THE IMPACT OF GENE CONVERSION ON INTRAGENIC VARIABILITY IN ASCOBOLUS IMMERSUS

"Selection of events associated with asymmetrical hybrid DNA formation"

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" تأثير التحول الجينى على الاختلافات داخل الجين "

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ملخص البحث

تم دراسة تأثير حدوث التحول الجينى الذى يحدث أثناء الانقسام الميوزى لمجموعة من الطفرات الواقعة داخل الجين ب٢ والذى يتحكم فى تكوين لـون الجراثيم فى الفطر الاسكى أسكوبولس أمرسس على الاختلافات الناتجة داخل الجين . انتخاب وتحليل الأسكيات التى تظهر انعزال مابعد الميـوزى (الانعزال الغير مندلى ٥ : ٣) وذلك باستخدام طفرة تقع فى وسط الجيـن وهذه الانعزالات تعكس تكوين ح د ن هجين من النوع الغير متماثل شـاملا جميع الطفرات المدروسة والموجودة فى صورة غير متماثلة أظهر أن كل أنـواع الاتحادات بين الطفرات المدروسة أمكن الحصول عليها وذلك عبر التحـول الجينى الذى يحدث أثناء الانقسام الميوزى وذلك بتكرارات عالية . وأن عمليـة الاصلاح لل ح د ن الهجين المعقد الذى تم تكوينه يمكن أن يكون له تفسيرات هامة . مثال . أن هذه الميكانيكية تكون ذات تأثير مطفر قادر على استبدال العديد من النيكليوتيدات فى خطوة واحدة . وكذلك يمكن لهذه الميكانيكية تفسير مصدر بعض صور التعدد الجينى فى عائلات الجينات المتعددة فى الكائنات المحددة النواة .

ABSTRACT

The effect of meiotic gene conversion of mutations located in the gene b_2, which controls spore pigmentation in the fungus Ascobolus immersus, on intragenic variability has been studied. Selection and analysis of asci showing postmeiotic segregation (5:3 non Mendelian segregation) at the level of an intermediate mutant site reflect asymmetric hybrid DNA formation. The hybrid DNA covering many heterozygous mutant sites, through the induction of meiotic gene conversion, gives all possible combinations between them. The in vivo processing of such complex hteroduplexes has many interesting implications. It offers a mutational mechanism capable of altering several nucleotides in a single step. It can also explain some of the genetic polymorphism in eukaryotic multigene families.

INTRODUCTION

Gene conversion may be defined as a unidirectional transfer of genetic information from one DNA duplex to another. At the molecular level, the initiation event of gene conversion is the hybrid DNA (h-DNA) formation (Holliday, 1964; Meselson and Radding, 1975). Hybrid DNA that is present on only one chromatid of the two interacting chromatids is called asymmetric h-DNA, while that which covers the same region of the two chromatids is called symmetric h-DNA. In mutant x wild-type crosses, the formation of h-DNA at the mutation site during the interaction between homologous chromatids, which leads to genetic recombination, seems to be a reasonable explanation for the observed non-Mendelian segregations (NMS); Pukkila, 1977.

The fungus Ascobolus immersus is an excellent organism for studying recombination, since more than 20 genes involved in spore pigmentation have been defined. In Ascobolus octospored asci are composed of four pairs of sister spores, each pair corresponding to one meiotic product. Hence, the occurrence of NMS of 5:3, aberrant 4:4 and 6:2 types can be easily detected. The 5:3 and aberrant 4:4

NMS reflect that the mismatch formed in h-DNA is not corrected and that the mutant and wild-type alleles segregate only at postmeiotic mitosis. The 6:2 NMS indicate that the mismatch formed in h-DNA is recognized and corrected leading to the transfer of a wild-type chromatid to a mutant or vice-versa. This hypothesis is supported by findings in Ascobolus (Leblon and Paquette, 1978) and in Sordaria (Yu-Sun et al., 1977).

The present study has been carried out with crosses involving up to six heterozygous sites in the b_2 gene. Selection for asymmetric h-DNA formation events was carried at the level of an intermediate mutant site which gives rise to all types of NMS, then the fate of h-DNA covering the flanking heterozygous sites was detected. Such in vivo processing of these complex heteroduplexes has many interesting implications as a natural source of considerable genetic diversity.

MATERIAL AND METHODS

Culture media: Details of culturing conditions have been reported previously by Paquette and Rossignol (1978).

