orientation; and Sanderson[7] has utilized a different methodology in reaching a conclusion in accord with ours.

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† Present address: Upsala College, East Orange, New Jersey.

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## *INFORMATION TRANSFER IN SALMONELLA TYPHIMURIUM**

BY KENNETH E. SANDERSON

BIOLOGY DEPARTMENT, BROOKHAVEN NATIONAL LABORATORY, UPTON, NEW YORK

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A considerable body of data suggests that information transfer, both transcription and translation, occurs in a polarized manner. In transcription this implies that information transfer begins at one end of the operon and proceeds to the other end. The polarity of operons may be determined using either operator[1] or polarity[2] mutants, which affect the transfer of genetic information in one direction from their site. Studies of these mutants in the histidine (*his*),[2] tryptophan (*try*),[3] and leucine (*leu*)[4] operons of *Salmonella typhimurium* have indicated the polarity of these operons with respect to other loci in the P22 transducing fragment in which each operon occurs, but the transduction methods used in these studies could not reveal the orientation of these transducing fragments with respect to the entire chromosome. Our investigations, which have used F-factor-mediated conjugation, have demonstrated that on the circular genetic map of *S. typhimurium*[5] the *his* and *try* operons

are polarized clockwise, and the *leu* operon counterclockwise. This indicates that the direction of information transfer is not the same in different regions of the chromosome of *S. typhimurium*.

*Materials and Methods.*—The minimal medium and supplements were as described in Demerec and Ohta.[6] Cells were mated in Difco Bacto Nutrient broth.

The donor strains HfrA (SR305) and SR315 were obtained from N. D. Zinder, while HfrB2 was isolated in this laboratory. The recipient strains *ara9 leu517 str-r* and *hisE35 metG319 purG302 str-r* were obtained from P. Margolin and P. E. Hartman, respectively; all other strains come from the stock collection of M. Demerec.

For conjugation analysis, overnight broth cultures of donor and recipient strains were diluted $^1/_{40}$ in broth and grown to mid-log phase on a shaker at 37°C. About $2 \times 10^8$ donor cells and $10^9$ recipient cells were mixed on a Millipore filter (25 mm diameter, 0.45 $\mu$ pore size), the liquid was drawn through the filter under vacuum, and the filter was placed on a plate of nutrient soft agar (0.75%) at 37°C for 5 min to permit the formation of effective contacts. The Millipore filter was then placed in 16 ml of broth at 37°C in a 250-ml Erlenmeyer flask, the cells were suspended in the broth, and mating was continued with gentle agitation.

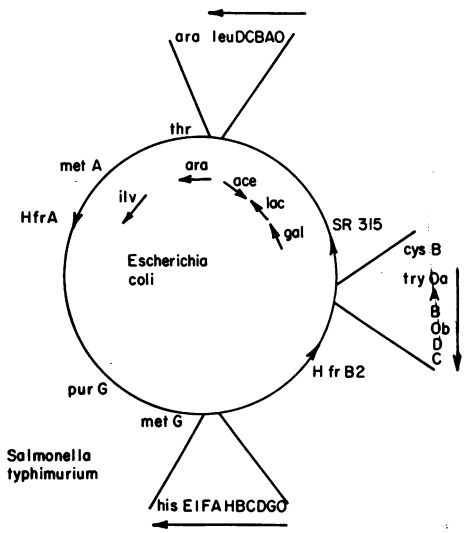


FIG. 1.—Genetic map of *Salmonella typhimurium* (outside the circle) and *Escherichia coli* (inside the circle) indicating the polarity of operons. Point of origin and direction of injection of *S. typhimurium* Hfr strains are shown.

For kinetic analysis, time zero was taken as the time at which the cells were drawn onto the Millipore filter. At various time intervals, samples of the mating mixture were diluted in broth, and the mated pairs were separated by shaking for 1 min with a vigorous shaking device. Samples were pipetted into 2.0 ml of soft minimal agar (0.75%), which was maintained at 45°C, and then poured onto minimal plates. Growth of the donor cells and plate mating were prevented by omitting from the minimal medium the supplements required by the auxotrophic donor strains, and by including streptomycin (1200 $\mu$g/ml) in the minimal medium to eliminate the streptomycin-sensitive donor cells.

