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SHORT PAPER

Consequences of double cross-over detections on the functional interpretation of segment '29' in *Podospora anserina*

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SUMMARY

Double cross-overs were detected in a few crosses involving segment '29' mutants of locus '14' in *Podospora anserina*. The occurrence of such recombination events made it necessary to reconsider the localization of three mutants. Use of an outside marker and the analysis of many other crosses allowed these mutants to be mapped unambiguously. The change in the localization of one of these mutants together with complementation tests on a new mutant leads to the conclusion that the polycistronic unit of transcription contains only four cistrons instead of the five previously thought to exist.

INTRODUCTION

A major concern of geneticists when dealing with gene structure and fine recombination phenomena is the reliability of genetic maps depending on the properties of the organism and the types of mutants under examination. This difficulty is generally overcome by the joint use of several methods so as to make up for the possible shortcomings of any one of them, no method being absolutely reliable.

This problem arises in *Podospora anserina* when considering the complex locus '14' and more especially the median segment '29', involved in ascospore pigmentation. Extensive genetic analysis was carried out in order to clarify the functional organization of this complex genetic segment and to study recombination phenomena. *P. anserina* and especially locus '14' proved particularly suitable material for this work. Owing to the ascus structure, the closeness of locus '14' to the centromere and the strong interference observed on most chromosomal arms, a very satisfactory method could be used for mapping the mutant sites of this locus (Marcou & Picard, 1967). When genetic analysis of a recombinant ascus (picked up from a 'mutant × mutant' cross) indicated that post-reduction had occurred for one mutant and pre-reduction of the other, it could then be assumed that a crossing-over had taken place between the two mutant sites, the post-reduced site being the farthest from the centromere.

Segment '29' displays the most conspicuous properties of a polycistronic unit of transcription (Picard, 1971). Comparison of genetic and complementation maps suggests that the unit consists of five genes. But more recent data lead us to question the method chosen for mapping the mutants in this segment. Genetic analysis revealed the occurrence of multiple reciprocal recombination events in a few crosses. A reliable genetic map is necessary to enable the interpretation of the functional structure of this segment to be tested and also to enable the recombination phenomena to be understood. Hence the need for some further experiments, including (*a*) analysis of more recombinant asci in some crosses, (*b*) use of an outside marker for distinguishing the asci corresponding to

double-cross-overs from those due to simple cross-overs, (*c*) a search for deletions that would provide independent information about the map.

In the present paper the authors have devoted their attention to the bearing of the observed data on the interpretation of the functional structure of segment '29'. How these results are connected with molecular mechanisms of recombination will be discussed in a following paper on recombination (Marcou & Touré, in preparation).

MATERIAL AND METHODS

The main properties of the ascomycete *Podospora anserina* as well as its techniques of culture, crossing and genetic analysis have been frequently described, as recently reviewed by Esser (1969). But the ascus structure seems of sufficient importance to be recalled here. The ascus contains four spores. Each one is formed around two non-sister nuclei of the postmeiotic mitosis so that both spores of each half-ascus are genetically identical.

The phenotypic properties and mutagenic origins of segment '29' mutants have already been described (Picard, 1971) except for those X-ray induced. Many mutants were obtained after X-ray irradiations (between 2000 and 30000 roentgens) of either mycelia or microconidia (strong doses being used in an attempt to obtain deletion-type mutations). RX mutants examined in this paper display either colourless or very slightly pigmented spores.

Mutants are quoted under the following abbreviations, based on their mutagenic origin: SP, spontaneous; UV, ultraviolet rays; NG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; ICR, IRC-170 (2-methoxy-6-chloro-9,3(ethyl-2-chloroethyl) amino propylamino quinoline dihydrochloride); RX, X-rays.

