STADLER SYMP. Vol. 8 (1976) University of Missouri, Columbia
pp. 159-169

DNA PACKAGING IN ISOLATED BACTERIAL NUCLEOIDS

(Chromosomes, DNA tertiary structure, nucleoids, DNA folding, DNA supercoiling, nascent RNA, membrane-DNA)

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SUMMARY

Properties of nucleoids isolated from Escherichia coli are described. Experiments which investigate the organization of DNA folding and supercoiling in the isolated chromosome are reviewed, and evidence is discussed suggesting a role for certain proteins and nascent RNA molecules in stabilizing the condensed DNA. A model of the conformational organization of the packaged DNA is presented.

INTRODUCTION

DNA as it exists in natural biological structures such as chromosomes is packaged in a compact state. The 3-dimensional organization of the DNA in these structures and the nature of the interactions which stabilize them are just beginning to be understood. It is not yet clear, for example, how precisely the tertiary structure of DNA is organized in the eukaryotic interphase nucleus or in the prokaryotic nucleoid. On the one hand, it is possible that there may be a precise long-range periodicity in the structure of the packaged DNA. On the other hand, the packaging may be more loosely organized so that there is a considerable flexibility in the long range DNA conformation. In either case, it seems most likely that there are severe restraints on the extension and rotation of the DNA or chromatin fibers, leading to sufficient order that tangling and other consequences of disorder are avoided. The recently discovered Nu bodies (see for review ELGIN and WEINTRAUB 1975) likely represent the first level of short-range conformational organization of eukaryotic DNA. Details of the interactions organizing longer range order are still to be elucidated. When the interactions responsible for the long range order are better understood, it will be possible to determine more exactly the chromosomal structure and its influences on genetic regulation.

Our approach to this problem has concentrated on studies of packaged DNA in bacterial nucleoids. Here I will review some of the progress that has been made toward an understanding of DNA tertiary structure in this comparatively simple chromosome.

NUCLEOID ISOLATION

Biochemical investigation of nucleoid structure required, first, the development of a procedure for isolating the nucleoids, which preserves the condensed state of the DNA. In the early 1970's it was observed that the DNA in lysates of *Escherichia coli* maintains non-viscous, particle-like properties if the cells are gently opened in solutions containing a high concentration of counterions. Any cation that has been tried (Li^+, Na^+, K^+, Mg^{++}, NH_4^+ or polyamines such as spermidine) enhanced the stability of the condensed DNA. It was then possible to separate the rapidly sedimenting, condensed DNA from other components of the

