Meetings

Control of Form in Cells

A conference, held in Estes Park, Colorado, 8–10 September 1969, gathered a group of scientists from divergent areas, held together by common bonds of interest in assembly processes that affect cell and organelle design. The program, organized by a committee headed by K. R. Porter and aided by T. F. Anderson, P. Satir, and L. G. Tilney, consisted of five sessions of which the first half dealt mainly with in vitro assembly processes in a variety of systems, such as tobacco mosaic virus (TMV), enzymes, T-even bacteriophages, ribosomes, flagella, cilia, and microtubules. The latter part of the conference touched on centriolar assembly, mitosis, membrane systems, and the role of microtubules and microfilaments in cell shape maintenance in vivo. A few papers on the genetic control of cell form and pattern ended the meeting.

D. L. D. Casper and A. Klug opened the program with illustrations of mechanisms of self-assembly. Casper pointed out how specific symmetrical structures can arise from regular bonding between identical subunits. Polymerization of the subunits is cooperative in that intermediate forms are found that serve as progressive nucleation sites for the final stable structure. The subunits are in equivalent or quasi-equivalent positions in the assembled structure. Nevertheless, polymorphism is made possible by slight changes in the subunits or in their interactions. Klug gave several detailed examples of self-assembly systems. He showed that the distortions of subunit packing in the Dahlemense strain of TMV, the helical form of bacterial flagella, and even position polymorphism in small icosahedral viruses are the result of compromises between ideal bonding conditions of identical single subunits and strains within the three-dimensional polymer. One interesting prediction of this approach is that motion of bacterial flagella could be caused by periodical conformational changes in the rows of subunits initiated at the flagellar base.

D. Richardson illustrated the self-assembly of a single molecule, the enzyme *Staphylococcus* nuclease (E.C. 3.1.4.7) whose primary structure and three-dimensional configuration are well known from x-ray diffraction analysis. It is possible to take this enzyme apart to obtain peptide fragments, which can be recombined. The fragments were structureless in solution, but on recombination the two parts form structure and regain enzymatic activity. This type of assembly system might lead to an understanding of the action of different portions of the polypeptide chain in molding the active site of an enzyme.

A very clear demonstration of self-assembly in vitro was given by W. Wood, presenting the assembly of bacteriophage T-4. Structural mutants of T-4 that have been genetically mapped are visualized by negative stain techniques and the structural defects are noted. Extracts from mutants with different defects are mixed and complementation is studied both as a rise in infectivity and as a morphological completion of the viral structure. In this manner, the stepwise nature of assembly can also be demonstrated. Each step has to be completed in sequence before further assembly can take place. L. Simon illustrated with excellent electron micrographs of sectioned material different steps during phage attachment to *E. coli*. One particularly interesting finding was that the central tube of the phage tail penetrates the cell wall but not the plasma membrane of the bacterium. Further, Simon demonstrated, using radioactive label, that contraction of the sheath is not enough to release the DNA into the bacterium, but that attachment must last for a certain period of time before DNA ejection will occur. Two types of basal plates were reported: one associated with contracted sheaths, the second with extended sheaths. A conformational change at the basal plate may initiate sheath contraction.

H. Koffler and A. Asakura discussed some questions concerning the assembly of bacterial flagella. Bacterial flagella are composed of flagellin that can be purified so that two fractions, A and B, are obtained. Koffler pointed out how reconstitution of morphologically perfect helical flagella can be formed by a single pure protein (A) alone. Reconstitution depends on environmental conditions that are species-specific, and in several cases no seeding was required. As Asakura showed, seeding is necessary in *Salmonella* flagella. Asakura prepared flagella fragments (seed) by treatment with high-frequency sound. These fragments show polarity on electron micrographs. Asakura labeled the seeds with antibodies and allowed flagellar assembly to take place. Only one end, the frayed end, added unlabeled monomers. This end is the growing end. This evidence suggests that bacterial flagella grow longer by adding subunits to their free ends, external to the cell, a conclusion that provoked some disagreement.