*Results.—Tryptophan:* The two operators for the tryptophan loci, *tryOa* and *tryOb*, each regulate the two structural genes shown on their right: *cysB tryOa tryA tryB tryOb tryD tryC*.[3, 7, 8] The strain SR315 donated loci at the following times: *cysB*, 10 min; *his*, 22 min; and *purC*, 35 min, and the frequency of recombination with numerous other loci confirmed the point of origin and direction of entry shown in Figure 1. Among *cysB*$^+$ recombinants from an interrupted mating of *cysB12 tryA3 str-r* ($F^-$) × SR315 *leu256 str-s* there was a highly significant increase in *tryA*$^+$ with increasing mating time (Table 1) in three independent tests. Among *tryA*$^+$ recombinants, there was no significant increase of *cysB*$^+$ with

TABLE 1

FREQUENCY OF THE UNSELECTED DONOR MARKER AMONG RECOMBINANTS SELECTED FROM THE INTERRUPTED MATING OF *cysB12 tryA3 str-r* ($F^-$) × SR315 *leu256 str-r*

| Mating time (min) | % *Try*$^+$ among *cysB*$^+$ Recombinants — Test No. | | | % *CysB*$^+$ among *Try*$^+$ Recombinants — Test No. | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1 | 2 | 3 |
| 10–11 | 22 (46)* | 36 (66) | 18 (11) | 94 (35) | 100 (13) | |
| 12–13 | | | 58 (145) | | | 93 (59) |
| 14 | 59 (49) | | | | | 84 (70) |
| 16 | | 68 (153) | 77 (22) | | 97 (131) | 95 (21) |
| 20 | 87 (57) | 71 (38) | | 94 (89) | 93 (38) | |
| 25–34 | 80 (100) | | 79 (47) | 99 (78) | | 94 (70) |
| Probability‡ | <0.01 | <0.01 | <0.01 | n.s. | n.s. | n.s. |

* The number shown in parentheses is the total number of recombinants observed.
‡ This indicates the probability that the differences between means could be due to chance. The means of tests marked "n.s." are not significantly different.

increased mating time in any of the three tests. These data show that in crosses involving SR315, *cysB*$^+$ enters the zygote prior to *tryA*$^+$. Interruption of mating at early times may disrupt the chromosome between *cysB*$^+$ and *tryA*$^+$, so that only *cysB*$^+$ enters the zygote; after longer mating time *tryA*$^+$ enters the zygote prior to interruption. Therefore, the order of loci on the SR315 chromosome is O-*cysB tryA*, as suggested previously,[9] and the *try* operon must be polarized clockwise, as shown in Figure 1.

*Leucine:* The order of loci in the *leu* operon is *ara leuD, C, B, A, O*.[4] HfrA of *S. typhimurium* donates loci at the following times: *ilv*, 7 min; *thrA*, 26 min; *ara*, 29 min; *leu*, 29 min; *proA*, 36 min. Recombinants from an interrupted mating of *ara9 leu517 str-r* × HfrA *hisD23 str-s* were analyzed (Table 2). The results indicate that *ara*$^+$ is proximal to *leu*$^+$ on HfrA, for in two out of three tests there is a significant increase in *leu*$^+$ among *ara*$^+$ recombinants when the length of the mating time is increased prior to interruption. Among recombinants selected for *leu*$^+$, none of the tests gave significant increases in *ara*$^+$ with increased mating time. Therefore, the order of loci on the HfrA chromosome is O-*thrA ara leu pro*, in agreement with the order for *ara leu* determined in *Escherichia coli*.[10] The *leu* operon must be polarized counterclockwise (Fig. 1).

*Histidine:* The order of loci in the histidine operon has been shown by transduction analysis to be *hisO, G, D, C, B, H, A, F, I, E*.[2, 11] From the interrupted conjugation of *hisE35 metG319 purG302 str-r* × HfrB2 *metA22 str-s*, the following times of entry were determined: *hisE*, 15 min; *metG*, 17 min; *purG*, 30 min. This order was confirmed by examination of unselected markers among the recombinants as a function of mating time. Among *his*$^+$ recombinants, there was a highly sig-

