3'-hydroxyl initiation points for new DNA synthesis (Wickner et al., 1972). One possible explanation of the lethality of polA12 recB21 strains is that in recB+ cells exonuclease V can also remove the RNA. In this connexion we have studied the ability of polA12 recB21 strains to join 'Okazaki pieces' at 41°C. We find that this double mutant is significantly slower than polA12 single mutants at joining these small DNA pieces. To explain the viability of polA12 recB21 sbcA and polA12 recB21 sbcB strains on this basis it is necessary only to propose that the ATP-independent deoxyribonuclease can also remove the RNA and that exonuclease I does some damage to the replicating machinery if joining of 'Okazaki pieces' is slowed down. The viability of polA12 recB21 double mutants is also consistent with this model in view of the recent finding by I. R. Lehman (personal communication) that the polA1 amber fragment has substantial amounts of 5'→3'-exonuclease activity when assayed in vitro.

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Possible Roles of the Enzymes of Recombination and Repair of Deoxyribonucleic Acid during Normal Growth

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Four enzyme activities are involved in the excision–repair of artificial lesions such as thymine dimers within DNA (for review, see Witkin, 1969). The first step is endonucleolytic attack at the site of a lesion, followed by excision of a number of bases, their subsequent replacement and final rejoining of the polynucleotide chains. These reactions are catalysed by endonuclease, exonuclease, DNA polymerase and polynucleotide ligase. The same four enzyme activities are involved in most models for recombination in both bacterial and eukaryotic organisms (Whitehouse, 1967).

There are, however, two important differences. The first concerns the recognition site of endonuclease. In the case of excision–repair, the artificial lesion itself is recognized by specific enzymes. Thus endonucleases specific for u.v.-irradiated DNA have been described (Nakayama et al., 1971; Carrier & Setlow, 1970). For recombination, initial endonuclease attack must occur within two DNA molecules at approximately the same place and time. It is highly unlikely that artificial lesions could direct this, and so some more normal recognition site must be envisaged. The second difference concerns the overall complexity of the recombination event. There seems no reason why a single-strand break or gap introduced into one chromosome cannot be efficiently repaired, thereby inhibiting recombination. Indeed, this probably occurs. Rather, for recombination to be successful, breakage–rejoining events must occupy a time sufficient to allow
complex strand exchange. It seems reasonable, then, to suggest a control feature, particularly control of the relative rates of excision and repair, to be essential for recombination without having any effect on excision–repair of artificial lesions. This allows considerable scope for mutations that are recombination-defective ({}_{rec^-}) while retaining full repair activity (for example, {}_{UV_{rec}}).

Consider, then, these four enzyme activities. All cells, both prokaryotic and eukaryotic, possess endonuclease, exonuclease, DNA polymerase and polynucleotide ligase during normal growth. Indeed, in many cases there are multiple forms of these enzymes. *Escherichia coli* has at least five exonucleases and three or four endonucleases (for review see Koerner, 1970). Three DNA polymerase activities have been detected in *E. coli*, two of which are not required for DNA replication (Kornberg & Gefter, 1971; Nusslein et al., 1971). Mammalian tissues possess at least four deoxyribonucleases (Lindahl et al., 1969).

Quite clearly these enzymes together are capable of repairing artificial lesions, but on the other hand it seems unlikely that cells accumulate sufficient lesions during normal growth to account for the multiplicity of enzyme forms. Because of this it is worth considering the possibility that these enzymes are intimately concerned with cell metabolism, and that our designation of them as enzymes of recombination or repair is a description of their secondary roles.

Previous studies of the mechanism of recombination as it occurs during transformation in *Bacillus subtilis* led to the suggestion that recipient cells, grown to the physiological state termed competence, display an aberrant degree of DNA turnover that results in the accumulation of single-strand gaps within the chromosomes of competent cells (Harris & Barr, 1971a,b). This has led to the development of the working hypothesis described in Scheme 1.

It is suggested that, during normal growth, single-strand breaks are continually introduced into chromosomes, causing unwinding and reannealing of the DNA. This sequence of events could cause the loss of a few bases, resulting in a requirement for limited 'repair-type' DNA synthesis (Scheme 1a). During competence, however, because of the growth procedure, it is suggested that there is an increase in exonuclease activities with resulting formation of large gaps (Scheme 1b). Moreover, a decrease in

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Scheme 1. **Possible role for 'repair-type' DNA synthesis within normal and competent cells**

Within both (a) normal and (b) competent cells local unwinding and reannealing of the DNA occurs, although in the latter case extensive repair is required for covalent rejoining of the polynucleotide strands.
the efficiency of repair would cause such gaps to exist for a finite time. A measurement of the activities of nuclease and polymerase within cells during growth to competence is described in Fig. 1. Competence is developed by a two-phase growth procedure (Harris & Barr, 1969). In the first growth phase cells are grown in a nutritionally rich medium to late exponential phase and then diluted tenfold into a nutritionally poorer medium. Maximum competence appears about 90 min later. Fig. 1 shows that both DNA polymerase and exonuclease activities increase in the second growth medium, and at the peak of competency.

Fig. 1. Activities of DNA polymerase and exonuclease within cells of *B. subtilis* during growth to competence.

Cells are grown for 4 h in the first growth phase and diluted tenfold into the second growth medium. Maximum competence appears at 90 min. Enzyme units: (a) DNA polymerase, specific activity = µg of dTMP incorporated into trichloroacetic acid-precipitable material/mg of protein; (b) exonuclease, specific activity = µg of native DNA (■) or denatured DNA (■) converted into trichloroacetic acid-soluble material/mg of protein.
of competence an imbalance in the activities of DNA polymerase and exonucleases results.

