MEETING REPORT

NUCLEIC ACID–PROTEIN INTERACTIONS

A Report on the Third Miami Winter Symposium held in Miami, Florida 18–19 January 1971*.

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1. Introduction

The title of the Third Annual Winter Symposium, sponsored by the University of Miami's Department of Biochemistry, was 'Nucleic Acid–Protein Interactions'. Included in the topics presented at the Symposium were studies on the mechanism of DNA replication and repair as well as studies on the control of transcription and translation.

Arthur Kornberg (Stanford) delivered the annual Feodor Lynen Lecture. This lecture, traditionally autobiographical, was entitled 'Enzymes, DNA and Membranes', reflecting Kornberg's past, present and future interests.

Kornberg's early interest in enzyme purification and nucleotidyl transferase reactions led to the first in vitro synthesis of DNA, and the elucidation of the reactions catalyzed by the DNA-dependent polymerase.

The role of the polymerase in DNA replication is not thought to be the same as the role attributed to it only one year ago. In fact, double-stranded circles of DNA are not active templates. Electron micrographs clearly show that binding of the polymerase is restricted to nicked regions in a homopolymer pair consisting of a chain of deoxyriboadenylate (4000 nucleotides long) annealed with 20 to 30 chains of deoxyribothymidylate (150 nucleotides long). Dimers created by the action of mercury on the polymerase also bind to these regions. Kornberg then described the multiple activities of DNA polymerase. The polymerase is one polypeptide chain (109,000 daltons) which may be cleaved into two fragments by controlled proteolysis. The fragments (75,000 and 36,000 daltons) have distinct enzymatic activities, and do not interact except in the presence of nicked DNA, to which they bind, and form a completely functional enzyme. The 75,000 dalton fragment contains the single disulfide and sulfhydryl group. It also has template and primer binding sites, the latter being involved in selecting the proper 3′-terminus in a nicked region of DNA. The 75,000 dalton fragment further contains the nucleoside triphosphate binding site – a recognition site that assures the fidelity of DNA synthesis. Also involved in the fidelity of replication in this fragment is a nuclease that degrades DNA sequentially from the 3′-terminus. The 3′-exonuclease can act on both frayed ends of double stranded DNA and on single stranded DNA. This nuclease performs a repair function as determined by elegant experiments in Kornberg's laboratory in which mismatched bases were introduced into the primer terminus. Oligo dT_{260} with one or several ^{3}H-dCMP residues at the 3′-terminus was annealed to poly dA_{4000}. The polymerase imme-

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diately removed the C-residues without removing T-residues. Furthermore, in this editing process, the mismatched region was removed before the polymerase pursued its synthetic course. A function of the 36,000 dalton fragment of the polymerase is to degrade DNA from the 5′- to the 3′-end. This activity stringently requires a double stranded DNA molecule and primarily produces mononucleotides, with occasional removal of di- and trinucleotides. This fragment has no polymerase activity.

To emphasize the repair functions of the DNA polymerase, Kornberg noted the studies with the mutant Pol A1 isolated in Cairn's laboratory. This mutant is sensitive to ultraviolet light, X-ray, alkylating agents and lacks the Kornberg polymerase. Also noted was the interesting work in the laboratory of Helinsky, who found the Pol A mutant to be deficient in colicins. In the laboratory of Julian Gross, it was found that rec A mutants, deficient in recombination, are not viable if they are also deficient in DNA polymerase.

Kornberg's current interest is in membrane-bound proteins and the unique problems associated with the delineation of their functions. He believes that the answers to these problems will depend on the solubilization and purification of the membrane proteins, even at the expense of their denaturation, and that the preservation of primary structure of these proteins will ultimately permit their native functional state to be restored. It was once thought that where structure begins, biochemistry ends, Kornberg concluded. This certainly is not indicative of our science of today and the future. Appropriately, the subject of the 1972 Symposium will include the topics of membranes and transport.

2. DNA replication and repair

R. Werner (Miami) reported studies on the in vivo replication of DNA in *E. coli*. Using very short pulses of ^{3}H-thymine, Werner found that high molecular weight DNA is formed prior to the appearance of low molecular weight DNA, suggesting that large DNA is a precursor of DNA fragments, and implying that Okazaki pieces are not the result of discontinuous DNA synthesis.

