Reports

Nature of Meromyosins

Following up the observation of Gergely (*1*) that a partial digestion of myosin with trypsin leaves its adenosine triphosphatase activity intact, Mihalyi (*2*) and Szent-Gyorgyi (*3*) isolated two large fragments of myosin from the digested mixture. The heavier of the two components carrying the adenosine triphosphatase activity was named H-meromyosin (H), the lighter component L-meromyosin (L) (*3*). Since these components are liberated in about equal weights and since H is about twice as heavy as L (L's molecular weight, 100,000) the myosin molecule must contain one H and two L components.

Since the meromyosins in their amino acid composition add up to myosin (*4*), they can be considered as the primary products of splitting, essentially unmodified by further digestion. In addition to trypsin other proteolytic enzymes such as chymotrypsin (*5*), subtilisin (*6*), and snake venom (*7*) have been found to split myosin, yielding products roughly similar to those produced by trypsin. Since these enzymes split ester bonds as well as peptide bonds, it is important to study the C-terminal groups of the split products to see whether those groups which appear correspond to the known specificity requirements of these enzymes.

Although the L-meromyosins produced by these enzymes appear very similar, they exhibit subtle differences. Gergely (*8*) found that L-meromyosin obtained with the digestion of chymotrypsin yielded 1 mole of phenylalanine as a C-terminal end group when studied with carboxypeptidase A (*8*). With the discovery of a new carboxypeptidase (*9*), specific for basic amino acids, it was possible to demonstrate the presence of a C-terminal lysine on the L-meromyosin produced by trypsin (*10*).

The appearance of these C-terminal groups is in agreement with the known specificity requirements of these proteolytic enzymes. The L-meromyosin produced by snake venom again is different since it has a C-terminal alanine (*7*). From these observations one must conclude that the meromyosins are the proteolytic split products of myosin and as such should not be considered as preexisting subunits of myosin. Nevertheless, since tracer studies show that the two fragments of myosin have different turnover rates (*11*), at least two subunits of some kind pre-existing in the muscle can be postulated.

For the arrangement of the meromyosins in the myosin molecule, Holtzer and Rice (*12*), from light-scattering evidence, proposed the arrangement LLH. In the interpretation of light-scattering data summation of shape considerations presents a difficult problem which can be solved only with simplifying assumptions.

The fact that both L- and H-meromyosin have "wounded" ends (*13*) supports the LHL arrangement. There are other chemical and biochemical facts that support this arrangement; here I want to point out one which appears quite decisive against the LLH arrangement.

The arrangement LHL is symmetrical and permits a molecular weight of the myosin monomer to be about 220,000, one-half of the current value of about 440,000.

The recent careful experiments of Ellenbogen and co-workers (*14*) show that the molecular weight of myosin from dog heart is about 223,000. Since myosin of twice this size, obviously a dimer, also has been obtained from heart muscle, an arrangement of the meromyosins in myosin (MW = ~ 440,000) must be such as to permit the halving of the molecule.

Evidence is mounting to show that myosin is more complex than the meromyosins would imply. Kominz in this laboratory isolated from myosin without the use of proteolytic enzymes a protein (about 12 percent myosin) rich in phenylalanine and very much different from the meromyosins (*15*). Alcohol denaturation was found to split L-meromyosin into a crystalline and noncrystalline component (*16*), showing the complexity of L-meromyosin.

To unravel the detailed composition of myosin is very important because it is becoming more and more apparent that without such knowledge the role of myosin in muscular contraction will not be understood.

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Soluble Deoxyribosidic Compounds in Relation to Duplication of Deoxyribonucleic Acid

One of the puzzling features of chromosome reproduction is the abruptness and relative rapidity with which the chromosomal substance is synthesized. Wherever the phenomenon has been adequately studied, whether in cells of plant, animal, or microorganism, the duplication of deoxyribonucleic acid (DNA) has consistently been found to occur during a comparatively brief interval in the life span of the cell. This behavior is in marked contrast to that of many other cell components which fluctuate in concentration or increase gradually in amount during cellular development.

The cause and course of DNA duplication is but little understood. Not even in phage-infected bacteria, where the

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].

