. A given controlling element is able to insert itself into a given gene or chromosomal region and produce a mutation thereby. Upon effecting the mutation, the episomal muteron can then transpose itself to another gene (or region) on another chromosome, and in turn, effect a mutation there. Such controlling elements can easily assure their transmission to progeny by attaching themselves to gametic chromosomes. During such a period, the episomal muterons would be in an inactive condition, wherein they would be incapable of effecting mutational activity.

McClintock, in fact, believes that certain other episomes in the nucleus have the regulatory ability to switch on or off the mutation inducing activity of the episomal muterons once such muterons make contact with the chromosomal gene. Furthermore she believes there is evidence for different regulatory episomes being specific respectively for different classes or groups of episomal muterons. The episomal muterons do not have the ability to switch themselves on or off, and once a/.....

a given muteron is switched off it must await the presence of a particular regulatory episome for it to be switched on de nova, even though the episomal regulatory element does not have to necessarily situate itself adjacent to the place where the episomal muteron is inserted. The absence of a particular regulatory episome, as a result of its becoming lost, may leave the episomal muteron permanently switched off until the particular type becomes present again, through a sexual fusion, or even through one of the other regulatory episomes itself undergoing mutation. In fact, it appears that regulatory episomal genes can mutate to episomal muterons and vise versa, though once a regulatory episome becomes transformed through mutation into an episomal muteron, it cannot switch on or off neither itself nor the activity of other motile muterons. The process whereby a regulatory episome becomes an episomal muteron can quite conceivably be effected through the action of a mutagenic enzyme produced by a non-motile chromosomal muteron. It is also known that a class of regulatory episomes are able to activate or inactivate chromosomal genes by inserting themselves in such genes. Though McClintock believes that the activities of such a class is itself under the control of still other regulatory episomes, it is quite conceivable also to see such a class as operating, at times, independently of other regulatory episomes. For example, one can see such a class as being divided into two sub-classes, one of which contains regulatory episomes which only inactivate active chromosomal genes, and another which only activates inactive chromosomal genes, by enabling such chromosomal genes to produce M-RNA. A specific type of inactivating episome could only inactivate a specific group of chromosomal genes, whilst only a specific activating episome could re-activate such a specific group by transposing itself from one inactive (or switched off) chromosomal gene to another. Such a class of regulatory episomes should be distinguished from the regulatory genes of the Jacob-Monod system, in so far as

the/.....

the regulatory genes of the Jacob-Monod system are chromosomal genes. It is such a system which would normally control the activity of the chromosomal muterons, but it is also possible, as well, that the episomal regulatory genes are capable of inactivating or activating chromosomal muterons.

Once activated specific chromosomal muterons could cause mutations in episomal regulatory genes, and in so doing, cause them to be converted into episomal muterons, or into episomal anti-muterons, the latter being either capable of negating mutations eventuated by episomal muterons or those wrought by chromosomal muterons. By the same token, some episomal muterons could be capable of converting chromosomal muterons into chromosomal anti-muterons, and vise versa. A specific chromosomal muteron could as well be mutated by a specific episomal muteron into a different chromosomal muteron, capable of producing another type of mutagenic enzyme, as a result. Of course, in this light, it is also conceivable of variant chromosomal muterons acting on one another via mutagens, with the result that they are all mutually converted into other divers types, capable of producing different mutagens as from before, with the ultimate result that an entire new set of genes, or regions thereof, are opened to mutational activity. Some of the latter may themselves be converted into chromosomal muterons, through such mutational activity, and in turn, either produce mutagens which open still other genes to mutational activity or produce mutagens which mutate muterons.

Also, we have seen that a few or even many chromosomal genes could be self-mutating muterons with the result that almost a non-terminating collection of self-mutating cycles is capable of being brought into existence. Such cycles could only be broken through the production of non-mutagenic enzymes, a change in temperature, or through the intervention of either chromosomal anti-muterons or episomal-anti-muterons. Moreover, such cycles could be induced by muterons mutating/.....

mutating genes into self-mutating chromosomal muterons. It is also conceivable that some of the mutagens produced by self-mutating muterons are specific for other genes, and when such genes mutate upon the action of such mutagens, they may as well become self-mutating muterons. These in turn could produce mutagens capable of inducing still other cycles of self-mutation, though some mutagens produced may transform some chromosomal muterons into chromosomal anti-muterons capable of breaking the cycle. Furthermore, it is feasible that not only could a group of self-mutating muterons be undergoing mutation as a result of the behaviour of their own respective self-produced and self-acting mutagens, but also as a result of these very same self-produced and self-acting mutagens acting, as well, on the self-mutating muterons in the group which are not their respective producers. However, such a mutual influence of self-mutating-muterons on one another during the cycles of self-induced mutation may not be general, but circumscribed to only a small group of self-mutating muterons.

A mutational cycle in an organism may not only be of the self-mutating-muteron variety, but include a system of many elements capable of inducing in one another an endless round of mutations. A simple example of such a system may include one episomal muteron, termed A, one episomal anti-muteron, termed B, two chromosomal muterons, termed F and C respectively, one chromosomal anti-muteron, termed D, and one normal structural gene - a gene which is neither a regulatory gene nor a muteron (or anti-muteron) - termed S_1. The cycle may commence by C inducing a mutation in S_1, only to be negated by the action of D. However, A may then induce another mutation in S_1, but this in turn may be altered to S_2. Upon this happening, C may effect a mutation in F, transforming it into F_x. F_x may then induce a mutation in S_2, causing it to become M_x a muteron. The muteron, M_x, may then act on C, transmutating it into C_x. C_x may, in turn, mutate A into A_x and A_x may in turn mutate M_x into M_z, still another muteron, but a/.....

a muteron capable of self-mutation. M_z may then proceed to mutate itself into M_{z1}. M_{z1} may then mutate itself into M_{z2}, M_{z2} into M_{z3}, and M_{z3} into M_{z4}, and so on. Upon the creation of M_{z20}, the gene would no longer be self-mutagenic, but act as a normal muteron.

The mutagens produced by M_{z20} may then act on A, converting it into A_2. A_2 may then act on D, converting it into a muteron, D_m. D_m may, in turn, act on A_2, converting it into A_3, and A_3 may then act on M_{z20}, converting it into Z, and this in turn ... and so the cycle would continue. The system would not involve all elements at all times and at one point may temporarily create a sub-self mutating muteron system which, in effect, ultimately proves to be a major factor in the continuance of the overall cycle. It is also important to add that mutagens produced by this particular cycle may as well influence other mutational cycles, and after a time, such a particular cycle may as well be influenced by cycles outside itself. The number of possible multifarious mutational cycles which could be in existence is high indeed, and time does not allow for all of their respective descriptions.

Though, it must be said that the same type of specificity applies to the interaction of muterons, as between the interaction of muterons and structural genes. A specific episomal muteron would be specific for a given chromosomal muteron by the virtue of the latter muteron having s specific shape in one of its regions, for which the episomal muteron has an affinity, and to which it is drawn as a result. However, we have described cases where a given episomal muteron is capable of causing mutations on a small number of different chromosomal genes. This would be possible if these chromosomal genes have in common a region with a similar shape. In such a case episomal muterons could best be described as being specific for only a particular group of chromosomal genes, albeit there may exist as/.....

as well a fair number of episomal muterons, each capable of causing a mutation in only one specific chromosomal gene, and in no other.