During short pulses, the relative amount of label found in Okazaki pieces varied with the nature of the precursor used. While nearly 70% of the incorporated ^{3}H-thymidine was found in Okazaki pieces, only 20% of the incorporated ^{3}H-thymine was found in these pieces, the remainder being high molecular weight DNA. In the presence of unlabeled thymidine, the incorporation of ^{3}H-thymine into Okazaki pieces was entirely suppressed. In view of these results, Werner proposed that Okazaki pieces arise from single strand nicks in both parental and newly synthesized DNA to act as swivel points for the rotation of the DNA helices during replication and transcription; indeed a large fraction of the thymidine-labeled Okazaki pieces arises from parental strands, as demonstrated by density-labeling experiments. Werner also suggested that the labeling of Okazaki pieces during short pulses with ^{3}H-thymidine represents, almost exclusively, repair synthesis, while thymine is used for DNA replication.

These results, together with data on the flow of thymine and thymidine through the intracellular nucleotide pools, led Werner to conclude that the precursor pools for replication and repair synthesis are separate, and that the precursors for DNA replication may not be the deoxyribonucleoside triphosphates, but some other form of high-energy deoxyribonucleotide.

Werner has introduced a reasonable doubt about what have been accepted as plausible mechanisms of DNA replication. It would be best to keep in mind the questions he raises as the further characterization of new polymerases is pursued.

C.C. Richardson (Harvard) reported studies on DNA replication in toluene-treated *E. coli* cells. These cells both replicate and repair DNA when provided with deoxyribonucleoside triphosphates and ATP, although only repair synthesis occurs in the absence of ATP. The deoxyribonucleoside diphosphates, but not the monophosphates, can replace the requirement for deoxyribonucleoside triphosphates. Replication is nearly completely inhibited by N-ethylmaleimide in toluene-treated cells.

Toluene-treated Pol A1 mutants, which lack the Kornberg polymerase, also replicate DNA in the presence of ATP and deoxyribonucleoside triphosphates. DNA polymerase II purified from these mutants and from wild type cells may be distinguished from the Kornberg enzyme (Polymerase I) by its elution pattern on ion exchange chromatography, by its sensitivity to

inhibition by N-ethylmaleimide, and by its inability to use poly d(A–T) as a template. Neither Polymerase I nor Polymerase II shows an in vitro requirement for ATP. Polymerase II represents 5–10% of the DNA polymerase activity of wild type *E. coli*. Although replication is abolished at restrictive temperatures in toluene-treated temperature-sensitive mutants, neither polymerase activity (Polymerase I or Polymerase II) is temperature sensitive in vitro. Whether Polymerase II is primarily involved in replication or repair synthesis remains to be determined.

Richardson also described studies on host restriction mechanisms in toluene-treated cells. The substitution of hydroxymethyl-dCTP for dCTP in the reaction mixture containing toluene-treated permissive host cells resulted in only a slightly reduced rate of replication, whereas when toluene-treated non-permissive cells were used, little or no DNA was synthesized. These results suggest that the restriction mechanism is present in the host cell before infection, and that it can recognize hydroxymethylcytidine residues in its own DNA as well as in the phage DNA.

The product of T4 gene 32, which is required for T4 replication and recombination, was shown by B.M. Alberts (Princeton) to bind to single stranded DNA. The binding is cooperative, and 32-protein undergoes spontaneous aggregation at high concentrations in the absence of DNA. Although 32-protein does not bind to double stranded DNA, it will bind to partially denatured T4 DNA, presumably at A–T rich regions. In the presence of 32-protein, the rate of renaturation of T4 DNA is increased 1000 fold at 37° and the in vitro rate of DNA synthesis is stimulated 5 to 10 fold with the T4-coded DNA polymerase. Alberts suggested that 32-protein aggregates are a structural part of the replication apparatus and proposed models for both continuous and discontinuous replication of T4 DNA in which 32-protein acts to unwind the double stranded template ahead of the polymerase.

J.E. Cleaver (San Francisco) discussed studies on the repair of UV damage in mammalian cells. Xeroderma pigmentosum (XP) is an autosomal recessive human disease in which there is an abnormally high incidence of actinic skin cancer. XP fibroblasts from dermal tissue are similar to normal fibroblasts in karyotype, growth parameters, lifetime in culture, and transformability by SV40 , but differ in their response to UV irradiation. These cells appear to be analogous to the uvr^- and hcr^- mutants of *E. coli*.

