

FILE PROGRESS REPORT

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1. Statement of Progress

Background: The general features of transformation are well known, and discussed in much detail in numerous reviews and texts (e.g., Stent (45), Watson (46), Hayes (32). A review by Hotchkiss and Gabor (33) cites 241 references through 1969, almost all of them relevant to the present discussion. We will then not undertake a monographic survey of complete bibliography here.

The specificity of transformation, e.g. the rejection of E. coli DNA by B. subtilis, pervades the literature. However, few published reports are directed at pushing the empirical upper limits, perhaps $<10^{-9}$, on the relative efficiency of gene transfer between these species, or similar situations. Undoubtedly, we have followed a common procedure in not bothering to publish such negative results in detail.

Gene transfer between B. subtilis and other B. spp. is greatly hindered, but nevertheless does occur (cf. Wilson and Young, 1972 (48), whose results concur with our own, R. Harris, unpubl.) The behavior of "hybrids" is consistent with the model of a requirement for regular duplex formation in local segments mentioned above (in contrast, for example, with a restriction and host-modification system as is reported for Hemophilus (Smith and Wilcox, 1970 (44)). Hotchkiss (33) has discussed the relative exclusion of some markers arising by mutation possibly involving heterogeneity at a single base pair. These findings help to delineate, rather than to solve, the problem of achieving molecular translocation involving arbitrary, alien sequences.

An overview of mutation in bacteria compared to eukaryotes also suggests a basic difference in the role of chromosome inversion and translocation. These processes dominate variety and species formation in eukaryotes; they occur only exceptionally in bacteria, and then perhaps mainly in relation to the integration and de-integration of episomes.

For this reason, in previous grant applications, I had postulated that translocation-mediating enzymes were a later evolution, connected with mechanisms of gene-regulation more complex than the sequential expression of markers in a linear (or circular, or simple multi-segmented) pattern - the concept associated with the operon model of gene regulation in prokaryotes. (One could also argue that eukaryote chromosomes have evolved multiple recognition sites, i.e., that this is one role of "redundant-sequence DNA" across which translocations could be sealed without invoking new enzymes. The report of trans-

locations between mouse and human chromosomes in somatic cell hybrids (Ruddle, 1972 (41)), tends to argue against such a role. For this reason, some of our previous efforts were directed at the examination of DNA-repair mechanisms of a eukaryote *in vivo*, that is in eggs of *Xenopus* injected with bacterial DNA. These studies, reported further in "4. Progress Report," have, however, been overtaken by Sgaramella's findings with T4-ligase.

This recent paper, which is appended, is the main foundation of our intended further work. Previous work had indicated terminal joining of two synthetic polynucleotides (Sgaramella et al, 1970 (43)), each of which had based-paired ends (a deoxynucleoside 5' phosphate paired with a complementary 3' hydroxyl), a condition of the ends that may be termed "flush". The present paper takes advantage of the known condition of phage P22 DNA as a flush clipped ensemble of segments which are terminally redundant, but produced as if by a random cut in a circular molecule. Therefore, almost every end is different, and the ease of terminal joining then argues against sequence-specificities for the ligase.

Furthermore, P22 did not join terminally to linearized SV-40 DNA, the latter having thus been shown to have not a clipped but a cohesive end, i.e., an overlapping simplex that would seek its complement on another strand. This SV-40 DNA (obtained by the action of a sequence-specific nuclease restriction enzyme on circular SV-40) could, however, be homo-oligomerized either with T4 or *E. coli* ligase, both enzymes being able to seal a contrived, doubly nicked duplex. The paper itself gives a more complete, and possibly clearer, account.

The problem of molecular translocation can then be reduced to that of securing flush ends on biologically interesting DNA. Alternatively, various restriction enzymes might have just the appropriate specificity to generate useful cohesive ends, or terminal polymerases may be used to add synthetic homo-polymer cohesive ends to existing DNA molecules, an approach also contemplated in the previously submitted applications in this series. Berg's group will be emphasizing the second and third of these approaches, and have, of course, already achieved an outstanding result with SV-40 and lambda; we will be concentrating on terminal joining.

In order to pursue the biological activity of the oligomers, we have developed a transfection system (see Progress Report) for P22 DNA. (In the light of unforeseeable hazards with derivatives of SV-40, we prefer not to pursue work with this as an animal virus in tissue culture). Although most promising, the transfection system needs further improvements (which we foresee should be possible) before we can efficiently test the oligomers for biological activity.

Summary: We tried various approaches to the chemical cross-linking molecules, the addition of synthetic cohesive ends, and alying the basis for identifying a terminal-looking enzyme in frog eggs. These false starts were superseded by the identification of terminal-joining activity in T4-ligase and its use in the formation of oligomers of P22 DNA (24).

We have also searched for deletion mutations in B. subtilis and were surprised to find that they are very rare, if they occur at all in our strain. Heterospecific transformations (B. subtilis x B. globigii) were studied and the model that sequence homology is required for efficient transformation was supported.

