# Fine structure analysis of the gene for $p$-fluoro-phenylalanine resistance in Aspergillus nidulans 

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Summary. - The gene ( $p f p$ ) for the resistance to $p$-fluoro-phenylalanine can be selected in the mutant as well as in the wild state. Fifteen $p f p$ mutants have been localized in a single locus by means of meiotic analysis. A strong negative interference has been detected, partially affected by the intensity of the intragenic selection. The negative interference is still stronger in intragenic mitotic recombination. It has been suggested that intragenic mitotic recombination is almost exclusively effected by a process «localized» to the gene involved (gene conversion?), while this process is paralleled by classic crossing-over in intragenic meiotic recombination.

Riassunto. (Analisi della struttura fine del gene per la resistenza alla p-fluoro-fenilalanina in Aspergillus nidulans). - Mutanti di Aspergillus nidulans resistenti alla $p$-flyoro-fenilalanina possono essere facilmente selezionati su un terreno contenente questa sostanza. Retromutanti e ricombinanti sensibili possono essere selezionati su un terreno contenente acido N -fenil-antranilico e L-3-ammino-tirosina (Fig. 1). L'analisi di una serie di incroci tra diversi mutanti resistenti $(p f p)$ ha permesso di localizzare quindici alleli nel locus pfp, immediatamente prossimale al locus ribo-1 sul I cromosoma, e di determinarne parzialmente l'ordine. Lo studio di numerosi diploidi parasessuali ha confermato l'appartenenza di tutte le mutazioni studiate ad un unico locus (cistrone).

Lo studio della ricombinazione meiotica intracistronica (Tab. 2) rivela una forte interferenza negativa non polarizzata. Tuttavia, quando la distanza tra gli alleli pfp selezionati è più breve si nota un significativo incremento del crossing-over nella regione ribo-1 - pfp, distale al locus in studio.

Lo studio della segregazione mitotica rivela una ancor più intensa interferenza negativa (Fig. 3). I cromosomi ricombinanti con marcatori esterni in combinazioni "parentali" sono di regola in notevole eccesso su quelli con marcatori esterni ricombinati, così da rendere impossibile l'ordinamento

[^0]dei geni sulla sola base della segregazione mitotica. Si avanza l'ipotesi che la ricombinazione intragenica avvenga attraverso due meccanismi, un" "localizzato", senza effetti sui marcatori esterni (gene conversion?), uno "generalizzato", riferibile al crossing-over classico. Nella ricombinazione meiotica i due meccanismi sarebbero ambedue operanti, mentre la ricombinazione mitotica sarebbe quasi esclusivamente di tipo "localizzato".

## INTRODUCTION

Researches on back-mutation and intragenic recombination are usually carried out on mutants from which wild type derivates can be easily selected, typically the auxotrophic mutants of moulds or bacteria.

However, such mutants cannot be selectively isolated in wild type populations, and it is usually an arduous task to collect them in large numbers. The same difficulties are encountered in reisolating mutant phenotypes as secondary mutants from back-mutants as well as in selecting mutant recombinants from crosses between wild phenotypes or from wild type heterozygous diploids. There are but few cases of mutations that can be selected forward and backward. The streptomycin resistant dependent mutants of some bacteria (e. g. E. coli, Hashimoto, 1960) are the best known example. In Aspergillus nidulans, Apirion (1962) selected fluoro-acetate resistant mutants, some of which are unable to grow on media containing acetate as the only source of carkon. Wild type back-mutants or recombinants can be isolated on the latter media. Another possibility of "for- and back -» selection is provided by those resistance mutants showing "collateral sensitivity " (Szybalsky, 1956). These mutants, together with the resistance to one drug, have acquired an increased sensitivity to another drug. Whatever the mechanism, they ideally fit the above mentioned requirements, provided they are fully recessive. In this paper, we have dealt with mutants of Aspergillus nidulans resistant to parafluorophenylalanine (pfp) which show a collateral sensitivity to aminotyrosine and phenylanthranilic acid, besides being leaky tyrosine requiring (Morpurgo, 1961) and suppressors of a mutation for nicotinic acid requirement (Morpurgo, unpublished). Forward and back-mutations as well as sexual and parasexual intragenic recombinations between different pfp mutants have been studied, and the possible mechanisms of their origin discussed.

## MATERIALS AND METHODS

Strains. - The ascomycete mould, Aspergillus nidulans, was used. The nutritional and colour mutations employed throughout the work are among those the location and origin of which were described in a review
paper by Käfer (1958). The same symbols have been adopted. The relevant markers dealt with were on chromosome I, and their symbols and location are as follows (deduced from Käfer, 1958 ; Morpurgo, 1962 a) :
(ribo- $1=$ riboflavin requirement; an-l $=$ aneurin requirement ; $y-1=$ yellow colour of conidia). The numbers under the line indicate distances in meiotic units. The $p f p$ gene is located at the immediate left of ribo-l (Morpurgo, 1962 a) which, together with an-1, was used in this paper as "outside marker " in intragenic crosses.

Media. - The complete (CM) and minimal (MM) media described in previous papers (Fratello, Morpurgo \& Sermonti, 1960) were used. For selection of $p f p$ mutants or recombinants, $p$-fluoro-phenylalanine ( pFP ) was added to the MM agar to a final conc. of 0.02 mM . If the medium is slightly acidified $(\mathrm{pH}=6)$, the background growth of sensitive colonies is almost abolished. For selection of pFP-sensitive back-mutants or recombinants, N -phenyl-anthranilic-acid (PAA) was added to MM agar to a final conc. of $0.01 \%(\mathrm{w} / \mathrm{v})$, and L-3-amino-tyrosine (AT) to a final conc. of $0.047 \%$ $(\mathrm{w} / \mathrm{v})$. Optimal selective conditions were obtained at pH 4.5 (Morpurgo, 1966). PAA was first dissolved in ethanol $(2.5 \%, w / v)$ and then added to the melted agar. In the first experiments, PAA alone was added to the MM. Addition of AT and reduction of pH clearly improved the selective conditions.

Mutagens and recombinagens. - Most of the mutations and backmutations adopted in this work were induced by treatment with U.V. light. Nitrogen mustard $\left(\mathrm{HN}_{2}\right)$, formalin ( HCHO ) and X-rays were also used. The procedures are those currently used in this laboratory (Fratello, Morpurgo \& Sermonti, 1960).

Perithecia analysis. - Conidia of nutritionally complementary strains were streaked on a rectangular piece of complete agar, placed on a thick layer of minimal medium in another Petri dish. When the two strains were $p f p, \mathrm{pFP}$ was added to the media ( 0.02 mM ) to improve growth and perithecia development and to prevent the sectoring out of sensitive clones. After two-three days of incubation at $30^{\circ} \mathrm{C}$, the dishes were sealed with scotch tape and further incubated at the same temperature until perithecia appeared. Fertility unfortunately was not very high, particularly when pFP was omitted and temperature was of $37^{\circ} \mathrm{C}$, as in usual crosses. After addition of pFP and reduction of temperature to $30^{\circ} \mathrm{C}$, about $70 \%$ of crosses between different pfp strains gave ripe perithecia. The perithecia were iso-
lated in separate small test tubes, the ascospores expressed in 0.5 ml of sterile water and the perithecia classified as "selfed" or "crossed", according to the segregation of colour markers. "Crossed" perithecia were then analyzed either separately or in pool. The percentages of "crossed" perithecia were always very high, often approaching $100 \%$.