Although XP cells repair many kinds of DNA damage to normal extents (e.g. X-ray, chemical mutagen, 5-bromouracil-UV photo products), they are more sensitive to UV light, show reduced host cell reactivation of irradiated viruses, fail to excise detectable amounts of cyclobutane dimers, and perform reduced levels of repair replication. These results are consistent with the hypothesis that the defect in the disease is at the initial excision step of the excision-repair pathway and that the affected enzyme is an endonuclease. Thus, mutagens which cause single strand breaks would be repaired normally in these cells.

Because damage induced by nitrosoguanidine is repaired in these cells, Cleaver proposes that this mutagen also causes single strand breaks. This is an interesting extrapolation from his well-studied system.

Unlike the situation in some malignant cells, XP cells are transformed at normal rates by SV 40 . Irradiation of recipient cells before infection, however, increases transformation and Cleaver speculates that XP cells might be more readily transformed by oncogenic viruses in the presence of sunlight. Many malignant cell lines display normal repair; therefore, the relationship of these studies to general carcinogenesis should be considered with some restraint.

3. Regulation of transcription

M. Ptashne (Harvard) discussed recent studies in his laboratory on the λ repressor and the repressor of the related phage λ^{434}_{imm}. In vitro, these repressors bind specifically and with high affinity to DNA containing the sites (operators) at which the repressor acts in vivo. Thus, λ repressor binds to DNA isolated from phage λ but not to 434 DNA, and the reverse is true for the 434 repressor. The binding of the repressor is somewhat diminished if the operator gene contains a point mutation, and is greatly diminished if an operator contains a double mutation, suggesting that mutations other than deletions can result in the O^c phenotype.

Both the λ and the 434 repressors bind to DNA as oligomers, probably as dimers, and these oligomers are in rapid, concentration-dependent equilibrium with

monomers. The λ and 434 monomers have molecular weights of 28,000 and 26,000 daltons respectively.

The λ repressor binds to two sites in the immunity region and appears to control two "early gene" operons which are read in opposite directions, beginning at the extremities of the immunity region. Purified λ repressor specifically and efficiently represses transcription of two classes of mRNA (7 S and 12 S) in an in vitro system when repressor is added to the system before RNA polymerase. Repression is not observed when λ repressor is added after RNA polymerase is bound to the template, a result suggesting that the repressor acts by preventing the binding of polymerase to the adjacent promoters.

I. Pastan (Bethesda) discussed recent studies in his laboratory on the role of 3′,5′-cyclic AMP (cAMP) and cAMP receptor protein (CRP) in the synthesis of inducible enzymes in *E. coli*. The in vitro synthesis of β-galactosidase activity has been shown to require both cAMP and CRP. The role of cAMP and CRP in the control of the transcription of *lac* genes was shown by the in vitro synthesis of *lac* mRNA with *E. coli* RNA polymerase using λh80d*lac* DNA as a template. The transcription of the *lac* genes was prevented by purified *lac* repressor, and this repression was reversed in the presence of the gratuitous inducer IPTG. Because the cAMP and CRP-dependent synthesis of *lac* mRNA was accomplished with purified proteins, Pastan proposed two possible sites of action of cAMP and CRP: they might modify RNA polymerase, allowing it to bind to the *lac* promoter, or they might bind to the *lac* promoter, allowing RNA polymerase to bind. The data, thus far, favor the second hypothesis, because a cAMP-CRP complex binds to *lac* DNA in the absence of RNA polymerase. Furthermore, the incubation of *lac* DNA, RNA polymerase, cAMP and CRP resulted in the formation of an initiation complex which was detected by its relative insensitivity to Rifampicin inhibition.

V.G. Allfrey (Rockefeller) discussed changes in chromosomal proteins associated with gene activation. The acidic phosphoproteins appear to play a role in positive control of transcription. The presence of acidic phosphoproteins in the chromatin correlates strongly with the RNA synthetic activity of the nucleus, and the rates of phosphorylation of these proteins are augmented at times of gene activation. An increase in the rate of ^{32}P incorporation into phosphoprotein precedes the increase in RNA synthesis in phytohemagglutinin-treated lymphocytes. The distribution of specific phosphoproteins was shown to vary in different tissues of the rat, and the synthesis of certain chromosomal proteins was shown to be influenced by hormones in the target tissue.