Mutants: The mutants used in the present study belong to stock 28 of Ascobolus immersus, and bear the CV_2A modifier allele of the b_2 gene (Girard and Rossignol, 1974). The origin and the phenotype of these mutants are indicated in Figure 1. Three other genetic markers were used, $vag._8$ (mycelial growth), $rnd_{1,2}$ (round ascospore shape) and mt (mating type). They are unliked to the b_2 gene (Nicolas et al., 1981).

Nature of Mutants: The frameshift mutation F_0, F_{16}, E_2 and E_1 lying in region F and E are assumed to correspond to small additions

or deletions of nucleotides (Leblon and Paquette, 1978). This assumption is based on the specificity of ICR_{170} mutagen demonstrated in both Ascobolus and Sordaria (Leblon, 1972; and Yu-Sun et al., 1977) and in Saccharomyces cerevisiae (Donahue et al., 1981). By extension the double frameshift mutants F_0F_{16} and E_2E_1 are assumed to correspond to (+1,-1) changes when compared with the wild-type sequence (Leblon and Paquette, 1978). The C_{234} mutation corresponds to a deletion in the middle part of the b_2 gene (Hamza et al., 1987). X_{15} is a type C mutant (gives both meiotic and postmeiotic segregations) and corresponds propably to a base pair substitution (Rossignol et al., 1979).

Multi mutant strain construction: The two double mutants F_0F_{16} and E_2E_1 were constructed separately. Strains with a single mutant site give a white spore phenotype, while the two double mutants F_0F_{16} and E_2E_1 have a pseudo wild-type phenotype and were obtained by crossing m_1 (F_0 or E_2) by m_2 (F_{16} or E_1) and by screening for 2 coloured : 6 white spore asci among the progeny of these crosses which give essentialy 8 white spore asci. The triple mutant F_0F_{16} X_{15} was obtained by crossing F_0F_{16} by X_{15} and screening for 3 coloured: 5 white (3C:5W) asci. The white spore of the mixed pair was then crossed by the wild-type. Among the progeny of the latter cross, 3C:5W and 5C:3W asci were isolated and the coloured spore of the mixed pair was then crossed by F_0. The existence of coloured spores with a F_0F_{16} genotype was thus determined, indicating that the parental white spore was actually $F_0F_{15}X_{15}$.

The F_0F_{16} $X_{15}E_2E_1$ strain was obtained by crossing $F_0F_{16}X_{15}$ by E_2E_1 and screening for 3c:5w and 5c:3w spored asci. The white spore of the mixed pair was then crossed by F_0 and F_1, respectively. The existence of coloured spores with a F_0F_{16} genotype in the cross of the white spore by F_0 as well as the existence of coloured spores with a E_2E_1 genotype in the cross of the same white spore by E_1 indicate that the parental white spore was actually $F_0F_{16}X_{15}E_2E_1$.

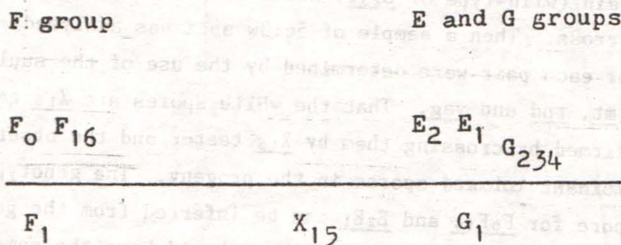

Fig. 1. Map of the b_2 mutants used. Regions F and E correspond to regions in which several frame-shift mutations belonging to the same group of intragenic suppression are located(Leblon and Paquette,1978).

Above the line: double site mutants with a pseudo-wild-type phenotype; under the line, mutants with a white spore phenotype. F_o and E_1 were isolated by ICR 170 mutagenesis. F_{16}, E_2 and F_1 were isolated by EMS mutagenesis. X_{15} was induce by X rays. G_1 and G_{234} are spontaneous mutations.

Testing the impact of gene conversion upon the intragenic variability: The effect of gene conversion upon the intragenic variability was tested in two different types of crosses. A set of wild-type and G_{234} strains were used. All these strains were obtained from crosses between wild-type and G_{234}.

