Committee V
East-West Perspectives on Science and Spirit: Time and Consciousness

DRAFT--5/15/91
For Conference Distribution Only

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TIME IN BIOLOGY: THE GENETIC CLOCKS

by

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The Eighteenth International Conference on the Unity of the Sciences
Seoul, Korea August 23-26, 1991

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Time in Biology: the genetic clocks.

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The idea about time has gone through a decisive cycle in the history of western civilization. It is evident that the meditations of Plato about time and cosmos gave rise to a wide and very rich tradition in our thinking. Today, scientists, philosophers and psychologists agree in recognizing that the idea of time derives from the ocurrence of changes; changes of our sensations or changes of our thoughts.

Biology is deeply concerned with the notion of time. In fact, time is inherent to all living processes, which may be considered as expressions of continuous changes controlled by multiple interactions between the genetic endownment of the organisms and the environment.

Living organisms, unicellular or pluricellular, develop their different characteristics and evolve within a time frame. The genome of each organism contains a program with information and instructions to be expressed at a certain time and for a certain duration. The physiology of genes is interwoven with the impact of environmental factors at every stage in development.

On the other hand living organisms are constantly changing as a result of spontaneous or induced genetic modifications. These changes are tested in different enviromental conditions and, as a consequence, organisms experiment evolution of their characters. In fact, the history of such evolution is incribed in the genome of the organisms.

The interest of biology on better knowing the processes that control time during the development and evolution of the organisms has given rise to Chronobiology, a new area of specialization in science, in which, it is possible to investigate or study the cellular and genetic aspects of those processes.

The biological notion of time.

The notion of time is a product of our hability to observe and to analyse nature. We are able to conceive the notion of time through our relation with sensible objects. This is a relative time, because what we really estimate is a time interval between two events. In this respect, Lowenhard has indicated that "the measurement of time is always implicit in that we utilize motions of constant velocity or periodic processes". Hence, time measurement is based in a comparison of moved distances or counting of cycles"(6). For such purpose, living organisms use internal periodic processes as time references. These processes may be considered as "internal clocks" that must have some sort of genetic dependence, which is interesting to know.

Scales for biological time

Our perception of the natural world and the language to express it have derived in a range of seconds to years. We are able to explain events taking place in seconds, such as the substrate induction of the sinthesis of an enzyme, like ß-galactosidase, in *Escherichia coli*. In fact, the formation of this enzyme takes 90 seconds after the addition of lactose to the culture medium of *E. coli*. During the interval, a very complex process takes place in the cell which includes the synthesis of the messenger RNA and the synthesis and excretion of the enzyme.

It is also possible to calibrate the activity of one molecule of the enzyme DNA polymerase I, which is able to catalyze the incorporation of one nucleotide/sec. to form DNA at a temperature of 37ºC.

We also can imagine and explain events that take place in 1/1000 of a second, such as the action potential of an axon in the nerve cell.

Nevertheless, nobody can have a personal notion about the meaning of $1/10^6$ of a second, and there is no doubt that there are living phenomena that take place in periods of time too fast to be perceived by our senses, even with the help of very powerful instruments.

On the other end, the time factor in biological evolution is evaluated in millions of years, which in human terms is, of course, a very slow process. Similarly, there are experiments in which the total time necessary to be completed exceeds the life of the scientist.

These limitations determine that the gap between what we are able to perceive and events that happen at very remote time scales is enormous.

Besides the previous considerations and since it is not possible to formulate explanations of nature in a fully consistent way without reference to the consciousness of the observer, a profound limitation lies in the concepts that are available to us to realize and express our understanding of nature. We, therefore, have a no "a priori" right to suppose that our present biological concepts will be appropriate to describe the behavior of natural events on a very vast time scale. The most we can expect is that concepts we have in contemporary biological knowledge will be useful as good analogies to explain or understand certain phenomena.

Nevertheless, it is amazing how far modern science has been able to go in dealing with time and space. In this respect, biologists are pushing the frontiers of their work of causes and effects further and further into the domain of what used to be called the spiritual. In this respect, very old biological questions, such as: What am I? or how we relate to the rest of the Universe? are being examined in a new perspective. As such, Biology is more than ever an intellectual, ethical and spiritual exercise.

How organisms perceive time?

The notion of time considers two different concepts.

a. The concept of succession.

b. The concept of duration.

The concept of succession is based upon the experience of continuous changes, in which the present turns into past. The concept of duration is an atribute of sensations. The threshold between simultaneous and succesion in man is around 30ms of separation between two events.

Man's nature has much in common with other organisms and much that is unique. Common to all is that their characteristics, processes and activities are coded in the genome and are goal directed. This is true in processes such as growth, maintenance of body integrity and repair of injuries.