Diploid formation. - Heterozygous diploids were obtained with the usual procedures of the parasexual cycle (Pontecorvo, 1953). All of them were of the type (chromosome I) :


Carrying two allelic pfp mutations, all the diploids were resistant to pFP and sensitive to PAA and AT.

Isolation of intracistronic mitotic recombinants. - Mitotic recombinants in the $p f p$ region were isolated by plating several millions of conidia from diploid strains pfp-A/pfp-B, onto minimal media containing PAA, AT and riboflavin.

Analysis of diploid segregant genotypes. - The analysis of the genotype of the intracistronic mitotic recombinants was carried out by haploidization and by classifying the second order segregants for the outside markers and for colour. As a rule, from each diploid pFP-sensitive segregant, two haploid types were obtained (only chromosome I was considered), one resistant and the other sensitive. The resistant haploid type (resistant strand) was most easy to obtain by point-transferring the segregant under examination onto complete medium supplemented by $\mathrm{pFP}(0.02 \mathrm{mM})$ which acted as inducer of haploidization (Morpurgo, 1961) as well as selective agent for resistant sectors. The selection of the sensitive haploid type (sensitive strand) was obtained by spot-transferring, or plating, spores of the segregant onto HCHO-supplemented complete medium. The second order segregants were detected thanks to their colour (yellow or dark green) contrasting with the pale green of the heterozygotes, and then checked for pFP-resistance and tested for the ribo-1 and an-1 markers.

Six types of sensitive segregant genotypes are to be expected on the selective medium, if we consider the pfp gene and its outside markers (Table 1). Genotype 4 is phenotypically recognizable from all the others because of its riboflavin requirement. Genotypes 1,2 and 6 can be distinguished, among the wild phenotypes, between themselves and from genotypes 3 and 5 on the basis of the "resistant strand" $(p p)$.

Table 1.
Expected genotypes of diploid pFP-sensitive segregants (1-6)
after mitotic crossing-over in the «pfp» gene (region B)

| Phase of cross * | Cross-over in B (complementary recombinant strands) | Cross-over in B (one recombinant, one parental strand) | C. o. in $\mathrm{A}+\mathrm{B}$ (complementary recombinant strands **) | C. o. in $\mathrm{B}+\mathrm{C}$ (complementary recombinant strands **) |
| :---: | :---: | :---: | :---: | :---: |
| I | 1 | 3 | 5 | 6 |
| $\begin{aligned} & \frac{\mathbf{r} \quad+\quad+}{+} 0 \\ & \text { reg. : A B } \quad \text { B } \end{aligned}$ | $\frac{+\quad+}{+}+\begin{aligned} & + \\ & \mathbf{r} \mathbf{p} \end{aligned}$ | $\frac{t+++}{t+p a} 0$ | $\frac{\mathbf{r}+++}{+\mathbf{p} \mathbf{p}} 0$ | $\frac{+++\mathbf{a}}{\mathrm{r} p \mathrm{p}+} \mathrm{O}$ |
| II | 2 | 4 | 6 | 5 |
|  | $\frac{\mathbf{r}++\mathbf{a}}{+\mathbf{p} \mathbf{p}+} 0$ | $\frac{\mathbf{r}++\mathbf{a}}{\mathbf{r}+\mathbf{p}+} \mathrm{O}$ | $\frac{+++\mathbf{a}}{\mathbf{r} \mathbf{p} \mathbf{p}+}$ | $\frac{\mathbf{r}+++}{+\mathbf{p} \mathbf{p}} 0$ |

* Abbreviations : $p f p=\mathrm{p}$; ribo- $1=\mathrm{r}$; an-1 $=\mathrm{a}$.
** The pFP-resistant strand (pp) is phenotypically indistinguishable (in haploid segregants) from one of the parental strands ( $+\mathbf{p}$ or $\mathbf{p}+$ ).

Distinction between genotypes 3 and 5 requires the analysis of both strands. As a rule, the complete analysis was carried out also for the genotypic characterization of the other four phenotypes. The double cross-over classes (5 and 6) show a parental arrangement of outside markers in both strands. The double-mutant resistant strand cannot be distinguished from the resistant parental strand through the phenotype analysis of the haploid segregants. Crosses in phase I, after cross-overs in $A+B$, give the same genotype (5) as crosses in phase II after cross-overs in $B+C$, and viceversa (Table 1). Crosses in phase I, after triple cross-over ( $A+B+C$ ), give the same genotypes (2 and 4) as crosses in phase II after a single cross-over (B). Crosses in phase II, after triple cross-over, give the same genotypes ( 1 and 3 ) as crosses in phase I after a single cross-over (B).

## RESULTS

Forward mutations and phenotype of mutants. - The spontaneous mutation rate for pfp is below $10^{-6}$, as observed by Morpurgo (1962b; 1966). Several morphological types appear after mutagen treatment (Fig. 1), varying in sporulation and intensity of the brown pigment produced. Only the well sporulating type of resistants was considered. Its distinction from any other

Fig. 1. - Forward and backward selection for $p f p$ mutations and their wild allele.
(Left) $p$-fluoro-phenylalanine-resistant mutants arising after plating of about $10^{7}$ conidia of a yellow sensitive
strain of Aspergillus nidulans onto a drug-supplemented minimal medium. The well sporulated (white in the photo)
colonies are mutant in the locus $p f p$.
(Right) $p$-fluoro-phenylalanine-sensitive recombinants arising after plating of about $10^{6}$ ascospores, from a cross
of Aspergillus nidulans involving the alleles $p f p-31$ and $p f p-83$, onto a medium supplemented by N-phenyl-anthranilic
acid and L-3-amino-tyrosine. Note the free segregation of the conidium colour marker ( $y /$ ).
type is clearcut. The rate of mutation after U.V. treatment (survival, about $1 \%$ ) approaches $0.1 \%$ of survivors. After 2 minutes of nitrogen mustard treatment at $24^{\circ} \mathrm{C}$, the rate of mutation was $0.18 \times 10^{-4}$.

Most of the well sporulating mutants are partially tyrosine requiring (Morpurgo, 1962 a) and show an increased sensitivity to phenylanthranilic acid and aminotyrosine. However, some mutants do not require tyrosine and are but moderately sensitive to the above mentioned drugs. The latter were not used throughout this work.

Back-mutations. - The spontaneous rate of back-mutation from $p f p$ to wild is low for most of the U.V. induced mutants. The mutations adopted in this work do not show a back-mutation rate higher than $10^{-6} . p f p$ mutants induced by HN-2 or obtained without treatment are very stable.

Sixteen U.V. induced back-mutants were crossed with the wild type. No pfp recombinant was ever obtained from such crosses, which indicates that the tested back-mutations were not of the nature of suppressors.

