Meetings

Gene Activation

Gene activation and its implications for growth and development were the subject of the first annual George H. Hudson symposium held at the State University College, Plattsburgh, New York, 22–23 April 1965.

Herbert Stern (University of California, La Jolla) surveyed the problems of development and, in particular, differentiation. He pointed out that the development of differences between cells is, in at least one important respect, caused by the differential reading of genes. The focus of the symposium was to be upon how such differential reading is effected. The symposium had to include not only the structure of the genetic material, but also the structure of the chromosome. The complexity of chromosomes in higher organisms points to regulatory devices which are absent in microorganisms. Moreover, the internal environment of the cell must be considered in attempting to understand the factors which lead to differential expression of gene activity.

Vincent Allfrey (Rockefeller University) reported on active and inactive states of chromatin. These studies had been initiated with the aim of answering the question, "Why do not all genes act?" Allfrey supported the concept that histones play a role in gene activation and deactivation. In isolated nuclei from the thymus lymphocyte, approximately 70 percent of the DNA was inactive in supporting RNA synthesis. Trypsin was used to remove about 70 percent of the histones from isolated nuclei; this treatment increased RNA synthesis. After removal or inhibition of the trypsin, the back addition of histone again inhibited production of RNA. It was pointed out that Huang and Bonner had observed histone inhibition of RNA polymerase activity in pea chromatin.

The view that histones regulate gene activation suggests that there is more histone on the repressed chromatin than on the active chromatin. However, data showed the ratio of histone on repressed to histone on active chromatin to be very close to one.

Active chromatin contained about two times as much nonhistone protein (including phosphoproteins) per unit of DNA as inactive chromatin. Since T. A. Langan had shown that the phosphoproteins have the ability to bind histones, their role in chromatin function was discussed.

The rate of synthesis of histones in nondividing cells is less than that of other nuclear proteins. Allfrey and his coworkers used ^{14}C-labeled acetate to show that acetylation of histones occurs in the nucleus. Other nuclear proteins did not become acetylated. Acetylation occurs after the synthesis of histone; there is a correlation between the amount of active chromatin and the percent of acetylation. If cells are triggered to produce increased amounts of RNA, there is a prior acetylation of the histones.

Allfrey concluded that most of the DNA in the nucleus is repressed. There is some evidence that histones play a role in this repression. Acetylation of histones may decrease the ability of the histones to inhibit RNA synthesis. Finally, when cells are triggered to divide, increased acetylation occurs before the increase in RNA synthesis.

Hans Laufer (University of Connecticut) discussed nuclear cytoplasmic interactions during chromosomal puffing. He reviewed the evidence which demonstrates that the giant puffs, or Balbiani rings, in giant chromosomes of insect nuclei represent sites of active genes. Laufer also discussed Clever's finding that injection of ecdysone activates certain specific puffs. Karlson has suggested this effect of ecdysone is direct, but Laufer did not think this was yet established.

In considering the significance of the puffing phenomena in the giant chromosomes of the insect *Chironomus tumi*, Laufer investigated the secretory products of the salivary gland. The secretion contained at least five specific enzymes—malic dehydrogenase, hyaluronidase, trehalase, peptidase, and esterase. These same enzymes were also detected in the hemolymph of the animal. Immunological and isotopic evidence shows that enzymes were not synthesized in the salivary glands, but were transported from the hemolymph into the gland cells by pinocytosis. The pinocytotic vesicles then traversed the gland cells and emptied the enzymes into the secretion on the other side of the cells. The whole transport system occurs only when specific regions of the chromosomes are puffed. In summary, operation of a cellular transport system is related to the activation or inactivation of specific genetic loci.

Herbert Stern reported on the control of enzymes during cell development. Stern used the lily to study events in the development of the meiotic cell. Evidence shows that there is a low ratio of ribosomal RNA to DNA as the cell goes through meiosis. There was no substantial change in either DNA or RNA polymerase throughout the meiotic cycle. Evidence indicated that RNA polymerase remained attached to the chromosomes at all times.

Thymidine kinase was synthesized at a predictable time just before DNA synthesis. This enzyme persisted for about 18 hours and then its activity dissipated. One polypeptide chain of thymidine kinase was present in constant amounts during the meiotic cycle and until the initiation of DNA synthesis in the post-meiotic cells. The difference in level of enzyme activity was caused by synthesis of a second chain and its subsequent removal.

A specific temporal control was involved in the synthesis of thymidine kinase. This was demonstrated by the finding that thymidine may be used to induce the synthesis of the enzyme, but only at a specific stage of development.

James Neelin (National Research Council, Ottawa) spoke about specificity of histones and gene repression. He referred to the existence of at least three types of histones (lysine-rich, moderately lysine-rich, and arginine-rich fractions) in most nuclei. A dis-

tinctive histone rich in serine was discovered in nuclei of chicken erythrocytes. Evidence, chiefly electrophoretic, was cited for differences in histone extracts from avian tissues. Lysine-rich histone seems ubiquitous; moderately lysine-rich histones are high in reticulocytes and thymus, but low in spleen, leukocytes, liver, and testis; arginine-rich histones are low in erythrocytes.