The presence of variegated muterons capable of inducing cycles of mutations may lead one to believe that the frequency of mutations is much higher than we suspect. The reason as to why one would not suspect a high frequency of mutations is simply that many of such mutations would not manifest themselves in physical or morphological terms, but in unobservable metabolic modifications which are not necessarily accompanied by morphological changes.[18] In fact, a high degree of mutational activity lasting continuously, over many long periods of the organism's adult life may be the rule rather than the exception. Any deleterous mutations, arising, could in many cases be negated through anti-muteron activity; the gene then could be mutated by another muteron, with the result that in this case, a beneficial mutation is produced. In the case of higher organisms such intense mutational activity may occur more often after the organism has passed through its embryological stages so as not to interfere with the developmental processes which are under genetic control.

Such developmental processes necessitate specific combinations of genes to be activated at specific times and in specific regions of the organism. Specific combinations of genes acting at specific times and regions results in cells of differential structure and chemistry at different times and in different regions, and such differentiation leads to the formation of different organs and organ systems.

At a very early stage development any group of cells has the potential or competence to development into any future organ of the mature organism. However, at/.....

18. Also, many genes occur in duplicate, one copy (allele) residing on each homologous chromosome. If mutations occur in only one member of each respective pair, with a subsequent inactivation of such mutant genes by controlling (regulatory) genes, and if, furthermore, the non-mutated copies continue to be active, then the existance of the new mutant genes would not be noted.

at a much later period of development a given group of cells loses such toti-potentiality, and thereby has its potential restricted or delimited to the capacity of only being able to follow one specific developmental pathway, out of many divers ones. Such delimation or restriction of developmental potential of a group of cells is believed to be due to many genes in those cells becoming permanently inactivated or switched off, genes, which if active, would give the cells the potential to follow other pathways leading to other organs.

An organ is the result of a temporal sequency of specific combinations of acting genes. During development, the specific combination of genes acting at any period of time is different from a combination acting at any other time. Each specific combination of genes acting in a cell produces a specific synthetic effect or product, that is to say, the effects or products produced by the genes in turn modify one another, producing a resultant effect, which is not usually the same as that produced by another combination. The same active gene could be a part of a temporal sequence of three different combinations, and produce a different effect in each. Actually, it would be more precise to say that the product produced by gene A becomes modified in three different ways when gene A is a part of three different combinations of acting genes. Therefore, three different gene combinations would serve to produce at three different times, three different products. The final modified or resultant product at one of these times would owe its existence, as a modified product, not to one gene, but to more than one gene. In this sense, the specific combination of genes must be regarded as a complex unit which effects into existence a specific modified product. For this reason, such a complex unit must in a sense be regarded as a gene. However, its existance as a complex unit would have temporal boundaries within the cell for at time t_1, it would exist, but at time t_3, it would cease to exist, being replaced by or changed into another specific combination of activated/.....

activated genes, or complex-unit. Because of the impermanent or transient or changing nature of such a complex-unit, I have decided to refer to such a unit as a fluxon. The fluxon would advert to another meaning of the term "gene".

A given cell may have two or more fluxons in existence at the same time, and they would be delimited from one another by virtue of their respective resultant products, in turn neither modifying one another nor working together as a team of enzymes on a series of chemical steps leading to some other product.[19] If such enzymes (products) did work together, then the fluxons producing them must, themselves, be regarded as making up even more complex fluxons. Moreover, one of the enzymes involved in the aforementioned sequence of chemical steps may also be involved in another sequence of steps leading to a different product. If this be tne case, the fluxon producing this enzyme must itself be regarded as making up two different complex fluxons. Also, in this connection, if the resultant products created respectively by three different fluxons eventuated in a temporal sequence, in turn, modify one another, after such fluxons are no longer in existence, the fluxons, at any rate, must be regarded as if in existence and as if in existence and as if making up a complex fluxon because of tne interaction of their respective products.[20]

At a given time, in a given region, a group of cells would have the same fluxons, while at another region, a group of cells would exist in which each member of the group would have the same fluxons as the others in the group, but the fluxons of this latter group would be variant from those of the first group mentioned. In addition, fluxons would be variant from region to region, whereby the/.....

19. Also, if the products of two or more different fluxons combined together to form a resultant complex product, different in properties from that of any one of the individual products, then such fluxons must be regarded as making up one complex fluxon as opposed to their being delimited from one another.

20. A fluxon may be equivalent, also, to one activated chromosomal gene if the product of that gene is not modified by other genes.

the chemical products produced in any one region would be different from most of those produced in any other region.

At time y, each of the cells of region K, could be seen as having fluxon 1, each of those of region L, fluxon 2, each of those at region M, fluxon 3. However, at time Z, each of the cells of region K, would have fluxon 10, each of those at region L, fluxon 20, and each of those of region M, fluxon 30. The existence of a given fluxon at a given time and space (region) would be determined or eventuated by the chromosomal regulatory genes of the Jacob-Monod system. In this connection, a substance or substances, produced in one region, P, could enter the cells of another region, U, and in turn induce specific operator genes to switch on (or off) the particular structural genes over which they exercise control, thereby producing new fluxons in that region, and as a result, new products. Some of these products may then move into another region of cells, R, and induce the creation of new fluxons within that region, by inducing certain (specific) operator genes to switch on the particular structural genes which they control, while inducing other specific operator genes to switch off other particular structural genes. Some of the products of the new fluxons in region R may then move into region P, and induce new fluxons there. Some of the new products produced in P may henceforth move into region U, and induce new fluxons to form in that region, wherein new products would soon ensue. Some of these products may enter region R with the induction of new fluxons, ensueing as a result . Some of the products of these latest fluxons of this region may then enter region P, again, and causate the formation of still newer fluxons in the region, and the products of this may enter region U ... and so on. Such a cycle may be one of many divers ones as may exist in developmental systems.

As was implied earlier, a cell is able, as well, to self-induce a temporal sequence of variant fluxons, therefore not requiring inducing-substances produced by/.....

by other cells of other regions. Such a system of self-induction would of necessity be cyclical. The cycle may begin by a fluxon A producing a substance X. X then induces the creation of three new fluxons, B, C, and D, and the termination of fluxon A. Fluxon C produces a substance, K, which induces the formation of fluxons S, T, V, and Y. Fluxons V and Y may then produce substances which cause the termination of fluxons C and D, and the induction of fluxons E and L. The substance produced by L may then induce the formation of still other fluxons and the termination of others ... and so on.

Specific substances would be drawn respectively to specific operator genes by virtue of the same mechanism which determines the specificity of mutagens; though, with regard to the preceding paragraph, it would seem as likely that a given substance could be specific for a particular group of operator genes rather than for just one particular operator gene. Of course, another given substance may only have an affinity for one particular operator gene.

