Department of Medical Genetics
New York State Psychiatric Institute, New York

Time Factor in Cytogenetics and Neoplasia

Mihály Bartalos

SUMMARY

In the complex interactions of genetic and environmental factors, time represents a coordinate. Thus, it is only logical to consider biological phenomena and features in the three dimensions of space and in the dimension of time. Gedda and coworkers devoted much attention to the genetic aspects of various chronobiological events in man, and performed an extensive series of experiments to probe the many ramifications of gene-related timing phenomena.

Time factors in cytogenetics and neoplasia are discussed by considering chronobiological events as manifestations of interconnecting oscillatory phenomena in living systems. These phenomena, in turn, are regarded as the products of negative feedback control processes.

Subjects under discussion include satellite association, nondisjunction, C-mitotic duplication, endomitosis, endoreduplication, and delayed fertilization. The different theories of carcinogenesis are briefly reviewed and the time factor, as it applies to postulated mutational changes in neoplasia, is discussed.

Recent interest in the genetic aspects of various chronobiological events in man is largely due to the studies of Professor Gedda and his coworkers. At this stage of our knowledge it is necessary to formulate new working hypotheses and to apply new strategies to this area of study. In the followings I will review some recent developments in theoretical biology and will attempt to provide a theoretical framework for the study of gene-related chronobiological events. This will be followed by an enumeration of some of the phenomena in cytogenetics and in carcinogenesis where chronobiologically oriented genetic studies may prove rewarding.

Time as a Coordinate

Biological phenomena generally are regarded as the results of a complex interaction between environmental influences and the genetic information of cells. In this interaction *space* represents three coordinates and *time* serves as the fourth coordinate (von Bertalanffy, 1952). Gene-environment interactions, when viewed in the perspective of time, assume a *direction*. This is exemplified by successive stages of development and by the successive activation and inactivation of genes during embryonic life. Gene-environment interactions are also characterized by *change*.

 Acta Genet. Med. Gemellol. (1971), 20: 350-358

In other words, it is a dynamic system. Furthermore, gene-environment interactions have a *continuity* over succeeding generations of organisms and cells whereby the genetic composition of the preceding generation and the environment shaped by it will provide the genetic information and the environment for the succeeding generation.

General System Theory and Cybernetic Principles

Our understanding of life phenomena was greatly advanced by von Bertalanffy's General System Theory (1952, 1969) which is gaining increasing acceptance in different branches of science (Meir, 1969). The General System Theory is concerned with the formulation and derivation of those principles which hold for systems in general, where " system " is defined as a complex of elements in interaction. The Theory distinguishes between open systems and closed systems. The importance of this distinction is underlined by the observation that the two principles of classical thermodynamics hold only for closed systems; i.e., to a system where there is an exchange of energy, but not matter, with the outside world (Prigogine, 1947). The living organisms represent open systems. Another of the basic tenets of the General System Theory is the recognition that in living organisms the large number of open systems are organized in hierarchical levels.

The functioning of these open systems and the laws governing their interrelationship became amenable to mathematical analysis following Wiener's formulation of the cybernetic principles (Wiener, 1948). According to cybernetic interpretations the functioning of hierarchically ordered open systems may be described as an hierarchical organization of negative feedback mechanisms. One property of this system is the generation of oscillations. Goodwin (1963) made pioneering efforts in establishing a sound mathematical basis for the time-dependent properties of interconnecting pathways of metabolism. He was especially concerned with the oscillating properties of interlocking systems, starting with the genetic locus and ending with the synthesis of a particular enzyme. The enzymes, in turn, were subjected to a mathematical analysis by Gander (1967) who showed that the physical properties of allosteric enzymes could generate and maintain relatively rapid oscillations in the concentrations of metabolites.

Oscillations and Chronobiological Phenomena

It is the inherent property of negative feedback control processes that they generate oscillations. It was proposed by Goodwin (1967) that the continuous on-going rhythmic activity of cells and organisms be called " talandric energy ". Talandric energy is regarded as a distinct type of activity of cells which is analogous to physical activity but distinct from it. Rhythmic activity, or talandric energy, was proposed to represent the basic driving force in inanimate systems (Goodwin, 1967).

It is of considerable interest that continuous oscillatory activities can produce biological rhythm or " chronobiological variations " (Sollberger, 1962). Such oscillatory activities where already used to explain such seemingly diverse phenomena as the cell division cycle, morphogenic movements, and chemotaxis (Goodwin, 1967).

