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On the Concepts of Chronon and Chronaxy and their Implications in Neoplasia

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Gedda (1965) recently proposed the term Chronon to designate the temporary character of the phenotypic expression of the gene. There are, indeed, good reasons to believe that during embryonic development several genes become active only at certain stages of development and that many of these genes may become metabolically inactive at subsequent stages of differentiation. But activation of previously " silent " genes is not limited to prenatal life. We may cite the well-known example of Huntington's chorea, which is a hereditary disease manifest mainly between 25 and 45 years of age (Bell, 1934). In addition to clinical and developmental genetics the concept of chronon appears to be important in cancer research as well as in basic biochemical genetics. The purpose of the present communication is to discuss the Chronon concept in biochemical terms and to indicate potential applications of this concept in some areas of research.

Recent advances in genetics and biochemistry opened up new avenues for thought. Subsequent experimentation on the molecular mechanism of gene regulation provided a molecular model for the time-dependent expression of genetic information, or chronon.

The substantiating demonstration by Huang and Bonner (1962) that in cells of higher organisms histones function as supressors of chromosomal ribonucleic acid synthesis is of far-reaching importance. Those fractions of histones which are rich in the amino acid arginine seem to fulfill this role (Allfrey *et al.*, 1963). According to our present concept arginine-rich histones regulate the activity of the genetic material by associating and dissociating with the nuclear DNA. DNA segments associated with arginine-rich histones do not participate in DNA-dependent RNA synthesis; genes on these segments are " turned off ". In the differentiated cells of a higher organism only a fraction of the genes are " turned on " at a given time (Littau *et al.*, 1963). It is apparent that time dependent activation and inactivation of genes may be the cornerstone of differentiation, thus the elucidation of the period of activity of a gene is of paramount importance in embryology.

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To designate the period during which a gene is expressed, i.e. engaged in m-RNA production, Gedda (1965) proposed the term " Chronaxy ". However, a designation is needed for the inactivated state of the gene, too. I propose the terms " positive chronaxy " and " negative chronaxy " for the designation of the metabolically active and inactive phases of the genes respectively. It is conceivable that genes which were active at some period of embryonic development, but subsequently become inactivated, can be reactivated again. This phenomena could be referred to as " recurrent positive chronaxy ". Similarly, " recurrent negative chronaxy ", then, would refer to the reversal of the " positive chronaxy " or the " recurrent positive chronaxy " state of genes. If an agent is able to cause recurrent positive chronaxy for some genes, the genome would be in an " uncoordinated chronaxy " state. Uncoordinated chronaxy would result also from " prolonged positive chronaxy " and for " prolonged negative chronaxy " of some genes. Theoretically, " recurrent positive chronaxy " and " uncoordinated chronaxy " may be implicated in the genesis of various maldifferentiation states, such as malignant neoplasia and congenital malformations.

It is of interest that the states of positive chronaxy and negative chronaxy of the genes have become subjects of experimental inquiries. It was reported (Davidson, 1965) that the action of at least some of the steroid hormones seems to be activation of genes by removing the blocking histones; i.e. induction of positive chronaxy. The reverse is true for the polypeptide antibiotic, actinomycin D (C1), which combines with the DNA and arrests DNA-dependent RNA synthesis (Hamilton *et al.*, 1963). According to the proposed terminology, this would represent an instance for artificial negative chronaxy.

In addition to negative and positive chronaxy we may encounter additional chronaxy states. If we assume, on the basis of present evidence, that steroid hormones act as gene-activators, it must follow that not all of the genes in negative chronaxy are vulnerable to their effect, since their increased production or administration would lead to devastating disharmony among the highly specialized cells of an organism. Thus some of the genetic material of some cells must be vulnerable toward this agent while others are not. To designate the vulnerability state of certain inactive genes toward a *given* agent the term " conditionally negative chronaxy " is proposed. A gene may be inert or in negative chronaxy state in respect to one agent while it may be susceptible or in a conditionally negative chronaxy state toward a different agent at the same time. Further experimentation may lead to the experimental establishment of a " conditionally positive chronaxy state " which would refer to the supressibility of certain genes by certain chemical agents.