Crosses between pairs of pfp mutants. - The result of some of these crosses are reported in Table 2. The recombinant ascospores were selected for resistance to phenylanthranilic acid and aminotyrosine, and the segregation of the outside markers was studied. Of the two non parental combinations of the outside markers $(r++a$ and ++++$)$, one is always in large excess on the other, thus clearly allowing to order the two mutations with respect to the outside markers, as follows :

$$
\begin{array}{ll}
\text { excess of } \mathrm{r}++\mathrm{a}, \quad & \text { excess of }++++ \text {, } \\
\text { genotype : } \frac{\mathrm{r}+\mathrm{PB}+}{+\mathrm{PA}+\mathrm{a}} & \text { genotype }: \frac{\mathrm{r} \mathrm{~PB}+}{++}+\mathrm{PA} \mathrm{a}
\end{array}
$$

The classes involving a double cross-over are much more frequent than expected by the product of the frequencies of the single events (in standard analysis) indicating a strong negative interference. This appears to be stronger when the pairs of mutations selected against within the $p f p$ gene are closer. The effect of negative interference is the apparent lengthening of both outside regions to approximately the same extent in each cross. This additional length goes from 6 units up to 20 units with the reduction of the intracistronic recombination fraction. The average difference between the enlarged lengths of the left and the right outside regions does not substantially differ from the difference between the standard length of the two regions, in the absence of negative interference (see Table 2).

The results reported in Table 2, together with other not reported observations, allow for a preliminary ordering of some mutations within the $p f p$ gene. The order is as follows :

[^1]Table 2.


[^2]Ann. Ist. Super. Sanità (1966) 2, 379-392.

The relative order within each group of mutations reported in brackets is still uncertain.

Intracistronic mitotic recombination. - All the heterozygous diploids synthesized between different pfp resistant mutants turned out to be resistant to pFP and sensitive to PA and AT, thus showing that all the isolated mutations occurred on one and the same gene. Not all the heterozygous diploids, however, were sensitive enough to be completely inhibited by PA and AT when plated at high density. From those giving practically no background growth on media supplemented by the latter antimetabolites, diploid mitotic segregants could be isolated by plating under such conditions scores of millions of diploid spores. The rate of appearance of ${ }_{\mathrm{p}}^{\mathrm{F}} \mathrm{F}$ sensitive segregants ranged within large limits, from $1 \cdot 10^{-4}$ to $2 \cdot 10^{-7}$, according to the diploid examined. Clonal effect cannot however be ruled out.

Two hundreds of diploid segregants have been classified according to their genotype. From most of them both members of the first chromosome pair have been detected and analyzed, while others have been classified only on the basis of the resistant strand, and considering only the six classes reported in Table 1 as possible. Some of the latter (figures in brackets in Table 3) could not be attributed with certainty to a given class and remained undecided between classes 3 and 5 sharing the $+p a$ strand. With negligible exceptions, all the tested genotypes fell within the six expected classes.

Contrary to what was observed among the meiotic recombinants, the most frequent segregant classes are usually those carrying on the recombinant first chromosome (the sensitive strand) the parental combinations of the outside markers ( $+a$ and $r+$ ), i. e. those which would have required a double cross-over. But in rare cases do the single cross-over classes appear to be the most frequent, and then in accordance with the assumed order of the pfp mutations involved, as deduced from the data of the meiotic analysis. While in the latter cases a classical cross-over may play the dominant part, obviously a different mechanism must be involved to account for the most common pattern of segregation.

In fact, the mitotic segregation of heteroallelic pFP-resistant diploids does not offer any criterium for the ordering of the pfp mutations with respect to the outside markers, since the segregants showing the non-parental associations of the outside markers are not regularly of the type expected on the basis of the order of the mutations involved. In fact, the order deduced from meiotic analysis being taken as granted, the overall rate of mitotic cross-over in the outside regions would amount to about fifty per cent on both sides of the pfp-r gene. This indicates that the reassortment of the outside markers is not directly related to the type of reassortment occurring within the pfp gene.
Tible 3.
Mitotic recombination in "pfp-x/pfp-y" diploids


## DISCUSSION

The kind of mutation adopted in the present work, allowing for the use of selective procedures in the detection of both wild and mutant types, provides a valuable instrument for the fine structure genetic analysis in moulds. A most favourable condition is met in the isolation of mitotic segregants with mutant phenotype from wild type diploids, since the selective agent is also effective as inducer of haploidization. As procedure was slightly modified in the course of the work, some estimates of recombination frequencies might be of scarce accuracy. Yet some conclusions can be drawn, and some general trends clearly observed.

A strong negative interference is observed in both meiotic and mitotic analyses, on both sides of $p f p$ gene, when intragenic recombinants are selected. At meiosis, this is generally stronger when the $p f p$ mutations involved in the cross are closer (Table 4). Yet, while the intensity of negative interference clearly decreases when the distance between the two $p f p$ alleles

Table 4.
Frequencies of extra cross-overs with increasing recombination fractions
Pooling of some data from Table 1.

| Range of recombination fractions$\left(\times 10^{-5}\right)$ |  | Percentage of recombinant classes (averages) |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Crossing-over |  |  |  | cross-over reg. A$(\%)$ | cross-over <br> reg. C <br> (\%) $\qquad$ |
|  |  | single, reg. B <br> (\%) | $\left\lvert\, \begin{gathered} \text { triple, regs. } \\ \mathrm{A}+\mathrm{B}+\mathrm{C} \\ (\%) \end{gathered}\right.$ | double, regs. $\mathrm{A}+\mathrm{B}$ (\%) | double, $\stackrel{\text { regs. }}{B+C}$ $\mathrm{B}+\mathrm{C}$ <br> (\%) |  |  |
| 0.2 . | 3 | 31 | 17 | 17 | 35 | 34 | 52 |
| 0.4-0.8 . . | 6 | 50 | 10 | 15 | 25 | 25 | 35 |
| 1.0-2.0 . | 9 | 55 | 3 | 14 | 28 | 17 | 31 |
| 3.0-4.4 . . | 4 | 65 | 7 | 5 | 23 | 12 | 30 |

increases, this trend is not so evident in the longer right side region pfp-an-1. A similar effect has been observed by Siddigi \& Putrament (1963) around the paba gene, on the right arm of chromosome I of Aspergillus nidulans. It is in the shortest (about 0.5 units) region at the left side of paba- 1 that the correlation between the interval of selection and the intensity of negative interference is more evident. Pritchard (1960) also observed a decrease in negative interference on the right and shortest (about 0.2 units) side with the increasing of the distance of the interval of selection in the ad-18 cistron on the right arm of chromosome $I$ in A. nidulans.

The correlation between interval of selection and negative interference may be due to the fact that the interested regions are very short, or have a special location or structure. Murray (1963) did not find any correlation of this type in Neurospora when studying the me-2 gene and adopting two not very closely linked outside markers.

We can assume that there is no structural or functional difference between the two outside regions around pfp (as well as around paba-1 and $\boldsymbol{a d}-18$ ), but that the closeness of one outside marker allows for the scoring on that side of a phenomenon not resolved on the other side. Negative interference could be more concentrated when the selected interval is shorter due to a variable length of the effective pairing region (Pritchard, 1955; 1960). If one outside marker is within the range of this variation, it would reveal it, which would not be observable by the use of a marker the distance of which from the selected region is outside this range. The negative interference would tend to be symmetric when its effect extends to a minute region not spanning either outside marker, and would appear asymmetric when it extends in such a way that one outside marker is encompassed, but not the other.

This interpretations is well compatible with the meiotic results of Siddigi \& Putrament (1963) as well as with ours, and would also explain the unsensitivity of the Murray system to the same effect.