RESULTS

For the present work more than 100 'mutant × mutant' crosses have been performed. The genetic analysis of several recombinant asci displaying reciprocal recombination demonstrates that post-reduction always occurs for the same site in a given cross. This means according to the selected method that a crossing-over has taken place between the two sites, the post-reduced site being the farthest from the centromere. However, analysis of five '$m_1 \times m_2$' crosses (Table 1) reveals the two following types of asci: the first exhibiting post-reduction for one site and the second post-reduction for the other site. One of these types must be produced by double cross-overs. Previous localization of mutants UV-135, UV-91, SP-14 and NG-192 (see Fig. 1*a*) then becomes questionable as such asci appear with appreciable relative frequency.

It is therefore necessary first to know which ascus type arises from a double cross-over and secondly to map precisely all mutants involved. A mutant unambiguously located in the region of UV-135 and SP-14 is required to solve these two problems. Preliminary data seem to indicate that NG-189 is such a mutant. Results of more extensive studies are given in Table 2. Mutant NG-189 always shows pre-reduction in the first five crosses, so that it must lie between the centromere and mutants UV-135, UV-91, NG-222, NG-207 and SP-315. When considering the two crosses involving SP-14 and NG-189, post-reduction for NG-189 can be noted in all of the 15 asci. Moreover, five of these asci have been isolated in a cross involving the outside marker *pumila* (*p*) (small sized spores) located on the same chromosomal arm as segment '29', 9 units away. In four out of these five asci simultaneous post-reduction of NG-189 and *p* is found, while SP-14 always displays pre-reduction. Therefore, SP-14 is closer to the centromere than NG-189 and also nearer than the other five mutants of Table 2.

It follows that double cross-over asci correspond to the β-type in Table 1. They appear less numerous than α type except perhaps in UV-91 × SP-14 cross. This β type of ascus

Table 1. *Exceptional "mutant × mutant" crosses exhibiting two types of reciprocal recombinant asci: postreduction occurred for the 'm_2' site in α type for the 'm_1' site in β type asci*

m_1	+
m_1	m_2
+	+
+	m_2

α

m_1	m_2
+	m_2
m_1	+
+	+

β

Crosses		No. of observed asci	No. of reciprocal recombinant asci	
m_1	m_2		α type*	β type
SP-14 × 20-315		500 000	10	1
SP-14 × NG-222		200 000	6	1
SP-14 × UV-135		650 000	5	2
SP-14 × UV-91		300 000	1	2
NG-192 × NG-136		250 000	4	1

* In such asci two spores display the wild phenotype and the other two are unpigmented. Some of them could not be analysed because many unpigmented spores failed to germinate.

can be interpreted in terms of the DNA hybrid model of Whitehouse (1967) as arising from 'reciprocal conversion', i.e. a gene conversion type of event without crossing-over which yields a wild-type chromatid and a double mutant chromatid. Such an explanation would be more likely if it were demonstrated that only two chromatids are involved in the recombination process leading to the β type of ascus formation. Whatever the origin of these asci may be, their occurrence raises the same mapping problem.

Many other crosses were performed in order to map UV-135, UV-91 and NG-192 with greater precision. They lead to the following conclusions (Table 3): (*a*) UV-135 is farther from the centromere than NG-177, (*b*) NG-183, and NG-170, belonging to complementation group IV with UV-135 (see Fig. 1*c*) are more distal than UV-135 and NG-193 with respect to the centromere, (*c*) UV-91 is more distal than NG-193. The new map position of UV-91 accounts more satisfactorily for preliminary recombination data on this mutant than the old one did (Touré & Marcou, 1970), (*d*) NG-192 is nearer the centromere than NG-261.

Supplementary data support the location of UV-135, NG-183 and NG-170 within group V. (*a*) UV-135 is mapped proximal with respect to UV-91 and to UV-115 (group V) (Picard, 1971). (*b*) Very few recombinants have been found in two-point crosses between mutants UV-135 and NG-193 (around 2×10^{-5}); NG-183, NG-170 and UV-29 ($\leqslant 3 \times 10^{-5}$); SP-315, NG-207, NG-222, UV-113 and UV-91 ($\leqslant 2 \times 10^{-5}$). On the other hand, each of these mutants recombines with a much higher frequency (from 0·6 to 2×10^{-4}) with all the other ones.