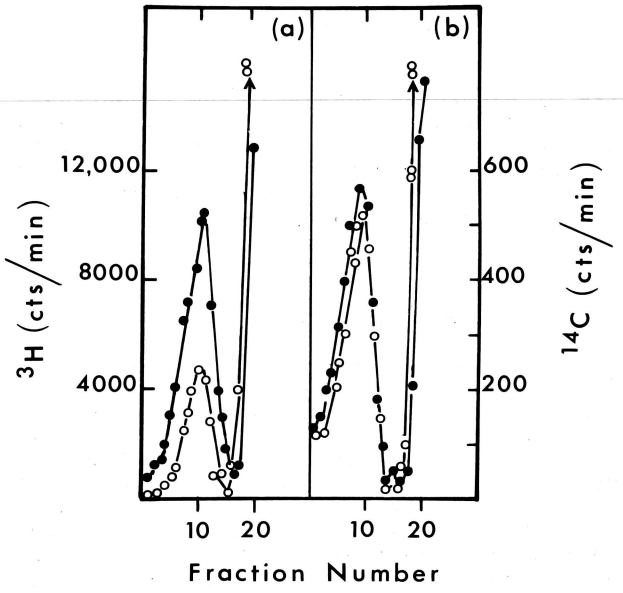

Figure 1. Isolation of nucleoids by sedimentation on sucrose gradients. Exponentially growing *E. coli* cells were labelled with [^{14}C] thymidine for 30 min and pulse-labelled with [5-^{3}H] uridine for 3 minutes prior to harvesting. They were lysed with lysozyme and the detergents brij, deoxycholate and sarkosyl in a solution containing 1.0 M NaCl using methods previously reported (GIORNO, HECHT and PETTIJOHN). The lysate was then layered on a sucrose gradient. A) The "membrane-free nucleoid" prepared by incubating the lysate at 24°C for 5 min prior to centrifugation (30 min at 17,000 rpm in an SW50 rotor). B) The membrane-associated nucleoid prepared by incubating the lysate at 4°C for 30 min. prior to centrifugation (7 min at 17,000 rpm). ^{14}C •; ^{3}H o.

lysate by centrifugation in sucrose gradients (see, for example, Fig. 1). The stabilizing effect of the counterions is believed to be attributable to shielding of charge repulsions of the densely packaged phosphate groups in the condensed nucleic acids, although this interpretation is not established. DNA in nucleoids isolated in the presence of the counterions can be unfolded by exposure to higher temperatures. The transition temperature (t_m) for the unfolding estimated from sedimentation or viscosity studies varies with the concentration of ions in the solvent. For example, at low salt concentrations (<0.10 M NaCl) the DNA unfolds at temperatures less than 40°C, while the unfolding occurs at 50-65°C in the presence of 1.0 M NaCl (FLINK and PETTIJOHN 1975). Small concentrations of multivalent counterion such as spermidine or Mg^{++} greatly enhance the stability of the structure. The use of these counterions has permitted nucleoid isolation in solvents of much lower ionic strength (KORNBERG and WORCEL 1974). Also, Dworsky (1975) has recently described a modified procedure for isolating nucleoids in solutions of lower ionic strength. It would be expected that many DNA bound proteins which normally are dissociated by high salt concentrations would remain attached to the DNA isolated by these methods.

MEMBRANE-ASSOCIATED AND MEMBRANE-FREE NUCLEOIDS

During lysis of bacteria the degree of disruption of the cell wall-membrane complex is dependent on the concentrations of lysozyme and detergents, the solvent used and the time and temperature of the lysis reaction. When the reaction is limited, a portion of the wall-membrane complex remains intact and co-purifies with the nucleoid. The sedimentation rate of these membrane-associated nucleoids is greater than that of the "membrane-free" nucleoid (Fig. 1). The amount of bound membrane is dependent on the extent of reaction with the lysing reagents, and nucleoids sedimenting at different rates generally in the range 7000-1600S can be obtained, depending on how much membrane-wall complex remains. When the reactions are allowed to proceed for some time at elevated temperatures, the amount of associated membrane becomes minimal, and the median sedimentation rate of the nucleoids isolated from rapidly growing cells is about 1600S (Fig. 1a). In Figure 1b it can be seen that nucleoids prepared from cells at lower temperatures which do not completely disrupt the membrane-wall complex need be centrifuged only about one fourth as long to reach a similar position in the gradient.

The amount of protein cosedimenting with the membrane-associated nucleoid is dependent on how much membrane was dissociated during lysis, but is always much greater than in the membrane-free nucleoid. The additional protein is primarily due to a membrane-wall protein of molecular weight about 36,000 daltons (Fig. 2). The "membrane-free" nucleoids have very little associated membrane-wall protein compared to the more rapidly sedimenting membrane-associated chromosomes; yet small amounts of these proteins are always detected sedimenting with the nucleoids. Although there is no evidence at this time that a small amount of the wall-membrane complex is essential for stability

of the nucleoid, it is difficult to exclude this possibility since no one has yet isolated nucleoids completely free of membrane-wall proteins. At least a portion of the remnant wall-membrane complex co-sedimenting with the 1600S nucleoid is unattached contaminating complex (Pettijohn, unpublished results; Worcel, personal communication). Whether or not very minor amounts are actually bound is uncertain. All of the results described here concerning the structure of the nucleoid were obtained with the so-called membrane-free nucleoid.