The asymmetrical negative interference reported by Siddiqi \& Putrament (1963) could be fully accounted for by this interpretation. Our present results do not reveal any significant asymmetry in recombination. An obvious asymmetry in recombination occurs at the me-2 gene of Neurospora (Murray, 1963). On the basis of a different criterium, asymmetric recombination was first demonstrated for the ascomycete Ascobulus immersus (Lissouba \& Rizet, 1960). The fact that asymmetric recombination occurs or not does not necessarily involve an intrinsic difference in the structure of the genes studied or in their duplication mechanism, as they are very likely the same in any Ascomycete (and perhaps in any organism); the different responses obtained with different systems could be due to the different location of the analyzed region with respect to the longitudinal discontinuities of the chromosome, which might well occur at intervals much longer than the length of one gene (Piperno, Carere \& Sermonti, 1966).

We have used the expression "negative interference» only to mean increased frequencies of recombination in the outside regions, coinciding with recombination between alleles at one locus. This was assumed to be a statistical consequence of the restriction of the recombination phenomena to short regions of "effective pairing" (Pritchard, 1955; 1960). This interpretation is not however compatible with a situation in which the rate of cross-over in one or both regions outside the recombinant locus exceeds
$50 \%$, as observed by Murray (1963). In such a case, a "return» or "switch back» cross-over was presumed to occur in the "superextended» region, connected with the interallelic cross-over, so that the double cross-over appears to be more frequent than the single one. A transfer of information accomplished through a double exchange cannot be formally distinguished from directed mutation or "gene conversion" occurring in the absence of cross-over.

The results of somatic segregation are strongly in favour of an interpretation based on gene conversion (or some sort of a return cross-over), the double cross-over parentally marked classes being usually in large excess over the presumptive single cross-over class (Table 3). This was also observed by Putrament (1964) when studying the paba-1 cistron in $A$. nidulans.

If two processes (classical cross-over and gene conversion) occur alternatively when intragenic recombination is determined, we need only to assume that the former is relatively more frequent in meiosis than in mitosis. In other words, the inhibition of pairing between homologous chromosomes during mitosis would not reduce the rate of the gene-confined recombination so severely as it does the process of somatic cross-over.

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# Polarized recombination in Streptomyces coelicolor 

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Summary. - A group of eleven mutations requiring histidine (his) for growth were mapped in a minute region (region his) on the linkage group I of Streptomyces coelicolor. Whenever an intra-region crossing-over was selected, the frequent occurrence of an external supplementary crossing-over at either side of the region was observed. The latter was preferentially on the (conventional) left side of the his region when the selected primary crossing-over was in the left half of the region, and vice versa when in the right half. The site and the frequency of the supplementary crossing-over measured the sense and the intensity of the "polarity" of the intra-region recombination, which was roughly a function of the site of the primary cross-over. It was assumed that the sites of the supplementary cros-overs marked two structural discontinuities on the DNA ribbon, defining a structural sub-unit, comprising at least five his genes, and possibly corresponding to an "operon».

Riassunto. (Ricombinazione polarizzata in Streptomyces). - Undici mutazioni per la richiesta di istidina (his) (Tab. 1) sono state localizzate in una minuta regione (regione his) sul primo gruppo di linkage dello Streptomyces coelicolor A3 (2) (Tab. 2, Fig. 1). In incroci tra diversi mutanti his si osserva di regola che, in coincidenza con il crossing-over selezionato entro la regione his, si verifica spesso un secondo crossing-over immediatamente esterno all'una o all'altra estremità della regione. Ciò risulta da una alta frequenza delle classi his ${ }^{+}$che presentano i marcatori esterni nelle combinazioni originali (Tab. 3). Nei ricombinati his ${ }^{+}$in cui è stato selezionato un crossingover nella parte sinistra della regione his, si constata con alta frequenza la presenza di un crossing-over supplementare esterno sulla sinistra della regione, mentre il reciproco si verifica nei ricombinanti $\mathrm{his}^{+}$che hanno comportato un crossing-over primario sulla parte destra della regione. La localizzazione (sinistra o destra) e la frequenza del crossing-over supplementare qualificano e misurano la "polarità " della ricombinazione primaria. Questa polarità è alta e (convenzionalmente) positiva nella zona sinistra della regione his,
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irregolare nella zona centrale e alta e negativa nella zona destra della regione (Fig. 1). Questo decorso è bruscamente interrotto se si oltrepassano le due estremità della regione, come risulta da incroci che importano la ricombinazione primaria tra paia di mutazioni a cavallo di una o dell'altra estremità (Tab. 4). I dati sono interpretati come indicativi della presenza di discontinuità lungo il nastro del DNA in corrispondenza con i luoghi del cros-sing-over supplementare. Due di tali discontinuità definirebbero la regione his comprendente almeno cinque geni. Si avanza l'ipotesi che il DNA (come già suggerito da Freese, 1958, Taylor, 1958, Kellenberger, 1960 e altri) sia suddiviso longitudinalmente in subunità strutturali. Ognuno di tali subunità potrebbe corrispondere ad una unità funzionale (un "operon»), e quindi ad una molecola di RNA messaggero (Ames \& Hartman, 1963).

Dedica. - A. C. e G. S. vogliono dedicare questo lavoro alla indimenticabile memoria di Rosella Piperno, che a questa ricerca ha dato tutta la sua passione e tutta la sua energia, sino agli ultimi giorni della sua giovanissima vita.

Genes with related functions show a tendency to occur in clusters in some Eubacteria and in the Actinomyces Streptomyces coelicolor (Hopwood, 1965). Such clusters of related genes have a special significance for the regulation of biosynthesis of related enzymes (Ames \& Hartman, 1963), and provide a tool for the structural analysis of short chromosome regions, comprising contiguous genes. In $S$. coelicolor a group of adjacent genes involved in histidine biosynthesis (his) has been localized to the immediate (conventional) right of the met-2 marker, ten units left of the arg-1 marker, on linkage group I (Hopwood \& Sermonti, 1962; Fratello, Khoudokormoff \& Russi-Polito, 1964 ; Hopwood, 1965 ; Carere, Khoudokormoff \& Piperno, 1965). In the course of analysis of the his region unexpected segregation patterns of the outside markers were repeatedly observed, indicating the occurrence of a structural discontinuity first on one side, then on both sides of the analysed region. The present paper deals with the formal aspect of the genetic analysis of the his region. The biochemical aspect of the research, already presented in a preliminary note (Fratello, Khoudokormoff \& Russi-Polito, 1964), will be the subject of separate papers.

## MATERIALS AND METHODS

General procedures, media, analytical methods, as well as marker symbols, are those already reported in a review paper (Hopwood \& Sermonti, 1962). All the mutant strains adopted trace back to Streptomyces coelicolor strain A 3 (2), also described in the quoted paper.