"Why should there be many histones if the specific repression of histones comes merely from their locus of action?" The answer was proposed that different histone fractions may have different functions, as well as different metabolic labilities. For example, it has been reported that synthesis of lysine-rich histone only occurs in phase with DNA synthesis. Serine-rich histones are not solely responsible for condensation of red cell nuclei, but may have a role in cessation of nucleic acid synthesis.

Francis T. Kenney (Oak Ridge National Laboratories) discussed steroid hormones in the control of genetic activity. Kenney agreed that hormones do control genetic activity, but thought the problem of whether hormones control gene activation directly, in the sense of the model of Jacob and Monod, still was unresolved. He also pointed out that there is no unambiguous evidence of substrate-mediated control of enzyme synthesis in animals.

Kenney summarized data showing that hydrocortisone induces synthesis in the rat liver of at least three enzymes, tyrosine transaminase, tryptophan pyrrolase, and glutamic-pyruvic transaminase. An increase in nuclear RNA synthesis before the increase in enzyme has been demonstrated. Kenney and his coworkers conclude that all three types of RNA (ribosomal type, DNA-like, and transfer type) are increased.

To resolve the paradox that the adrenal steroids stimulate synthesis of specific proteins while having a general stimulatory effect upon RNA synthesis, Kenney pointed out two possible explanations. One is that the methods of RNA determination so far employed are too insensitive to show specific changes and the other is that specificity may be at the cytoplasmic level. Kenney concluded that we still have much to learn about the hormonal control of enzyme induction. He concluded that gene activation in the sense of the Jacob and Monod model either is not operative or is insufficient to explain all the data about hormone-induced RNA and protein synthesis.

Gunther Eichhorn (National Institutes of Health) reported on metal ions as determinants of nucleic acid structure. He pointed out that metal ions can react with nucleic acids at either one of two locations. They may bind at the negatively charged phosphate groups where their presence stabilizes DNA or, under more drastic conditions, cleave the phosphate-deoxyribose bond. Ions may also bind to the electron donor groups on the bases of DNA. In this latter position the metal ions destabilize DNA by disrupting the hydrogen bonding between the two strands. As evidence for the above, Mg^{++}, which binds to phosphate, increases the melting temperature of DNA; Cu^{++}, which binds to the bases, decreases the melting temperature as followed by optical absorbance at 260 $m\mu$.

The denaturation of DNA under the influence of Cu^{++} could be reversed by the addition of a large amount of $NaNO_3$. The denaturation and renaturation effects can be explained in the following manner. Cu^{++} is interposed between the two strands of DNA and thus holds the two strands in register, while destroying interactions between the stacked bases. Since the bases are held in register, the native helix can reform when the ionic strength is increased.

The question of whether metal ions are involved in gene activation was considered. Although Cu^{++} produces unwinding, and sodium ions produce rewinding of the DNA helix, there is no evidence that these effects occur in vivo. Evidence for specificities in ionic interactions with polynucleotides was noted.

In summary, Eichhorn thought that metal ions are probably involved in gene activation. He reemphasized that metal ions interact with polynucleotides in many ways.

Herbert Stern summarized the symposium by pointing out the difficulties in accounting for the temporal parameter of development by a progressive linear reading of DNA. This might work in bacteria which have a closed loop of DNA, but plainly cannot occur in higher organisms. Sequential reading of chromosomes by the pure device of a transcription enzyme moving along a tape is not possible. Furthermore, chromosome translocations do not interfere with development in a way that would be expected if the order of gene reading was determined by their gross ordering as the chromosome. The sequential reading of genes which is a characteristic of cell development thus requires a mechanism more elaborate than that inherent in the geometry of a tape.

In considering the possible role of metallic ions discussed by Eichhorn it was pointed out that although subtle intermolecular changes caused by intracellular shifts in ion concentration are usually disregarded, the capacity of cells to regulate intracellular diffusion is poorly understood. The work of Laufer indicates that there are gene loci that control diffusion. The possibility that ions may have specific effects on gene activity poses the problem of how to explain control of localized diffusion. Until recently there has been little investigation of possible connections between transport systems and developmental biology. It was pointed out that diffusion control in general is a very important feature of ordering in higher systems.

The consideration was raised that in higher organisms there may be a need for more than one level of control for activating genes. For example, it may be necessary to activate large regions of genetic material in some circumstances, whereas small regions may be activated in others. The acetylation of histones might play a role in the latter case.

In considering histones and possible histone specificity, Stern pointed out that it is most improbable that a system would evolve where all of the histones were doing nothing but neutralizing phosphate groups. Stern reemphasized the importance of intercellular communication within multicellular organisms by means of hormones. Hormones, plant or animal, stimulate RNA as well as protein synthesis. The unresolved problem is the chain of molecular recognitions—which component of a cell sees the hormone, and upon which component of a cell does the primary receptor act. Do hormones primarily stimulate a preset pattern of gene transcriptions or do they primarily set patterns?

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