Since Pavlov, it has been demostrated that many animal species are able to perceive sensations, memories and even emotions. It is also accepted that higher animals are conscious beings. In this respect conscious drives or activities in man and higher animals, such as satisfaction of hunger, sexuality, etc. have the time component in its expression.

While most animals seem to live in a perpetual present time, human beings are able to categorize time in three orders or levels: past, present and future and as a consequence we enjoy a higher level of consciousness, the self-consciousness with the inherent capacity of thinking, creative imagination and subjective knowledge. Because of this advantage, a very important question arises; whether time corresponds to an objective reality or is a creation of our brain?. If our notion of time is of a subjective nature, it also corresponds to an objective reality, which, according toJ.M.R. Delgado, would be "what exists and happens in the universe, regardless of our presence or existence" (3).

Consciousness or awareness seems to be restricted to living organisms equipped with nervous systems and is a phenomenon which accompanies certain modes of information processing. Any organism which is confronted with the task of its adaptation to a very complex environment has to integrate a large amount of information in a way that makes possible the production of a holystic response at a certain time.

What is the cellular basis of consciousness?

The behavior of living organisms, unicellular or pluricellular, follow a cyclic pattern. Biological clocks may be partially explained on the basis of: learning, heredity, and interaction with external stimuli derived from the cosmos activity, etc. So, we have "biological clocks" governing behavior, some of which have gone through an evolutionary process of adaptation to the rotatory movements of the earth.

If an organism finds useful to repeat a certain phenomenon every 1/1000 of a second or every spring, it will do so, provided it has internal systems capable to measure time and internal systems sensitive to clock temporal signals from the environment.

To be aware of a certain object or phenomenon the neural system of an organism has to separate the bits of information related to the object or phenomenon from all other information that is being taken. This is a very complex task to be performed by neurons that may be scattered in the brain. These cells must coordinate their bits of information in to one coherent response or impression. In this respect J.M.R. Delgado has indicated that "there is a kind of intrinsic biological clock providing a neuronal timing mechanism and the continuous occurrence of cyclic activities for estimation of the passage of time" (3).

The capacities of neurons to do this work must have some genetic dependence. Genetic instructions for governing the development and functioning of nerve nets for consciousness must be encoded in the genome. Nevertheless this is a very complex process, which was formulated by S.Benzer in 1973. He expresed: "When the individual organism develops from a fertilized egg, the one-dimensional informations arrayed in the linear sequence of the genes on the chromosomes controls the formation of a two-dimensional cell layer that folds to give rise to a precise three-dimensional arrangement of sense organs, central nervous system and muscles. These elements interact to produce the organism's behavior, a phenomenon whose description requires four dimensions at least. Surely, the genes, which so largely determine anatomical and biochemical characteristcs, must also interact with the environment to determine behavior. But how?" (2).

The existence of behavioral rhythms in pluricellular organisms present many interesting questions. One is related to the possibility that biological clocks could be localized in a special center or structure within an organ, or if they are present as parts of each cell. Experiments done in cardiac tissue, have suggested the presence of oscillators in each cell which seem to be regulated by more powerful mechanisms located in special cells. These oscillators give the appropriate pace for the more weak oscillators present in each cell.

Genetic clockwork.

Research done in the last 15 years has revealed the existence of genes involved in regulating biological rhythms. In *Drosophila melanogaster*, the genetically famous fruit fly, there is a gene called *per* (per periodic), which regulates at least three timed events:

a. the cyrcadian (24 hrs.) activity-rest cycle.

b. the courtship song of the male fruit fly.

c. the time in which the adult fly will hatch from its pupal cocoon.

size and the composition (1200 aminoacids, approximately) of the protein product of this gene. The cells of the eye and optic lobes of the brain of Drosophila have the capacity to make such protein.

It has also been proved that the amount of protein made by the per gene influence the timing of the events indicated. The more protein the animal has, the faster its biological clock ticked.

Now, how this protein regulates a biological clock?. It seems to influence transmission among nerve cells. If this is so, per mutations would alter biological rhythms by modifying the rate of electrical conductance among nerve cells, specifically devoted to timing. The study of the gene per in Drosophila melanogaster has been useful to reveal mechanisms used by the fly to synchronize behavior and metabolism with daily enviromental changes determined by the rotation of the Earth (5).

Recently, neuroscientists have recorded oscillating waves of synchronous electrical potentials with a frequency of, roughly, 40 hertz that may be the basis for visual awareness in the brains of cats. These 40 hertz oscillations of scattered neurones have a time scale corresponding with that of attention flitting from one object to another. The neurons may stay phase-locked for several hundred milliseconds, which would allow them to make and break their liaisons in, roughly, the same period that a person's attention moves from one subject to the next (4).