The inactivation or activation of structural genes may not only be attributable to their being switched off or on by operator genes. For example, a fluxon of one chromosomal gene may produce polypeptides which are able to attach themselves length-wise to five different sequences of bases corresponding to five specific genes, termed A, B, C, D, and E, respectively. By attaching themselves to the bases of these genes, they prevent such genes from producing M-RNA. Since the genes are hindered from producing M-RNA, they must be regarded as inactivated. The inactivation of these five genes by the process results in a new combination of genes interacting together, and therefore a new fluxon. However, at a later period of time, a chromosomal gene may produce an enzyme capable of severing the protein from the bases of genes A and D only, thereby activating those genes, with the resultant creation of a new fluxon. Still, at a/.....

a later period of time an enzyme may be produced which is capable of severing the protein from the bases of gene C, allowing for its activation, while another fluxon produces a protein which attaches to the basesof gene D again, resulting in its inactivation.

The activating enzyme which severs the protein from a gene's bases would have a specificity for that particular gene, or maybe even for two or three additional genes. The mechanism of this specificity would be the same as that on which the specificity of mutagenic enzymes is based.

The action of a mutagenic enzyme on a particular chromosomal gene, making up a particular fluxon, would result in the creation of a new fluxon, not normal to the organism. For example, if a fluxon is composed of the acting genes, A, B, and C, and if a mutagenic enzyme acts on C mutating it to D, the fluxon A-B-D, would thenceforth result, and in turn, a new product. Such a mutational event, and tne consequent production of anabnormal fluxon (that is a fluxon which is not only unique for tnat particular organism but is temporally and spatially out of place, as far as tne normal developmental pattern is concerned) may very well result in a slightly abnormal phenotype.

Moreover, a mutagenic enzyme would act on a given operator gene, mutating it to tne O^c state. In such a state the O^c gene cannot be induced to switch off the particular structural genes which are under its control. If the existence of a particular fluxon calls for the switching off of ~~these~~ two structural genes, such a particular fluxon could not come to be, and in its place, therefore, an abnormal fluxon would be produced.

As we have recently seen, new fluxons, as a part of normal development, may be created by specific enzymes activating specific genes through the severing of proteins/.....

proteins from the bases of such genes. At a specific time, a given normal fluxon might be created due to the severing of a protein from the bases of a specific gene, S, by an enzyme produced by a particular gene, P. However, gene P may mutate to Z as a result of muteron activity. The consequence of the mutation would be that the gene may produce a different enzyme. The enzyme, being different, may be specific for a gene other than S, let's say T, and would then proceed to sever a protein from T's bases thereby activating it. The activation of T rather than of S would serve at that time to produce an abnormal fluxon. The creation of abnormal fluxons must be regarded as the creation of complex-mutations. The basis of such complex-mutations would ultimately rest on mutations having been induced in chromosomal genes. As such, these complex-mutations (abnormal fluxons) would most likely appear again at the corresponding developmental stage(s) of the organism's progeny.

Upon an organism having achieved its developmental maturity, certain chromosomal genes would have become permanently inactivated, while other chromosomal genes would have become permanently active. Yet, what delimits one active chromosomal gene, especially when both are adjacent to one another? Earlier in this paper, a gene was defined in one sense as being an arbitrary section of a chromosome, and as we know, each section of a chromosome corresponds to a specific sequence of purine and pyrimidine bases. If this is so, then each specific and adjacent gene making up a chromosome would be equivalent to a specific sequence of bases. But, since these specific sequences of bases are continuous with one another, for in as much as they are but parts of an overall continuum of a specific sequence of bases, which corresponds to the chromosome as a whole, what would serve to delimit or demarcate one sub-sequence from another? Enzymes may be the agencies involved in such demarcations. Different enzymes would be inserted/.....

inserted by other genes at specific points or positions along the chromosomal continuum of bases, thereby sectioning off specific sub-sequences of bases. The transcription of a particular sub-sequence (gene) into a particular chain of M-RNA would then commence at one enzyme, and proceed unidirectionally to the position of another enzyme, where such transcription would be hindered and in turn, terminated. The specific M-RNA thus produced would then proceed to the ribosomes. The particular code of the M-RNA would there be translated into a specific polypeptide chain made up of specific amino acids arranged in a specific order. Sectioning in a fair number of cases may also be eventuated by enzymes inserting small polypeptides of three or four amino acids at different positions along the continuum of bases. Such would hence also serve as commencing and terminating points for M-RNA transcription. The small polypeptides could be obtained through the degradation of proteins.

As long as the enzyme and small polypeptide demarcators remained fixed in their positions they would be transmitted along with the chromosomes to the next generation, and by the same token, the particular base-sequences demarcated by these fixed delimitors would as well be transmitted. The size or length of these different sections (genes) would not necessarily be equal, and some may consist of thousands of bases, while others may only consist of one hundred. The result would be the production of polypeptide chains of different length.

However, not all of the enzyme and small polypeptide demarcators may remain respectively in one fixed position throughout the organism's lifetime, but be moved from one position to another by a specific class of enzymes, the re-positioning enzymes, with the result that new sections are produced made up of groups of new base sequences. These latter groups of base-sequences would be different from any particular base-sequence group contained in a section that existed before the re-positioning. The result of such re-positioning would be shown in the production of/.....

M-RNAs different in bases sequence from those produced before. The following diagrams may make the preceding more clear:

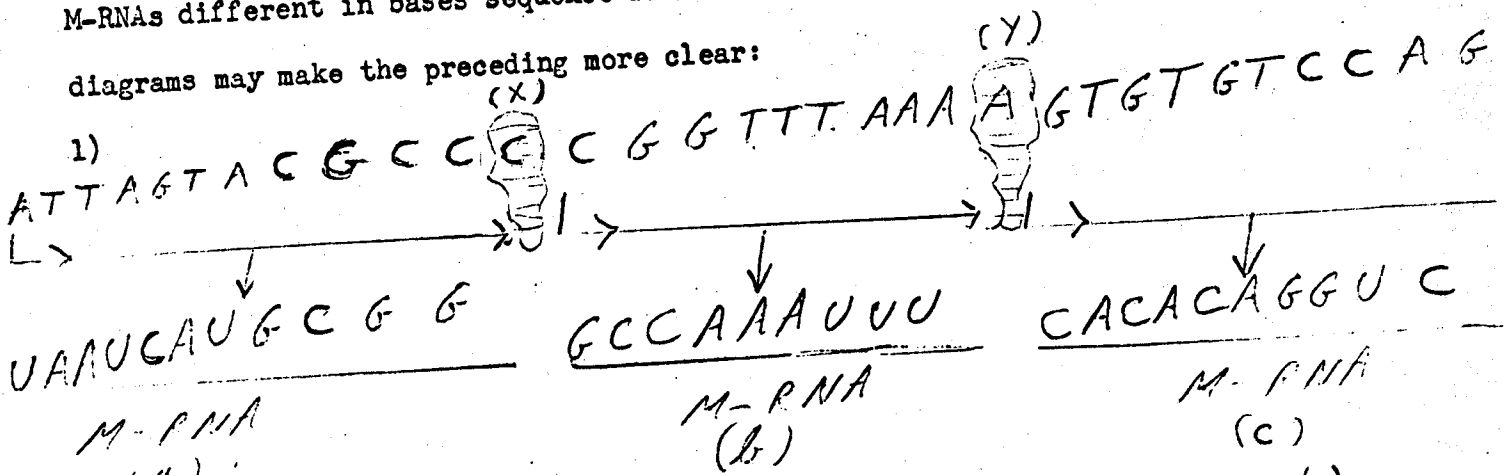

Diagram one is symbolic of one chromosomal continuum of bases with enzymes (x) and (y) inserted respectively at different points along the continuum. The formation of M-RNA (a) is shown as commencing at the beginning of the chromosome and terminating at enzyme (x). The formation of M-RNA (b) is shown as commencing at enzyme (x) and as terminating at enzyme (y). The transcription of M-RNA (c) is shown as commencing at enzyme (y) and as terminating at the end of the chromosome.