The symbols of the histidine-less (his) mutations adopted in the present work, their mode of origin and the phenotypes of the mutants are listed in Table 1. In the last column of Table 1 the abbreviated symbols of the adopted strains, carrying the various his mutations, are also listed. Markers not relevant to analysis have been omitted. Three marker genes have been

Table 1.
Some characteristics of histidine-less mutants of «Streptomyces coelicolor».

| Mutations * | Gene and complementation group | $\begin{gathered} \text { Induced } \\ \text { by } \end{gathered}$ | Growth on L-histidinol | Accumu- <br> lations ** | Available combinations with outside markers *** |
| :---: | :---: | :---: | :---: | :---: | :---: |
| his 1..... | A | X-rays | - | 3 | $h, m h, l h . h a, l h a$, mha |
| his 2. . . . . . | B | u. v. | $+$ | 2 | $h, m h$ |
| his 9. . . . . . . | Ca | u. v. | $+$ | 1 | $h, l h, h a$ |
| his 10. | Ca | u. v. | $+$ | 1 | $h, h a$ |
| his 11. . . . . . | B | u. v. | $+$ | 2 | $h, l h, h a$ |
| his 14. | Cb | u. v. | + | 1 | $h, h a$ |
| his 116 . . . . . . . | F | u. v. | + | 1 | $h, l h, h a$ |
| his 120 . . . . . . | A | ${ }_{32} \mathrm{P}$ | - | 3 | $m h$ |
| his 123. | F | u. v. | $+$ | 1 | $h, l h, h a, m h$ |
| his 127. | Ca | u. v. | $+$ | 1 | h, lmh, ha |
| his 128. | Ca | u. v. | + | 1 | $h, m h, l h . ~ h a ~$ |
| his 129 . . . | I | u. v. | + | 1 | $h, h a$ |
| his 132 . . . . . . | A | unknown | - | 3 | $m h$ |

* Those with code number inferior to 100 have been obtained by D. A. Hopwood.
** $1=5$-amino-1-ribosyl-4-aminoimidazole-carboxamide; $2=$ imidazole glycerol; $3=$ histidinol.
*** Abbreviated symbols. Markers other than met-2 (m), his (h), arg-1 (a) and leu-1 (l) omitted.
adopted: met-2 (requirement of methionine), leu-1 (requirement of leucine) and arg-1 (requirement of arginine), whose shortened symbols are respectively $m, l$ and $a$. As shortened symbol for his, $h$ is used. The location of the marker genes in respect to the his-1 mutation is as follows (Hopwood \& Sermonti, 1962; Hopwood, 1965) :

| leu-1 | met-2 | his-1 | arg-1 |
| :--- | :--- | :--- | :--- | :--- |
|  | (10) | 3 | 11 |

The numbers under the line indicate the distances in map units.
The distance leu-1 - met-2 has not yet been determined with accuracy.

Preparation of strains. - To have the same his mutation in different combinations with the outside markers, a strain carrying a given his mutation in coupling with one marker was crossed with a his ${ }^{+}$strain bearing another marker. This procedure caused unexpected difficulties since, in at least two established cases, the isolated strain showing the histidine requirement coupled with a new marker turned out to bear an altogether new his mutation. This was possibly due to the selection of a his spontaneous mutation, either present in the his ${ }^{+}$strain, or occurring in the course of the cross. After this discovery, a very careful check was made on the identity of the presumed corresponding his mutations borne by different strains, and some strains had to be discarded. This check was subsequently considered as a fundamental precaution whenever new associations of markers were to be adopted.

Calcultation of distances of his mutations from met-2 : Method 1. Ratio : met- 2 - his- $X$ recombinants to heteroclones (Carere, Khoudokormoff \& Piperno, 1965; Hopwood, 1965). This ratio was calculated in crosses of the type:

$$
\text { met }-2+\times+ \text { his }-X
$$

after plating the spores of the mixed suspension on a medium lacking both methionine and histidine. To eliminate one possible source of variability the same met- strain ( 158 met-2 arg-1 phe-1 str-1) was used in all crosses. This method was based on the assumption that, while the frequency of the true haploid recombinants ( $\mathrm{met}^{+} \mathrm{his}^{+}$) would be a simple function of the distance met- 2 - his- $X$, the frequency of the heteroclones (heterozygous: met $^{-}$his $^{+} /$met $^{+}$his $^{-}$) should be substantially independent of it . The latter was thus used as a reference value. The same criterion was adopted by Hartman, Hartman \& Serman (1960), when using the ratio: true transductants to abortive transductants, for the evaluation of minute distances in Salmonella.

Method 2. Ratio: met-2 - his- $X$ recombinants to met- 2 - arg-1 recombinants. This ratio was calculated in crosses of the type:

$$
\text { met }-2++\times+ \text { his- } X \text { arg-1, }
$$

by plating spores of the mixed cultures on a medium containing histidine alone, the met ${ }^{+}$arg $^{+}$recombinants being classified as $\mathrm{his}^{-}$or his $^{+}$. The ratio of the latter class ( met $^{+}$his $^{+}$arg $^{+}$) to the total number ( his $^{-}+$his $^{+}$) should be a simple function of the ratio of the recombination frequencies between met- 2 and his- $X$, and met-2 and arg-1.

Again, to eliminate a possible cause of variability the same met strain (121 met-2 ura-1 str-1) was used in all crosses. Being the met-2 - arg-1 distance known, and equal to 14 map units (Hopwood \& Sermonti, 1962), the relative met-2 - his- $X$ recombination frequencies could be converted into map units.

Ordering of pairs of his mutations in respect to the outside markers. This has been established in crosses of the type:

$$
\begin{array}{ccc}
\text { met-2 } & \text { (his-X) } & + \\
\hline+ & \text { (his-Y) } & \text { arg-1 }
\end{array}
$$

The spores of the mixed cultures were plated on a medium lacking histidine, and the resulting his $^{+}$colonies classified for the outside markers. An excess of met ${ }^{+}$his $^{+}$arg $^{+}$over met ${ }^{-}$his $^{+}$arg $^{-}$segregants was considered indicative of the following arrangement of the mutations (abbreviated symbols) designed ad phase $I$ :

while an excess of the class met his ${ }^{+}$arg was indicative of phase II:

$$
\begin{array}{lcc}
m & + & h-X \\
\hline+h-W & + & a
\end{array}
$$

When possible, the order of a pair of his markers deduced from one cross, was checked by crossing strains (properly checked, see earlier paragraph) with the reversed coupling of the markers. The opposite phase was always obtained, i.e.
cross 1: $\frac{m h-X++}{+\quad+h-Y a}\left(\right.$ phase I) $;$ cross $2: \frac{m+h-Y+}{+h-X+a}$ (phase II).

RESULTS
met $^{+}$his ${ }^{-} \times$met $^{-}$his $^{+}$crosses
Mapping of his mutations according to their distance from met-2. The data obtained using the procedures outlined under MATERIALS AND METHODS are listed in Table 2. The two procedures gave compatible results as far as the order of the mutations was concerned. However, the distances estimated according to one method were not equal to the distances estimated according to the other. More data are required to attempt and interpretation of this discrepancy. The most obvious difference between the two estimates concerns the location of his-11, the his mutation closest to met-2. According to Method 1, his-11 appears very close to met-2 (their estimated distance is about 3 per cent of the distance between met- 2 and the most distal his mutation, his-123), according to Method 2 his-11 appears farther from met-2, at about 2 map units ( 39 per cent of met-2 - his-123). It is possible that the met ${ }^{+}$arg $^{+}$selection (required by Method 2) favours the likelihood of exchanges in the his region.