COMPONENTS OF THE ISOLATED NUCLEOID

An isolated nucleoid seems to contain the entire DNA compliment of the *in vivo* nucleoid. The average DNA content per singlet or doublet nucleoid (Fig. 3) is respectively 9.1±1.0 x 10^{-9}µg or 15±2 x 10^{-9}µg (HECHT, TAGGART and PETTIJOHN 1975), which is equivalent to 2.2 or 3.6 genome equivalents of DNA per structure respectively. The latter figure is in close agreement with the average DNA content per *E. coli* cell grown under similar conditions (KUBITSCHEK and FREEDMAN 1971). Sedimentation studies of

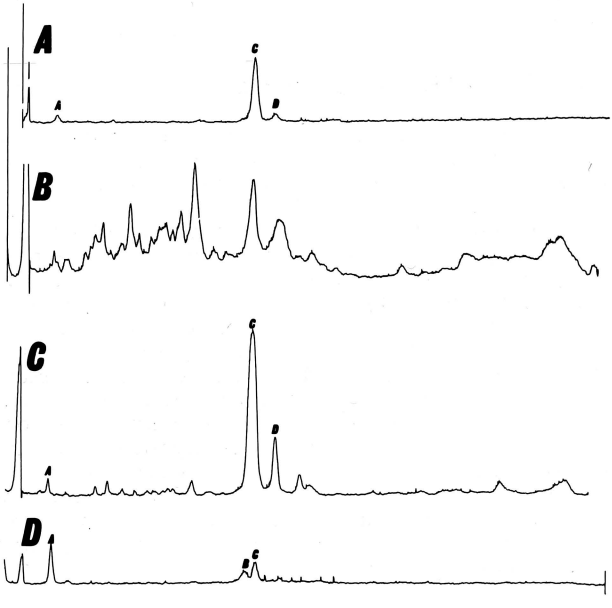

Figure 2. Electrophoretic analysis on SDS-containing polyacrylamide gels of proteins of the isolated nucleoids. The above are densitometer scans of autoradiograms obtained from the gels containing ^{14}C-labelled proteins. a) proteins from isolated condensed T4 DNA particles; b) total proteins of *E. coli*; c) proteins from isolated membrane-associated nucleoids; d) proteins from isolated membrane-free nucleoids. Direction of electrophoresis was left to right. Molecular weights of bands C and D were respectively 36K and 31K, determined by comparison with T4 protein markers.

the DNA of the nucleoid after it has been unfolded by treatment with strong ionic detergents suggest that its molecular weight is similar to that of the intact bacterial chromosome (STONINGTON and PETTIJOHN 1971; WORCEL and BURGI 1972; DRLICA and WORCEL 1975).

The proteins of the membrane-free (1600S) nucleoid are composed primarily of the subunits of core RNA polymerase (STONINGTON and PETTIJOHN 1971). For example, in the electrophoretic analysis shown in Figure 2d the major protein band (A) has a mobility indistinguishable from that of the ββ' subunits of RNA polymerase. The more minor bands (C and D) were previously shown to be those of the major cell envelope proteins. These latter bands are much more prominent in the proteins of the membrane-associated nucleoid (Fig. 2c) and also in proteins associated with condensed T4 DNA (Fig. 2a) isolated from T4 infected *E. coli* cells by a procedure analogous to the nucleoid isolation (S. HAMILTON and D. PETTIJOHN, submitted for publication). Band A, the RNA polymerase subunits, can also be seen among the proteins of the membrane-associated nucleoid and the condensed T4 DNA. Comparison with the analysis of total *E. coli* proteins (Fig. 2b) shows that the proteins of the nucleoids are a highly selected fraction of the total cellular protein. Although the envelope proteins and RNA polymerase subunits are the only ones clearly observable in the analysis of membrane-free nucleoids, very minor bands corresponding to other proteins can be more clearly seen when higher concentrations of protein are analyzed. The identity of these more minor protein components is unknown.