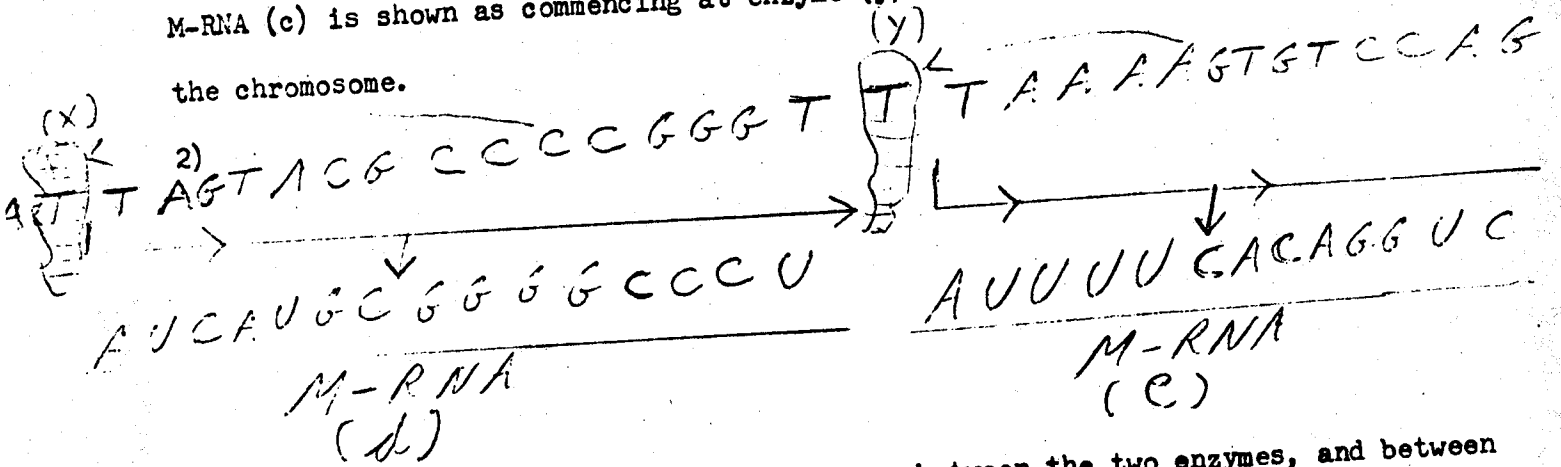

Diagram two shows that the sequence of bases between the two enzymes, and between an enzyme and the end of the chromosome is different from before, as a result of the enzymes demarcators having been re-positioned by other enzymes (enzyme re-positioners). The result is manifested in the transcription of only two M-RNAs, (d) and (e), different in their respective codes, and different, as well, in their codes from the three M-RNAs produced before the acts of re-positioning. A chromosome would have thousands of more bases than depicted in the diagrams, and/.....

and the re-positioning may involve more than two enzyme (or polypeptide) demarcators.

The permanent removal of some of these demarcators would result in longer sections, and as a result of such a lengthening of sections, new overall base-sequences would have come to reside between the demarcators remaining. The creation or inclusion of new base-sequences between given demarcators can, thence, not only come about through the re-positioning of such demarcators, but through the permanent removal of some, as well. If a specific gene is equivalent to a specific sequence of bases, as defined by the specific positions of two enzymes, or by the specific position of one enzyme and the one end of the chromosome, the re-positioning of those enzymes or the permanent removal of one or the other of those enzymes would mean the termination of the existence of that specific gene. Yet, the very termination of that specific gene would be commensurate with the creation of new base-sequences between some of the demarcators as a result of the re-positioning, and such a demarcation of new base sequences would be equivalent to the creation of new genes.

The re-positioning of some demarcators, or their removal, and the concomitant termination or creation of particular genes, which is the result, may occur within certain periods of development, and, as a normal aspect of such development.

At certain periods of development repositioning-enzymes would be produced which would reposition the enzyme and polypeptide demarcators. Each of the re-positioning enzymes would be capable of moving only one specific demarcator, and to only one specific position. Each specific period would see the production of only two or three re-positioning-enzymes. After having brought about the re-positioning at a particular period the two or three repositioning enzymes would be degraded.

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At a particular period of time, therefore, certain genes would be terminated, while others would be created in the very termination of the former. At a later period of time, the latter would, as well, be terminated, and others created as a result. The existence of some particular chromosomal genes in a given organism would thus be transient. The existence of some particular chromosomal genes, and the length of time allowed their respective existences, would ultimately be determined by, or conditional on, the existence of still other genes, in as much as these latter genes would be bringing into existence the re-positioning-enzymes. Most chromosomal genes I suspect would not be transitory in nature but remain in existence throughout the life of the organism, in so far as the majority of demarcators defining their respective existences would normally remain permanently fixed in their respective positions, except during the times of chromosomal replication, when they would be moved temporarily from their respective positions so as to allow the replication to proceed. As for the new or replicated chromosomes produced, a new assortment of demarcators would be inserted into the normal positions.

The termination or creation of some genes as due to the re-positioning of demarcators cannot be regarded as mutations since the "terminated gene" would reappear again at a specific period during the development of the progeny. In the progeny, its existence would soon be terminated again, only to reappear de nova at a specific time in the development of the progeny's off-spring ... and so on. The termination and creation of the same transient genes at the same particular times would be repeated again and again in succeeding generations. The continual, though, short existence of the <u>same</u> transient genes in subsequent generations illustrates a lack of mutational activity as far as these genes are concerned.

The phenomenon of a transient gene being created and then terminated after

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a short period of time is highly analogous to a non-transient gene being switched on, or activated, at a certain period of time, and then switched off at a later period of time. When a non-transient gene is in the inactive state, it is as if the gene does not exist, since there would be no way of detecting its existence while it is inactive. When such a gene is switched on it is as if it were created anew, since its existence may now probably be detectable. When such a gene becomes switched off de nova, it would be as if its existence was terminated. From the point of view of a gross observation, the distinction between a transient gene and one which at one moment is switched on, and at a later moment is switched off, would not be clear. In fact, because of our imperfect perception, what in some cases we refer to as a gene being activated may be in reality a transient gene. A transient gene would refer to still another meaning which can be applied to the term 'gene'. The length of a transient gene or section may be as small as to only include three nucleotides, or what is also referred to as a codon. In some cases it may even be as small as to only include two nucleotides. The M-RNAs of codon length may then be joined end to end by certain enzymes, forming, thereby, a longer M-RNA or a new sequence. More in this regard shall be elucidated soon.

