ENZYMES

New Phosphorylations

from our Molecular Biology Correspondent

THE enzymatic phosphorylation of protein side chains is a phenomenon that has long been familiar to enzymologists, but is only now surfacing in molecular biology as an apparently widely distributed, though largely unexplained, control mechanism. The phosphorylation of histones is well known, and recently Allfrey and his colleagues (Teng *et al.*, *Biochem. Biophys. Res. Commun.*, **41**, 690; 1970) have reported that several of the numinous acidic proteins of chromatin rapidly incorporate labelled phosphorus, which turns up as phosphoserine and phosphothreonine residues. A large proportion of the phosphoproteins—the distribution of which has been found to be tissue specific—bind to endogenous DNA, but not to DNA from an unrelated species. These findings clearly offer a new point of application in the study of transcriptional control in animal cells.

Phosphorylation of side chains has also been observed in ribosomal proteins of rabbit reticulocytes by Kabat, who suggested that this process arrests activity *in vivo*. He has now defined the system in more detail (*Biochemistry*, **10**, 197; 1971). Transfer *in vitro* of the labelled γ-phosphorus from ATP to serine and threonine side chains occurs primarily to three components, two of which are identifiable ribosomal proteins. The kinase which catalyses this reaction resides in the ribosome, and is extracted from it by washing with buffers of high ionic strength. The specificity of the kinase is evidently low, for it will efficiently phosphorylate chicken erythrocyte histones. Neither of the two ribosomal phosphoproteins can be easily removed by washing, and one of them especially concentrates in the run-off monomers. The next aim must presumably be to identify the function of this ribosomal protein.

Another, and different, context in which phosphorylation has been suggested to play a part is in the action of DNA-dependent RNA polymerase. Martelo *et al.* (*ibid.*, **9**, 4807; 1970), with a view apparently to explaining the microheterogeneity found in preparations of the *Escherichia coli* enzyme, have tried the effect on it of the AMP-dependent muscle protein kinase, an enzyme that is implicated in regulation of other activities. The introduction of the kinase and ATP into a system containing T4 DNA and the polymerase led to a large enhancement of activity, the more so when 3′,5′ AMP was also present. A similar effect was caused by an erythrocyte protein kinase, which is presumably the same as Kabat's enzyme. Radioactive phosphorus is transferred from ATP to the polymerase, where it finds its way into the σ-factor. Indeed, isolated σ-factor can also be phosphorylated with beneficial results on the efficiency of transcription. It is not, of course, by any means obvious that this process is of physiological relevance, but it at least raises the possibility of transcriptional control by selective phosphorylation of one σ-factor, if several of these are in fact present.

It may be remarked in this connexion that a different ATP-dependent modification of *E. coli* RNA polymerase has also just been reported (Chelala, Hirschbein and Torres, *Proc. US Nat. Acad. Sci.*, **68**, 152; 1971). They find that incubation with ATP, magnesium and a cellular fraction results in progressive but reversible inactivation, the reactivation being also dependent on the presence of magnesium. A γ phosphorus label on the ATP is not incorporated into the protein, however, showing that there is no question of phosphorylation. Labels in the α phosphorus and the carbon of ATP do appear in the protein: the inactivation is therefore apparently caused by adenylylation. This is also a phenomenon previously recognized by enzymologists: a thoroughly studied system is the glutamine synthetase of *E. coli*; the function of which is controlled by specific adenylylation of tyrosine under the action of another enzyme, adenylyl transferase (see Hennig, Anderson and Ginsburg, *ibid.*, **67**, 1761; 1970). Chelala *et al.* have shown that in RNA polymerase it is not the σ factor that is modified, but rather one or more of the several core proteins.

The relation of phosphorylation to the operation of the glycogen phosphorylase system has exercised a number of illustrious enzymologists; the most recent chapter has now been added by Fischer and his co-workers (*J. Biol. Chem.*, **245**, 6642; 1970, *sqq*), and this I propose to write about next week.

Phage Under Scrutiny

IN next Monday's *Nature New Biology* a paper by Harrison, Caspar, Camerini-Otero and Franklin most happily and unusually reconciles X-ray diffraction and electron microscopy, and in so doing goes far towards defining the structure in all its complexity. PM2 phage is a marine species, which like some animal viruses contains lipid. The electron micrographs show it to consist of roughly spherical bodies, some 600 Å in diameter, which on closer inspection are seen to be almost certainly icosahedral. There appear to be protruberances at the corners, as in some other icosahedral viruses. The most striking features of PM2, however are the two concentric lines, 40 Å apart, which run round the circumference, with a stain-free intervening region. This is evidently a unit membrane, very similar to that seen in myelin and other systems. Round the outer edge of the membrane there appears to be another layer, consisting of protein.

The X-ray diffraction pattern shows on the one hand a series of regular reflexions at low angles, which correspond to the packing pattern of spheres, and on the other six features at wide angles. One of these is the 3.4 Å reflexion associated with B-form DNA, one is the characteristic diffuse 10 Å ring due to protein, and a broad maximum at about 4.5 Å corresponds to the packing of hydrocarbon chains in the liquid-like phases formed by lipids. This leaves three larger periodicities to be explained. To solve the phase problem Harrison *et al.* use sucrose to change the electron density of the solvent and solvent-penetrable parts of the structure. The electron density map which they obtain shows a deep well at 220 Å, corresponding to the hydrocarbon sandwich in the bilayer, with peaks due to lipid head groups at 200 and 240 Å. Outside this shell there is a broad peak which diminishes as sucrose is added. This presumably corresponds to an open or fenestrated protein shell through which solvent can percolate. There are four protein species in PM2 phage, and the total amount of protein that would be required to construct an outer shell is such that it must contain at least the preponderant species. This has a molecular weight of 34,000, so that if spherical, each molecule would be 30 Å across. A reflexion corresponding to about this value is in fact present in the X-ray pattern. The structures at the corners presumably account for another protein. From arguments of electron density, volume and composition it emerges that the membrane layer is probably penetrated at intervals by protein.

The electron density in the interior of the particle is too great to be accounted for solely by the DNA, and at least another protein component must therefore be associated with it. An electron density maximum at around 185 Å suggests, that this is at least partly in the form of an internal shell of subunits. The overall diameters of the virus and the lipid bilayer, that emerge from the X-ray diffraction analysis, are in remarkably good agreement with the corresponding dimensions in the electron microscope pictures, showing that there is no significant distortion during the fixing and staining procedures, a result that should give comfort to electron microscopists. Further analysis of the X-ray data has enabled Harrison *et al.* to define features of the structure in considerable quantitative detail.

© 1971 Nature Publishing Group