Table 2.
Calculation of distances between «met-2» and the «his» mutations.

| Method 1. |  |  |  |  | Method 2. |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Type of cross * : <br> ratio : | met + his $-\times$ met - his +met + his $+\quad$ recombinantsheteroclones |  |  |  | $\begin{aligned} & \text { Type of } \\ & \text { cross ** }: \text { met }+ \text { his }- \text { arg }-\times \text { met }- \text { his }+ \text { arg }+ \\ & \text { ratio }: \frac{\text { met }+ \text { his }+ \text { recombinants } \times 100}{\text { met }+ \text { arg }+ \text { recombinants }} \end{aligned}$ |  |  |  |  |
| his allele | Code of cross | Recombinants ( $\mathrm{N}^{\circ}$ ) | Heteroclones ( $\mathrm{N}^{\circ}$ ) | Ratio | Code of cross | $\begin{array}{\|c} \text { his }^{+} \\ \text {re- } \\ \text { combin- } \\ \text { ants } \\ \left(\mathrm{N}^{\circ}\right) \end{array}$ | $\begin{gathered} \text { Total } \\ \text { re- } \\ \text { combin- } \\ \text { ants } \\ \left(\mathbf{N}^{0}\right) \end{gathered}$ | Ratio | Map units o met-2) |
| his 11. | HZ 5 | 118 | 260 | 0.45 | HC 164 | 41 | 324 | 12.6 | 1.8 |
| his 2 | HZ 2 | 162 | 233 | 0.69 |  |  |  |  |  |
| his 129 | HZ 11 | 121 | 50 | 2.42 | HS 10 | 99 | 559 | 17.7 | 2.5 |
| his 1 | HC 32 | 455 | 129 | 3.52 | HP 56 | 34 | 190 | 17.8 | 2.5 |
| his 128 | HZ 10 | 52 | 10 | 5.20 | HP 77 | 33 | 142 | 23.2 | 3.3 |
| his 127 | HP 14 | 94 | 18 | 5.22 | HP 58 | 76 | 314 | 24.2 | 3.4 |
| his 9 | HC 57 | 423 | 52 | 8.13 |  |  |  |  |  |
| his 10 | HP 30 | 450 | 54 | 8.33 | HC 167 | 41 | 162 | 25.3 | 3.6 |
| his 14 | HC 199 | 115 | 13 | 8.84 | HS 27 | 132 | 510 | 25.8 | 3.6 |
| his 116 | HC 55 | 452 | 43 | 10.51 | HP 81 | 89 | 283 | 31.4 | 4.5 |
| his 123. | HC 56 | 131 | 9 | 14.55 | HP 82 | 62 | 188 | 32.9 | 4.6 |
|  |  |  |  |  |  |  |  |  |  |

* The met-his+ strain was always the same strain 158 met-2 arg-1 ura-1 phe-1. Selective medium : minimal medium plus arginine, uracil, phenylalanine.
** The met - his + arg + strain was always the same strain 121 met-2 ura-1 str-1. Selective medium : minimal medium plus histidine and uracil.
- Taken the distance met-2-arg-1 as equal to 14 (Hopwood \& Sermonti, 1962).

The order of the genes estimated according to either Method is such that non complementing mutations are always contiguous to each other. This aspect of the research will be discussed elsewhere.

$$
\text { his }-\mathrm{Y}^{+} \text {his- } \mathrm{X}^{-} \times \text {his- } \mathrm{Y}^{-} \text {his- } \mathrm{X}^{+} \text {crosses }
$$

Classes of his ${ }^{+}$recombinants with marker genes recombined: ordering of his mutations. Many pairs of his mutants have been "crossed » and plated on media lacking histidine, and the resulting his $^{+}$recombinang colonies classified for the outside markers. Usually met-2 ( $m$ ) and arg-1 (a) have been employed, either in phase I or phase II and, when possible, in both phases.

Sometimes leu-1 ( $l$ ) has been used instead of met-2. Among the four possible combinations of outside markers ( $m^{+} a^{+}, m^{-} a^{-}, m^{+} a^{-} m^{-} a^{+}$), only those carrying the marker genes recombined permit a determination of the order of the two his markers involved in the cross relative to the marker genes themselves (see MATERIALS AND METHODS). On the basis of the relative frequencies of these classes (Table 3) thirteen his mutations have been ordered. The ordering as deduced from crosses of opposite phase was always consistent (for some apparent exceptions see Preparation of strains in the section MATERIALS AND METHODS). All the crosses examined gave an unequivocal order for the genes examined, which was in turn perfectly consistant with the order deduced by the methods based on the calculation of the distances of the various his mutations from met-2 (Table 2). From data of Tables 2 and 3 the his mutations can be arranged in the following order :
met-2, his-11, -2, -129, (-132, -120), -1, -128, -127, -9, -10, -14, -116, -123, arg-1

The relative order of his-132 and his-120 cannot yet be established on the basis of the reported data.

Classes of his ${ }^{+}$recombinants with marker genes in parental combinations: "polarity" of the crosses. Whenever a his ${ }^{+}$recombinant carries the outside markers in a parental combination, an additional exchange has to be assumed on one side of the primary exchange in the his region. The total frequency of the parentally marked classes, which should not exceed $14 \%$ (distance between met-2 and arg-1), is in most crosses higher than $30 \%$ and often exceeds $50 \%$ reaching peaks of more than $90 \%$. This large excess of the parentally marked classes is usually accompanied by a high rate of the triple cross-over class. A remarkable feature of the high incidence of the additional exchanges is their polarity. Frequently one parentally marked class greatly exceeds the complementary one, thus indicating a tendency of the additional exchange to be predominantly on one side of the his region, a phenomenon which was designated as "polarized recombination" (see Murray, 1960), although it should be made clear that the polarity does not concern the primary exchange between the selected alleles, but a second exchange coincident with the former.

For the sake of description the his region has been divided into two subregions of the same length, one ( $\alpha$ ) comprising mutations his-11, -2; -129; $-132,-120,-1$ (three cistrons), the other ( $\beta$ ) comprising mutations his -128 , $-127,-9,-10 ;-14$ (two cistrons). Mutations his-116 and his-123, owing to their extreme position with respect to the others, as well as for their special behaviour in crosses [see "Crosses of the type his- $X$ arg-1 $\times$ leu- 1 his-(116 or 123] have been considered separately. From data in Table 3 it may be argued that in crosses concerning his alleles in the sub-region $\alpha$ the extra
Segregation of outside markers in crosses involving pairs of «his» mutations.