If a gene producing a specific re-positioning enzyme, X, was mutated by a muteron, a different type of re-positioning enzyme, y, would be produced by the gene, as a result. Being different, it may move demarcator P to position R, whereas normally at the time enzyme x, if it had been produced instead, would have been specific for demarcator S, and would have moved it to position T. The result of this production of a different (mutant) re-positioning enzyme with specifities different from that of a non-mutant re-positioning-enzyme would be the causation (creation) of transient genes which are not otherwise created at that specific period of development, if at all. Such abnormal transient genes produced/.....

produced by a mutant re-positioning-enzyme must be regarded as being themselves mutations. Different groups of mutant re-positioning-enzymes produced during variant periods of development would cause the formation, in those respective periods of development of many abnormal transient genes or mutations. As such, these mutant re-positioning-enzymes must themselves be regarded as mutagens, and the mutant genes producing them, as muterons. Also in some cases, a few mutant-demarcator enzymes may be produced which are capable of re-positioning themselves. This, as well, would result in the production of abnormal transient genes. It is quite possible that two or three mutant genes (muterons) may together contribute to the formation of one mutagenic-repositioning enzyme (or mutagenic-demarcating enzyme). For this reason, these genes (muterons) must be regarded as making up a fluxon, and in this case, a fluxon becomes equivalent to a complex-muteron.

One may wonder as to the specific mechanism whereby three or four active genes working as a fluxon (or complex muteron) may produce some given protein or enzyme. One possible variation of such a mechanism may be as follows: A gene A produces polypeptide A. Gene B produces a polypeptide B. The two chains combine producing a two-chain resultant product E. Gene C produces a polypeptide which happens also to be an enzyme, C. C then acts on E converting it into another type of protein, S, which happens also to be an enzyme capable of acting on C. S then acts on C, modifying it into protein X. X then combines with S, producing still different protein, Z, as a result. Z would be the final resultant product of the genes A, B, and C.

It appears that the production of Z involved the alteration of the primary amino-acid sequences of the respective polypeptides, after such primary sequences were/.....

were formed on the ribosomes.

"There are a number of good examples among the proteins which illustrate processes of alteration which occur after formation of primary amino-acid sequences. A case in which a good deal of detail is known is that of chymotrypsin. This proteolytic enzyme is derived from the zymogen chymotrypsinogen through specific actions of proteolysis. Activation can occur slowly through the action of trypsin to give α-chymotrypsin or by a fast sequence involving auto-catalysis through the steps:

1) Chymotrypsinogen $\xrightarrow{\text{Trypsin}}$ π Chymotrypsin

2) π Chymostrypsin $\xrightarrow{\pi\text{Chymotrypsin}}$ δ Chymotrypsin

... Here it may be presumed that the single primary chain of chymotrypsinogen is that designated by DNA through M-RNA, but this is not the functional enzyme. Full activity is achieved, however, by the splitting of one peptide bond (Ileu-Arg) [by trypsin] giving two chains linked by cystine. The autocatalytic action of π-chymotrypsin on itself [meaning that one given complex molecule of π-chymotrypsin acts on another given complex molecule of π-chymotrypsin] simply shortens one chain - [producing δ-chymotrypsin] ... and ... α chymotrypsin is produced by a second break and a peptide loss to give three separate chains bond together ...

Although different in details, conversions of trypsinogen to trypsin, pepsinogen to pepsin, and fibrenogen to fibren - in blood clotting - all involve the same principle, that of limited and specific proteolysis of nonfunctional protein to entities with recognisable and measurable functions. In these cases, then, part of the synthesis of specific enzymes occurs after designation of primary sequences with participation of other enzymes and products or intermediates (autocatalysis), and all components and reactions participate individually and collectively in metabolic control.

With increasing recognition of the participation of macromolecules as substrates in reactions (such as the above) which alter or yield biological specificity, it should be profitable to give serious consideration to other possible kinds of reactions [involving enzymes acting on other enzymes or proteins] that might be significant in this regard. In proteins there numerous possibilities which include amidation and deamidation of carboxyl groups in side chains [which would serve to convert one specific amino acid into another], other substitutions such as alkylation or acylation of side-chain hydroxyl or amino groups [which also serve to convert one particular amino acid into another], actual changes in side changes such as one-carbon transfers to interconvert glycene and serine, and alteration of primary chains by transpeptidation. These and similar phenomena remain to be discovered, though a recent unconfirmed report [by Zubay in 1962] suggests the occurrence of deamidation after protein synthesis."[21]

21. Wagner, Genetics and Metabolism

The phenomena of enzymes or proteins serving as substrates for as yet other enzymes, and the resultant creation of new enzymes or proteins, with new amino acid sequences, as a consequence of their role of substrates shows very clearly that not all amino acid sequences are determined by M-RNA molecules through the process of translation. Even though one or two enzymes acting on a protein are themselves produced by genes, and therefore by M-RNA templates, the actual modification effected by these enzymes on the third enzyme cannot be said to correspond to any physical existing template or ribonucleotide code. In fact the modification corresponds to or is based upon certain enzymes having the properties of being able to act on a specific substrate in a specific way. Yet, even more important, an enzyme (or enzymes) has the property to create over and over again the same type of modification in different molecules of the same pro :tein substrate. This capacity on the part of an enzyme to bring about identical alterations in different molecules of the same substrate seems to manifest some type of mnemonic code inherent in the enzyme structure which has the enzyme duplicate past actions. Quite reasonably, such a mnemonic code as embodied by the particular enzyme may in some sense be regarded as the particular template which corresponds to the particular alteration created. From this point of view, the particular enzyme which effects an alteration on another enzyme or protein must to a fair degree be regarded as the template on which such an alteration is patterned. If a particular enzyme, effecting an alteration in a protein, is itself a direct product of one particular gene, the mnemonic code of that enzyme must be regarded as a further extension of the gene's code though an extension which does not actually exist until the enzyme is brought into being, and which does not exist in terms of a nucleotide sequence.

Different alterative-enzymes each of variant mnemonic codes could bring about respectively different alterations on different protein or enzyme substrates or/.....

or as has been implied, different alterative-enzymes could each effect respectively, and in turn, a particular, or characteristic, alteration on the same protein or enzyme substrate. Each particular alteration effected would be patterned or determined by the particular mnemonic code. Moreover, the particular protein substrate to be acted upon, the particular regions where such chemical actions would take place, and the particular type of chemical action eventuated, would all be determined or patterned by the given mnemonic code. In this connection, enzyme specificity, in general, may be the expression of the given mnemonic code. As such, the code may be related to the particular shape of the enzyme, as determined by the particular configuration taken on by enzyme's hybridized electron orbitals.

Would the possibility of alterating-enzymes having some type of mnemonic code give them the status of genes? One of the major characteristics of a chromosomal gene is that it has the ability to make duplicates or copies of itself (or its code). We have seen how an enzyme X can act on a protein Y as a substrate, and convert, or alter such a protein into an enzyme Z. We have also seen how an enzyme X can act on another molecule of Enzyme X and convert such an enzyme into a protein F. Can it not also be possible that an enzyme X can act of a protein G and convert or alter such a protein into another molecule of enzyme X? Moreover, can it not be possible that an enzyme X is able to act on a particular class of five or six different protein substrates, and change all of such into molecules of enzyme X? Furthermore, can it not be possible that an enzyme is able to act on a protein T, and convert that protein into enzyme V, which in turn has the capacity to act on a protein P, converting it into a molecule of enzyme X? The mnemonic code of enzyme X would, in this light, be quite versatile, for not only could it effect directly, or indirectly, a small number of variant reactions which would, in effect, lead to the creation of more molecules of enzyme X, but the/.....

the two or three metabolic reactions which are required for the normal functioning of the organism. A small number of enzymes may create additional molecules of themselves in the manner of enzyme X.