Nascent RNA chains are also bound to the isolated nucleoids. When cells are pulse-labelled with ^{3}H-uridine for 10 sec or less, nearly all of the labelled nascent RNA cosediments with the nucleoid (PETTIJOHN, STONINGTON and KOSSMAN 1970). When longer labelling times are used as in Figure 1, the amounts of RNA released from the nucleoids are substantially higher. As shown in Figure 1, there is more ^{3}H-RNA bound per membrane-associated nucleoid than per membrane-free nucleoid. Electrophoretic analysis of the labelled RNA has shown that the additional RNA in the membrane-associated nucleoids is made up mostly of the rRNA precursors p16 and p23 plus 23S (HAMILTON and PETTIJOHN, unpublished result). Previous studies (HAYWOOD 1971) have shown that newly synthesized rRNA precursor molecules in *E. coli* are preferentially bound to membrane in cell lysates. It is therefore likely that the additional RNA in the membrane-associated nucleoid is bound to the membrane-fragment rather than to the DNA. Worcel and Burgi (1974) found the same amount of RNA bound to the membrane-free and membrane-associated nucleoids. It is likely that the amount of bound RNA is dependent on the amount of wall-membrane complexed with the nucleoid. As estimated from sedimentation rates, the membrane associated nucleoids of Fig. 1b have more wall-membrane complex than that analyzed in Worcel's laboratory.

UNFOLDING THE DNA

The state of condensation of the DNA in the nucleoids can

be studied by several techniques. The nucleoids can be visualized by fluorescence or electron microscopy (Fig. 3). The fluorescence microscopy technique has the advantage of maintaining an aqueous environment, while dehydration is necessary in the electron microscopy which may lead to DNA spreading or aggregation. Conversely, greater resolution is available using the electron microscopy. Either technique shows particles having dimensions similar to those of the nucleoid observed *in vivo*. In DNA spreading techniques such as the Kleinsmidt method are used, the DNA becomes more extended and the dimensions of the observed structures are much greater than those of the cells from which they were isolated. The microscopy techniques can be used to examine the state of condensation of the nucleoid as the DNA is unfolded.

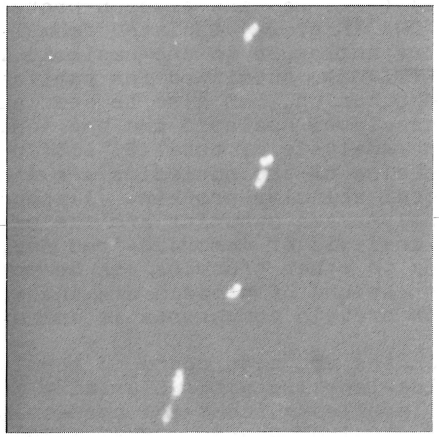

Figure 3. Fluorescence microscopy of the isolated bacterial nucleoid. Ethidium bromide was the fluorescent chromophore. The maximal size of the observed particles was 0.5 to 1.5 μm. Many doublets which appear to be two closely linked particles were observed.

Unfolding of the DNA is also indicated by a very large increase in the viscosity of solutions containing the nucleoids (STONINGTON and PETTIJOHN 1971). This technique provides a sensitive assay for changes in the condensation of the DNA; however, there are certain anomalies in the measurements which are not understood. Gel-like behavior in the unfolded DNA has been observed (BOWEN and ZIMM, personal communication), and anomalously large specific viscosities are obtained (DRLICA and WORCEL 1975). Until these effects are better understood, viscosity measurements should be interpreted cautiously and at most used as qualitative indications of the state of condensation of the DNA.

Sedimentation studies have also been used to follow the unfolding of the DNA (PETTIJOHN and HECHT 1973). As the DNA unfolds, its frictional coefficient increases and its rate of sedimentation falls. For example, it was observed that nucleoids treated with various concentrations of RNase acquire sedimenta-

tion rates between 160O and 160S, depending on how great the exposure to RNase; the more RNA hydrolysis that occurred, the smaller the sedimentation rate. These changes were primarily attributable to DNA unfolding, since there were no detectable breaks in the DNA and mass changes due to release of RNA fragments would not be sufficient to account for the change. Also, the viscosity of DNA dramatically increased after the RNase exposure, and microsopic examination indicated a more expanded conformation. These and other findings have demonstrated that certain RNA components of the isolated nucleoid are essential for its stability.