We know that genetic information is subject to changes to produce mutations. One mutant affecting per gene, is called per^{s} (s for short) and is characterized for having a clock running faster than normal. The activity-rest cycle last 19 hrs; the rhythm of the wingbeat courtship song is reduced from 60 seconds to 40 seconds, and the hatching is advanced from dawn to predawn.

Another mutant, called per^{l} , (l for long) has a slower than normal clock. The activity-rest cycle takes a total of 30 hours; the courtship song last 80 seconds, and hatching is a late morning event.

A third mutation, called per^{0} (0 for nothing) is characterized by arrhythmic or random timing patterns on all three events.

Further studies conducted with these mutations have made clear that no single tissue acts as a unique centralized pacemaker for these three timed functions. On the contrary, a series of independent timers seem to use the product of per gene, a protein.

The control of the activity-rest cycle has been located at the eye and optic lobes in the brain tissue of the fly. The control for the courtship song has been associated with nerve tissue in the chest.

Dr. Michael W. Young and collaborators, at the Rockefeller University, N.Y., were able to clone the per genes in 1984 (1). They also have deciphered its sequence of nucleotides. This information has allowed them to deduce the

Visual awareness could be considered a model for consciousness in general. It is possible to imagine that the synchronous firing of neurons is not only a mechanism to focus attention of our senses, but to develop ideas and thoughts in our conscience without an external sensitive origen

These data are illustrative of the progress being achieved to uncover the mechanisms through which cells perceive time. Nevertheless, much remains to be learned about time as a form of perception inherent to the nature of the mind.

The significance of time in biological evolution.

The darwinian theory of evolution by natural selection has served as a great unifiying principle in Biology. Biological evolution may be considered as a history of the variation of living characters through an interpretation of the phenotypes we find today.

The outstanding progress in scientific knowledge achieved in the last few decades has made possible new interpretations about the theory of evolution on the basis of better information of what is called time.

Since living organisms are the result of very dynamic interactions between the genome and the environment, it is possible to consider that the history of all organisms is inscribed in the genetic material, which may be considered as a molecular clock of evolution. Mankind is a product of an evolutionary history. In the same way, life and mind may be considered as products of a historical process of evolutionary development.

Where do we come from?. Molecular clocks in evolution.

In the process of evolution, animals adapt slowly to new environments by changing their genes. Multicellular eukaryotic organisms, including man,have evolved homeostatic mechanims that buffer the majority of their cells from direct interaction with their external environment. Due to this condition, hominoids seems to be slower in evolution than other organisms.

Until 3.5 - 4 billion years ago there was no life on earth. Between 0.5 - 1 billion years ago living organisms presented only primitive nervous systems. Only 2 - 3 million years ago, hominoids primates appeared with brains that were becoming large in relation to their body size.

Until three decades ago our understanding of human origin had been built up almost entirely through the study of fossils ancestors found in Africa. The main idea worked out from those studies was that we shared a common lineage with gorilla and chimpance. A divergence between humans and african apes was estimated by most paleontologists to have taken place 20-30 million years ago.

Around 1960, it was discovered that rates of molecular changes may be used as clocks to estimate time in evolutionary changes.

In fact, evolution proceeds through changes in the genome carried by DNA. Part of these changes are also manifested in the composition of proteins coded by different genes. This sort of molecular evolution is characterized by constancy in rate/year, providing to science a sort of molecular clock for estimating time in evolution.

These molecular clocks has been calibrated and dated from comparisons of DNA proteins and molecules obtained from living human beings, living gorillas and living chimpaces. Information from proteins has been obtained by using inmunological tests and aminoacid sequencing. Information from DNA has been collected by different techniques: DNA hybridization, nuclear and mitochondrial DNA sequencing. The method of DNA hybridization allows to compare the organization of, around, three billions of nucleotides pairs in the hominoid nuclear genome.

In the case of mitochondria, the same is possible for the 15.000 nucleotide pairs that are the basis for the genome of this cellular organelle. The advantage in doing evolutionry inquiries in this organelle is, that, for some reason, it evolves at five to ten times the average rate of evolution of nuclear DNA. So, with mitochondrial DNA we have a "faster clock" for timing evolutionary changes in hominoids.

Using these techniques and as a result of the work of many scientists it has been possible to estimate that the divergence between humans and african apes may have taken place around 5 million years ago (7).

But more precisely than this estimation, it has been possible to find that the human genome binds or hibridize 20 percent stronger to the chimpance genome than with the gorilla genome. This binding is also stronger as compared with the hibridization between the genomes of chimpance and gorilla. These results suggests that the gorilla line split off around one million years earlier than the split of human and chimpaces lines.

<u>What to expect</u>?