TABLE 2

FREQUENCY OF THE UNSELECTED DONOR MARKER AMONG RECOMBINANTS SELECTED FROM THE INTERRUPTED MATING OF *ara9 leu517 str-r* ($F^-$) × HfrA *hisD23 str-s*

| Mating time (min) | % *Leu*$^+$ among *Ara*$^+$ Recombinants — Test No. | | | % *Ara*$^+$ among *Leu*$^+$ Recombinants — Test No. | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1 | 2 | 3 |
| 30–32 | 80 (10)* | 81 (52) | | 94 (16) | 87 (62) | |
| 35–36 | 85 (26) | 85 (104) | 77 (110) | 94 (32) | 90 (103) | 98 (124) |
| 50 | 96 (28) | 92 (39) | 84 (75) | 96 (25) | 97 (29) | 98 (62) |
| 60 | 100 (46) | 97 (95) | 85 (75) | 95 (64) | 94 (53) | 95 (198) |
| Probability‡ | <0.05 | <0.01 | n.s. | n.s. | n.s. | n.s. |

* The number shown in parentheses is the total number of recombinants observed.
‡ This indicates the probability that the differences between means could be due to chance. The means of tests marked "n.s." are not significantly different.

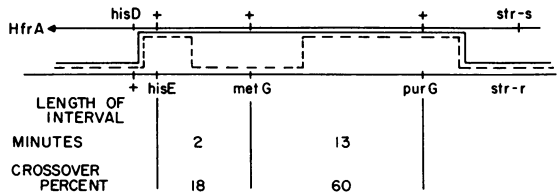


FIG. 2.—Cross *hisE35 metG319 purG302 str-r* ($F^-$) × HfrA *hisD23 str-s*, used to determine the order of the *his* operon. The order *hisE metG purG* had been determined by time of entry data. The excess of $his^+$ $met^+$ $pur^+$ recombinants (shown by solid line) over $his^+$ $met^-$ $pur^+$ recombinants (dotted line) indicated the order of the *his* loci to be as shown in the figure.

nificant increase in frequency of $metG^+$ from 28 to 38 per cent at early mating times to 65 per cent at later times, and $purG^+$ did not begin to appear among $hisE^+$ $metG^+$ recombinants until after 25 min of mating. Among $purG^+$ recombinants, the crossover per cent in the *hisE metG* interval (2 min in length) was 82/453 (18%), while in the *metG purG* interval (13 min in length) it was 273/453 (60%). HfrA *hisD23 str-s*, with the point of origin and direction of injection as shown in Figure 1, was crossed with *hisE35 metG319 purG302 str-r*, and $hisD^+$ $hisE^+$ $purG^+$ *str-r* recombinants were selected and tested for methionine requirement (Fig. 2). The results show the order of these loci to be *hisD hisE metG*, for 46 out of 55 $hisD^+$ $hisE^+$ recombinants (84%) were $metG^+$, indicating that the crossover from $hisD^+$ to $hisE^+$ in the zygote must be to the donor strand on the *metG* side. If the order were *hisE hisD metG*, the frequency of $metG^-$ among these recombinants should be greater than the frequency of $metG^+$. The orientation derived for the *his* loci agrees with the conclusions of two studies which used *col*-factor-mediated conjugation.[9, 12] If the orientation of the *his* loci with respect to *metG* is considered together with the order of the histidine operator and structural genes,[2] the *his* operon is polarized in a clockwise direction (Fig. 1).

*Discussion.*—The genetic data reported above on orientation of transducing fragments, when considered together with previously reported information on the polarity of operons, indicate that the *try* and *his* operons of *S. typhimurium* are polarized clockwise, the *leu* operon counterclockwise. Polarity is assumed to indicate the direction of transcription of genetic information from the DNA in the form of messenger RNA (mRNA). Thus the direction of information transfer is opposite in different operons.

It is generally assumed that the DNA-dependent RNA polymerase will transscribe in only one direction on a single strand of DNA. If that assumption is correct, transcription must be from one strand of the double-stranded, antiparallel DNA of the chromosome in some operons and from the other strand in others, and some mechanism must operate by which the RNA polymerase selects a particular strand to copy in any one operon.

Two hypotheses to explain bipolarity have been suggested:[8] the polarity of each operon may be determined independently from that of its neighbors; the chromosome may be made up of a number of units which are larger than an operon, within which all operons are polarized in the same direction.

The second alternative is supported by studies of transcription in bacteriophage which have shown that *in vivo*[13] and *in vitro* if intact DNA of $\phi$X174[14] or undenatured DNA of T4[15] is used as primer, the mRNA is copied from one of the two strands of DNA. Therefore, all the operons of the bacteriophage may be assumed to be polarized in one direction. The molecular weight of DNA of T4 is approximately five per cent of that of *S. typhimurium;* therefore, multi-operon units of

substantial size which are polarized in the same direction apparently exist in T4 and might be expected in *S. typhimurium*.