In addition to recurrent positive chronaxy and/or uncoordinated chronaxy we can conceive of further genetic mechanisms which can be implicated in malignant tumors. If mutations of the structural genes are responsible for the development of certain types of malignancies, such mutations may occur on genes in positive chronaxy. In this case, a more or less immediate malignant transformation would be expected. If, however, such mutations affect genes which are in conditionally negative chronaxy state, then drastic changes in the homeostasis, i.e. puberty, stress, preg-

nancy, menopause, etc. may initiate tumor development by turning these genes into positive chronaxy. In addition, such " tumor-genic " mutations may affect genes which are in negative chronaxy and thus will not be manifested during the life time of the individual. If such genes, however, behave as if in conditionally negative chronaxy toward some unphysiological agents, their application to the individual may initiate tumor growth. It is conceivable that some chemical carcinogens, especially those with a chemical structure resembling steroid hormones, may initiate cancerous transformation by this mechanism. While it must be apparent that these mechanisms do not cover the whole range of carcinogenic mechanism at the genetic level, such as genetic transfer, etc., they have been discussed as an extension to the concepts of Chronon and Chronaxy of Gedda to illustrate the usefulness of these concepts in the study of gene regulation. Since a fuller understanding of gene regulation in man seems to be essential for future attempts to change the genetic expression in man, as it may be indicated in certain types of neoplasia and in some inherited genetic diseases of man, the creation of a terminology for the newly uncovered phenomena seems to be desirable.

Summary

The concepts of Chronon and Chronaxy of Gedda are discussed in the light of recent biochemical-genetic knowledge. It is proposed that the term « positive chronaxy » and « negative chronaxy » be employed to designate the period during which a gene is in active and inactive state, respectively. Other terms proposed are « recurrent positive chronaxy », « recurrent negative chronaxy » and « uncoordinated chronaxy ». It is pointed out that the different chronaxy states of genes may be subject to modification by certain agents. If a given agent is able to change a gene from a negative chronaxy state into positive chronaxy state, the gene toward this agent behaves as being in a « conditionally negative chronaxy state ». In the same sense, we can speak also of a « conditionally positive chronaxy state » of a gene. It is proposed that mutations facilitating malignant transformation of cells can, theoretically, occur in both the active and inactive segments of the DNA. If active segments of the DNA are affected, i.e. those in positive chronaxy state, the effects of such mutations would be expressed immediately. If, however, genes in negative chronaxy state have similar mutations, their effect would be expressed only if these genes become activated by some agents, either physiological or unphysiological. Malignancies facilitated by hormonal inbalance may represent instances or mutated gene activation by physiological agents while tumors caused by carginogenic substances with a structure resembling steroid hormones could be examples of gene activation by unphysiological agents.

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RIASSUNTO

Vengono discussi i concetti di « chronon » e di « cronassia » formulati da Gedda, alla luce delle recenti acquisizioni della genetica biochimica. Si propone che i termini di « cronassia positiva » e « cronassia negativa » vengano adoperati per indicare il periodo durante il quale un gene si trova rispettivamente nello stato attivo e inattivo. Altri termini proposti sono « cronassia ricorrente positiva », « cronassia ricorrente negativa » e « cronassia non coordinata ». Si suggerisce che i differenti stati della cronassia dei geni possano essere soggetti a modificazioni da parte di alcuni agenti. Se un dato agente è in grado di modificare un gene da uno stato di cronassia negativa ad uno di cronassia positiva, nei riguardi di tale agente il gene si comporta come in uno « stato di cronassia condizionalmente negativa ». Analogamente, si può anche parlare di uno stato di « cronassia condizionalmente positiva » di un gene. Si suggerisce che le mutazioni che facilitano una trasformazione maligna delle cellule possano in teoria verificarsi sia nel segmento attivo che in quello inattivo del DNA. Se sono colpiti segmenti attivi del DNA, cioè quelli nello stato di cronassia positiva, gli effetti di tali mutazioni verrebbero ad essere immediatamente espressi. Se, viceversa, simili mutazioni vengono a colpire geni in stato di cronassia negativo, il loro effetto verrebbe ad essere espresso soltanto quando tali geni venissero attivati da alcuni agenti, a carattere sia fisiologico che non fisiologico. I tumori facilitati da squilibrio ormonale possono rappresentare casi di attivazione di geni mutati da parte di agenti fisiologici, mentre i tumori causati da sostanze carcinogene con una struttura simile a quella degli ormoni steroidi, potrebbero essere esempi di attivazione genica da parte di agenti non fisiologici.