P. Traub discussed the assembly of ribosomes from *E. coli*. The ribosome illustrates that the principles of self-assembly apply to reasonably complex cell organelles. The ribosomal protein is very heterogenous. For example, there are at least 20 different proteins in the 30*S* ribosomal subunit. Traub demonstrated that these proteins, together with 16*S* RNA, will self-assemble to give a particle that will combine with intact 50*S* subunits and function in protein synthesizing systems. Reconstitution is apparently a first order reaction, with a temperature-dependent activation step as the rate limiting process.

R. Stephens introduced the discussion of in vitro disassembly and assembly of microtubules; he used purified sperm-tail proteins from sea urchins. Recently some heterogeneity has been recognized in microtubular protein and Stephens clearly demonstrated that at least two proteins (which differ from actin) can be isolated from the outer doublet microtubules: A-tubulin and B-tubulin, probably associated with subfibers A and B, respectively. The polymerization of microtubules from subunits is similar in many respects to the polymerization of bacterial flagella. Apparently, a preformed microtubular fragment is a necessary seed. The existence of another protein that might link microtubules together was suggested.

L. Tilney, P. Satir, and E. Dirksen talked about assembly of cilia and centrioles in vivo. Tilney concentrated on the role of microtubules as a cytoskeleton. The axopods of *Echinosphaerium* contain two interlocking coils of microtubules built up in 12 sectors.

Cooling or hydrostatic pressure depolymerizes the microtubules and the axopods collapse. When the environment is returned to normal, the axopods reform as the microtubular arrays develop. The pathway of reformation of the complex array suggests that morphogenetic information present in the tubules themselves and the bridges between them determines the sector superstructure. Satir introduced the amoeboflagellate *Naegleria gruberi* to illustrate both assembly of cilia and concomitant cell shape change. When placed in distilled water, *Naegleria* form two to four cilia (flagella) *de novo*. Axonemal mictrotubules probably polymerize in an external bud by self-assembly processes. Assembly is stepwise and under nuclear gene control. Actinomycin D and mercaptoethanol prevent cilia formation if added before critical transition points. E. R. Dirksen gave an electron-microscopic description of centriole or basal body replication in the mouse oviduct. She showed that short centrioles appear around a dense material—a "condensation body"—in oviduct cells. These grow to mature centrioles while the dense material disappears. Electron-microscopic autoradiography during this period revealed that tritiated leucine could be found in association with centrioles and centriole precursors, but nucleic acid precursors were not incorporated.

R. McIntosh added a note of exciting controversy to the conference by introducing his concept of the mechanism of chromosome movement in the mitotic apparatus. He suggested that the movement is caused by microtubules sliding past each other. According to this hypothesis, as the mitotic apparatus forms, microtubules of opposite polarity grow from opposite sides of the spindle to lie alongside one another. By anaphase, these tubules have slid, so that their head ends no longer overlap near the poles, while their tails are pulled to the center cleavage furrow or cell plate, and then remain slightly overlapped. The hypothesis predicts that in anaphase there will be twice as many continuous microtubules near the cell plate as near the poles, while in metaphase, the number will be equal in the two regions. McIntosh and collaborators counted the tubules in single cells in different stages of mitosis and could confirm the prediction qualitatively, although the exact ratio in anaphase was more nearly 3:2. B. Brinkley reported similar studies performed independently with similar results.

The next two speakers dealt with the assembly of membranes and it became apparent that this problem was not as easily attacked as any of the earlier subjects. As pointed out by G. Guidotti, different membranes are quite different biochemically and functionally, and the biochemistry of membrane protein is not well understood. Two models of membrane structure were considered: the unit membrane (lipid bilayer) model and a lipoprotein subunit model. Neither model can be applied generally to all membranes. W. Stoeckenius criticized the subunit model developed especially for mitochondrial cristae membranes because the negative stained membrane particles are a single enzyme, adenosine triphosphatase (E.C. 3.6.1.3), and other presumed subunits appear to have unit membrane structure. Only the structure of myelin seems settled. Casper showed that the most detailed x-ray diffraction patterns of myelin correspond to the bimolecular leaflet membrane model.