According to this line of thought, living organisms may be considered as a hierarchical collection of interacting open systems having nonlinear-oscillatory characters with the ability of self-replacement of its own components and ability of self-replication of the entire system.

Inductions of Chronobiological Changes

It follows from the preceding discussions that changes in chronobiological parameters may result from changes in the oscillatory parameters (Tab. I). Oscillatory parameters, on the other hand, may be altered either by changes in the *Zeitgeber* (external events, such as light, temperature, etc., with which oscillations of the system may be synchronized), or by structural changes of the components of the oscillatory

Tab. I. Changes in oscillatory parameters

1. Changes in the *Zeitgeber*	Environmental
2. Structural changes of the components of the oscillatory system	
2.1. External influences	
2.2. Inherited changes	Genetic

systems. The structural changes can involve different components of the oscillatory systems, such as hormones, enzymes, and genes. Such structural changes result, either from external influences, such as ionizing radiation, or may be inherited as a mutational change.

It follows from the foregoing considerations that chronobiological changes may result from either external influences or from changes in the genetic material. Thus, when chronobiological changes are considered as phenotypic features, the role of genetic factors in their causation should be established. Once the etiological role of genetic factors is ascertained, the abnormality may be subjected to formal genetic analysis.

Genetic Changes and Chronobiological Variations

In regard to the nature of the genetic change, one may envision three basic genic alterations that may lead to chronobiological variations (Tab. II):

(1) The timing of the activation or inactivation of a gene is altered.

(2) There is a change in the nucleotide sequence of a structural gene resulting in the production of an altered protein. Such a protein molecule may have altered physico-chemical properties causing alterations in the molecule's oscillatory parameters. Altered oscillatory processes, in turn, may cause chronobiological changes at the gross phenotypic level.

(3) The timing of gene activation is correct and the nucleotide sequences are coding for the correct information, but the *informational lifetime* of a structural gene is altered. Such a decreased informational lifetime may result (*a*) from inherent variations in the chemical properties of the constituents of the DNA molecule leading to early decomposition, or (*b*) from faulty functioning of the DNA repair mechanisms preventing proper restitution of damaged DNA, or (*c*) from the co-occurrence of both of these mechanisms (Gedda and Brenci, 1969).

Tab. II. Possible genetic changes as causes of chronobiological variations

1. Single Gene Defect
   - 1.1. Altered timing of activation or inactivation of a gene
   - 1.2. Nucleotide substitution leading to the production of a protein with altered catalytic properties and/or with altered functional lifetime
   - 1.3. Altered informational lifetime of the gene
2. Multiple Gene Defects
   - 2.1. Additive
     - 2.1.1. Affecting proteins which have diverse function
     - 2.1.2. Affecting proteins which participate in the same metabolic pathway
     - 2.1.3. Affecting different polypeptides of the same protein molecule
   - 2.2. Superimposed

In addition to changes involving structural genes, alterations of controller genes likewise may lead to chronobiological variations. Furthermore, alterations of both the structural and control gene may be present at the same time and thus their effects may be superimposed.

Beside such *superimposed gene defects* one may consider the possibility of an *additive gene defect*, meaning the concomitant change of more than one structural gene or of more than one controller gene leading to compound changes in chronobiological phenomena.

Additive gene defects may be of three kinds: (1) the proteins influenced by the affected genes have diverse functions; (2) the affected proteins participate in the

same metabolic sequence; and (3) the affected genes influence polypeptide chains which are constituents of the same protein molecule.

While many chronobiological events are influenced by changes of a single gene, others may be appreciably altered only after the accumulation of a certain number of strategically placed mutations. Such a mechanism was indeed proposed to be operative in aging (Gander, 1967) and carcinogenesis (Burch, 1963).

Gene Action in Time

In a cell, at a certain time, a gene is either active or inactive metabolically. The state of activity, however, may not need to be permanent; it may change from active to inactive and vice versa. The temporal succession of gene activity states was called *chronaxy* by Gedda (1965). Later Bartalos (1967) proposed that the metabolically active phase of a gene be called *positive chronaxy* and the inactive one *negative chronaxy*. Other proposed terms included *recurrent positive chronaxy* and *recurrent negative chronaxy* referring respectively to the reactivation of a previously active gene which was subsequently inactivated and to the reinactivation of a previously inactive gene that subsequently became activated. A gene, whose state of activity is out of phase with the rest of the genome is in an *uncoordinated chronaxy state*. Furthermore, a chronaxy state may be *conditionally positive* toward an agent meaning that the gene is in the active state but could be rendered inactive by the agent in question under proper conditions. Conversely *conditional negative chronaxy* refers to the potential vulnerability of an inactive gene toward an agent which could act as the gene's activator.