Four operons of *E. coli* are thought to be polarized counterclockwise: lactose (*lac*);[16] arabinose (*ara*);[17, 18] galactose (*gal*);[19, 20] and isoleucine-valine (*ilv*).[21] Based on the map locations for $O^c$ mutations, the loci for acetate mutants in *E. coli* are polarized clockwise.[22] Figure 1 shows that three operons, *try* and *his* of *S. typhimurium* and *ace* of *E. coli*, are polarized clockwise, while five operons, *leu* of *S. typhimurium*, *lac*, *ara*, *ilv*, and *gal* of *E. coli*, are polarized clockwise. There is some indication of grouping of operons polarized in the same direction, but far more operons must be studied.

Data from transformation using hybrid DNA molecules[23] suggest that the genetic information for enzymes of tryptophan biosynthesis in *Bacillus subtilis* is located on the opposite DNA strand from the information for histidine biosynthesis. Our data, which indicate that in *S. typhimurium* the *try* and *his* operons are polarized in the same direction, suggest that the genetic information of the two operons is on the same DNA strand in this organism. An inversion of the *try* or *his* region in one of these organisms would explain these observations.

*Summary.*—The orientation of three transducing fragments of *Salmonella typhimurium* which contain known operons was determined on the chromosome by F-factor-mediated conjugation. The tryptophan and histidine operons are polarized clockwise, for the structural genes are located clockwise from the operator gene, while the leucine operon is polarized counterclockwise. This indicates that the direction of information transfer is reversed in different operons.

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Abbreviations used: *ace*, acetate; *ara*, arabinose; *cys*, cysteine; *gal*, galactose; *his*, histidine; *ilv*, isoleucine-valine; *lac*, lactose; *leu*, leucine; *met*, methionine; *pur*, purine (adenine); *str-r*, streptomycin-resistant; *str-s*, streptomycin-sensitive; *thr*, threonine; *try*, tryptophan; *O*, operator; 0, point of origin of Hfr.

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## *EVIDENCE BEARING ON THE COINCIDENCE OF EXCHANGE AND DNA REPLICATION IN THE OÖCYTE OF DROSOPHILA MELANOGASTER**

BY RHODA F. GRELL AND ANN C. CHANDLEY†

BIOLOGY DIVISION, OAK RIDGE NATIONAL LABORATORY, OAK RIDGE, TENNESSEE

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The first investigation into the time of crossing-over in the Drosophila female was made by Plough.[1] From his results he estimated that crossing-over took place in "the very early oöcytes, just after the last oögonial division." Recent studies of the time of the last DNA replication in animal germ cells place this synthesis almost exclusively at the premeiotic interphase immediately preceding meiotic prophase.[2–4] These findings are consistent with the possibility that crossing over and DNA synthesis are coincident.

In the present experiment, the time relationship between crossing-over and DNA replication in the Drosophila oöcyte has been examined. The induction of an increase in crossing-over by elevated temperature has been used as a genetic "marker" for the time of crossing-over. Incorporation of tritiated thymidine has been used as a cytological "marker" for the time of DNA replication. The results show that, within the limits of resolution permitted by the method, the two processes are close or coincident in time.

*General Method.*—Heat treatments have been shown to increase the frequency of crossing-over in the proximal regions of chromosome two.[1] Once the interval between heat treatment and the deposition of affected eggs is determined, this time can be read on a cytological timetable (see below) to provide information about the stage of cytological development at which heat treatment produces its genetic effect. The assumption is made that the genetic effect of heat treatment results from alterations which occur at the time of normal crossing-over. Although no direct evidence exists for this assumption, it seems to be a reasonable one.

The cytological timetable is based upon detection of incorporated $H^3$-thymidine which provides a means for identifying those germ cells which were completing their *last* DNA replication at the time tritiated thymidine was available, and hence must have been in the oöcyte stage. Tritiated thymidine incorporated into oögonia is expected to be diluted by subsequent replication, and such oögonia will, in general, follow behind the front of heavily labeled oöcytes. The progression of the heavily labeled oöcytes through successive stages until maturity is expected to provide information about: (1) the time interval from the last DNA replication to maturity, and (2) the time interval between the last DNA replication and any maturation stage. In the present experiments labeled oöcytes in heat-treated and untreated females progressed through the ovarioles at the same rates so that data from both could be combined. A detailed description of the cytological studies is in preparation by Ann C. Chandley and will be published separately.