The role of microfilaments in cell differentiation was discussed by H. Holtzer and R. Cloney. In certain cells microfilaments can serve the functions that are generally attributed to microtubules in determining cell shape and position. It appears that there may be several size classes of microfilaments with perhaps several functions. H. Holtzer and H. Ishikawa described a class of 100-Å filaments that is present in several types of chick embryo cells. These microfilaments will bind heavy meromyosin after appropriate treatment, and arrowhead structures can be seen in thin sections of this material. This suggests that these filaments are a single class of actin-like protein that is essential for development of many cells. R. Cloney dealt more specifically with the role of microfilaments in contraction and cell shape change during tail resorption in ascidian metamorphosis. At the apical surface of the cells that actively change shape to pull the tail in, a mat of 40- to 70-Å microfilaments was found. As the tail is resorbed the mat thickens. Presumably, the microfilaments are the active elements in the resorption process. This resorption could be reversibly blocked by hydrostatic pressure and treatment with cyanide.

An important part of the program centered around the role of microtubules in the development and maintenance of cell form. One question discussed was: are all microtubules alike in form and function? The answer appears to be negative. There may be several size classes of microtubules, but one group of microtubules ($\simeq$ 240 Å in diameter) functions similarly in development and maintenance of shape and in cytoplasmic streaming in many cell types. O. Behnke presented evidence to indicate that there are several classes of microtubules with regard to stability to various treatments. In blood cells, the marginal bundle of microtubules can be depolymerized without the cell becoming spherical. Another interesting question is whether there is a class of microfilaments that is a precursor for or a product of microtubules. R. B. Wuerker showed that 70-Å microfilaments found in astrocytes do not seem to be a direct breakdown product of microtubules. The globular subunits comprising the filaments and tubules are probably different.

The next two speakers dealt with control of form in plants. P. Green showed that polystyrene beads placed equidistantly on the surface of a growing algal cell move apart as the shape and form are changed by deformation (torsion) and expansion of the cell surface. Several different mechanisms of expansion may be employed to give the same result. It is possible that differences in expansion rate can induce an initial alignment of microfibrils in the cell wall or of cytoplasmic microtubules. Colchicine treatment can change elongated cells into round spherical cells. Such cells lose oriented cell wall structure, perhaps because their microtubules are depolymerized by the treatment.

A. Løvlie introduced a series of mutants such as "bubble" and "slender," isolated from the green alga *Ulva mutalis*, in which either the timing or the plane of cell division is altered and the ability of cells to differentiate is lost. The bubble mutation acts through a cytoplasmic factor producing a "maternal effect" on the direction of the early planes of cell division. Although centrioles are present in these cells, they do not occur at the spindle poles. Further study of this system may yield a series of genes affecting gross morphogenetic processes through a sequence of simple organelle assembly steps.

T. Sonneborn ended the conference by discussing the formation and maintenance of pattern as illustrated by the ciliated cortex of *Paramecium*. The cortex is an extremely specific and stable system, consisting of thousands of units (basal bodies) knitted together in a very specific pattern, which is maintained

unaltered generation after generation. When the cell divides, this pattern is duplicated unit for unit. The control of this process remains unknown. Preliminary electron-microscopic autoradiography failed to implicate any DNA component in the basal bodies, and Sonneborn concluded that the need for such is unnecessary, since numerous possibilities exist within inherent self-assembly systems.

Finally, R. D. Hotchkiss was called upon to summarize the events of the meeting. Hotchkiss pointed out that in the systems discussed, regardless of complexity, three component steps apparently are essential in the control of cell form. The first one of these is acquisition of unit. The unit and its specificity are determined by the DNA template. The second step involves the orientation of the unit to its neighbors. The more specific sites of recognition a morphological subunit possesses, the more precise the fit in the final orientation of the units to each other. During assembly, the units are funneled by local and cooperative interaction into a precise structure. The last step allows rearrangement of the complex, perhaps for function.

We are still a long way from understanding how a highly complex process such as duplication of the ciliate cortex takes place. But it was generally agreed that some of the fundamental rules of assembly that provide a theoretical basis for the solution of such problems were brought forth in stimulating and challenging ways in this meeting.