DNA-binding studies indicate that many of the acidic chromosomal proteins bind specifically to the DNA of the species of origin. Using RNA polymerase of *E. coli*, Allfrey showed that RNA synthesis is increased 72–90% by rat kidney nuclear proteins when DNA from rat kidney or rat liver was used as a template, although no stimulation was observed with calf thymus DNA as a template. Phosphoproteins also block the repression of RNA synthesis by histones.

The relationships between phosphorylation (which is rapid and readily reversible) and RNA synthesis suggest that certain chromosomal proteins might have functions analogous to bacterial and viral sigma factors, directing the attachment of RNA polymerases to specific sites of initiation on the chromosome. This analogy is strengthened by the fact that phosphorylation of *E. coli* sigma factors by protein kinases from animal tissues has been found to stimulate RNA synthesis; indeed, the sigma phosphorylation reaction is itself stimulated by cyclic AMP.

A. So and K. Downey (Miami) reported studies on the effects of the formation of a single phosphodiester bond on the stability of the DNA–RNA polymerase complex, utilizing the d(A–T) copolymer as a template for poly r(A–U) synthesis. By using as substrates a complementary dinucleotide and a single ribonucleoside triphosphate (e.g., ApU and ATP, or UpA and UTP), the synthetic reaction could be limited to the formation of a single phosphodiester bond, which was shown to be sufficient to stabilize the DNA-enzyme complex to dissociation by high ionic strength. Phosphodiester bond formation, and the resultant stabilization of the DNA-enzyme complex, was dependent on sigma factor, the subunit of RNA polymerase required for chain initiation. Incubation of the DNA-enzyme complex with preformed dinucleotides or with single ribonucleoside triphosphates was ineffective in stabilizing the complex to dissociation by high salt. The enzyme-catalyzed formation of the trinucleotide ApUpA, but not the formation of UpApU, was reported to protect the enzyme against inhibition by the antibiotic Rifampicin.

J. Hurwitz (Albert Einstein) discussed mechanisms of termination of transcription. During in vitro transcription, RNA chains may be released from the DNA template either by high ionic strength (0.2 M KCl) or by rho factor, a protein described by Roberts. Salt-mediated termination also results in the release of RNA polymerase and the subsequent reinitiation of RNA synthesis, and one observes repetitive transcription of the same regions of the T4 genome. Over 90% of the T4-coded RNA chains released during salt-mediated termination were reported to contain uridine as a 3′-hydroxyl terminus, suggesting that RNA polymerase may recognize a specific termination sequence on the DNA template.

Rho factor, a protein of 200,000 daltons, effects termination of transcription by binding to the DNA template and blocking the chain elongation reaction and, thus, causes production of an RNA of decreased chain length. In rho-mediated termination, RNA polymerase is not released from the template and is consequently not available for reinitiation. Hurwitz postulated the existence of an anti-rho factor which would function to release the enzyme from the DNA-enzyme-rho factor complex and allow reinitiation of transcription.

4. Protein-tRNA interactions

K. Muench (Miami) reported on the subunit structure and substrate binding sites of purified Trp-tRNA synthetase of *E. coli.* The protein has a molecular weight of 75,000 daltons and consists of two identical subunits of 37,000 daltons. The dimer was shown to have two binding sites for $tRNA^{Trp}$, and for Trp-ATP. Titration with DTNB revealed two sulfhydryl groups per dimer, both protected by ATP and tryptophan. The enzyme was tentatively assigned two active sites per dimer, demonstrated to be independent by kinetic studies.

The formation of Trp-ATP, a reaction previously reported for the Trp-tRNA synthetase of beef pancreas, has not been reported for any other aminoacyl-tRNA synthetase, and the physiological significance of this unusual reaction has not been elucidated. Finding a role for Trp-ATP, if it exists in vivo, may be a rewarding pursuit, as the control of tryptophan biosynthesis appears to be unique in *E. coli*; perhaps Trp-ATP serves a control function.