It was also found that the DNA unfolds when nucleoids are treated with ionic detergents or other reagents which dissociate or degrade proteins on the nucleoid. This has led to the suggestion that certain protein components of the nucleoid are essential for its stability (PETTIJOHN et al. 1973; DRLICA and WORCEL 1975).

SUPERCOILED DNA IN NUCLEOIDS

Worcel and Burgi (1972) first demonstrated that the effects of intercaled ethidium bromide on the nucleoid were characteristic of supercoiled DNA. A biphasic transition in the sedimentation rate of the nucleoid is observed when its sedimentation rates in different concentrations of ethidium are compared. The sedimentation rate is minimal at a certain critical concentration of ethidium where the supercoils are removed. At concentrations above and below this critical concentration negative or positive supercoils exist, making the structure more compact. When single-strand breaks are introduced into the DNA of the nucleoid with DNase, the breaks provide swivels which relax the supercoiling. It was found that many breaks per chromosome were required to relax all of the supercoiling (WORCEL and BURGI 1972; PETTIJOHN and HECHT 1973). This finding suggested that the chromosome is segregated into separate domains, like those that occur in the nucleoid sites, which restrain the rotation of the double-helix (see Figure 4). A nick in one domain relaxes the supercoiling only in that domain, since rotation about the nick cannot be propagated to adjacent domains.

Nucleoids partially unfolded by reaction with RNase show the same biphasic transitions in ethidium bromide as the completely folded nucleoids (PETTIJOHN and HECHT 1973). This finding indicates that some unfolding of the DNA can occur without detectably relaxing the supercoiling. Likewise it appears that partial or complete relaxation of supercoiling can occur without affecting the DNA folding. Nucleoids reacted to various extents with DNase so that only a part of the supercoiling is relaxed sediment at different rates between that of completely relaxed and completely supercoiled structures. In the presence of the critical concentration of ethidium (just sufficient to unwind all supercoils) the nucleoids nicked or unnicked sediment at the same rate. Thus the unfolding is not detectable in these partially relaxed chromosomes.

It has also been demonstrated that nucleoids which were par-

tially unfolded by RNase require fewer single-strand breaks in the DNA to relax their supercoiling (PETTIJOHN and HECHT 1973). Thus even though the partial unfolding achieved with RNase did not relax the supercoiling, it did affect the manner in which the supercoiling is stabilized or segregated in the isolated chromosome.

A MODEL OF THE DNA IN ISOLATED NUCLEOIDS

The model described in Figure 4 accounts for all the properties of the nucleoid described above. Certain features of this model, including the formation of loops of independent supercoiling, were first proposed by Worcel and Burgi (1972). The focus here is on the participation of RNA molecules in stabilizing the structure. It is proposed that an RNA molecule bound to two or more separate sites on the isolated chromosome can define the position of a DNA fold and also restrain the rotation of the double helix at the sites of attachment. Extensive rotation of the DNA would be limited by the possible coiling of the RNA about the DNA or would require breaking the RNA-DNA interaction. The DNA restrained by many different RNA molecules in this fashion would be segregated into a series of domains defined by the sites of RNA binding. Nicks in the DNA would relax only the domains in which they occur (Fig. 4E). After partial hydrolysis of the linking RNA molecules, unfolding occurs as adjacent domains