Gedda (1965) pointed out that each gene has a chronological dimension. The original information content of the gene does not have to remain unchanged, but may have a given period of existence. That period of time during which the original information content of the gene remains intact is called *chronon* (Gedda and Brenci, 1969). This term refers to the potential availability of the original genetic information regardless whether the gene is engaged in transcription, duplication, or is in an inactive, repressed state.

The degree of the informational stability of the gene is referred to as *ergon* (Gedda and Brenci, 1969). It is considered to be the resultant of the individual stabilities of the constituent nucleotides of the gene. The ergon as an *energy of stability* was distinguished by these authors from an *energy of information*. Two genes with dissimilar nucleotide sequences may code for identical information as the result of the degeneracy of the genetic code. Gedda and Brenci (1969) pointed out that the same two genes however, may have different ergons because of the different stability of their constituent nucleotide pairs. Beside variations in nucleotide sequence, other factors influencing a gene's ergon include the redundancy of the genetic information and the efficiency of the genetic repair and/or correcting mechanisms.

Some Chronobiological Phenomena in Cytogenetics

A failure of coordination in the timing of various intracellular processes seems to account for several cytogenetic phenomena. In *C-mitotic duplication* chromosome duplication, spiralization and dissolution of the nuclear membrane take place, but because of failure of the spindle mechanism, the chromosomes do not move to the opposite poles. Eventually, the duplicated chromosome set is reconstituted within a single nucleus. This process is called C-mitotic in recognition of the fact that changes of the same kind can be induced by colchicine. In *endomitosis* (Geitler, 1939) duplication and spiralization of the chromosomes occur without dissolution of the nuclear membrane. After separation of the sister-chromatids the division does not proceed further and chromosome despiralization takes place. In the case of *endoreduplication* (Levan and Hauschka, 1953) chromosome duplication takes place during the interphase without any visible signs of mitotic activity. It would appear that in all of these instances incoordination of intracellular events leads to incompleted cell division cycles.

Abnormalities in the separation of chromosomes during cell division may manifest themselves in *chromosome or satellite association, anaphase lagging* and *nondisjunction.* The facts that satellite association may lead to anaphase lagging or nondisjunction and that satellite association have a significant genetic determinant (Gedda and Brenci, 1969) suggest that a search for genetically determined chronobiological phenomena may be rewarding in all three of these conditions.

In animal experiments *overripeness of eggs* due to delayed ovulation resulted in the birth of offsprings, several of which had chromosomal abnormalities (Witschi and Laguens, 1963). It is conceivable that a similar timing defect may exist in man and is operative in some cases of human aneuploidy.

An important puzzle is the mechanism by which the incidence of chromosome aneuploidy is increased among the offspring of older mothers. What makes some zygotes to experience chromosomal maldistribution while children born latter to the same mothers escape this fate? Would eventually all zygotes show chromosomal maldistribution if fertilization is delayed long enough? Is the mechanism observed in older mothers the same as that described in cases of familial predisposition to aneuploidy?

Aspects of Genic Chronology and Tumors

In view of our present knowledge it can be safely assumed that malignant transformation of cells is accompanied and preceeded by changes in the genetic material of the cells. Such changes can be brought about by several means, such as ionizing radiations, chemicals, and certain viruses, or they may be inherited.

A mutation which has the potential of causing malignant transformation may, theoretically, occur (1) on metabolically active chromosome segments, or (2) on *temporarily* inactivated chromosome segments (which have the potential to be acti-

vated during major changes in homeostasis, such as occur during puberty, pregnancy or menopause) and finally (3) such mutations may occur on chromosome segments which are inactive and are destined to remain inactive under physiological conditions. In this latter case a mutation would remain inconsequential as long as the affected segment is not turned-on by certain drugs or other unphysiological agents.

It may be added that carcinogenesis may involve not only cancer promoting mutations but that genes may exist which can counteract the carcinogenic effects of other genes within the same genome. In such case the development of cancer would depend not only on the presence of cancer promoting mutations but also on the balance between cancer-promoting and cancer-inhibiting genes. While such a system has been described (Hitotsumachi et al, 1971) the inhibitory genes are not incorporated into our scheme since they do not affect our basic conclusions while they would make our discussion considerably more complicated.

