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Mutational Analysis of a Regulatory Gene for Morphogenesis in *Schizophyllum*

(eukaryotic regulatory gene/sexual morphogenesis/fungal incompatibility/Bβ structure and function)

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ABSTRACT **Knowledge of the structure and function of a complex gene regulating sexual development in the mushroom, *Schizophyllum commune*, has come from the analysis of various mutations in a chromosomal region known as the *Bβ* incompatibility gene. This gene is one of two linked genes, *Bα* and *Bβ*, that together comprise the *B* factor which regulates a developmental sequence known as the *B*-sequence. The *B*-sequence is normally "turned off" unless alleles of different specificity of either *Bα* or *Bβ* are combined in a heterokaryon resulting from the mating of two individuals. Various mutations have been found in the *Bβ* gene that range in effect from dominant, *always-on*, through a number of intermediate stages of changes in specificity and function, to recessive, *always-off*. There is evidence that the *Bβ* gene operates via a positive control mechanism and involves at least four functions, one of which has been shown to be separable by recombination.**

Four genes regulate sexual morphogenesis in higher fungi such as *Schizophyllum commune*. The genes are multiple-allelic and are linked in two pairs, *Aα–Aβ* and *Bα–Bβ*, termed the *A* and *B* incompatibility factors. Together they control a developmental sequence that converts two self-sterile, haploid strains into a fertile heterokaryon, the dikaryon, that corresponds to the diploid phase of other organisms (1). The morphogenetic progression comprises a series of events that are assignable to two distinct and complementary sequences, the A-sequence and the B-sequence, regulated by the *A* and *B* factors, respectively.

Each monosporus isolate is haploid and thus carries a single allele in each of the four loci; such unmated strains exhibit no sexual expression. Sexual development occurs only when two strains with allelic differences in both factors are mated. This consists of a series of morphologically distinct events (Fig. 1 *a–e*), and each factor controls a part of the series (2). The A-sequence comprises nuclear pairing (*b*), conjugate division (*c*), and formation of hook cell (*c* and *d*), and converges with the B-sequence in fusion of hook cell (*e*). The B-sequence consists of nuclear migration (*a*) and fusion of hook cells (*e*). Sexual development thus requires the operation of both A- and B-sequences, or *A-on–B-on*. The converse, no sexual expression in unmated haploid strains or in matings homozygous for *A* and *B*, is *A-off–B-off*.

Profound biochemical alterations underlie the operation of the sequences and suggest shifts in the spectrum of genes expressed in the morphogenetic progression (3).

The present report is concerned with regulatory activity of the *B* factor and the status of the B-sequence, *B-off* or *B-on;* consequently, the status of the A-sequence can be essentially ignored. The terms *"off"* and *"on"* as used here signify only the inactivity or operation of the developmental sequence; they are not meant to imply anything about the mode of action of the incompatibility factors.

The two genes of the *B* factor, *Bα* and *Bβ*, each with nine known alleles (4), appear to be functionally identical and to act independently. Thus, the matings *B α1-β1* x *B α1-β2*, *B α1-β1* x *B α2-β1*, and *B α3-β4* x *B α5-β6* lead to a common result: to "turn on" the B-sequence and the characteristic *B-on* morphology. Matings homozygous for a single *B* factor, e.g., *B α3-β2* x *B α3-β2* fail to turn on the B-sequence, and the phenotype remains *B-off*.

The manner in which the genes of the *B* factor operate to control the B-sequence of sexual morphogenesis is unknown, but a study of the mutative capabilities provides some clues. All of many mutant alleles recovered in a system designed to select for a change from *B-off* to *B-on* were constitutive for the B-sequence (5, 6). The selective system would also have revealed mutations to new wild-type alleles capable of interacting with the progenitor allele to turn on the B-sequence, but no such mutation was found. Each of the mutants in isolation has a continuously operating B-sequence, e.g., the mutation of wild allele *Bβ2*$\dashrightarrow$*Bβ2(1)* (the number in parentheses representing a type of mutation) renders the B-sequence always on and makes the mutant allele interactive with all wild *Bβ* alleles, the progenitor *Bβ2* included. The *B-always-on* phenotype is characterized by stunted growth, irregular branching, disrupted septa, and continuous nuclear migration; when the A-sequence is also operative, *B-always-on* elicits fusion of hook cells (7).