It is therefore tempting to suggest that events occurring during competence are precisely those that occur during normal growth, although the net effect is exaggerated because of an imbalance in rates of degradation and resynthesis. As such, investigation of the nature of single-strand gaps within competent cells serves as a model for intracellular events during normal growth. Previous studies indicated that the single-strand gap was an intermediate in the transformation event (Harris & Barr, 1969, 1971b). Any preferential ability of certain genes to undergo transformation is therefore an indication of preferential formation of a gap within that gene of the host chromosome. Erickson & Braun (1968) demonstrated a phased appearance of the ability of genes to be transformed, this phased appearance being in the same direction as DNA replication. Table 1 describes an example of phased appearance of single-strand gaps within the chromosomes of competent cells.

As an assay of those genes that possess single-strand gaps, the ability of such DNA to act as a donor in transformation studies was examined. Since only a single strand of donor DNA is integrated, but either strand may be integrated, any molecules of DNA that possess a single-strand gap will display a deficiency in biological activity for that gene compared with genes that retain both strand complements [for example in Scheme 1(b) the trp gene would be expected to be biologically deficient compared with leu and his]. In Table 1 cells of B. subtilis 168M (trpC2) were grown to competence. The ability of these cells themselves to be transformed to tryptophan-independence was tested and found to be maximal at 70 min. Within these cells therefore at 70 min there should be a large number of gaps within the trp gene and less at 100 min. Table 1 demonstrates that at 70 min the trp gene is highly deficient in biological activity, whereas the met gene achieves maximal biological deficiency at 90 min. The direction of replication of the B. subtilis chromosome is origin-trp-his-met-thy-terminus.

We have also found that the ability of genes to undergo transformation can be affected by phenotypic expression of the gene within the recipient cell. The addition of excess of leucine to cells growing to competence preferentially depresses the ability of the leu marker to undergo transformation.

Although other explanations are possible, particularly in view of the complex nature of the transformation process, the simple explanation for both the above observations lies in the concept that single-strand breaks and local unwinding occur in front of the replication fork, as well as within regions of the genome undergoing transcription. Innumerable models for DNA replication and gene transcription incorporate these events.

Table 1. Effect of time of competence on biological activity of DNA extracted from recipient cells

<table>
<thead>
<tr>
<th>168M DNA prepared from cells at</th>
<th>Trp+</th>
<th>His+</th>
<th>Met+</th>
<th>Thy+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stationary phase</td>
<td>1250</td>
<td>2530</td>
<td>1400</td>
<td>3420</td>
</tr>
<tr>
<td>70 min in second growth medium</td>
<td>460</td>
<td>1714</td>
<td>1125</td>
<td>1455</td>
</tr>
<tr>
<td>90 min in second growth medium</td>
<td>360</td>
<td>1300</td>
<td>660</td>
<td>980</td>
</tr>
<tr>
<td>100 min in second growth medium</td>
<td>660</td>
<td>1723</td>
<td>1230</td>
<td>1970</td>
</tr>
<tr>
<td>Competence of 168M cells:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transformants/ml at 70 min</td>
<td>180 × 10^3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transformants/ml at 90 min</td>
<td>90 × 10^3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transformants/ml at 100 min</td>
<td>20 × 10^2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Vol. 1
The sequence depicted by Scheme I(a) predicts that during normal growth two types of DNA synthesis are occurring simultaneously, 'replicative' semi-conservative DNA synthesis and non-conservative 'repair-type' synthesis. That both types of DNA synthesis are indeed occurring simultaneously was demonstrated by preparing cells of B. subtilis labelled with $^{15}$N and $^2$H$_2$O, growing subsequently in medium containing $^{14}$N and $^2$H$_2$O, and examining the density profiles of pulses of radioactive precursors within CsCl density gradients. It was found that 'repair-type' DNA synthesis does occur during normal growth, but can only be detected if thymine is used as precursor. A 10 min pulse of thymine is found within both 'replicative' and 'repair-type' DNA synthesis, whereas a 10 min pulse of thymidine is found almost exclusively in 'replicative' synthesis. Further, it has been found that caffeine selectively eliminates the 'repair-type' synthesis and 6-(p-hydroxyphenylazo)uracil the 'replicative' synthesis (Fraser et al., 1972).

Extensive studies by Brown (1971) have clearly demonstrated the specific inhibition of DNA replication by 6-(p-hydroxyphenylazo)uracil. We have obtained evidence that throughout the entire growth cycle there is a relatively constant rate of 'repair-type' synthesis, which increases slightly in stationary phase. At this time, also, there is a large increase in activity of an ATP-dependent exonuclease, suggesting a possible correlation between the amount of repair and nuclease activities.

At concentrations that specifically inhibit repair or replication it is found that caffeine is bacteriocidal whereas 6-(p-hydroxyphenylazo)uracil is bacteriostatic. We have found a similar tendency with nalidixic acid, a preferential repair inhibitor, and novobiocin, a preferential replication inhibitor. It would seem, then, that inhibition of replication is bacteriostatic whereas inhibition of repair is lethal. Indeed our studies indicate that the lethal event of thymineless death may well be inhibition of 'repair-type' synthesis (R. N. Buick & W. J. Harris, unpublished work).

In summary, then, we believe that we have evidence for the occurrence of 'repair-type' DNA synthesis during normal growth. This synthesis occurs throughout the growth cycle, preferentially within regions of the genome that have not recently been replicated, and inhibition of it is lethal to the cell. The possibility is raised that the amount and extent of this repair is controlled by the activities of deoxyribonucleases. Indeed, fluctuations in cellular nuclease activities could be an important control feature. It is suggested that these reactions arise in association with replication and transcription, and that it is these events that are the initial events for genetic recombination.

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Fraser, L., Mitchell, A. I. & Harris, W. J. (1972) Biochem. J. 129, 49–50