The development of malignant tumors, in general, need considerable time from the first application of a carcinogenic agent until the appearance of the tumor. The reason for the delayed appearance of malignant tissues is not known, but in some cases it seems to be related to differential cell line selection. An intriguing proposition was made by Burch (1963) who concluded from his data that in adult human beings malignant tumors generally develop from cells " containing a total of four specific (nuclear) gene mutations, one of which is often inherited, the remainder originating in one or more somatic cell lines. " Burch further suggested that two out of the four mutations probably affect homologous genes at a particular locus and the remaining two mutations probably affect homologous genes at another locus. It is evident that if more than one of these mutations are inherited by a person, then he only needs to accumulate two more mutations during his lifetime in order to develop cancer. Thus the inheritance of one or more genes with additive carcinogenic potential could account for cases with increased tumor susceptibility. The mechanism proposed by Burch (1963) is analogous to the earlier discussed *additive gene defects.*

An interesting recent observation is the description of transplacental carcinogenesis in man (Herbst et al, 1971; Greenwald et al, 1971). These reports describe several instances where administration of stilbestrol to pregnant mothers for the purpose of preventing threatened abortions resulted in the development of vaginal adenocarcinoma in their female children when they reached adolescence. Noteworthy features of these observations are that (1) stilbestrol is not a natural product but a synthetic nonsteroidal estrogen; (2) this type of tumor is exceedingly rare in the general population; (3) the mothers did not develop carcinoma; and (4) the time required for the appearance of the tumors is rather long.

While the mechanism of carcinogenesis is not known in this instance, clinical data suggest a certain parallelism to one of our previously mentioned mechanisms. It is possible that the genetic material of the unborn child is affected by the stilbestrol but the genetic change remains masked because the affected genes are in an inactive state. At the time of puberty, however, when there is a change in homeostasis, the altered genes become unmasked and thus malignant transformation and proliferation ensues.

Conclusions

A fresh look at the gene with special regard to its temporal aspect is indicated. The knowledge gained by such studies would contribute to a better understanding of biological phenomena and may open up new avenues of inquiry into disease mechanism. The potential usefulness of chronobiological studies in cytogenetics and carcinogenesis was illustrated by selected examples.

References

Bartalos M. (1967). On the concepts of chronon and chronaxy and their implications in neoplasia. Acta Genet. Med. Gemellol., **16:** 21-26.

Burch P. R. J. (1963). Human cancer: Mendelian inheritance or vertical transmission? Nature (Lond.), **197:** 1042.

Gander J. E. (1967). Some possible interrelationships between steady-state kinetics and time. Ann. N. Y. Acad. Sci., **138:** 730.

Gedda L. (1965). Application de la génétique à la pratique médicale. (La constitution au point de vue de la génétique - Le "chronon": concept et application au service de la médecine). Acta Genet. Med. Gemellol., **14:** 1-12.

Gedda L., Brenci G. (1969). Biology of the gene: the ergon/chronon system. Acta Genet. Med. Gemellol., **18:** 329-379.

Geitler L. (1939). Die Entstehung der polyploiden Somakerne der Heteropteren durch Chromosomenteilung ohne Kernteilung. Chromosoma, **1:** 1.

Goodwin B. C. (1963). Temporal Organization in Cells. Academic Press, New York.

Goodwin B. C. (1967). Biological control processes and time. Ann. N. Y. Acad. Sci., **138:** 748.

Greenwald P., Barlow J. J., Nasca P. C., Burnett W. S. (1971). Vaginal cancer after maternal treatment with synthetic estrogens. New Eng. J. Med., **285:** 390.

Herbst A. L., Ulfelder H., Poskanzer D. C. (1971). Adenocarcinoma of the vagina: association of maternal stilbestrol therapy with tumor appearance in young women. New Eng. J. Med., **284:** 878.

Hitotsumachi S., Rabinowitz Z., Sachs L. (1971). Chromosomal control of reversion in transformed cells. Nature (Lond.), **231:** 511.

Levan A., Hauschka T. S. (1953). Endomitotic reduplication mechanisms in ascites tumors of the mouse. J. Natl. Canc. Inst., **14:** 1.

Meir A. Z. (1969). General system theory. Developments and perspectives for medicine and psychiatry. Arch. Gen. Psychiatry, **21:** 302.

Prigogine I. (1947). Étude Thermodynamique des Phénomènes Irreversibles. Liège. (In L. von Bertalanffy, 1952).