Another possible process whereby an enzyme could produce a duplication of itself may be much more indirect than the previous ones mentioned, and may require the help of other enzymes which are not indirectly or directly created by the enzyme to be replicated. For example, the reader will recall that different M-RNA molecules of codon length may be produced by transient genes. It is possible, therefore, that, in some cases, the enzyme to be replicated, which we shall call Z, works together with certain other enzymes in joining together these M-RNA molecules (or codons) into a much longer molecule of M-RNA, having a specific sequence of bases, which when translated would give rise to a molecule of enzyme Z. In commencing the synthesis of such a large M-RNA molecule of a specific code, an enzyme A would only be capable of joining codon X to Codon A, giving rise to the specific sequence of bases, T. Another enzyme, only capable of joining codon P to sequence T would then proceed to join P to T, giving rise to sequence U. Another enzyme, capable of joining codon E to sequence U will then proceed to do so, producing sequence V. Another enzyme capable of only joining codon X to sequence V, then proceeds to do so, giving rise to sequence W ... and so on. In this way, a specific M-RNA template could be produced through the activities of specific enzymes. It is as if the mnemonic codes of the respective enzymes involved are combined somehow into a complex code which corresponds to the specific template of M-RNA to be produced.

The process whereby some enzymes are able to duplicate themselves is of course dependent on the existence of certain protein (or M-RNA codon) substrates. Yet, a chromosomal gene cannot make or produce a way of itself without the existence of certain substrates, namely specific nucleotides. In fact, a chromosomal/.....

chromosomal gene, as we have seen earlier cannot effect the duplication of itself without the assistance of certain enzymes, the DNA polymereases. Since an enzyme, such as enzyme X may be able to produce replicas of itself, such an enzyme, thereby, would fulfill the major characteristic of a chromosomal gene, and moreover would not be as dependent on extrinsic factors (i.e. DNA polymereases) as chromosomal genes are in carrying out the production of such duplications.

Another characteristic of a chromosomal gene is that it can be transmitted to the progeny. Enzyme X and the other enzymes which might produce copies of themselves, could be transmitted to the progeny via the egg cytoplasm, or they can attach themselves to the chromosomes of the germ cells, and be transmitted with such chromosomes to the progeny. Once in the zygote they could disengage themselves from the chromosomes, enter the cytoplasm, and there proceed to produce replicas of themselves by acting on the protein substrates existent in the cytoplasm, thereby, producing a sizable pool of such duplicates. As the zygote cleaves into daughter cells, two or three replicas, such as of enzyme X, would be existent in each of the daughter cells. In each of these daughter cells, a duplication, such as of a molecule of enzyme X, would proceed to create more replicas of itself by acting on the protein and enzyme substrates present in the cytoplasm. In each cell produced through mitoses, a duplicated enzyme, such as of enzyme X, would proceed to produce three or four more duplications of itself. Once produced, one or two of these duplicated enzymes would then proceed to effect one or two specific metabolic reactions[22] which would be but a small part of the total metabolic reactions required for the cell's survival. During mitotis, the remaining duplicated enzymes would proceed into the daughter cells via the cytoplasm, where in turn they would form copies of themselves ... and so on.

22. These duplicating enzymes may also contribute to the formation of demarcator-enzymes by acting on protein substrates. Also they may join small polypeptides together so as to form long amino acid-sequences (or proteins).

If a particular chromosomal gene happens to produce an enzyme capable of creating copies of itself, and if furthermore, such a gene becomes lost from the genome of the species, the enzyme could, nevertheless, be maintained throughout future generations of the species by virtue of its capacity to produce replicas of itself, and by virtue of the replicas having means of transmission. In fact, many enzymes capable of producing duplications of themselves may have continued to maintain their respective existences through the generations long after their particular initial creators (specific chromosomal genes) ceased to exist in the genome.

It would seem from what has been said that certain enzymes thus take on the status of being genes, though genes, different in structure from that of chromosomal genes, and having a code which cannot be regarded as a specific sequence of nucleotides. The possible existence of this new class or system of genes, which I shall refer to as genezymes, should not appear strange when one recalls that even though the chromosomal genes (or transient genes and fluxons) ultimately determine "the recurrence in successive generations of like forms of metabolism", it is the same enzymes in those successive generations which directly effect such recurring metabolic forms into existence through the action of specific catalysis. The only distinction between these enzymes and the genezymes would be that the latter would be able to maintain their respective existences independently of chromosomal genes.

If some genezymes do effect the recurrences of some metabolic patterns in successive generations, it would stand to reason that modification or alteration of such genezymes into other specific genezymes by enzymes would serve to cause alterations in those matabolic patterns, and these altered patterns would be maintained as such in subsequent generations as long as the altered enzymes produced/.....

produced copies of themselves. Such altered genezymes must be regarded as mutations, and the enzymes effecting the particular alterations must be regarded as mutagenic enzymes produced by muterons (or fluxon-muterons). In fact, a few genezymes may be capable of altering some other genezymes into different gene-:zymes. As such, the genezymes inducing the mutations must be regarded as genezyme-muterons. They may effect alterations by acting directly on the genezymes to be mutated, by producing mutagenic enzymes from protein substrates, or by effecting specific chemical reactions which would result in the production of non-enzymic chemical mutagens capable of bringing about amidation, deamidation, alkylation, and acylations of specific amino acids.

One or two genezyme-muterons may even be capable of eventuating specific mutations in chromosomal genes or effect alterations in the base sequences of the M-RNA molecules. The latter type of alteration would be maintained as a mutation in subsequent generations as long as the particular genezyme responsible for the alteration in the particular M-RNA is maintained through the subsequent generations. Genezyme-muterons, by acting on chromosomal genes, M-RNA molecules, and on genezymes would be one of the main agents residing within an organism capable of inducing a high frequency of mutations.

The existence of the genezyme-muterons plus the other numerous agents capable of determining or directing or controlling mutational processes within an organism makes it that more likely that a fair degree of mutational activity lasting for major periods of the organism's lifetime is the rule rather than the exception. If such is the rule, the organism would, during these periods, be constantly changing its genotype. Because of the fair degree of mutational activity, each change in the long succession of graded changes would be of a significant degree. More important, such a series of changes in genotype through the lifetime would be an evolution. As such, this would be an example of evolution not taking place/.....

place over a large number of successive generations, but within the life span of one organism. It is quite possible that the degree of evolution effected by some organisms within their respective life times, through the intense and enduring activity of their respective muterons may be comparable to the degree of evolution which normally in others of relatively less muteron activity would require thousands of generations.