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Cross-Reactions of Oncogenic Viruses

At a working conference called to assess the current knowledge of viral antigens of tumor viruses, scientists from the National Cancer Institute and from many cooperating laboratories informally presented relevant information on the C-type leukemia and sarcoma viruses and on herpesviruses suspected of being oncogenic.

On the first day, the speakers discusssed the antigens of the viruses that are associated with leukemia and sarcoma in chickens, mice, and cats, as well as the antigens of the C-type particles associated with certain leukemias and sarcomas of man. Although no virus has been consistently isolated from bovine leukemia, attempts to find such an agent were described.

The avian leukosis viruses have been classified into four subgroups (A–D) on the basis of neutralization, interference tests, and host range. Although reactions between envelope antigens of the members of groups B and D have been reported, viruses of the different subgroups generally do not cross-react serologically. Several group-specific antigens have been reported when the internal structure of the virus is exposed; one antigen has been characterized as a polypeptide of low molecular weight. The Rous sarcoma viruses RSVα(0) and RSVβ(0) have radically different envelope antigens and are derived from cells previously considered nonproducers. These viruses fail to infect most chicken cells, but RSVβ(0) can be replicated in quail and turkey cells. No host is known for RSVα(0).

Antigens found in the membranes of cells infected by the murine leukemia-sarcoma complex of viruses were omitted from this discussion because they have not been classified as cellular or viral in origin. Evidence for a type-specific envelope antigen and a group-specific internal antigen, probably a low-molecular-weight polypeptide common to all virus strains isolated, was presented. Since results of animal protection and neutralization tests from different laboratories were conflicting, it was agreed that it was premature to divide these viruses into subgroups on the basis of their envelope antigens.

The viruses isolated from spontaneous and induced leukemia of cats showed a common antigen. Using three techniques, two groups from separate laboratories have not yet been able to confirm a reported cross-reaction, demonstrated by immunodiffusion, between a minor group-specific component of feline leukemia virus and a similar component from murine leukemia virus. When sufficient amounts of feline leukemia-sarcoma viruses uncontaminated by unrelated cat viruses become available, the nature of the antigens of this newly isolated group of viruses can be better defined. Demonstrated oncogenicity of feline sarcoma viruses for canine and primate hosts make this virus group extremely important for future study.

The finding that some human tumors contain a common antigen suggested that some of these malignancies may be viral in origin. C-type virus particles have been seen, on electron microscope examination and after culture of cells from human osteosarcomas and liposarcomas. With extracts of the sarcomas as antigen, complement-fixation tests detected antibody more often and in higher titer in the serums of sarcoma patients and their immediate families than in other groups. The C-type particles have also been found in plasma sediments (obtained by high-speed centrifugation) of patients with various leukemias. Serums of patients often reacted with malignant bone marrow cells in immunofluorescence tests; serums of normal individuals did not. Although the nature of these antigens in human tumors remains obscure, their viral nature cannot be excluded.

Much discussion centered around a second goal of the conference—cross-reactions between oncogenic viruses of man and other species. Many of the participants described their attempts to find human serum antibodies to the various oncogenic C-type viruses isolated from different animal species. Although a few positive findings were reported—for example, some human serums apparently neutralize avian and murine viruses—the results were mostly negative. It was agreed that such studies should be pursued with additional serological tests until an unequivocal answer is obtained.

On the second day, the speakers discussed several types of herpesviruses which have been associated with various malignant states of animals and man. Because these viruses are not always present in malignant cells in vivo but often appear after the cells are cultured, the problem of determining whether they are passengers or tumor-producing viruses has been difficult. The following herpesviruses were discussed: two human strains, one isolated from Burkitt's lymphoma [Epstein-Barr virus (EBV)], and the other believed to be associated with cervical carcinoma (*Herpes hominis II*); and three animal strains, those isolated from kidney adenocarcinoma of frogs (Lucké), from Marek's disease of chickens, and from a lymphoproliferative disease of primates (*Herpes saimiri*).

Certain herpesviruses associated with tumors—Burkitt's lymphoma, Lucké frog adenocarcinoma, and Marek's disease—resemble cytomegaloviruses and can be placed in the group whose DNA has a low guanine-cytosine content. These viruses grow poorly and are difficult to transmit by cell-free filtrates. Based on biochemical analyses, nine