Sollberger A. (1962). General properties of biological rhythms. Ann. N. Y. Acad. Sci., **98:** 757.

Von Bertalanffy L. (1952). Problems of Life. An Evaluation of Modern Biological and Scientific Thought. Harper & Brothers, New York 1960.

Von Bartalanffy L. (1969). General System Theory: Foundations, Developments, Applications. George Braziller, New York.

Wiener N. (1948). Cybernetics. The Technology Press, Cambridge, Massachusetts.

Witschi E., Laguens R. (1963). Chromosomal aberrations in embryos from over-ripe eggs. Dev. Biol., **7:** 605.

RIASSUNTO

Nelle complesse interazioni dei fattori genetici ed ambientali il tempo rappresenta una coordinata. Diviene quindi logico considerare i fenomeni biologici nelle tre dimensioni dello spazio e nella dimensione del tempo. Gedda e collaboratori hanno dedicato molta attenzione agli aspetti genetici dei vari eventi cronobiologici nell'uomo, conducendo una lunga serie di esperimenti per chiarire le numerose ramificazioni dei fenomeni temporali collegati al gene.

Vengono discussi i fattori temporali nei settori della citogenetica e delle neoplasie, considerando gli eventi cronobiologici come manifestazioni di fenomeni oscillatori fra loro collegati nei sistemi biologici. A loro volta, tali fenomeni sono considerati come prodotti di processi di controllo di un feedback negativo.

Sono discussi casi di satelliti, non-disgiunzione, duplicazione C-mitotica, endomitosi, endoduplicazione e fecondazione ritardata. Le diverse teorie della cancerogenesi vengono brevemente passate in rassegna e viene discusso il fattore tempo nella sua applicazione alle variazioni mutazionali postulate nelle neoplasie.

RÉSUMÉ

Dans les complexes intéractions des facteurs génétiques et ambiants, le temps représente une coordonnée. Il est par conséquent logique de considérer les phénomènes biologiques dans les trois dimensions de l'espace ainsi que dans la dimension du temps. Gedda et ses collaborateurs ont dédié beaucoup d'attention aux aspects génétiques des différents événements chronobiologiques chez l'homme, conduisant une longue série d'expérimentations afin de mieux comprendre les nombreuses ramifications des phénomènes temporels reliés au gène.

Les facteurs temporels dans les secteurs de la cytogénétique et des néoplasies sont discutés considérant les événements chronobiologiques en tant que manifestation de phénomènes oscillatoires entreliés dans les systèmes biologiques. Ces phénomènes sont à leur tour considérés comme des produits de procès de contrôle d'un feedback négatif.

Sont discutés des cas de satellites, non-disjonction, duplication C-mitotique, endomitose, endoduplication et fécondation retardée. Les différentes étapes de la cancérogénèse sont brièvement examinées, et le facteur temps est discuté dans son application aux variations mutationnelles postulées dans les néoplasies.

ZUSAMMENFASSUNG

Die Zeit ist eine Koordinate in dem komplexen Wechselspiel zwischen Umwelt und Vererbung. Es erscheint daher logisch, die biologischen Phänomene nicht nur in den drei Raummassen sondern auch in ihrem Zeitmass zu betrachten. Gedda u. Mitarb. haben den genetischen Aspekten der verschiedenen chronobiologischen Ereignisse beim Menschen grosse Aufmerksamkeit gewidmit, in dem Versuch, durch weitläufige Serienuntersuchungen die zahlreichen, gengebundenen Verzweigungen der zeitlichen Phänomene zu klären.

Dabei erörtern sie die Bedeutung der Zeitfaktoren auf dem Gebiet der Zellgenetik und der Neoplasien, indem sie die chronobiologischen Ereignisse als Äusserung von Schwankungsfaktoren ansehen, welche in den biologischen Systemen miteinander verbunden sind. Diese Phänomene werden ihrerseits als Ergebnis von Kontrollprozessen eines negativen feedback angesehen.

Es werden folgende Fälle besprochen: Satellite, non-Dysjunction, C-Mitosen-Duplikation, Endomitosen, Endoduplikation und Befruchtungsverzögerung. Dabei werden auch die verschiedenen Krebstheorien kurz gestreift und der Zeitfaktor erörtert, der auf die bei Neoplasien vermuteten Mutationsvariationen angewandt wird.

MIHÁLY BARTALOS, M. D., 722 West 168th Street, New York, N.Y. 10032, USA.