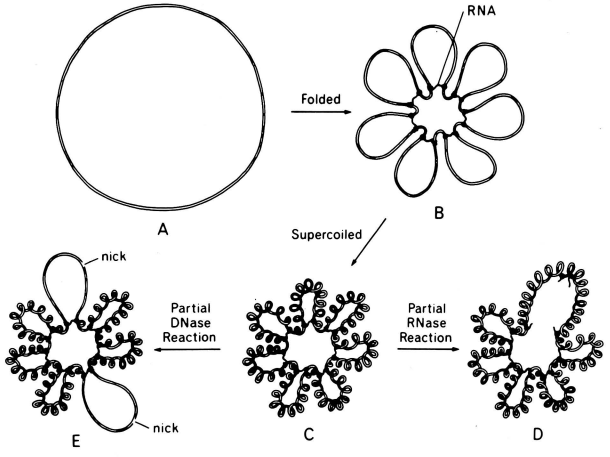

Figure 4. A model for the organization of DNA in the bacterial nucleoid. A) the circular completely unfolded, relaxed DNA; B) the chromosome containing only folds restrained by RNA-DNA interactions; seven domains are thus defined (the actual number is greater); C) folded and supercoiled DNA in the nucleoid; D) the partially unfolded DNA of the nucleoid obtained by hydrolysing some of the RNA; E) chromosome with two single-strand breaks in the DNA, relaxing only the domains in which the nicks occur. See text for details.

coalesce (Fig. 4D). Because of this coalescence of domains, a single nick in the DNA can now relax more of the supercoiling, explaining the finding that fewer nicks are required to relax the partially unfolded DNA. Independence in the relaxation of folding and supercoiling is also possible. Nicks in the DNA relax supercoiling but should not affect folding since they need not influence the RNA-DNA interaction. Nucleoids partially unfolded after reaction with RNase would lose no supercoiling, since neighboring RNA-DNA sites would continue to limit the rotation of the DNA.

It is proposed that the critical RNA chains are nascent RNA molecules. This proposal is derived from the finding that inhibition of RNA synthesis by rifampicin or other inhibitors prior to isolation of nucleoids results in the DNA unfolding after it is released from the cell during lysis (PETTIJOHN and HECHT 1973; DWORSKY and SCHAECHTER 1973). Analysis of this phenomenon has suggested that it is the elimination of the nascent RNA chains that is critical. One of the sites of attachment of the stabilizing RNA to the folded DNA would therefore be via its associated RNA polymerase at the 3' end of the nascent RNA. The nature of the other site(s) of attachment cannot be specified. It is possible that it may involve the formation of an RNA-DNA hybrid between a region(s) of the nascent RNA chain closer to its 5' end. Richardson (1975) has found that supercoiled viral DNA molecules can form hybrids with extensive regions of their nascent RNA chains. Recently we have also isolated from the nucleoid complexes between the DNA and nascent RNA chains which are resistant to RNase and have other properties expected of RNA-DNA hybrids (HECHT and PETTIJOHN 1976). It is possible that these RNA-DNA interactions represent the second binding sites of the stabilizing RNA molecules.

When proteins are dissociated from the nucleoid by treatment with ionic detergents or other protein denaturants, the DNA unfolds. This would be expected from the above model, since the release of RNA polymerase molecules could destabilize the 3' attachment site of the nascent RNA chains. However, it is also possible that other proteins may participate in stabilizing DNA folds. Indeed, some recent evidence points to this possibility (DRLICA and WORCEL 1975).

It should be emphasized that the model applies only to the *isolated* nucleoid. There is no evidence as yet that the observed interactions also occur in the cell. It is difficult to exclude the possibility that the observed RNA-DNA interactions are the result of some fortuitous association occurring during lysis of the cells.

NUMBER OF SUPERCOILED DOMAINS

Potentially the number of domains in a chromosome can be calculated from the number of single-strand breaks required to relax all the supercoiling in the chromosome. Application of Poisson statistics to this problem requires knowledge of the size distribution of the domains and the assumption that the nicks

occur randomly in the DNA. Assuming equally sized domains and random distribution, the number of domains relaxed by DNase hits has been estimated in the range 50±40 per genome equivalent of DNA (WORCEL and BURGI 1972; PETTIJOHN and HECHT 1973). The wide range is attributable to uncertainties in measuring precisely the single-strand molecular weights of the nicked DNA, difficulties in measuring simultaneously the supercoiling and number of nicks in nucleoids (since the rate of nicking is not totally reproducible from time to time) and uncertainties in the above assumptions.