The multimutation strain F_0F_{16} X_{15} E_2E_1 was crossed by each test strain (wild-type or G_{234}) and a sample of 1000 asci was counted in each cross. Then a sample of 5c:3w asci was analysed. The sister spores of each pair were determined by the use of the suplimentary markers mt, rnd and vag. That the white spores are X_{15} genotype was confirmed by crossing them by X_{15} tester and the observation of no recombinant coloured spores in the progeny. The genotype of the white spore for F_0F_{16} and E_2E_1 can be inferred from the genotype of the sister coloured spore. The spore should have the same genotype since it is known that the F_0F_{16} and E_2E_1 markers used give no post meiotic segregations (Rossignol et al., 1979).

The genotype of coloured spores for F_0F_{16}, E_2E_1 and G_{234} was determined by crossing the coloured spore with F_1 and G_1 (Hamza et al., 1987). The distinction between E_2E_1, and G_{234} was inferred from pink (E_2E_1) versus brown (G_{234}) spore phenotype.

RESULTS AND DISCUSSION

In the two sets of the crosses $F_0F_{16}X_{15}E_2E_1$ with wild-type and G_{234}, the NMS pattern of the X_{15} mutation was established. The results were compared for the individual classes of NMS asci 6C:2W, 5C:3W and aberrant 4C:4W (Table 1). Statistical analysis showed that the frequency of 6C:2W asci is significantly higher in crosses with G_{234} than with wild-type. In Table 1, the absolute frequencies of aberrant 4C:4W were corrected according to, (Paquette, 1978),

Table (1): Conversion pattern of X_{15} mutants in crosses of $F_0F_{16}X_{15}E_2E_1$ with wild-type and with the deletion G_{234}.

Cross	N	6c : 2w	5c : 3w	ab. 4c:4w
$\frac{F_0F_{16}X_{15}E_2E_1}{+\ +\ +\ +\ +}$	6000	103	160	31 (46.5)
Freq. / 1000		17.2	26.7	5.2* (7.8)
$\frac{F_0F_{16}X_{15}E_2E_1}{+\ +\ +\ G_{234}}$	5000	158	102	12 (18)
Freq. / 1000		31.6*	20.4	2.4 (3.6)

N : number of total asci observed.

ab: aberrant, in parentheses, estimated number of aberrant 4 : 4 asic are given.

* different is significant at .05 level.

because only 2/3 of these events are observed. The results are in agreement with the previous observations obtained by Hamza et al. (1987). The excess of 6c:2w asci in crosses with G_{234} is at the expense of the 5c:3w asci because G_{234} imposes its NMS pattern on the closely linked mutations and gives preferential conversion toward the donor strand which is G_{234} in this case (Hamza et al., 1986). The decrease in aberrant 4:4 NMS frequency in crosses with G_{234} is also the result of the fact that G_{234} blocks the propagation of the symmetrical h-DNA at its border toward the high conversion end of the b_2 gene (Hamza et al., 1981).

Ascus Analysis: Samples of intact asci with 4 oval : 4 round ascospores were analysed in each series of crosses. A total of 45 and 38 asci of the type (5c:3w) were analysed in the progeny of the crosses $F_0F_{16}X_{15}E_2E_1$ with wild-type and G_{234}, respectively. Both coloured and mixed pairs of spores were analysed. The genotype of pairs of white spores was assumed to be $F_0F_{16}X_{15}E_2E_1$.

The analysis showed that in crosses with wild-type, 15 types of asci can be detected while in crosses with G_{234} only 9 types were found (Fig. 2, and Table 2). In all the asci tested X_{15} was found to segregate postmeiotically giving rise to 5c:3w asci. In crosses with wild-type, 7 types of asci showed a Mendelian segregation of the double mutant F_0F_{16}, 4 types of asci showed a conversion of F_0F_{16} to wild-type and 4 types showed a conversion to mutant. While in crosses with G_{234}, only 3 types of asci showed a Mendelian segregation of F_0F_{16}, 3 a conversion to wild type and also 3 types showed a conversion to mutant. In all types of asci, segregation of E_2E_1 was observed (Fig. 2). At the DNA strand level, all possible combinations of F_0F_{16}, X_{15}, E_2E_1 and their wild type alleles were observed in both crosses with wild-type and G_{234}. The frequency of different combinations ranged from 3.9-28.3% of postmeiotic segregation events in crosses with wild-type and from 2.0-43.4% in crosses with G_{234} (Table 3).

Figure 2: Genotypes of different classes of 5c : 3w asci in the crosses $F_0F_{16}X_{15}E_2E_1$ with wild-type and G_{234}*.