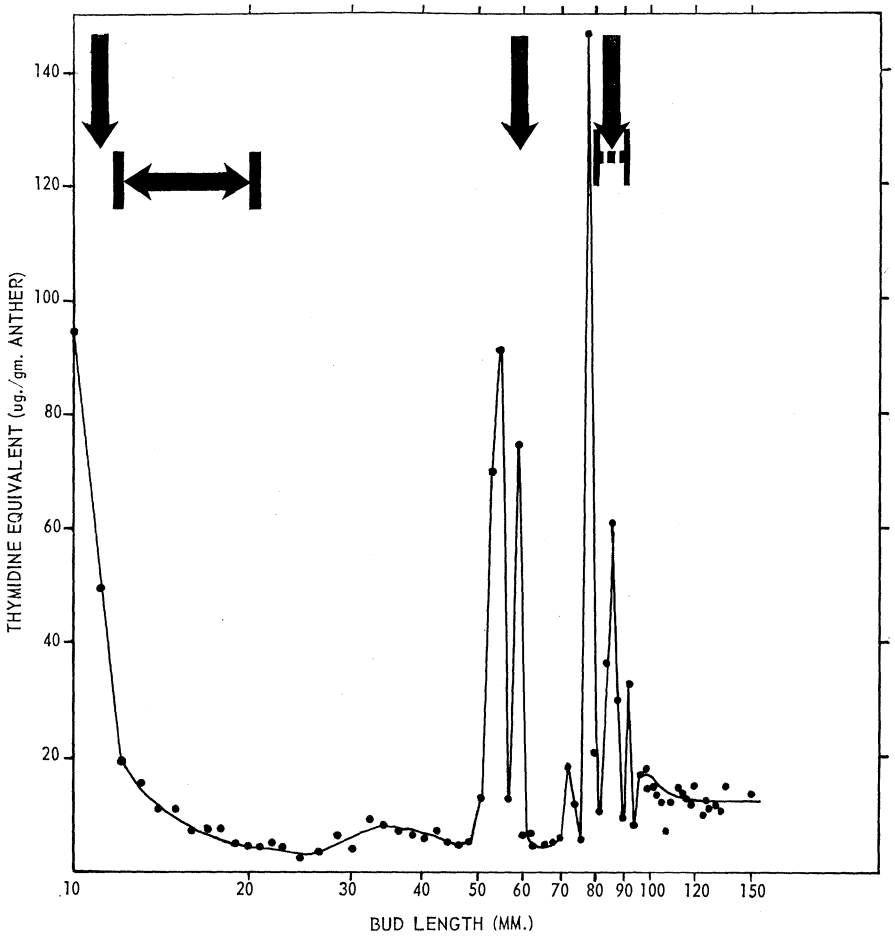

Fig. 1. Variations in soluble deoxyribosidic compounds with anther development. Methanolic and trichloroacetic acid extracts were incubated at *p*H 9.0 with a commercial preparation of alkaline phosphatase for 20 hours at 37°C. *Lactobacillus acidophilus* R-26 was the test organism. Vertical arrows indicate periods of DNA synthesis with sporogenous tissue as established by Ogur *et al.* (*4*) and by Taylor and McMaster (*5*). Microsporocyte meiosis and microspore mitosis were checked cytologically. The third period of DNA synthesis is marked with a bar because of the uncertainty covering its precise occurrence. Tapetal DNA synthesis is indicated by a horizontal arrow.

immediate stimulus to DNA synthesis is known and controllable, is the chain of events entirely apparent. It could be assumed that the whole complex of synthetic pathways from deoxyribose production to deoxyribonucleotide polymerization is tightly integrated and activated as a unit, whether by a viral or an intracellular agent. But the fact, so clearly demonstrated by Schneider (*1*), that pyrimidine deoxyribosides occur in blood and in various nonproliferating organs of mammals suggests a slightly looser association between precursor formation and ultimate polymerization. It is conceivable that one of the mechanisms by which a cell limits chromosome reproduction is by controlling the availability of soluble deoxyribosidic compounds.

This report (*2*) on the occurrence of deoxyribosidic substances in anthers of *Lilium longiflorum* var. Croft bears on the latter point. The suitability of anthers for the study of mitotic processes has previously been discussed (*3*). It need only be indicated here that during the interval of development studied there are three periods of DNA synthesis (*4, 5*). The first precedes microsporocyte meiosis and tapetal cell mitosis; the second precedes microspore mitosis; and the third occurs during pollen maturation. In the brief account of experiments which follows it is shown that immediately prior to each instance of DNA synthesis there is a marked accumulation of soluble deoxyribosidic compounds.

Buds of appropriate length which had been stored at −30°C were extracted first with 0.05*M* methanolic formic acid and then with 5 percent trichloroacetic acid. Each of these extracts was analyzed for deoxyribosidic material before and after treatment with mucosal phosphatase. The microbiological assay technique of Hoff-Jørgensen as modified by Schneider (*1, 6*) was employed. To guard against the possibility that growth-promoting factors other than deoxyribosidic ones were present in the extracts, parallel assays with added thymidine were run for each developmental stage. No synergistic effects were observed. Both methanolic and trichloroacetic extracts, whether or not they were treated with phosphatase, shared a common pattern with respect to location of peak concentrations of the deoxyribosidic compounds. The extracts differed qualitatively (*7*). The quantitative features of the pattern are illustrated in Fig. 1, in which the deoxyribosidic contents of the phosphatase-treated extracts were summed and the values so obtained were plotted against length of the bud.

There are three outstanding peaks in concentration of deoxyribosidic compounds during anther development, and each of them is associated with an interval of DNA synthesis. The first of these precedes microsporocyte meiosis and tapetal mitosis; for technical reasons buds of lengths less than 10 mm were not collected, and hence a phase of ascending concentration has to be assumed. The second peak precedes microspore mitosis, and the third coincides with the beginning of the interval of DNA synthesis in the maturing pollen.

Two properties of each of the peaks are distinctive: (i) the level of concentration is some 25 times that of the base one, and (ii) the duration of the high levels is of the same order as that of DNA synthesis. It may be inferred from these results, as Schneider has already done for animal tissue (*1, 8*), that DNA is formed from soluble deoxyribosidic compounds. It may also be inferred that the chain of events leading to DNA duplication begins with an abrupt activation of enzymes metabolizing these soluble deoxyribosidic compounds. Whether the accumulation of such compounds is of itself sufficient to initiate polymerization, or whether the two processes are separately activated, remains to be learned from enzymatic studies (*9*).

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