Several secondary mutations induced in mutant *B-on* alleles of *Bβ*, e.g., *Bβ2(1)*$\dashrightarrow$*Bβ2(1-1)*, *Bβ2(1-2)*, etc. (the second number in parentheses representing the type of secondary mutation) have been recovered in a system designed to select for a change from *B-on* of the primary mutant to *B-off*. These, when paired with wild-type alleles, show varied effects upon the ability to interact with the spectrum of wild *Bβ* alleles and also upon the course of the B-sequence (8–11).

The characterization of these secondary mutations in the *Bβ* locus indicated its function to be complex but gave no hint

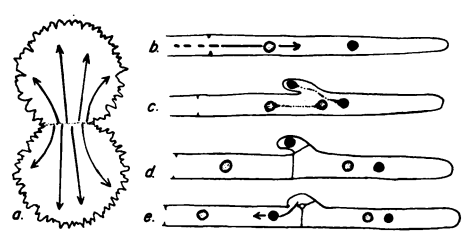

FIG. 1. Stages in sexual morphogenesis in *Schizophyllum*. (*a*) Reciprocal nuclear exchange and migration in mating of wild-type strains. (*b*) Pairing of migrant and resident nuclei in apical cell. (*c*) Conjugate division with formation of hook; one daughter nucleus in hook. (*d*) Unfused hook cell, the final stage of *A-on–B-off* development. (*e*) Fused hook cell, the final stage of *A-on–B-on* development.

of the structure of the locus. Further dissection of function and clarification of structure of the $B\beta$ gene is sought here in the more comprehensive analysis of its mutative capabilities.

MATERIALS AND METHODS

The strains of *S. commune* used were either taken directly or generated from cultures in the authors' collection at Harvard University. These include strains carrying a primary $B\beta$ mutation originally provided by Y. Parag (5), various *B* factors from nature (12), and recombinant *B* factors generated by Y. Koltin (13).

The media and routine procedures used are those developed in earlier studies with *Schizophyllum* (14).

A fruiting dikaryon,

$$\frac{dm\ A\alpha 4\ +\ +\ A\beta 6\ B\alpha 3\ B\beta 2(1)\ nic\text{-}2}{dm\ A\alpha 1\ pab\ ade\text{-}5\ A\beta 6\ B\alpha 3\ B\beta 2(1)\ nic\text{-}2},$$

provided the spores used for the induction of mutations: (*a*) The primary mutation, $B\beta 2(1)$, was made homozygous so that the progeny would be uniformly *B-on* in morphology and homogeneous for the locus under test. (*b*) Homozygosity for the morphological mutation *dome* (*dm*) restricted colony size of all progeny to allow dense plating with a minimum of intermingling. (*c*) The auxotrophic mutation *nic-2* served as a marker and for nutritional forcing in later tests. (*d*) The mutations *pab* and *ade-5* selected on the plating medium against survivors carrying one of the *A* factors. Because of linkage of $A\alpha$ and *pab*, only about 1% of occasional juxtaposed colonies could interact to form fertile dikaryons that resemble in vigor the mutants being sought.

A dense suspension of spores was x-irradiated at a dosage of 180,000 R (100 kV, unfiltered; Norelco, model M 100) to give 95–98% mortality. The suspension of treated spores was then plated at a dilution to give 200–300 survivors per 90-mm plate. The plating medium was minimal (14) with nicotinic acid, and a total sample of 1.4×10^6 survivors was screened.

The initial screening depended upon the visual recognition of vigorously growing, dense colonies among the poorly growing *B-on* mutants. Only well-isolated colonies were selected, transferred to complete medium, and carefully examined microscopically. Those exhibiting a lack of sexual expression, *B-off* morphology, were retained for study.