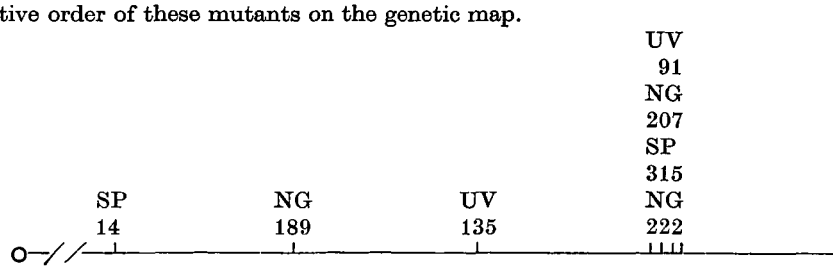
From the whole of these data the genetic map has to be altered in the way shown in Fig. 1(*a*, *b*). Change in map position of mutant UV-135 is the only one which requires a new comparison between genetic and complementation maps. The effect of this change of position on the interpretation of the functional structure of segment '29' make it necessary to confirm the localization of mutant UV-135 by another method. Hence, the search for deletion-like mutants: such mutants would, *a priori*, display either no or very weak pigmentation. Twenty-three X-ray-induced mutants exhibiting this phenotype were obtained in segment '29'. Complementation tests between these mutants and the other mutants of segment '29' were carried out. The observed data can be seen

Table 2. *Two- and three-point crosses involving mutant NG-189 performed in order to obtain map positions of mutant SP-14 with regard to UV-135, UV-91, NG-207, NG-222 and SP-315*

(*a*) Experimental data.

Crosses m_1	Crosses m_2	No. of observed asci	No. of reciprocal recombinant asci: α type	No. of reciprocal recombinant asci: β type
NG-189	UV-135	1 000 000	3	0
NG-189	SP-315	440 000	3	0
NG-189	NG-222	240 000	2	0
NG-189	UV-91	250 000	2	0
NG-189	NG-207	100 000	2	0
SP-14	NG-189	600 000	10	0
SP-14*p*	NG-189*p*$^+$	500 000	5	4 (post-reduction of outside marker '*p*') / 1 (pre-reduction of outside marker '*p*')

(*b*) Relative order of these mutants on the genetic map.



either in Fig. 1(*c*) or in its legend. Recombination tests showed that all X-ray-induced mutants recombine well with the other mutants except RX-2, RX-3 and RX-25. The first two either fail to recombine or yield very few recombinant asci with mutant UV-29. On the contrary they produce many recombinant asci when crossed with adjacent mutants such as UV-115 and NG-193. As for mutant RX-25, it recombines with all mutants except UV-135 and NG-193, which are very close to one another.

The conclusion is that none of the X-ray-induced mutants correspond to deletion. However, the comparison between complementation and recombination data obtained with RX-25 affords very important information on the functional structure of the segment as discussed hereunder.

DISCUSSION

The above data fit in with the general frame of the interpretation of segment '29' as a polycistronic unit of transcription, but raise a question as to the strict relation established between the number of complementation groups and the number of genes. This aspect of the results is the only one to be discussed here.