F_0F_{16}	X_{15}	E_2E_1	F_0F_{16}	X_{15}	E_2E_1	F_0F_{16}	X_{15}	E_2E_1
F_0F_{16}	$X_{15/+}$	E_2E_1	F_0F_{16}	$X_{15/+}$	+ +	F_0F_{16}	$X_{15/+}$	+ +
+ +	+	+ +	+ +	+	E_2E_1	+ +	+	+ +
+ +	+	+ +	+ +	+	+ +	+ +	+	+ +
	(a)			(b)			(c)	

F_0F_{16}	X_{15}	E_2E_1	F_0F_{16}	X_{15}	E_2E_1	F_0F_{16}	X_{15}	E_2E_1
+ +	$X_{15/+}$	E_2E_1	+ +	$X_{15/+}$	+ +	+ +	$X_{15/+}$	+ +
F_0F_{16}	++	+ +	F_0F_{16}	+	E_2E_1	F_0F_{16}	+	+ +
+ +	+	+ +	+ +	+	+ +	+ +	+	+ +
	(d)			(e)			(f)	

F_0F_{16}	X_{15}	E_2E_1	F_0F_{16}	X_{15}	E_2E_1	F_0F_{16}	X_{15}	E_2E_1
+ +	$X_{15/+}$	E_2E_1	F_0F_{16}	$X_{15/+}$	E_2E_1	F_0F_{16}	$X_{15/+}$	+ +
F_0F_{16}	+	E_2E_1	F_0F_{16}	+	+ +	F_0F_{16}	+	E_2E_1
+ +	+	+ +	+ +	+	+ +	+ +	+	+ +
	(g)			(h)			(i)	

F_0F_{16}	X_{15} +	E_2E_1	F_0F_{16}	X_{15}	E_2E_1	F_0F_{16}	X_{15}	E_2E_1
F_0F_{16}	$X_{15/+}$	+ +	F_0F_{16}	$X_{15/+}$	E_2E_1	+ +	$X_{15/+}$	E_2E_1
F_0F_{16}	+	+ +	F_0F_{16}	+	E_2E_1	+ +	+	+ +
+ +	+	+ +	+ +	+	+ +	+ +	+	+ +
	(j)			(k)			(l)	

F_0F_{16}	X_{15}	E_2E_1	F_0F_{16}	X_{15}	E_2E_1	F_0F_{16}	X_{15}	E_2E_1
+ +	$X_{15/+}$	+ +	+ +	$X_{15/+}$	+ +	+ +	$X_{15/+}$	E_2E_1
+ +	+	E_2E_1	+ +	+	+ +	+ +	+	E_2E_1
+ +	+	+ +	+ +	+	+ +	+ +	+	+ +
	(m)			(n)			(o)	

* In crosses with G_{234} the genotypes are E_2E_1 and G_{234} in stade of E_2E_1 and ++

Table (2): Distribution of different 5C : 3W asci in the crosses F_o F_{16} X_{15} E_2 E_1 with wild-type and G_{234}

Class of asci	F_o F_{16} + +	X_{15} +	E_2 E_1 + +	F_o F_{16} + +	X_{15} +	E_2 E_1 G_{234}
	N	%	abs	N	%	abs
a	15	33.3	8.9	10	26.3	5.4
b	1	2.2	0.6	0	0.0	0.0
c	1	2.2	0.6	7	18.4	3.8
d	1	2.2	0.6	0	0.0	0.0
e	4	8.9	2.4	0	0.0	0.0
f	1	2.2	0.6	1	2.6	0.5
g	2	4.4	1.2	0	0.0	0.0
h	2	4.4	1.2	3	7.9	1.6
i	2	4.4	1.2	0	0.0	0.0
j	3	6.7	1.8	1	2.6	0.5
k	6	13.3	3.6	4	10.5	2.1
l	2	4.4	1.8	3	7.9	1.6
m	1	2.2	0.6	1	2.6	0.5
n	2	4.4	1.2	8	21.1	4.3
o	2	4.4	1.2	0	0.0	0.0

N, number of asci in each class .

abs, the absolute frequency of each class of asci .

Table (3) : Frequency of different spore genotypes in the two pairs of spores implicated in the DNA duplex interaction in the two crosses $F_o\ F_{16}\ X_{15}\ E_2\ E_1$ with wild-type and G_{234} .