These isolates were then subjected to test matings with a series of wild-type *B* factors to specify any alterations in the B-sequence (10). These test matings, as well as all crosses, opposed unlike *A* factors (henceforth omitted for simplicity); the A-sequence was thus always on. The first component of the B-sequence, nuclear migration, occurs independently of the A-sequence, but the second component of the B-sequence, hook-cell fusion, requires the prior achievement of the early stages of the A-sequence; effects upon hook-cell fusion can thus be determined only when the A-sequence is *on*. Whenever nuclear migration failed to occur in a test, the mating was nutritionally forced to determine the presence or absence of hook-cell fusion in the resulting heterokaryon.

The mating tests identified a frequent class of "modifier" mutations that lie outside the *B* factor and suppress the *B-on* morphology (7), four classes of secondary $B\beta$ mutations that had been previously reported (8–11), and secondary $B\beta$ mutations of new types. All isolates of the last category as well as numerous representatives of previously known types were then outcrossed with a strain having a wild-type *B* factor, and samples of 400–600 progeny of each cross were screened for *B-on* segregants. The absence of *B-on* progeny located each mutation in or very near the $B\beta$ locus. Test matings of the progeny confirmed the expected segregation of 1:1 for the parental mutant and wild-type *B* factors. A comprehensive series of tests was subsequently performed to analyze the effects of $B\beta$-dependent ($B\alpha$-*off*) and $B\alpha$-dependent ($B\beta$-*off*) interactions: in each case, the *B*-mutant strain (wild-type for the morphological marker *dm*) was mated with strains carrying (*a*) all known $B\beta$ alleles in association with the progenitor $B\alpha 3$ allele, and (*b*) known $B\alpha$ alleles in association with either the mutant $B\beta$ allele itself or a wild $B\beta$ allele with which the mutant allele could not interact.

Several spontaneous mutations from a large sample of untreated spores of a similar dikaryon were also characterized.

RESULTS

A wide variety of *B-off* isolates resulting from secondary mutations of the $B\beta 2(1)$ primary mutant allele were recovered among 1.4×10^6 survivors of x-irradiation. Reversion to the wild progenitor $B\beta 2$ allele represented one extreme, several *B-always-off* isolates the other extreme. Between these extremes were nine additional types of mutations that showed graded degrees of loss of function. All together, *B-off* isolates numbered 393; 300 were extra-*B* factor modifier mutants (7) (five of which were of a type previously unknown to be described elsewhere), 78 were secondary $B\beta$ mutants of the four types previously described (8–11), and 15 were secondary $B\beta$ mutants of six previously unknown types. The frequency of secondary $B\beta$ mutations here was 6.6×10^{-5} as compared to a frequency of 6.8×10^{-8} for primary mutations induced in $B\alpha$ and $B\beta$ by x-rays, as reported by Koltin (6).

From an untreated sample of 7×10^6 progeny, 134 *B-off* isolates were recovered. Of these, 121 were extra-*B* factor modifier mutants, 12 were secondary $B\beta$ mutants of the commonest known type, and one was a new type that also occurred in the x-irradiated sample.

Secondary $B\beta$ mutations were thus about 36 times more frequent in the treated than in the untreated sample.

The Secondary Bβ Mutations. The characterization of the various types of secondary $B\beta$ mutations depends upon their interactions with various wild-type *B* factors so constituted as to test allelic interactions at both $B\alpha$ and $B\beta$ loci. These interactions as well as the corresponding interactions of the original $B\beta 2$ allele and the primary mutant $B\beta 2(1)$ allele are detailed in Fig. 2. The number of mutations of the several classes in the sample of 1.4×10^6 survivors of x-irradiation and the status of the B-sequence of each class of mutants are also given.