We have made a detailed analysis of the effects of chlorine on DNA, but eventually concluded that its effects on DNA breakage were incidental to effects on proteins, and that the latter was the principal site of cell damage. However, chlorine is a (feeble) mutagen, and has an enigmatic effect on the burst size as well as viability of treated phage (λ). It therefore deserves further study as a possible environmental pollutant of biological consequence.

A variety of other incidental findings are indicated in the list of published titles.

The most salient findings have already been outlined under "background" and in the Sgaramella paper (1972, (24)), which should be regarded as part of this application.

A range of other studies avowedly of lesser importance is detailed in the list of publications.

Work not yet published includes:

i. (R. Harris). A study of the specificity of a nuclease produced by B. globigii and not by B. subtilis. This nuclease attacks B. subtilis DNA more rapidly than that from B. globigii and may therefore resemble "restriction enzymes" in specificity. The DNA from different hybrids is being examined to look into the role and nature of a host-modification system. However, there is no evidence of such an enzyme with differential specificity in the competent strain of B. subtilis as might be hypothesized to account for the specificity of DNA in transformation.

ii. (I. Majerfeld). The cross-linking of DNA with various agents, of which glyoxal and glutaraldehyde appear to be the most promising. (The rationale was that chemically linked DNA might be copied with DNA polymerase with a mere skip across the link in the template. However, material isolated so far has not been well enough defined for a cogent

test of the concept). The work was interrupted by Mrs. Majerfeld's emigration to England with her husband prior to the completion of her Ph.D. research. She may, however, resume it at Sussex.

iii. (B. Brandt and J. Wachtel, in press). On the hypothesis that eukaryotes possessed an enzyme for molecular translocation, we tried to develop a system for studying the effect of frog egg enzymes, in vivo, on injected bacterial DNA. Following Gurdon, (31), DNA synthesis was demonstrable on the injected templates. However, it proved to be too difficult to recover workable amounts of "repaired" DNA from injected eggs, and attention was then directed to the enzymes in the extracts. These have been shown to contain a DNA-polymerase with variations in template specificity similar to those reported for the intact eggs by Gurdon. That is, *Xenopus laevis* DNA, undenatured (but treated with DNase I) was a preferred primer, compared to *E. coli* DNA or to poly d(A,T). *E. coli* polymerase I prefers denatured DNA from various sources. Larvae and immature ovaries yielded enzymatic activities with still different patterns of preference. However, on 50-fold purification, the egg extract enzyme was less discriminating, perhaps owing to the removal of a DNase-inhibitor. A ligase has also been demonstrated in these extracts, but has not yet been characterized, i.e. for terminal joining activity.

iv. (V. Sgaramella and H. Bursztyrn, manuscript in preparation). Transfection in P22. The more or less fortuitous amenability of P22 DNA to terminal joining by T4 ligase naturally led to an inquiry on the biological activity of the oligomers. Published work in transfection with P22 DNA has been remarkably discouraging, efficiencies about 10^{-9} having been reported with spheroplasted hosts (28). In order to attempt correlative transduction, we would prefer intact bacteria. Attempts to condition *Salmonella* by cold shock and $CaCl_2$ which has given spectacular results with *E. coli* (29) were unrewarding. However, supernates of shocked *E. coli* cells were found to condition an R strain of *S. typhimurium* LT2 to a low rate of transfective competence. (The supernate factor is, of course, under close study). Subsequently it was found that limited treatment of P22 DNA with exonuclease would further augment the efficiency of transfection to a level now in the range of 10^{-7} to 10^{-8} . We have not evidently exhausted the possibility of further improvement which would furnish another valuable tool. These rates are still too low to expect the transduction of bacterial markers to be observed - nor have we done so as yet.

Staffing: (1968 - 1972)

Joshua Lederberg, Principal Investigator, Professor of Genetics

Professors A.T. Ganesan and Walter Bodmer (until the latter left Stanford for Oxford in December 1970) also played important consultative roles in this work; however, their research is supported and reported independently.

Sergio Barlati, Research Associate 1966-1969

Orio Ciferri, Research Associate. (Professor of Genetics, University of Pavia, Italy. Dr. Ciferri played an active role in our research and was in the Department of Genetics at Stanford from July to December 1966, September to December 1971 and October to November 1972).

Vittorio Sgaramella, Research Associate 1971 - present

Alan Duffield, Sr. Research Associate 1970 - present

Silvano Riva, Research Associate 1969 - 1970

Katherine Shih, Postdoctoral trainee
NIH training grant 1969 - present

Bruce Brandt, Postdoctoral trainee
NIH training grant 1970 - 1972

2. Publications

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Majerfeld, I., Barlati, S. and Ciferri, O., 1970. Tryptophanless Death in Bacillus subtilis. J. Bact. 101, 350-354.

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- Harris-Warrick, R.M., Lederberg, J., 1977. Interspecies Transformation in Bacillus: Mechanism of Heterologous Intergenote Transformation. J. Bact. 133, 1246-1253.
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