Acquisition of New Capsular Type by Pneumococcus, a Multifactor Transformation[1]

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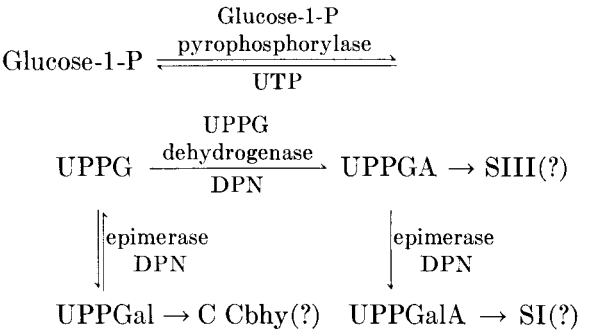
Transformation of noncapsulated or of partially capsulated mutants of pneumococcus type III with the desoxyribonucleates (DNA) of pneumococcus type I is followed by the appearance of two phenotypes: type I cells and doubly capsulated type I-III cells (Austrian and Bernheimer, 1955). The formation of type III polysaccharide by the I-III phenotype suggests that the noncapsulated mutant of pneumococcus type III retains its capsular polymerizing system but may be unable to carry out a preliminary biochemical step essential to the formation of a component of the polysaccharide. In the absence of any evidence that type I pneumococci can synthesize type III polysaccharide, this hypothesis seems to be a tenable one. The restitution of the synthesis of a normal amount of type III capsular material in the I-III phenotype may result from one of two possibilities. Transformation of the mutant type III strain with DNA from type I cells may be followed by modification to normality of that part of the genome controlling type III polysaccharide synthesis, or the simultaneous presence within the pneumococcal cell of the mutant type III genome and of the normal type I genome may somehow bring about augmented production of type III capsular material. To discriminate between these two possibilities, noncapsulated pneumococci derived from a strain of capsular type II were transformed with DNA from cells of the I-III phenotype. Three pneumococcal variants were recovered in this experiment: cells of the mutant type III phenotype, type I cells and rare type I-III cells. Normally capsulated type III cells were not observed. The results of the experiment indicate, therefore, that the augmentation of type III polysaccharide synthesis by cells of the I-III phenotype results from the simultaneous presence within them of the mutated type III genome and of the normal type I genome and not from restoration of the mutant type III genome to the normal state.

To explain further these findings, attention was directed toward an examination of possible common biochemical pathways involved in the synthesis of type I and of type III capsular polysaccharide. Type I polysaccharide includes galacturonic acid and an acetylated hexosamine among its components. Type III polysaccharide is composed of glucose and of glucuronic acid. Because the uronic acid group is the only recognized unit of chemical similarity in these two polymers, an investigation of the uronic acid metabolism of pneumococcus was undertaken. The results of the study are shown schematically in the diagram.



From capsulated type III cells, the two nucleotides, uridine pyrophosphoglucose (UPPG) and uridine pyrophosphoglucuronic acid (UPPGA) were isolated (Smith, *et al.*, 1957a) whereas only the former nucleotide was found in noncapsulated mutant type III cells. In addition to the two nucleotides present in capsulated type III cells, uridine pyrophosphogalacturonic acid (UPPGalA) was recovered from capsulated type I cells (Smith, *et al.*, 1957b). Two mutant noncapsulated variants of pneumococcus type I were studied also. From one, only UPPG could be isolated whereas from the other, UPPGA could be recovered in addition to UPPG. UPPGalA was absent from the cells of both noncapsulated variants of pneumococcus type I. Examination of the interaction of DNA of the noncapsulated mutant of pneumococcus type III with that of each of the two noncapsulated mutants of pneumococcus type I showed that augmentation of type III polysaccharide synthesis occurred only when recombination took place between the type III mutant and DNA of the type I mutant producing UPPGA or when the reciprocal reaction involving these two strains

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was carried out. DNA from the noncapsulated mutant of pneumococcus type I failing to produce UPPGA was without effect.

Cell-free extracts of capsulated type I and type III pneumococci and of the noncapsulated variant of type I pneumococcus which contains UPPGA are capable of oxidizing UPPG to UPPGA in the presence of DPN. Similar extracts of the noncapsulated mutant of pneumococcus type III and the noncapsulated variant of pneumoccocus type I from which UPPGA cannot be isolated lack this capacity. Extracts of capsulated pneumococcus type I can also convert UPPGA to UPPGalA by a reaction of epimerization which requires the presence of DPN (Smith, *et al.*, 1958).

From the observations reported, it may be concluded that transformation of a noncapsulated pneumococcus derived from one capsular type to an heterologous capsular type may involve the incorporation of genetic material controlling an entire biosynthetic pathway. Transformation of the noncapsulated mutant of pneumococcus type III to capsular type I is accompanied by a minimum of three new biochemical activities on the part of the transformed cell: oxidation of UPPG to UPPGA, epimerization of UPPGA to UPPGalA and polymerization of type I capsular polysaccharide. The fact that capsular transformation requires the acquisition of multiple biosynthetic capabilities and, by inference, the introduction of a relatively large unit of DNA may account for the low frequency of its occurrence in comparison to that of the acquisition through transformation of certain other characters, such as drug resistance. In addition it has been shown that an intermediary product of one biosynthetic pathway (type I polysaccharide) may be diverted into a second biosynthetic pathway (type III polysaccharide) to be utilized in the formation of the end-product of the latter. Noteworthy also is the fact that the genetic structures controlling synthesis of a specific polysaccharide behave as though they were closely linked whereas linkage between the genetic determinants of the two capsular components of doubly capsulated strains is apparently of a much less stable order.

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