Human evolution may be considered as an outgrowth and continuation of biological evolution. Darwinian scientists have assumed that human nature is constantly changing. Nevertheless, it is also accepted that changes in human nature are so infinitely slow that behavioral scientists, for all practical purposes, can assume that it is not changing.

Consciousness and ethical concerns appeared at some time in the evolutionary process. Nevertheless, there is still no way to date at present, even approximately, the <u>emergence of human mind</u> with its self-awareness.

The morphological destiny of man is linked to his rationality. In fact, man, also, adapts himself by changing his environment to fit his genes.

Since man is capable of modifying its environment, he is able to escape to natural selection processes. In this sense human evolution has trascended biological evolution, because the human evolutionary process is now governed to a certain degree by its spirit which bringing out an adaptation of physical characters to spirit. In this context it may be easier, or perhaps, more difficult to forecast the future evolution of mankind.

Although we may accept the existence of genetic clocks for development, differentiation and evolution of living matter, the open ended and perverse nature of time in science, also, presents features outside the realm of a strict scientific treatment. This does not mean that they will be so in the future. It is possible to hope that science, within its limitations, will uncover some of the answers for these questions.

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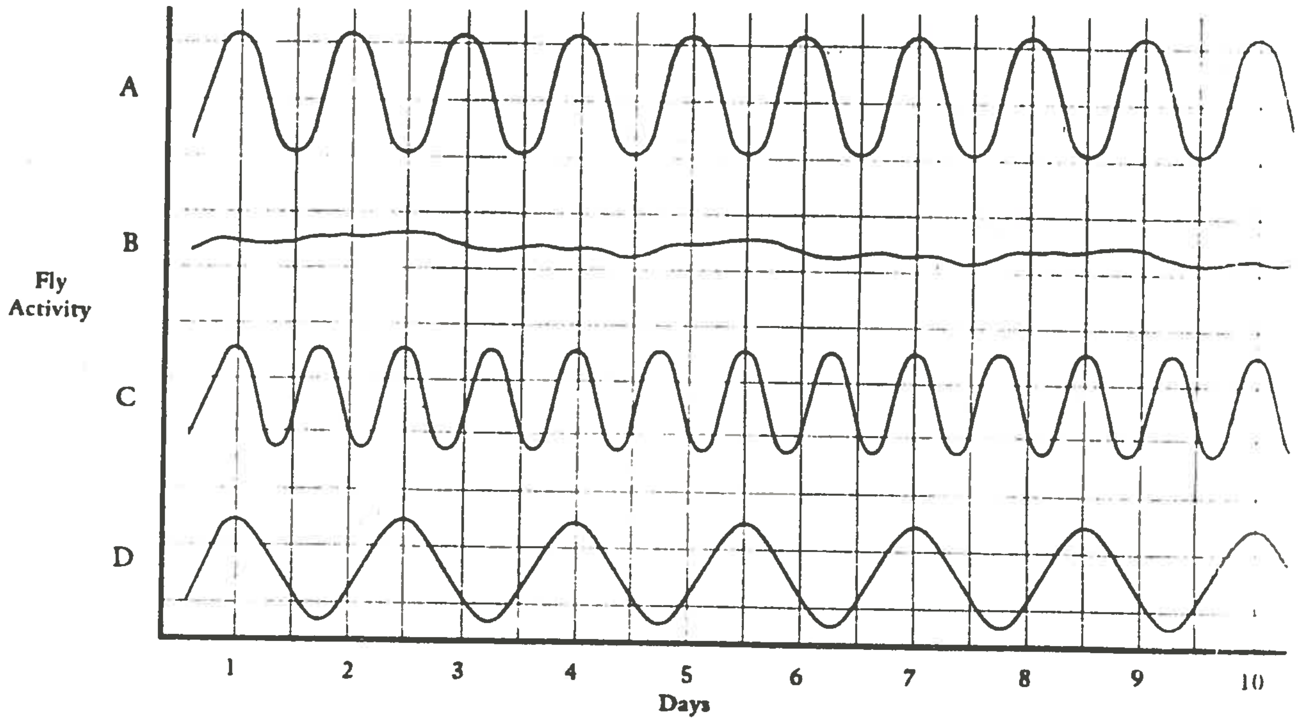

Fig. 1.

GRAPH A. When present in normal amounts, the per gene helps promote a rhythmic wave of alternating activity and inactivity.

Graph B. The arrhythmic timing pattern of the mutant fly strain, pero, which lacks a working per gene.

Graph C. The pers mutation causes a faster running biological clock with peaks of activity every 18 rather than the usual 24 hours.

Graph D. The amount of per protein a fly makes affects the speed of its biological clock. Specially engineered per genes have been introduced into the fly generating protein in varying concentrations. Shown is the activity of a fly making 20 times less protein than normal. It has about half as many cycles as the hyperactive mutant fly, pers in Graph C.