It is necessary to alter the assumed number of genes in segment '29' for two reasons:

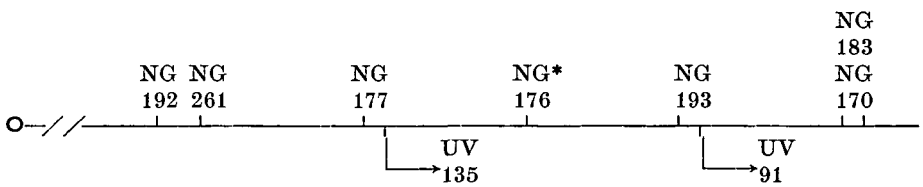
(1) The map position of mutant UV-135 is now absolutely certain as all the data agree on this point. It is farther from the centromere than SP-14 and NG-177: it lies very near to NG-193 and two other mutants belonging to complementation group IV are localized on the right of NG-193, close to UV-29 (Fig. 1*b*). Three mutants of complemen-

Table 3. '*Mutant* × *mutant*' *crosses performed in order to localize mutants UV-135, UV-91, NG-183, NG-170 and NG-192*

(*a*) Experimental data.

Crosses		No. of observed asci	No. of reciprocal recombinant asci	
m_1	m_2		α type	β type
NG-177 × UV-135		200000	1	0
NG-176 × NG-183		140000	1	0
NG-193 × NG-183		200000	2	0
NG-193 × NG-170		300000	2	0
UV-135 × NG-170		200000	1	0
NG-193 × UV-91		280000	3	0
NG-177 × UV-91		250000	3	0
NG-192 × NG-261		450000	3	0

(*b*) Relative order of these sites on the genetic map.



* Mutant NG-176 is known to lie between NG-177 and NG-193 (previously published data).

tation group IV are thus localized among mutants belonging to complementation group V. The assumption that groups IV and V correspond to two different genes is thus highly improbable.

(2) RX-25 exhibits a new complementation pattern. It is the only mutant (among 65 complementing mutants) which does not complement with mutants both of group IV and V. Moreover, preliminary recombination data show that it definitely lies very close to NG-193 on the genetic map.

When these two types of information were still lacking, complementation group IV seemed to correspond to one distinct gene. It contains indeed six mutants (1 UV, 3 NG and 2 EMS induced) which display an homogeneous complementation pattern: they do not complement each other but they yield total complementation when associated with all other mutants, especially those of group V.

If group IV actually corresponds to a single distinct gene, the mutants localized between UV-14 and NG-193 on the genetic map must then belong to a sixth gene (see Fig. 1*b*, *c*). These last mutants would be polar and antipolar, except NG-189, which would only be antipolar. Such phenomena would not be unlikely if the products of genes V and VI (but not gene IV) are only functional in a multiprotein aggregate (Bauerle & Margolin, 1966; Shaffer, Rytka & Fink, 1969). However, such an assumption becomes doubtful with mutant RX-25. If its complementation pattern is to fit into the frame of this hypothesis, it has to be imagined as a double mutant or a small deletion overlapping genes IV and VI. Reversion experiments might solve this problem.

All the results can be accounted for by another more simple hypothesis: mutants of complementation groups IV and V belong to the same gene coding for a polypeptide chain which is active as a multimere protein (owing to intragenic complementation). Groups IV mutants which strongly complement with those of group V could correspond

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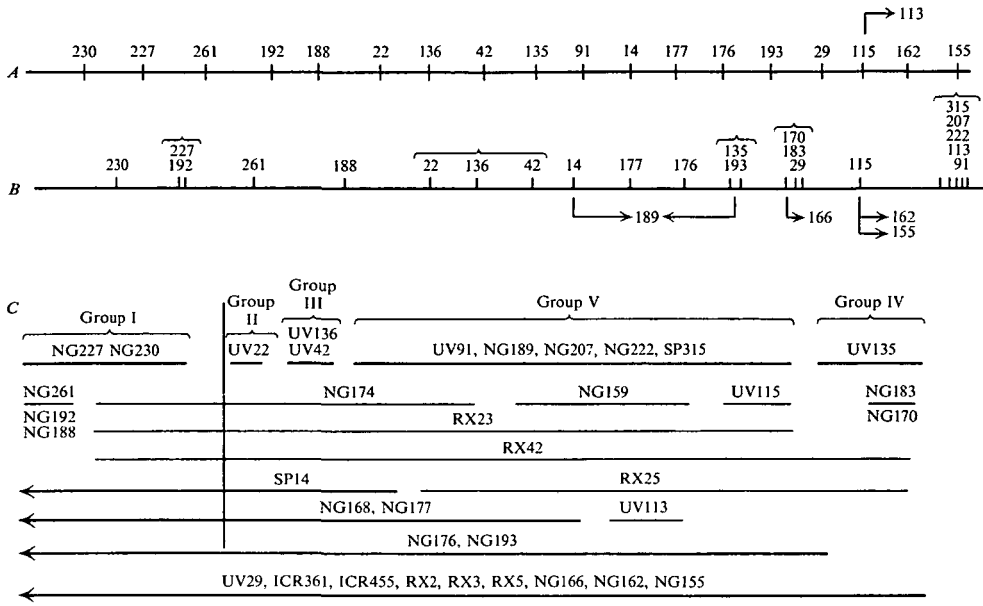

