[CANCER RESEARCH 28, 1823–1824, September 1968]

Summary of Discussion on Developmental Biology of Normal Cells

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In this part of the symposium we have heard current ideas and experiments concerning components that bring order to the replication of genetic material and to events preceding cell division. We attempt to distinguish these from other components which, although necessary, do not seem to have a coordinating function in the replication cycle. It is convenient to visualize the coordinating subsystem as a circular chain of causation, with branches, to show that some of the events having unique temporal relations to the cycle may nevertheless not determine the subsequent steps. Disorders of such a system can be imagined in the form of permanent blockades which, although eventually lethal, appear to be normal for some kinds of differentiated cells; or they may be imagined in the form of temporal displacements that would cause the system to dismantle itself, as would happen for instance, if cytokinesis preceded chromosome replication. Finally, of particular interest in the context of neoplasms, are disorders in which the system remains functional, but may be thought of as isolated from controls evolved to arrest the cycle when that is appropriate to the role of the cell in an organism. The speakers discussed a number of real entities that can be fitted into the foregoing framework.

The replicon concept, first derived from experiments with bacteria, is the principal framework of speculation in regard to the replication of the genome. Pardee discussed the structural integrity of the bacterial replicon, the attachment of the growth fork and replicating complex to the cell membrane, the dependence of septum formation on DNA synthesis, the independence of DNA synthesis on septum formation, the frequent, though not universal, existence of a unique starting or finishing point in the chromosome, the dependence of reinitiation on new protein synthesis, and the completion of already initiated cycles in the absence of new protein synthesis. The most certain generalization that can be made is that the initiation of a growth fork in DNA, and its progression along the chain, are shown by experiment to be clearly separable; that is, each can be blocked independently of the other. Moreover, it is the initiation step that sets the pace in the sense that inability to provide deoxynucleoside triphosphates is not the usual limitation on DNA synthesis. The mechanism of reinitiation is therefore a central problem.

The hypothesis that comes most readily to mind is that initiation may be caused by passage of the growth fork over a certain locus, or by doubling of a certain locus. As Lark pointed out, however, cycling (in the sense of initiation of new growth forks) continues on time when DNA synthesis is blocked. If this were true for all the cells in an asynchronous population, it would mean that neither the passage of the growth fork nor the consequent abrupt change in marker frequency can be the timing agent. On the other hand, if it is true for only some of the cells, namely, those which have already doubled a critical locus at the moment of blockade, then it would mean that the replication of that locus is in some way a trigger, necessary but not sufficient, for the production of new replication complexes. This raises the question of the relationship between the doubling of a locus and its transcription. For a locus that is constantly being transcribed, it is obvious that doubling of the gene will augment its product. A more interesting possibility is that passage of the growth fork may be followed by a brief burst of transcription consequent to the displacement of a repressor from its binding site. Even if the repressor were not displaced, one of the daughter genes would be temporarily derepressed. As to the completion of the round of replication, it is an obvious prerequisite to chromosome separation, but we can dismiss the notion that completion *per se* is the signal for renewal of the replicating complex.

A corollary of the replicon concept is that a random segment of DNA introduced into a cell does not replicate. The truth of this generalization is born out in all the circumstances leading to the production of linear clones. Conversely, when an adventitious segment of DNA does replicate indefinitely, it is found either to have integrated with the resident genome, or to have carried with it the essential genetic elements of a replicon, as in the case of episomes and viruses. This emphasizes the requirement for structural contiguity of the system in cells, and the added difficulty of achieving it in cell-free systems. The DNA polymerases thus far isolable are not believed to replicate genomes, but rather to be repair enzymes; they prefer a single-stranded template and yield a biologically inactive product. If it were possible to isolate the replicon polymerase, it should (ideally) respond only to intact templates bearing the recognition features of the replicon.

Prescott reviewed the evidence (which can be taken as conclusive) that in contrast to bacterial chromosomes, which are single replicons, eukaryote chromosomes comprise many, each being the segment of DNA associated with a given initiation point. During the S period these are activated in a determined order as shown by the characteristic times of labeling of different chromosomal regions. This characteristic is probably an autonomous property of the chromosome segment, since it was seen not to be affected by chromosome rearrangement. The continuity of the chromosome, to judge from experiments on enzymatic degradation, resides in the DNA. If this is true, then the delineation of the different replicons must reside in local sequence specificity. The question arises whether the boundary

sequences are different in those activated at different times. The apparent autonomy of the replicons favors that interpretation. Moreover, the proteins that interact with the different regions are, one may surmise, made in the same way as proteins in general and are probably diffusible until they find their sites of action. In order for the sites to be distinguished from one another they must have different identifying sequences. The number of different sequences required may be much smaller than the number of replicons, since many replicons may be activated simultaneously; in any case, it appears inevitable that the multireplicon system in eukaryotes demands multiple specificities among the recognition sequences in DNA. With what proteins do these specific regions interact? After rejecting histones, two categories of answers remain: different polymerases that match the sites, or a number of regulatory proteins. This is the sort of question that could be approached by means of genetic analysis, for example, with temperature-sensitive mutants.

It is not known whether replication of one region is required for that of another. The problem is analogous to that of reinitiation in bacteria; it comprises the same alternatives. On the one hand, replication itself may set in motion the gene actions that lead to the next initiation; on the other hand, the replications may be only results, not causes. The cascade transcription model mentioned by Prescott, for example, is quite noncommittal in regard to whether the putative succession of transcriptions has any dependence on the succession of regional S periods it is imagined to coordinate.

Little was settled about G_1 and G_2. Inhibition experiments in G_1 seem equally compatible with a single initiator of S, or a complex system; the method has inherent limitations. The normal arrest of regulated cells in G_1 marks this as the stage in which the coordinating system has an interface with the peripheral features of the cell that are sensitive to the regulating conditions, such as cell to cell contact. A failure of regulation can be imagined at either level.