Such an evolution of genotypes taking place through an organism's lifetime could be quite independent of environmental influences or pressures, and would be inner-determined or inner-controlled by virtue of the organism containing within itself, muterons, anit-muterons, and regulatory genes acting in a specified and non-random manner. The fact that many mutations occur only at specific times and in specific genes, the likelihood that the activities of many muterons can be controlled or regulated, the likely possibility that many muterons have a specificity of action and that such specificity can be modified in a controlled manner, the possibility that mechanisms exist which can negate deleterous mutations and replace them with beneficial mutations, the possible existence of mutational cycles, indeed would show or imply that mutational activity is more organized than chaotic. In this paper descriptions of models of possible muteron interactions, as well as models depicting the possible interactions between muterons, and anti-muterons, showed that such systems could be of organized natures. Moreover, it became clear that mutational systems have some mechanisms in common with developmental systems, the latter being known to be quite organized.

Even though such inner determined mutational activities may represent a degree of organization, many accidents may, nevertheless, occur. In fact, the result of some inner-directed mutational activities may be sudden disorganization within certain parts of the organism.

A/.....

A good example of disorganization suddenly occuring within an organism would be the phenomenon of cancer, whereby some cells lose the normal control devices which keep such cells from dividing. As a result, the cells divide without cessation, and such unceasing division is chaotic and causes a dis-:organization of the tissues or organs in which such cells exist. There is evidence which shows that cancer may be due to some type of alteration within a gene. For example

> "When a cancer cell divides, the two progeny cells are usually morphologically identical to the parental cell. The factor(s) that gives cancer cells their essential quality of unrestrained growth is thus regularly passed on from parent to progeny cells. These changes persist not only in tumors growing in intact animals, but also in tumor cells growing in tissue culture. Hundreds of generations of growth can occur in tissue culture without appreciable reversion to a normal state. The permance of such changes is shown not only by perpetuation of a typical morphology, but also by the ability of progeny cells to cause new tumors when injected into a tumor-free animal of genetic composition similar to the one from which the original tissue was obtained. (Thus) the heritability of the changes allowing unrestrained growth makes us consider the possibility that an alteration has occurred at the cnromosomal level."[23]

Many of such mutations leading to cancerous cells could be due to muteron and/or genzyme muteron activity. The fact that some viruses have been found to cause some cancers in higher organisms may indirectly support the possibility that the majority of cancers in higher organisms may be specifically due (in large part) to the action of episomal muterons residing in the nucleus.

The best known of the cancer causing viruses is the RNA virus, Rous sarcoma. This virus induces tumours in chickens. Also, some DNA viruses can induce tumours. One of the best known is polyoma, and when injected into newborn mice, such a virus can cause a variety of cancers.

> "When a polyoma virus enters a susceptible cell it may suffer two possible fates. Most commonly, it multiplies like a conventional virus and produces a very large number of new virus particles. The site of polyoma reproduction within its/.....

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23. Watson, Molecular Biology of the Gene.

> its host cell is the cell nucleus. A single nucleus may become filled with millions of particles, a process during which the normal nuclear functions are disrupted and the host cell necessarily dies (a lytic infection). Much more rarely, the virus enters the cell and forms no new particles. Instead, the infected cell becomes transformed into a morphologically distinguishable cancer cell ... the transformed cell then begins to multiply in the disorganized and easily identifiable fashion of a cancer cell ... no infectious polyoma particles are present in transformed cells. It is, of course, not surprising that the nuclei of transformed cells are not filled with viral particles. This would probably result in cell death. The fact is, however, that no particles at all can be detected. It appears as if the virus enters the cell and then vanishes. This phenomenon immediately raises the question of whether only the polyoma chromosome is present, perhaps integrated into one of the host chromosomes."[24]

Such an integration may involve the virus DNA having inserted itself into the host chromosome or becoming a continuous part of such chromosome through the process of transduction. The process of transduction would be in keeping with the benaviour of lysogenic bacterial viruses. No matter the exact manner by which the virus impinges on the host chromosome, the result of such an impingement would most likely be a mutation in the chromosomal gene (or genes) with which the virus is making contact.

The similarity between such mutator-viruses and the motile episomal muterons residing in the nucleus is quite evident, especially when one realizes that viruses are in effect nothing but motile pieces of genetic material surrounded by some protein.

In fact, some of the cytoplasmic episomes, such as the milk factors and the sigma particles, are believed to be types of viruses. Even more interesting is that the milk factors are capable of inducing mammary tumours. If some cytoplasmic motile episomes are capable of moving, once and a while, to the nucleus, and when in the nucleus, capable of inducing mutations which lead to cancer, then it/.....

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24. Ibid.

it is quite reasonable to believe that the episomal muterons which reside all the time in the nucleus are the major agents of cancer induction. Of course, the chromosomal muterons and the genezyme-muterons may also effect some mutations which lead to cancerous cells.

It is quite possible that a high number of mutations which would have given rise to cancerous cells are negated by episomal anti-muterons. This would imply that the frequency of cancer in populations would be higher than it actually is if it were not for the anti-muterons.

The results of some experiments performed by Nieu would seem to supply some type of basis for the existence of cancer negating agencies composed of genetic material. Nieu took some RNA from non-cancerous cells and injected it into cancerous cells. Soon after doing this, he observed that the cancerous cells became transformed into normal cells. It would seem reasonable to conclude that the RNA somehow negated the mutations which made the cells cancerous. As such, it would have behaved in the manner of the episomal anti-muteron postulated to have the capability of negating mutations which could or have given rise to cancerous cells. It is quite feasible that a fair number of episomal anti-muterons are composed of RNA.

The possible existence of episomal anti-muterons capable of negating, specifically, mutations which determine the existence of cancerous cells may have definite medical implications. If an extract of such hypothetical anti-muterons could be prepared, and in turn injected into human cancerous tissue, such tissue may very well be transformed into normal tissue. In fact, any muteron capable of transforming a cancer-mutation into a non-cancer-mutation would be a potential anti-cancer agent.

The/.....

The possibility that cancer is the result of an organism having attempted to evolve within its own life time seems quite reasonable in light of what has been said. That a fair number of organisms have inherent within them safeguards, or controls, against the occurrence of cancer, or controls capable of negating cancer soon after it is created, also, seems feasible. Not all cells within an organism may have such safeguards, however, nor may all of such controls be equally effective in negating cancer-mutations. Even so, the existence of such controls has a deeper meaning, for in as much as they would allow an organism to engage in a high degree of mutational activity without allowing such mutational activity, in fair part, to bring harm to the organism in question.

Even though some organisms may be capable of evolving many genotypes within a lifetime, the continuation of such an evolution would, nevertheless, necessitate the existence of numerous subsequent generations. The creation of new generations would mean the creation of new genotypes as a result of new gene combinations coming together during successive sexual fusions. Yet, the creation of new genes in the germ and somatic cells, and hence new genotypes, would continue to occur within the respective lifetimes, and such creation, in most part, if not all, would be eventuated by inner-directed mutational activity.

Even mutations occurring in somatic cells, while not in the germ cells, could, nevertheless, be transmitted to progeny. Such mutant genes could produce M-RNA templates, and these M-RNA molecules could then move via the blood stream to the germ cells into which they would enter through the help of enzymes that would sever small portions of the respective cell walls. Once in the cytoplasm of the germ cells, the M-RNA molecules would then proceed to produce DNA molecules corresponding in base sequence to those of the different M-RNA base sequences (Wagner does cite possibilities where M-RNA could synthesize DNA molecules).

Once/.....