With one exception, the secondary mutations share several features: They are *B-off* and thus have characteristic *B-off* morphology. They are totally nonreactive in all combinations with each other. When mated with the progenitor mutant $B\beta 2(1)$, nuclear migration, the first event of the B-sequence, fails to occur, but fusion of hook cells, the second event, is normal. The exception, $B\beta 2(1\text{–}0)$, is a revertant to the wild pregenitor allele, $B\beta 2$, and shares only the first of these characters. It recombines normally with $B\alpha$ alleles with a frequency of about 2% and forms *B* factors with different $B\alpha$ alleles that are indistinguishable from wild *B* factors with the same $B\alpha$–$B\beta$ constitutions.

$B\beta 2(1\text{–}3)$ is a repeat of a mutation previously reported

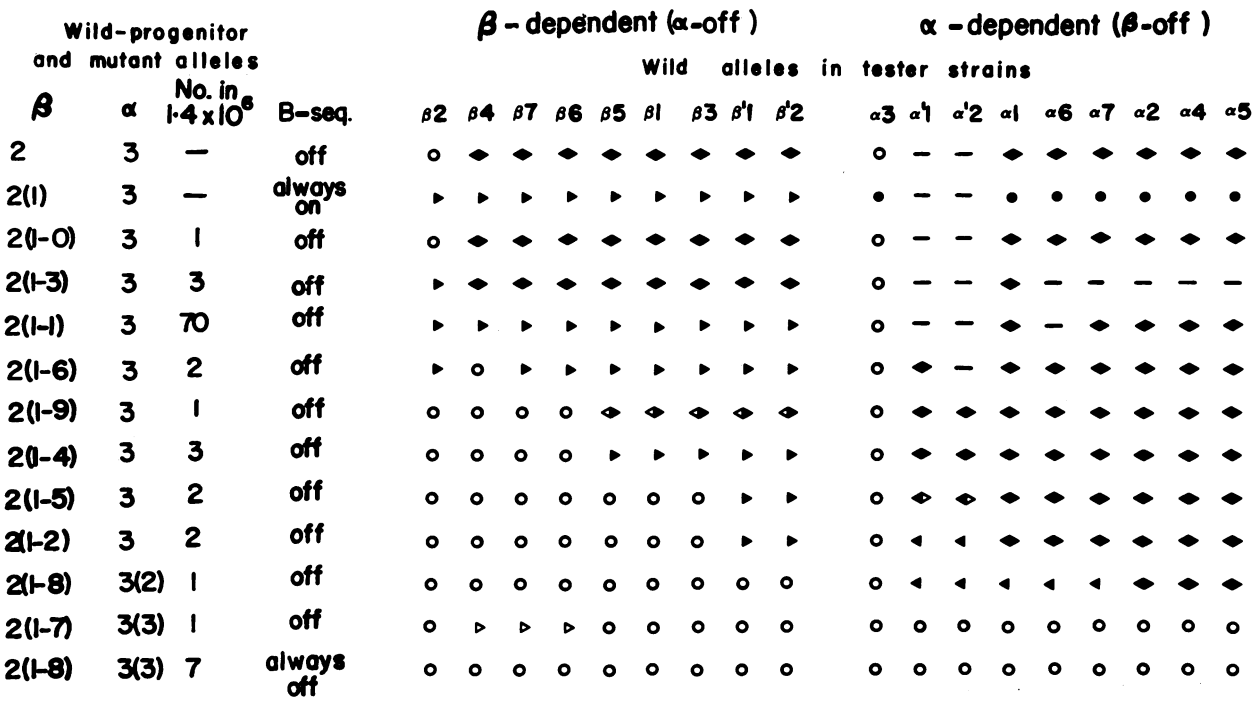

FIG. 2. Interactions of mutant and wild-type $B\alpha$ and $B\beta$ alleles. The wild progenitor B factor, B $\alpha 3$–$\beta 2$, illustrates the pattern of wild-B x wild-B interactions. The mutant factor B $\alpha 3$–$\beta 2(1)$ is the immediate progenitor of the mutations below. B-sequence (column 4)—Status in mutant strains; where *off*, the B-sequence can be turned on to the extent shown by interactions at right. Key to symbols—*Arrows* indicate nuclear migration: unilaterally to the right into tester, ▶ or ▷; unilaterally to left into mutant, ◀; bilaterally into mutant and tester, ◆, ◇, or ◈. *Circles* indicate no muclear migration, ● or ○. *Solid symbols* signify fusion of hook cells and formation of *B-on* heterokaryons; *open symbols*, unfused hook cells and formation of *B-off* heterokaryons. A *dash* indicates no test—. $B\beta$-dependent group—Interactions between mutant $B\beta$ and wild $B\beta$ alleles; requires $B\alpha$-*off* (see *text*). $B\alpha$-dependent group—Interactions between $B\alpha$ of mutant and $B\alpha$ of tester; requires $B\beta$-*off*. The classes of mutations as well as the series of $B\alpha$ and $B\beta$ alleles are so ordered to emphasize the gradations in loss of B-factor function.

(10). It is interactive with the wild progenitor $B\beta 2$ and differs from a new normal $B\beta$ allele only in its unilateral-donor behavior in nuclear migration when mated with $B\beta 2$. This mutation represents the closest approach yet achieved to the generation of a new allele equivalent to those found in nature—or conversely, a near approach to reversion to the original wild allele.

$B\beta 2(1\text{–}1)$, the most common of the mutations, was also the first to be reported (8). This mutation shares with $B\beta 2(1\text{–}3)$ the ability to interact with all $B\beta$ alleles but is unilateral-donor in all $B\beta$-dependent interactions.