Studies on the origins of the genetic code were presented by J.C. Lacey and S.W. Fox (Miami). Thermal proteinoids, prepared by heating a dry mixture of amino acids, were reported to form particulate complexes with homopolynucleotides when the proteinoids carried a net positive charge. Although in some cases anti-codonic relationships were observed in particle formation (i.e., lysine-rich proteinoids complexed preferentially with poly U), the secondary structure of the homopolynucleotide was found to be an important factor in these interactions.

Proteinoid-polynucleotide particles of varying compositions were tested for their ability to bind aminoacyl-adenylates and, in these experiments, codonic relationships were observed (i.e., Pro-AMP preferentially bound to a proteinoid–poly C particle). Lacey discussed the possible significance of proteinoid-polynucleotide particles as evolutionary precursors of contemporary ribosomes.

F.C. Neidhardt (Michigan) showed that under certain growth conditions aminoacyl-tRNA synthetases are subject to high rates of inactivation in the intact cell. It is possible that the conformation of the active enzyme is such a strained one, that after functioning there is a significant probability of irreversible denaturation. The evidence that supports this view is the fact that the conformation of several well-studied synthetases can be profoundly altered by interactions with their ligands. Furthermore, synthetases are unusually susceptible to mutations that increase their thermolability.

Recent work with a new temperature-sensitive mutant suggests that synthetase inactivation is related in an interesting manner to the catalysis of aminoacylation by these enzymes. Isotope experiments showed that the valyl-tRNA synthetase is inactivated at the same rate valyl-tRNA is being formed. Extracts of this mutant can be incubated in the presence of valine and ATP for prolonged periods at 40° without damage to the enzyme. The addition of tRNA to such a mixture induces a rapid inactivation of the enzyme. The valyl-tRNA synthetase is stable in this strain at 40° under non-growth conditions but unstable during growth. From studies with this mutant Neidhardt believes that *E. coli* is programmed to maintain a functional excess of valyl-tRNA synthetase.

K.B. Jacobson (Oak Ridge) presented a novel molecular basis for the suppression of a vermilion mutant of *Drosophila melanogaster.* The biochemical lesion

in the vermilion mutant is a deficiency in tryptophan pyrrolase activity which appears to be due to the specific sensitivity of the mutant enzyme to one of the three isoacceptor forms of uncharged $tRNA^{Tyr}$. In the suppressor mutant, this particular form of $tRNA^{Tyr}$ ($tRNA^{Tyr-2}$) is not present. The tryptophan pyrrolase of the vermillion mutant is restored to 75% of the wild type activity by the addition of RNase T1. This 'activated' enzyme is again inhibited by wild type tRNA but not by suppressor tRNA. Because the total amount of $tRNA^{Tyr}$ is the same in the *spr* mutant as in the wild type (as peak number 2 diminishes, peak number 1 increases) it appears that the mutation is not in a structural gene for a particular $tRNA^{Tyr}$ but in an enzymatic step that converts a precursor to $tRNA^{Tyr-2}$.

This exciting work is related more to the participation of tRNA in the control of the synthesis or the assembly of enzymes than it is to more usual mechanisms of suppression observed in microorganisms. Jacobson's findings are relevant to recent, separate studies in which (1) histidyl-tRNA was found to bind to the first enzyme in the histidine operon, (2) both phenylalanyl-tRNA and $tRNA^{Phe}$ form a complex with the first enzyme in the bacterial pathway for synthesis of aromatic amino acids, and (3) leucyl-tRNA is bound to an immature form of threonine dehydratase and prevents the maturation of the enzyme to its active form. As recently as two years ago, the debate concerning the site of repression of enzyme forming systems by tRNA was limited to interactions with DNA or with RNA. Few investigators would have entertained the notion of an interaction with the products of translation.

5. Conclusion

The papers presented at the symposium clearly reflected the rapid growth of knowledge in the area of nucleic acid-protein interactions, and emphasized the variety and complexity of the regulatory processes that ultimately depend on highly specific interactions of proteins with nucleic acids. The solutions of many of the problems of molecular biology await a better understanding of the nature of these interactions.

The success of the Symposium was enhanced by an ever increasing interest by the visitors in the intellectual rather than the meteorological climate of the meetings, and the fact that the subjects of the allied PCRI Winter Symposium were tumor viruses and reverse transcription. This led to a stimulating interaction between the participants in both Symposia.