Once produced the DNA molecules would move into the nuclei of the germ cells. Sections of the chromosomes corresponding to the length of the non-mutant genes would then be excised by enzymes. In their places, the DNA molecules corresponding to the mutant genes of the somatic cells would then be respectively inserted. Each one would now be residing in the place where its non-mutant allele had been. Being now parts of the germ cell chromosomes, such duplicate somatic cell mutations could then be transmitted to progeny.

The likely possibility that most of the variant genes transmitted from generation to generation have been eventuated by inner directed mutation either in the germ cells or in the somatic cells, or both, and the fact that the major mechanism of evolution depends on the existence of such variant genes would seem to imply that evolution has an inner-parameter, meaning that evolutionary change through time is to some degree effected or determined from within, that not all evolutionary change is dependent on merely the random shuffling of different genes togetner so as to give randomly new combinations, nor on merely selection pressures, nor on merely random sampling. The very variation which can be the source of new combinations, the very variation on which selection pressures operate may ultimately be controlled from within, may ultimately be dictated from within by muteron and genezyme-muteron systems.

Not only the variation between genes, but the number of chromosomal genes existent today may have ultimately been determined by inner agents. The creation of polyploid organisms (which means the increase in the number of genes existent in such organisms) may have been due in part to genes and/or genezymes determining the production of chemicals[25] which destroy the mitotic spindles created during successive mitoses. Moreover, the phenomen of polyploidy may also have been due/.....

25. Such chemicals may be acenaphthene, sulfanilamide and colchicine.

due in part to genes, controlling the replication of chromosomes (DNA) in the fertilized egg, having been mutated by muterons to such a specific state whereby they permit[26] chromosomes to replicate frequently without the corresponding existence of self clearage. After such a series of replications the mutation may then have been negated by an anti-muteron into the normal state.

The increase in the number of genes in a polyploid organism means that three or four or five identical copies etc. of the same gene would exist in the same organism. However, different muterons could then effect different mutations within each set of identical genes with the result that variant genes are produced within each set. In this way not only would the number of genes be increased but the number of variant genes as well. The source of both phenomena in this case would have come from within. Even the increase in the number of chromosomal genes alone would result in a new combination or genotype (gene) through the creation of identical chromosomal genes. The creation of such a new combination or genotype would constitute a fluxial-mutation, also determined from within.

In many organisms non-reciprocal translocations between homologues effected through the agencies of mutagenic enzymes could have led to gene duplications on the same chromosome with the result that the progeny of such organisms would have had a greater number of genes than the parental generation. Many of these duplicated genes could then have been mutated by muterons into variant forms. This process, as like the other, would be a good example of how the major mechanism of evolutionary change is generated from within. In point of fact, it is known that the existence of the variant genes (alleles) producing the variant hemoglobins is ultimately based upon repeated non-reciprocal translocations having/.....

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26. Hayes has referred to genes which appear to control the initiation of DNA replication. He calls such genes, replicator genes. It would appear that other specific mutations in such genes play some role in cancer.

having taken place between certain homologous chromosomes through the generations.

The irreversibility of evolutionary divergence may also, in great part, be determined from within. For example, different races or populations, though capable of interbreeding may to a fair degree differ respectively in the types of muterons each has. Because of these respective differences, mutations induced in one race, for most part, probably would be variant from those induced in another race. If the muteron activity is intense and continuous over the generations of the respective populations such populations or races in a short time would have come to differ greatly in their respective genes. The result of such great genetic differences would be that the two populations would be unable to interbreed as before; they would have become reproductively isolated.

> "The attainment of the species status, that is the advent of reproductive isolation between populations is, biologically considered an event of fundamental importance. This is because evolutionary divergence becomes irreversible at this stage."[27]

The irreversibility of such evolutionary divergence as we have just seen can be completely based upon the differential effects produced by muterons, and thus can be entirely determined from within. It would seem therefore quite likely that speciation in general is to a fair degree inner-determined.

In some situations new resultant species can be indirectly created from a cross between two different species. The allotetraploid Raphanobrussicia is a good example of such resultant species created indirectly, in that

> "it is fertile, true breeding, and to a considerable extent isolated reproductively from its progenitors, the radish and the cabbage."[28]

The direct result of such a cross is the production of an allodiploid radish-cabbage hybrid. However, such a hybrid is unable to produce viable gametes, and this/.....

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27. Dobzhansky, The Principles of Genetics.

28. Ibid.

this attests to the reproductive incompatibility or isolation of the two progenitors. If, however, the hybrid is transformed from an allodiploid to an allotetraploid, a new species is thereby formed, capable of producing viable gametes. Such a transformation is effected through the respective doublings of the radish complement of chromosomes and the cabbage complement of chromosomes. If such a transformation was brought about through the activities of muterons (or genezyme-muterons), then such a transformation must be regarded as having been inner determined. It is quite possible in this light that in many cases the sudden creation of new resultant species, such as Raphanobrassicia, may have had a major inner-component.

It is fairly reasonable to believe that evolutionary change can occur over thousands of years quite independently of any environmental factors, especially when, as Stern points out, a great many mutations are neutral as far as survival is concerned. It is known that evolutionary change can occur independently of selection pressures over a certain period of time through the process of random drift, brought about by random sampling in successive generations of small populations. However, evolutionary changes maintained by random sampling alone cannot go on for an indefinite period of time, in as much as specific genes (alleles) tend towards fixation, with the result that new genotypes can no longer be created in subsequent generations. However, constant and intense mutational activity, wrought by muteron systems, coupled with the process of random sampling, would keep fixation from occurring, and the result would be that random drift could continue for thousands of years. Hence, constant muteron activity coupled with random sampling could maintain in small populations evolutionary change for great lengths of time, quite independently of the environmental factors which always have been believed to be necessary for any evolution to have occurred over great amounts of time. In this light, cases/.....

cases of microevolution may exist which came about quite independently of the external environment, though quite dependently on the inner-environment composed of muteron systems, and on the process of random drift, which in itself, would require for its maintainance through time, the activities of the inner environment.

By stipulating the possibility that evolution has an inner component I by no means tend to imply that evolution has some pre-determined end. The existence of such an end remains an open question, and a discussion pertaining to this question has remained beyond the scope of this paper. One of the major points I should make, however, is that evolutionary processes, by virtue of having a possible inner-mechanism capable of acting in some organized manner may have a degree of inner order as opposed to complete inner-randomness or chance; not only would evolution have come to pass through the random activity of the external agents of mutation, the external pressures of environment, and random sampling, but through the organized activity of internal mutation agents, as well. Not only could such inner mechanisms have created greater numbers of variant chromosomal genes, but could have eventuated, as well, supplementary systems of heredity, such as the genezymes. The creation of such additional avenues of transmission from within would allow for that many more avenues on which inner-directed change would operate.

From such would result the enchancement of the inner component of evolution. The question as to what length of time evolution has had an inner-parameter as well as an outer may never be answered. But the fact some viruses are capable of self-induced mutations may lead one to suppose that the inner-parameter emerged with the first emergence of life itself.

The article shows knowledge in many areas, and there are a number of interesting ideas in it. The blueprints for ontogenesis, carcinogenesis, and orthogenesis are worked out with a lot of imagination, but with no concern about whether Nature intends to follow them!

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