The use of gamma irradiation to introduce single-strand breaks into the DNA can alleviate most of these uncertainties. The rate of nicking the DNA is constant in time and reproducible so that numbers of nicks can be more accurately measured at any dose; since there is no diffusion problem as there is with DNase, the assumption of random nicking seems more justified (indeed the measured single-strand molecular weights show random distribution). Recent measurements using gamma irradiation to relax supercoiling in the nucleoid has indicated 160±40 domains per genome equivalent of DNA (assuming equal-sized domains)(LYDERSEN and PETTIJOHN, in preparation). We prefer to continue using the term "domain" rather than "loop" for the units of segregation determined by these physical-chemical methods, since it has not been demonstrated that there is a one-to-one equivalence between the number of loops observed under the electron microscope (KAVENOFF and RYTER 1976) and the number measured chemically.

ACKNOWLEDGEMENTS

The work from this laboratory has been carried out in collaboration with Drs. O. Stonington, C. Kossman, T. Stamato, R. Hecht, R. Giorno, I.Flink, K. Lydersen, J. Carlson and S. Hamilton and Ms. E. Miles. This research was supported by grants from the U. S. Public Health Service (GM 18243 and training grant GM 00781) and from the National Science Foundation (GB 43359). This is publication No. 657 of the Department of Biophysics and Genetics.

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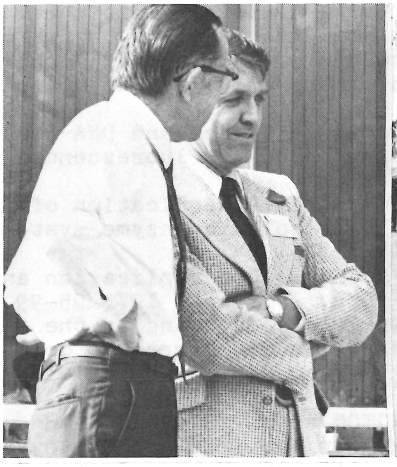

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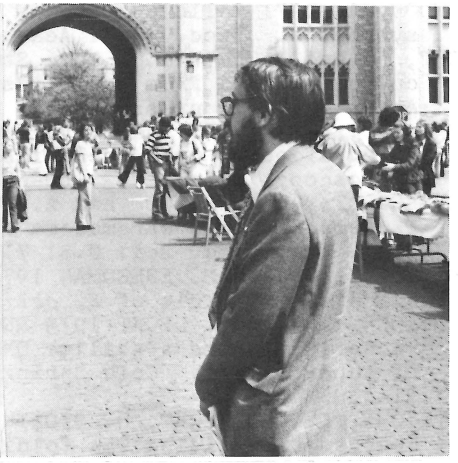

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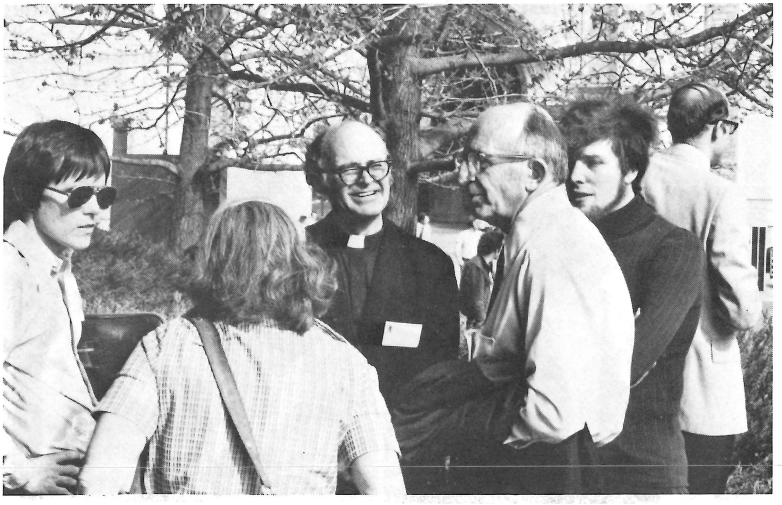

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