| Code of cross | Genotypes of parents * | hisgenes andcistrons | Phase <br> of <br> cross | Classification of his prototrophs according <br> to exchanges <br> (marker genes recombined) $\mid$ (parentally marked classes) |  |  |  | Percent his prototrophs |  | Polarity (difference: \% c. o. in A minus $\%$ c. o. in C) |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |  |  | with <br> c. o. <br> in A <br> (\%) | with <br> c. o. <br> in C <br> (\%) |  |  |
|  |  |  |  | Single <br> c. $o$. in $\qquad$ | Triple c. 0 . in $\mathrm{A}+\mathrm{B}+\mathbf{C}$ | Double c. o. in $A+B$ | Double c. $o$. in $\mathrm{B}+\mathrm{C}$ |  |  |  |  |
| Sub-region $\alpha$ |  |  |  |  |  |  |  |  |  |  |  |
| HC 46 | h11 $a \times m$ h2 | $B \times B$ | II | $\begin{array}{llll}m & a & 120\end{array}$ | $++18$ | + a 51 | $m+6$ | 35 | 12 | + | 23 |
| HC 158 | $l \mathrm{h11} \times \mathrm{hl29a}$ | $\mathrm{B} \times \mathrm{I}$ | I | $++16$ | $l \begin{array}{lll}l & a & 0\end{array}$ | $l+144$ | - a 7 | 86 | 4 | $+$ |  |
| HG 14A | $\mathrm{h} 11 \mathrm{a} \times \mathrm{m} \mathrm{hl}$ | $B \times \mathrm{A}$ | II | $\begin{array}{llll}m & a & 20\end{array}$ | $++1$ | +a 61 | $m+10$ | 67 | 12 | + | 55 |
| HF | h11 $a \times m \mathrm{hl}$ | $B \times \mathrm{A}$ | II | $\begin{array}{llll}m & a & 63\end{array}$ | $++27$ | + a 122 | $m+18$ | 64 | 19 | + | 45 |
| HG 2 | h11 $a \times m \mathrm{hl}$ | $\mathrm{B} \times \mathrm{A}$ | II | $\begin{array}{llll}m & a & 11\end{array}$ | $++11$ | + a 253 | $\boldsymbol{m}+0$ | 96 | 4 | $+$ | 92 |
| HG 25A | $\mathrm{h} 11 \mathrm{a} \times \mathrm{m} \mathrm{hl}$ | $\mathrm{B} \times \mathrm{A}$ | II | $\begin{array}{llll}m & a & 43\end{array}$ | t.t 26 | + $a 61$ | $m+8$ | 63 | 24 | + | 39 |
| HG 25B | $m$ hll $\times$ hla | $B \times \mathrm{A}$ | I | $++89$ | $m \begin{array}{llll}m & a & 5\end{array}$ | $m+181$ | + a 26 | 67 | 25 | + | 42 |
| HP 6 | $m \mathrm{~h} 2 \times \mathrm{h} 129 \mathrm{a}$ | $\mathbf{B} \times \mathrm{I}$ | I | $t+138$ | $\begin{array}{llll}m & a & 1\end{array}$ | $m+6$ | + a 5 | 5 | 4 | - | 1 |
| HS 61 | $m \mathrm{~h} 2 \times \mathrm{h} 129 \mathrm{a}$ | $\mathrm{B} \times \mathrm{I}$ | I | $++50$ | $\begin{array}{llll}m & a & 9\end{array}$ | $m+13$ | + a 12 | 15 | 14 | $+$ | 1 |
| HC 132 | $m \mathrm{~h} 2 \times \mathrm{h} 129 \mathrm{a}$ | $B \times I$ | I | $++93$ | $\begin{array}{llll}m & a & 4\end{array}$ | $m+12$ | + a 10 | 13 | 12 | + | 1 |
| HW 19 | $m \mathrm{~h} 2 \times \mathrm{h} 1 \mathrm{a}$ | $B \times \mathrm{A}$ | I | $++142$ | $\begin{array}{llll}m & a & 5\end{array}$ | $m+11$ | + a 10 | 10 | 9 | + | 1 |
| HC 135 | $m \mathrm{~h} 2 \times \mathrm{h} 1 \mathrm{a}$ | $\mathbf{B} \times \mathbf{A}$ | I | $\pm+52$ | $\begin{array}{llll}m & a & 5\end{array}$ | $m+23$ | + a 13 | 30 | 19 | + | 11 |
| HC 4/5 | $\mathrm{h} 2 \mathrm{a} \times \mathrm{m}$ h1 | $\mathrm{B} \times \mathrm{A}$ | II | $\begin{array}{llll}m & a & 63\end{array}$ | $++18$ | $+a 245$ | $m+8$ | 81 | 8 | + | 73 |
| HP 7 | $\mathrm{h} 129 \mathrm{a} \times \mathrm{m}$ h120 | $\mathrm{I} \times \mathrm{A}$ | II | $\begin{array}{llll}\boldsymbol{m} & \boldsymbol{a} & 39\end{array}$ | $++0$ | + a 231 | $\boldsymbol{m}+0$ | 86 | 0 | $+$ | 86 |
| HW 29 | h129 $a \times m \mathrm{hl}$ | $\mathrm{I} \times \mathrm{A}$ | II | $\begin{array}{llll}m & a & 99\end{array}$ | $++4$ | + a 48 | $\boldsymbol{m}+0$ | 34 | 3 | + | 31 |
| HW 13 | h129 a $\times l \mathrm{hl}$ | $\mathrm{I} \times \mathrm{A}$ | II | $\begin{array}{llll}l & a & 10\end{array}$ | $++4$ | + a 130 | $l+4$ | 91 | 5 | + | 86 |
| HC 43 | h129 a $\times$ m h132 | $\mathrm{I} \times \mathrm{A}$ | II | $m \begin{array}{llll}m & a & 231\end{array}$ | $++1$ | + a 57 | $\boldsymbol{m}+0$ | 20 | 0 | + | 20 |
| HC 44 | h129 a $\times$ m h132 | $\mathbf{I} \times \mathrm{A}$ | II | $\begin{array}{llll}m & a & 72\end{array}$ | $++0$ | + a 53 | $m+0$ | 32 | 0 | + | 32 |
| HC 34 | h129 a $\times$ m h132 | $\mathrm{I} \times \mathrm{A}$ | II | $\begin{array}{llll}m & a & 47\end{array}$ | $++0$ | + a 250 | $\boldsymbol{m}+0$ | 84 | 0 | + | 84 |
| HW 15 | $m \mathrm{~h} 120 \times \mathrm{hla}$ | $\mathrm{A} \times \mathrm{A}$ | I | $++100$ | $\begin{array}{llll}m & a & 36\end{array}$ | $m+36$ | + a 21 | 37 | 29 | + | 8 |
| HG 00 | $m \mathrm{~h} 120 \times \mathrm{hla}$ | $\mathrm{A} \times \mathrm{A}$ | I | $++13$ | $\begin{array}{llll}m & a & 0\end{array}$ | $m+4$ | + a 2 | 21 | 10 | $+$ | 11 |
| HC 187 | $m$ hl20 $\times$ hl a | $\mathbf{A} \times \mathbf{A}$ | I | $+ \pm 131$ | $\begin{array}{llll}m & a & 1\end{array}$ | $m+14$ | +al | 10 | 1 | $+$ | 9 |






 $\times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times$


$$
\begin{aligned}
& \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times \times
\end{aligned}
$$

exchanges occurred predominantly on the left side (A) of the sub-region, while in crosses between alleles in sub-region $\beta$ the extra exhcanges occurred predominantly on the right side (B) of the sub-region. In crosses involving his alleles in both regions the polarity was variable and usually lower. When two alleles were involved in crosses of reciprocal phases (see crosses: his-11 $\times$ his-1, his- $2 \times$ his- $1, \quad$ his- $129 \times$ his-132, his- $11 \times$ his- $9, \quad$ his $-1 \times h i s-9)$, the phenotype of the parentally marked class in excess was the opposite in the two cases, so that the additional exchange occurred on the same side irrespective of the phase - , usually at a comparable rate.

If we consider the whole his region we can conclude that the selected cross-over was frequently accompanied by an extra cross-over external to the region, occurring on the side closest to the more distal his allele involved. The extra cross-over acted as a switch-back exchange, restoring the parental arrangement of the outside markers around the his ${ }^{+}$allele closer to the middle of the region (so that the marker alleles in excess were those in coupling with the distal his ${ }^{-}$allele).

The sense and the extent of the polarity has been measured as the difference: percent exchanges in A minus percent exchanges in B. A positive value means excess of exchanges on the left, a negative value means excess on the right. The figures give the extent of the polarity. The values for the polarity in different crosses are listed in the last column of Table 3.