Fig. 1. Genetic and complementation maps of segment '29'.

(*A*) Genetic maps previously published.

(*B*) Genetic map taking into account present data. Distances shown between sites are arbitrary. Crosses between mutants grouped under the same bracket give very few or no recombinant asci. In these cases their relative order has not been determined. Mutants under the map are only partially localized: for instance 166 stands to the right of 29; 189 lies between 14 and 135.

(*C*) Schematic complementation map. Only a proportion of the mutants (36 out of 91) are indicated so as to bring out the main features of this map. Moreover two positive complementation responses are shown as negative: NG-176 × UV-115 and RX-25 × UV-113. However, all the mutants located on the genetic map are marked on the complementation map. Among the 23 X-ray-induced mutants, six appear here. The remaining 17 are scattered as follows: 3 belong to complementation group I, 9 to group V and 5 are non-complementing mutants.

to mutations affecting the stability of the multimere. This instability would be the primary reason for the lack of activity of these mutants, whereas interactions with differently altered polypeptide chains (group V mutants) provide a proper and stable multimere configuration. Such mutants have been described in *E. coli* (Schlesinger, Torriani & Levinthal, 1963) and in *Neurospora* (Fincham & Stadler, 1965). Group IV mutants could alternatively be the consequence of mutations leading to a greater fragility of the polypeptide chain at a precise point. Such a property would explain positive complementation responses between group IV mutants and some 'polar' mutants according to the mechanisms known in β-galactosidase (Ullmann, Jacob & Monod, 1968).

If group IV and V mutants belong to the same gene, the unit of transcription is then made of four instead of 5 genes. But this assumption opens up a new problem. Apart from two genes where many mutants are known (gene I with 24 mutants and gene IV–V with 38 non-polar and 27 polar mutants), there are two genes with few – too few – mutants. Gene II contains only one mutant and gene III two mutants. All three are UV-induced. These two 'genes' could correspond to a peculiar genetic region which would be insensitive to most mutagens except ultraviolet rays. New UV-mutagen tests are needed

to support this argument. However, these three mutants could belong to gene V and show the same complementation properties as group IV mutants. If such is the case, the polycistronic unit of transcription would only contain two genes.

Whatever the number of genes (probably 4 or 2), a polycistronic unit of transcription *does* exist on the level of segment '29'. Indeed, all genetic and biochemical analyses carried out with a number of genes in many organisms (for instance, Case & Giles, 1960; Fincham, 1967) show that non-complementing mutants are scattered along the whole genetic map of single genes but that they display an asymmetrical localization in the case of polycistronic units of transcription. Up to now nine non-complementing mutants of segment '29' have been localized (1 UV-, 3 NG-, 2 ICR- and 2 RX-induced) out of 19 such mutants (1 UV-, 3 NG-, 5 ICR-, 1 SP- and 9 RX-induced). They all lie at the distal end of this segment with respect to the centromere, to the right of mutant NG-193.

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