[CANCER RESEARCH 28, 1821-1822, September 1968]

Developmental Biology of Normal Cells: Biology and Biochemical Aspects[1]

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The accumulated evidence summarized here by Prescott (3) and elsewhere by others (2) leaves little doubt that the capacity of a cell to traverse its life cycle is dependent upon a sequence of transcriptions and translations. The degree of coupling of these events has been investigated in our laboratory, and the results may be of interest to those attending this symposium engaged in studies of fundamental control mechanisms as they apply both to normal and neoplastic cells. Through the use of life-cycle analysis technics (4) and a refined cell counting methodology, and by developing the argument that times exist in the cellular life cycle when specific requirements for macromolecular synthesis have been completed, we have described a series of biochemical markers in late G_2 (10). Chinese hamster cells in the last 2 hours of their life cycle divide successfully in the presence of 2 μg/ml of actinomycin D, indicating that no RNA biosynthesis essential for division occurs during this interval. In cells treated with protein-synthesis inhibitors (1 μg/ml of cycloheximide or 50 μg/ml of puromycin), a similar point can be observed 1 hour prior to division but still clearly in G_2. These points in the life cycle are interpreted as the respective ends of RNA and protein synthesis essential for division. The interval between the markers contains a unique class of cells which have completed transcription but not corresponding translation and must now traverse a significant portion of the life cycle, including late G_2 and all of M, to divide. These cells traverse successfully only so long as they are permitted to synthesize protein and stop immediately upon addition of protein-synthesis inhibitors (8). If cells were held in cycloheximide for 2 hours or less, they divided successfully upon removal of the inhibitor, indicating that appropriate messengers remained intact. Cells inhibited for periods exceeding 3 hours can be divided into two classes. After removal of the inhibitor, those trapped between the markers (transcription completed) failed to divide, presumably due to messenger decay, while cells located earlier in the life cycle than the actinomycin marker and, therefore, still capable of transcribing, divided successfully. Cells between the markers clearly cannot retranscribe and appear to have lost access to specific segments of their genome (8).

The relationship between transcription and translation has been shown by an experiment in which protein but not RNA synthesis was inhibited. No cells continued to transcribe while translation was inhibited (8). We know from both net synthesis (5) and isotope incorporation studies (8) that some RNA synthesis continues until the cell enters mitosis and that inhibition of functional RNA synthesis, therefore, must be quite specific.

These results lead us to conclude that traverse of the life cycle is the result of a series of highly specific and tightly coupled transcriptions and translations constituting an undetermined fraction of total messenger synthesis and related translation, and that the specific translation must, in each instance, be completed before the cell can proceed to the next transcription. Stent (6–8) and Cline and Bock (1) have recently reviewed evidence and have suggested models for a translational control sequence, and we now feel that our data support their views for translational control of progress through interphase in mammalian cells.

These markers have been observed in several established cell lines, and we consider them to be fundamental properties of the cell unrelated to the cell's history of presence or absence of neoplasia. Obviously, we would like to subdivide the life cycle further and to obtain precise times for biochemical events in G_1. These are currently conspicuous by their absence, and it may be that, since G_1 appears to be completely elastic (3, 9), there is no good reason to anticipate useful markers in G_1 against which specific events can be timed. In no case has it been possible to alter the timing of S, G_2, and mitotic events (9). Our view then is that fundamental control of the life cycle and, certainly, of the reproductive phases S, G_2, and M is rigidly impressed in a transcription-translation sequence in which the terminal events occur after the cell no longer has access to specific segments of its genome.

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