The next five mutations listed in Fig. 2, $B\beta 2(1\text{–}6)$, $B\beta 2(1\text{–}9)$, $B\beta 2(1\text{–}4)$, $B\beta 2(1\text{–}5)$, and $B\beta 2(1\text{–}2)$, have the ability to discriminate among wild $B\beta$ alleles, a feature earlier described for $B\beta 2(1\text{–}2)$ (15) and $B\beta 7(1\text{–}4)$ (11). The patterns of reaction–nonreaction indicate relative differences in specificities of the wild $B\beta$ alleles: note the bilateral nuclear migration–unilateral hook-cell fusion pattern in $B\beta$-dependent interactions of $B\beta 2(1\text{–}9)$ as compared to the strictly unilateral nuclear migration *plus* hook-cell fusion of $B\beta 2(1\text{–}1)$, $B\beta 2(1\text{–}6)$, $B\beta 2(1\text{–}4)$, $B\beta 2(1\text{–}5)$, and $B\beta 2(1\text{–}2)$. The latter two are of particular interest in that $B\alpha$ function appears to be affected; this effect, however, must result from some impairment associated with the mutant $B\beta$ allele, as $B\beta 2(1\text{–}2)$ associated by recombination with several wild $B\alpha$ alleles elicits the same specific responses (15).

Effects of the last three mutations extend over the entire B factor and their location could not be resolved by recombination. $B\alpha 3(2)$–$\beta 2(1\text{–}8)$ gives no evidence of any function attributable to $B\beta$, and $B\alpha$ function also appears altered to the extent that it interacts unilaterally with certain members of the series of $B\alpha$ alleles. The lesion thus appears to involve both genes of the B factor. A combined sample of 1025 progeny of four crosses between this mutant and wild strains yielded no $B\alpha$–$B\beta$ recombinants in contrast to about 2% recombinants in crosses involving the same wild B factors with the progenitor B factor, $B\alpha 3\text{–}2(1)$. The $B\alpha$-effect cannot be rigorously located in the $B\alpha$ locus, but the failure to obtain $B\alpha$–$B\beta$ recombinants and the altered $B\alpha$-dependent interactions most likely reflect a primary mutation in the $B\alpha$ locus, $B\alpha 3(2)$, unlike the primary mutation $B\alpha' 1(1)$ described earlier (6).

B $\alpha 3(3)$–$\beta 2(1\text{–}7)$ reveals no $B\alpha$ function and has very restricted $B\beta$ function: it is able only to donate nuclei to three $B\beta$ alleles, but no fusion of hook cells follows. The result of $B\beta$-dependent interactions is thus the formation of typical *B-off* heterokaryons. Its location in the B factor was resolved in a mating with the progenitor $B\beta 2(1)$ mutant (see below).

