Meetings

Cytoplasmic and Environmental Influences on Nuclear Behavior

From 31 August to 3 September 1966 a symposium on "Cytoplasmic and Environmental Influences on Nuclear Behavior" was held at Woods Hole, Massachusetts, under the auspices of the Society of General Physiologists to determine whether knowledge of genetic control mechanisms and related phenomena of higher organisms (eukaryotic cells) was sufficiently advanced to be related to concepts of regulation in bacteria (protokaryotic cells). Hopefully, the achievements in the molecular genetics of bacteria would serve as models or guideposts in considering mechanisms in cells with a nuclear envelope.

It was evident early, from reports on chromosomal and cellular reproduction, that the differences between the cell types with respect to some control mechanisms, at least, might be greater than the similarities. The bacterial DNA synthesis period is generally continuous from one division to the next and this is a reason that regulation of chromosome replication, once triggered by division, is thought to be largely autonomous within the chromosome. In eukaryotes, however, DNA synthesis is usually preceded and followed by nonsynthetic periods and, thus, the cytoplasm is often thought to be controlling —at least in the initiation phase. A cytoplasmic influence on DNA synthesis initiation in amebae was shown by L. Goldstein (Philadelphia) and D. M. Prescott (Boulder) but, surprisingly, the cytoplasm also seems to be involved in the termination of DNA replication. It now becomes important to determine the basis of the very rapid transition from a cytoplasmic state (premitotic) in amebae that does not sustain replication to one (postmitotic) that does.

Probably extranuclear influences are important for the initiation of meiosis, since it is a distinct kind of differentiation in multicellular organisms and, moreover, the influences must act on controls unlike those of bacteria. Nothing, however, could have prepared one for the dramatic discovery, reported by H. Stern (La Jolla), that in the first meiotic prophase of lily pollen cells a small amount of DNA synthesis occurs a good deal after the apparently last typical mitotic cell cycle has ended. This DNA is essentially similar to the bulk of lily chromosomal DNA, except for a higher guanine-cytosine content. The late synthesis might represent repair of chromosome breaks associated with crossing-over or a specially delayed replication important for the onset of meiosis. Stern's persuasive experiments, including the demonstration that meiosis was blocked when the late DNA synthesis was inhibited, showed that here was an important mechanism in meiosis control.

That DNA synthesis regulation probably is central for the regulation of some kinds of genetic expression was shown by R. W. Dutton (La Jolla), who discussed many questions and answers dealing with antibody production but spoke mainly about his own experiments with model systems in vitro. He showed that rabbit spleen cells could be induced to give a highly specific and quantitatively significant immune response but, after an antigenic stimulation, first must initiate DNA synthesis and proliferate. They thus differ not only from bacteria, which undergo induced protein synthesis in multiplying or nonmultiplying cultures, but also from cells undergoing "classical" kinds of development, in which differentiation presumably begins only after the cessation of cell division.

The first step in gene expression, transcription, was considered in three different reports. J. Warner (New York) reviewed the work by his group on HeLa cells, thereby providing an RNA glossary. He noted that there are at least eight known RNA's: 45*S*, 32*S*, and 90*S* (H.S.) in the nucleus, and 28*S*, 16*S*, 5*S*, transfer (4*S*) and messenger RNA (mRNA) in the cytoplasm, but he expects more will be found. (Interestingly, the three nuclear RNA's of eukaryotic cells have not been found in bacteria.) Particularly noteworthy is the recently discovered 90*S* RNA (also cited by Nemer) which Warner thinks is converted to mRNA by specific enzymic cleavage—perhaps a heretofore unsuspected control mechanism.

Fertilization and the onset of cleavage in sea urchin embryos is accompanied by the development of polyribosomes active in protein synthesis. Whether this is due to activation (perhaps by the removal of a "masking" protein) at the level of maternal ribosomes or of already existing mRNA is unclear. Newly synthesized RNA is at first predominantly mRNA and then, as maternal ribosomes become depleted towards gastrulation, ribosomal RNA synthesis predominates. M. Nemer (Philadelphia), in describing these developmental changes, suggested that coordination in the use of the prefertilization mRNA and newly synthesized mRNA may be a regulatory device. The existence in the unfertilized egg of a "dormant" mRNA emphasizes the probability that the controls are different from those in bacteria.

The puffing pattern in *Chironomus tentans* salivary chromosomes is a visible expression of genetic transcription associated with differentiation. U. Clever (Lafayette) described the sequence of puffing in normal development, its induction by the hormone ecdysone, and the effect on puffing patterns of inhibitors of protein and RNA synthesis. He proposed that some early puffs are dependent on RNA synthesis more or less directly induced by ecdysone, whereas later puffs, with accompanying RNA synthesis, are dependent on some product—possibly an unstable protein —resulting from earlier puff activity. Proteins may also influence puff control by involvement in transport of RNA from the chromosome.

RNA transport out of the nucleus (possibly another facet of genetic regulation) was considered several times during the symposium and often, as in Clever's work, proteins were implicated. Thus, Warner reported that in HeLa cells proteins are involved in the transport of ribosomal RNA subunits and Bell (M.I.T.) noted that in developing chick feather follicles, mRNA is attached to protein moieties (perhaps 40*S* or 60*S* ribosomal subunits) before being transported to the cytoplasm. In reporting on the dynamics of protein

interchange between nucleus and cytoplasm in amebae, Goldstein and Prescott also suggested that some nuclear proteins may serve in RNA transport. One class of nuclear proteins (about 40 percent of the nuclear total) is 50 times more concentrated in the nucleus than in the cytoplasm, yet is constantly shuttling back and forth between the two compartments; these proteins could be involved in transcription regulation. All the remaining nuclear proteins are in a class that also leaves the nucleus but at a relatively slow rate. Evidently many of these latter proteins also return to the nucleus after a time and may be part of nascent ribosomes that serve as carrier material.