Cross	Spore genotype	Frequency	%
$\frac{F_o\ F_{16}\ X_{15}\ E_2\ E_1}{+\ +\ +\ +\ +}$	$F_o\ F_{16}\ X_{15}\ E_2\ E_1$	23	12.8
	$+\ +\ X_{15}\ E_2\ E_1$	7	3.9
	$+\ +\ X_{15}\ +\ +$	8	4.4
	$F_o\ F_{16}\ X_{15}\ +\ +$	7	3.9
	$F_o\ F_{16}\ +\ E_2\ E_1$	51	28.3
	$+\ +\ +\ E_2\ E_1$	15	8.3
	$+\ +\ +\ +\ +$	48	26.7
	$F_o\ F_{16}\ +\ +\ +$	21	11.7
$\frac{F_o\ F_{16}\ X_{15}\ E_2\ E_1}{+\ +\ +\ G_{234}}$	$F_o\ F_{16}\ X_{15}\ E_2\ E_1$	17	11.2
	$+\ +\ X_{15}\ E_2\ E_1$	3	2.0
	$+\ +\ X_{15}\ G_{234}$	10	6.6
	$F_o\ F_{16}\ X_{15}\ G_{234}$	8	5.3
	$F_o\ F_{16}\ +\ E_2\ E_1$	25	16.4
	$+\ +\ +\ E_2\ E_1$	5	3.3
	$+\ +\ +\ G_{234}$	66	43.4
	$F_o\ F_{16}\ +\ G_{234}$	18	11.8

Hybrid DNA formation in the b_2 gene was found to be polar with a preferential initiation region toward the high conversion end to the F region (Hamza et al., 1981). The extension of h-DNA in the b_2 gene was argued by Rossignol and Haedens (1980). These facts support the assumption that when selection for h-DNA formation at X_{15} level was made, the great majority of these events cover both F_0F_{16} and E_2E_1 sites. Previous studies in Ascobolus showed that h-DNA is the obligatory intermediate of gene conversion and confirmed that independent mismatch corrections appear along the same h-DNA tract in the b_2 gene (Kalogeropoulos and Roddignol, 1980). In the present study experiments were performed using mutations which have different conversional pathways (Hasting et al., 1980; Hamza et al., 1986).

The X_{15} mutation was used to detect the meiotic events where h-DNA was formed and persisted. The 5c:3w asci reflect mainly the asymmetrical h-DNA formation with $F_0F_{16}X_{15}E_2E_1$ as the recipient DNA duplex. Analysis of 5c:3w asci led to the following conclusions:

1- all possible combinations between F_0F_{16}, X_{15} and E_2E_1 were observed.
2- The detection of these events in asci showing NMS of X_{15} argued for a h-DNA formation followed by mismatch correction leading to either conversion or restoration of the mutant sites implicated in the h-DNA stretch.
3- The absence of some types of asci when crosses with wild-type and G_{234} are compared is in agreement with the properties of the known conversional pathways.

The present results as a whole show the impact of gene conversion which occurs via h-DNA formation and following mismatch correction on intragneic variability. Previous studies showed that heteroduplexes can be formed in vivo by DNA strand exchange between partial

homologous but not identical sequences (Radding, 1978). Heteroduplexes can be prepared in vitro, transformed into living cells and their in vivo correction can then be studied (Dohet et al.,1985). These studies have been carried out with simple heteroduplexes carrying one or a few nucleotide mismatches. Little is known about the correction of more complex structures involving many noncomplementary nucleotides, this mechanism could be capable of generating considerable diversity. At the same time this idea can explain some of the polymorphism in the eukaryotic multigene families. Correction of complex heteroduplexes may be used as a practical means of engineering genetic variants. One of its interesting chracteristics is that all features of the primary structure common to both parents are conserved in the variants.

In higher eukaryotes, many sequences are only partially homologous and differ in many nucleotides. It has been often postulated that they can undergo crossing over and gene conversion due to their partial homology. If h-DNA is involved in any of these genetic exchanges, it must be in the form of complex heteroduplexes. The processing of these complex heteroduplexes has two interesting implications:

1- It may be a source of considerable genetic diversity. At least some variations could be attributed to gene conversion.
2- The correction of complex heteroduplexes offer a mutational mechanism capable of altering several nucleotides (amino acids) in a single step in different sites of DNA duplex (polypeptide chain). Such mechanisms can have important evolutionary significance.

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