RÉSUMÉ

Les concepts de « chronon » et « chronaxie » (Gedda, 1965) sont discutés sur la base des récentes acquisitions de la génétique biochimique. L'on propose que les termes « chronaxie positive » et « chronaxie négative » soient employés pour indiquer la période pendant laquelle un gène se trouve, respectivement, dans l'état actif ou inactif. D'autres termes proposés sont « chronaxie recourante positive », « chronaxie recourante négative » et « chronaxie non coordonnée ». L'on suggère que les différents états de la chronaxie des gènes puissent être sujets à des modifications de la part de certains agents. Si un agent donné est capable de modifier un gène,

d'un état de chronaxie négative à un de chronaxie positive, vis-à-vis de cet agent le gène agit comme s'il se trouvait en « état de chronaxie conditionnellement négative ». Egalement, l'on peut aussi parler d'un « état de chronaxie conditionnellement positive ». L'on suggère l'hypothèse que les mutations facilitant une transformation maline des cellules puissent théoriquement avoir lieu aussi bien dans le segment actif que dans le segment inactif de l'ADN. Si des segments actifs sont atteints — c'est-à-dire des segments en état de chronaxie positive — les effets de ces mutations seraient immédiatement exprimés. Si, au contraire, des gènes en état de chronaxie négative sont atteints, leur effet n'est exprimé que lorsque ces gènes sont activés de la part de certains agents, à caractère physiologique ou non physiologique. Les tumeurs facilitées d'un déséquilibre hormonal peuvent représenter des cas d'activation de gènes mutés de la part d'agents physiologiques, tandis que les tumeurs causées par des substances carcinogènes, à structure semblable à celle des hormones stéroïdes, pourraient constituer des examples d'activation génique de la part d'agents non physiologiques.

ZUSAMMENFASSUNG

Im Licht der neuesten Errungenschaften der biochemischen Genetik werden die von Gedda formulierten Begriffe « Chronon » und « Chronaxie » erörtert. Es wird vorgeschlagen, für die Zeit, während der sich ein Gen in aktivem bzw. inaktivem Zustand befindet, die Ausdrücke « positive Chronaxie » und « negative Chronaxie » zu verwenden. Ausserdem werden folgende Bezeichnungen vorgeschlagen: « positive rekurrente Chronaxie », « negative rekurrente Chronaxie » und « nicht koordinierte Chronaxie ». Man nimmt an, dass die verschiedenen Chronaxie-Stadien der Gene durch irrgendwelche Erreger Veränderungen erfahren können. Wenn ein Erreger imstande ist, ein Gen vom positiven ins negative Stadium zu überführen, so benimmt sich dieses Gen dem Erreger gegenüber so, als ob es sich in einem « bedingt negativen Chronaxiestadium » befände. Ebenso kann man auch von der « bedingt positiven Chronaxie » eines Gens sprechen. Man nimmt an, dass die Mutationen, welche eine maligne Transformation der Zellen förden, theoretisch gesehen sowohl im positiven als auch im negativen Segment der DNS stattfinden können. Wenn aktive, d. h. in positivem Chronaxiestadium befindliche DNS-Segmente davon betroffen werden, so dürften sich die Wirkungen einer solchen Mutation sofort äussern. Wenn diese Mutationen hingegen Gene in negativem Chronaxiestadium betreffen, so dürfte ihre Wirkung erst dann zum Ausdruck kommen, wenn die Gene durch physiologische oder auch nicht physiologische Erreger aktiviert würden.

Es ist möglich, dass die durch mangelndes hormonelles Gleichgewicht in ihrer Entwicklung begünstigten Tumore Fälle darstellen, in denen Gene, welche durch physiologische Erreger einer Mutation unterworfen worden waren, aktiviert wurden. Diejenigen Tumore hingegen, welche durch krebserzeugende Substanzen mit den Steroidhormonen ähnlicher Struktur bedingt wurden, könnten Beispiele für eine Genaktivierung durch nicht physiologische Erreger darstellen.

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Note to Dr. Bartalos paper

Dr. Bartalos' concepts and proposed complementary definitions are certainly fit, but only concern one single aspect of Chronon, as defined by Gedda, i.e. the temporal succession of genic activations and inactivations; namely, the gene «chronaxy».

A complete interpretation of the concept of Chronon should first of all take into account the temporal interval between the first activation and the last inactivation of the gene action; namely, the total period of erogation of the genic information itself. The reason is that Chronon differs from the usual concept of a control of the gene action, realised through the operon's relais. Actually, the Chronon postulate is that the last inactivation be not considered as such, but rather to represent the exhaustion of the energetic quantum representing the information of a structural gene.

Chronon therefore not only represents the temporal modalities of erogation of the genic information, but also the potential time of the information itself, and therefore the time of existence of the gene as a functional unit.

Ed.

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