Another view of transport from nucleus to cytoplasm was provided by A. R. Stevens (Boulder) from electron microscopic, autoradiographic, and other kinds of evidence. The famous helices found in the *Amoeba proteus* nucleus apparently contain RNA and not DNA as formerly believed. The helices arise near nucleoli shortly after telophase and when "mature" are found in the honeycomb-like openings in the nuclear envelope. Later in the cell cycle they appear in the cytoplasm—suggesting that they pass through nuclear envelope pores to the cytoplasm. Because of their size, localization, and the presence of RNA, Stevens suspects the helices are a form into which nascent ribosomes are packaged for transport to the cytoplasm.

The site of nuclear protein synthesis was debated several times during the symposium. B. Schultze (Cologne), in the only direct report on the subject, showed that the amount of nuclear protein synthesis is proportional to the nuclear volume and interpreted this as reflecting the nuclear synthesis of nuclear proteins. But, since the amount of nuclear protein synthesis generally is also proportional to the amount of cytoplasmic protein synthesis, perhaps nuclear proteins are synthesized in the cytoplasm and move rapidly to the nucleus.

The control of more specific gene expressions was considered in four contrasting reports. S. Hennen (Bloomington) studied interspecific "nucleocytoplasmic hybrids" created by implanting diploid blastula nuclei from one frog species into enucleate eggs of another frog species. Nuclei replicating in cytoplasm of a distantly related species are unable to develop beyond the gastrula stage and display marked chromosome alterations, which are not reversed when back transferred to original species cytoplasm. Hybrids between closely related species develop into tadpoles that exhibit characteristic deficiencies but nuclei from these promote normal development when back transferred and, significantly, have normal karyotypes. J. C. Mounolou (Gif-sur-Yvette) considered interactions between cytoplasm and nucleus in the determination of yeast mitochondrion phenotype, which is controlled by a chromosomal gene and a series of heritable epistatic cytoplasmic factors—presumably located in the mitochondrion itself. Mounolou demonstrated that cytoplasmic factor mutations reflect major alterations in mitochondrion DNA and he is trying to determine in greater detail the nature of these apparently irreversible mutations. Also of interest is how the translation products determined by these genes interact to express the mitochondrion phenotype.

The control by the environment and the cytoplasm of the expression of the so-called immobilization antigens on the cilia of *Paramecium aurelia* has long been studied, and in some respects—such as the expression of one antigenic type leading to the suppression of all other antigens—has been a great puzzle. In dealing with only a few features of this genetic system, I. Finger (Haverford) showed that once a particular ciliary antigen is induced, its continued production apparently is promoted by a positive feedback involving the already produced antigen. The suppression of all other antigenic phenotypes apparently is due, in part at least, to the liberation into the culture medium of repressors of all genetic loci determining immobilization antigens, except that which is being expressed.

J. R. Sadler (Denver) was invited to discuss bacterial control mechanisms to illustrate the models or guideposts mentioned in this report's first paragraph but took the conservative position that there is too much ignorance of genetic control mechanisms in bacteria to provide help in understanding controls in eukaryotic cells. Sadler dealt with questions of: positive versus negative control of enzyme synthesis; the target for the regulator gene product (the repressor), that is, questions about operator loci; whether the repressor acts at the level of transcription or translation; the nature of the regulator gene product. With regard to the last, his studies with Novick show that the repressor is an allosteric protein that interacts with inducers and thereby undergoes a structural change. Surprisingly, the repressor is growth unstable (that is, is inactivated by cell growth), which is interesting not only in itself but also because: (i) Clever speculated about unstable proteins involved in the regulation of chromosome puffs, (ii) the prefertilization "masking" of mRNA considered by Nemer may be due to unstable proteins, and (iii) some of the nuclear protein behavior described by Goldstein and Prescott may reflect instability of proteins.

Sadler made one point that might serve as a symposium keynote. In discussing the difficulties in determining whether repressors acted at the chromosome during transcription or at the ribosome during translation, he noted that a major obstacle was that bacterial transcription and translation were intimately related and often difficult to separate for analysis. That such is not the case for eukaryotes is indicated by the existence of a nuclear membrane that separates the two functions, and is emphasized by the oft-repeated symposium observation that, except for one or two cell types, few complete ribosomes are found in the nucleus.

In his closing remarks, D. M. Prescott drew a model to unite much of the information in terms of nuclear interactions with various extranuclear factors and emphasized that the molecules acting in these controls must not only recognize the appropriate cistron or replicon but must do so at specific times in the cell life cycle. In calling attention to the generality of some of the ideas, he noted that the 14 reports dealt with 9 taxonomically widely different species, which he took to be an indication of the unity of the biological problems under consideration.

The symposium proceedings will be published in February 1967, as *The Control of Nuclear Activity*, L. Goldstein, Ed. (Prentice-Hall, New York). The symposium was, and the volume will be, dedicated to Merkel H. Jacobs on the occasion of the 20th anniversary of the Society of General Physiologists, of which he was a founder. A National Science Foundation grant provided needed financial assistance for participants' expenses.

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