In Fig. 1 the polarity values (values from different crosses concerning the same pair of his alleles were averaged) are plotted against the middlepoint of the region of the primary cross-over (B). The latter-is given as the point intermediate between the location of the two his alleles involved in each type of cross, as deduced from the ratio : recombinants to heteroclones (Method 1, Table 2). An evident correlation appears between the polarity and the position of the selected cross-over. When the latter goes from the left, to the middle, to the right end of the region, the polarity gradually turns from highly positive to neutral to highly negative.

Allelic specific behaviour. - Although the polarity of a his ${ }^{-} \times$his $^{-}$cross is an approximate function of the position of the selected his alleles (Fig. 1), an allelic specificity in determining the extent of the polarity is often also evident. For instance, when the mutant allele his- 2 is involved, the polarity is always reduced. On the contrary, when his-129 is the left mutant allele, the polarity is very high, the cross-overs on the right side region being held practically to zero.

Crosses of his markers with markers external to the his region.
Crosses of the type leu-1 met-2 arg-1 $\times$ his-X. As already noticed, met- 2 appears very close to the left end of the his region. Crosses have been per-
formed selecting for recombination between met-2 and the his alleles at the very left of the region, using leu-1 and arg-1 as outside markers to observe the polarity. This was clearly negative and the closer the his allele involved


Fig. 1. - Polarity of crosses as a function of map location of the selected his alleles. Polarity is given as difference: extra-exchanges in "A" (left) minus extra-exchanges in " C " (right). Average position of cross-over is taken to be the mid point between the selected his alleles. The map position of the his alleles is calculated by Method 1 (see text) giving their relative distance from met-2. Solid circles refer to crosses involving the allele his- 2 .
was to the left end of the his region, the higher the polarity (Table 4). The previously mentioned allele specific behaviour was not observed. The transition between the his alleles and met-2 marked, therefore, a shift point in the polarity of the crosses thus supporting the occurrence of a discontinuity in that region.

Crosses of the type his-X arg-1 $\times$ leu-1 his-(116 or 123). The right end of the his region was assumed to lie between his-14 and his-116, mainly on the basis of the location of the mutations his-116 and his-123, which are somewhat separated from the other his mutations (exspecially according to Me thod 2). This assumption is supported by the polarity observed in crosses
Table 4.


* Abbreviated symbols : l=leu-1; m=met-2; $h=$ his; $a=$ arg-1.
Crosses involving one allele in the «his» region and another external to it.
Types of crosses : $\frac{\text { leu-1 met- } 2+\ldots \text { arg-1 }}{+\quad+\quad \text { his }-\mathrm{X}+} ; \frac{\text { leu-1 }+ \text { his- } \mathrm{Z}+}{+ \text { his-X }+ \text { arg-1 }}$
involving his-9 or his-10, at the very left of the his region, together with his-116 or his-123. The polarity, which is strongly negative at the right end of the his region, shifts to high positive values when the selection involves the presumed "external» alleles (Table 4). The behaviour is parallel (and reciprocal) to that observed at the left end of the his region when met-2 is involved in the selection. The opposite behaviour of the polarity at the two ends of the his region is thus emphasized.


## DISCUSSION

Polarized recombination, as manifested through the asymmetry of the parentally marked classes in fine structure analysis has been commonly observed in moulds (Calef, 1957; Pritchard, 1960 ; Stadler, 1959 ; Murray, 1960 ; Siddiqi \& Putrament, 1963). This is the first report of polarized recombination in a bacterium, and the first instance of such an analysis extended to a series of adjacent genes.

Polarized recombination is generally attributed to the occurrence of discontinuities in the genetic material. According to Murrays' (1960) speculation "if the genetic material consists of sub-units (e.g. DNA molecules) it can be visualized that the recombination patterns for particular intragenic markers will be influenced by the position of these markers relative to the end of the sub-units ».

The his region analyzed in this present paper very likely occupies the whole length of such a sub-unit. The polarity of recombination is strong at both ends of the segment and low and irregular in the middle region. The ends of the sub-unit are defined as sites of concentration of a secondary (switch-back) cross-over coincident with a primary exchange selected within the sub-unit.

The polarized recombination has been interpreted in some recently proposed molecular models (Holliday, 1964: Whitehouse \& Hastings, 1965), by postulating «linkers» of unspecified nature scattered along the genetic material and defining a series of structural sub-units.

The occurrence of «linkers» joining DNA sub-units, or fibrils, was also postulated on the basis of mechanical considerations (Freese, 1958), of cytological observations (Kellenberger, 1960), as well as to provide models for the interpretation of some genetic anomalies (Taylor, 1958; Stahl, 1961 ; Lissouba et al., 1962; and also Holliday, 1964; Whitehouse \& Hastings, 1965).

The size of the his sub-unit in S. coelicolor can roughly be estimated on the basis of the available information. From the data of the present paper it appears about 1.8 map units long. The total length of the map of S. coelicolor A $3(2)$ is hard to estimate. Two segments of respectively 60 and 70
units are well mapped (Hopwood, 1965), but they are probably joined by two regions each corresponding to more than fifty recombination units (Hopwood, 1965; Sermonti, 1965 ; Sermonti, Bandiera \& Spada-Sermonti, 1966). A minimal estimate of the total map length is thus 250-300 units. The amount of DNA per nucleus of S. coelicolor is comparable to that in one nucleus of Escherichia coli (Serlupi-Crescenzi, personal communication), around $7 \times 10^{9}$ daltons. The size of the sub-unit would thus be of the order of $\frac{1}{200}$, i.e. c. $3.5 \times 10^{7}$ daltons, or $5 \times 10^{4}$ nucleotide pairs, an estimate somewhat in excess of the estimate of the length of the his operon (Ames \& Hartman, 1962) in Salmonella ( $1.3-2 \times 10^{4}$ nucleotide pairs). In terms of expanded DNA molecules, the length of the his region would be around $10 \mu$, or perhaps a lower value, if the possible under-estimation of the total map length in $S$. coelicolor is considered.

This size is quite comparable with that assumed for the sub-units of Freese (1958) ( $5 \mu$ ) and for the fibrils of Kellenberger (1960) $(3 \mu)$, and it is also in good agreement with the length of the loops of the lampbrush chromosomes of the amphibians (Callan \& Lloyd, 1960), also considered to be DNA sub-units.

More interesting still, the length of the his sub-unit appears comparable to that $(16.3 \mu)$ of the DNA of a small temperate phage (Ris \& Chandler, 1963), and of a molecule of messenger RNA (Rich, Warner \& Goodman, 1963).

The classic work of Ames \& Hartman (1962) on the his operon in Salmonella has shown that the whole group of adjacent his genes operates as a single physiological unit, probably producing a single RNA messenger molecule (Ames \& Hartman, 1963; Martin, 1963). The hypothesis may now be put forward that such a physiological unit corresponds to a structural sub-unit in the DNA ribbon.

Dedication. - A. C. and G. S. wish to dedicate this paper to the unforgettable memory of Rosella Piperno who, to the very last days of her young, vanishing life, devoted all her passion and energy to this work.

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[^0]:    (*) Borsista del Centro Internazionale di Chimica Microbiologica.

[^1]:    ribo-1, pfp-: $(108,50),(31,51,103,105),(109,81,104,11,79),(3,77), 83$, an-1.

[^2]:    * On the hypothesis : single $(B)$ : double $(B+C)=$ double $(A+B):$ triple $(A+B+C)$. An asterisk marks significant chi squares $(p<0.05)$. ** In brackets when 20 , or less, recombinants were tested.
    *** In the absence of interference.