B $\alpha 3(3)$–$\beta 2(1\text{–}8)$ has no detectable $B\alpha$- or $B\beta$-function; it is nonreactive in matings with all wild strains and, in nutritionally forced matings, yields only *B-off* heterokaryons. Forced matings with the primary mutant $B\beta 2(1)$ with its consitutive B-sequence, however, result in hook-cell fusion in the heterokaryon and completion of the life cycle. A summed sample of 675 progeny of crosses between all seven mutants of this class and $B\beta 2(1)$ mates yielded *only B-always-off* and

Bβ2(1)-always-on isolates. Segregation for the parental *A* factors was normal. *B-off* to *B-on* segregants occurred in a ratio of 1:1 in only a single cross; it averaged 1:2 in all crosses. Germination of spores was poor, 45–94% (average 69%), as compared to 85–98% (average 90%) in crosses involving other secondary mutants.

A selective system was developed for one of the two possible reciprocal products of *Bα–Bβ* recombination: Mass samples of 10,000–15,000 progeny per plate from the cross *B α3(3)–β2(1–8)* x *B α1–β2(1)* were screened for evidence of nuclear migration into the mutant tester *B α1–β2(1–2)*. The tester will not accept nuclei from either of the parental strains, but it should accept nuclei from any strain carrying a functional *Bα* other than *Bα1* in association with *Bβ2(1)*. No evidence for any functional *B* factor other than the parental *B α1–β2(1)* was found in a sample totalling 116,000 progeny from crosses involving two different *B α3(3)–β2(1–8)* mutants. It was concluded either that no recombination had occurred or that the *Bα* of the secondary mutant was inactive. This suggests a third type of primary mutation, *Bα3(3)*, which because of a like phenotype re *Bα*-dependent function, is designated as being present also in the preceeding mutant, *B α3(3)–β2(1–7)*.

Resolution of the Commonest Secondary Bβ mutation? One mutation that appears to belong to the *Bβ2(1–1)* group has been resolved structurally into two parts: *Bβ2(1)* and *Bβ-functionless* or *f*. The *f* mutation blocks *Bβ*-control of the B-sequence and may be characterized as follows:

(*a*) In association with the primary mutation *Bβ2(1)*, it elicits *B-off* morphology and precisely duplicates the total range of interactions of the commonest secondary mutation, *Bβ2(1–1)*, with all wild *Bβ* alleles.

(*b*) It is separable from *Bβ2(1)* by recombination at an overall frequency of 0.8% (14/1685). The association of parental specificities for *Bα* and *Bβ* in recombinants for *Bβ-f* locates the site for *f* distal to *Bβ* specificity.

(*c*) The mutation has no effect upon the specificity of wild *Bβ2* but imposes unilateral-donor behavior in matings of *Bβ2* with all other wild *Bβ* alleles. Thus in all cases where activation of the B-sequence is dependent upon *Bβ–Bβ* interaction, *f* blocks the acceptance of nuclei in mating.

(*d*) When homozygous, the *f* mutation completely blocks interaction between two otherwise interacting *Bβ* alleles, e.g., *Bβ2(1)* ▶ *Bβ2*, *Bβ2(1) f* ▶ *Bβ2*, and *Bβ2(1)* ● *Bβ2 f* are interactive as shown, but *Bβ2(1) f* ○ *Bβ2 f* do not interact (symbols as in Fig. 2).

(*e*) The mutation *f* has no effect when B-sequence activity is dependent upon *Bα–Bα* interaction. When *Bβ* is inactive, as in *Bβ2 f* x *Bβ2(1) f*, interaction between two *Bα* alleles results in normal bilateral mating. The action of *f* is thus *Bβ*-locus specific.

(*f*) All efforts to reassociate the *f* mutation with *Bβ* alleles other than the progenitor *Bβ2* have been unsuccessful, and only a single recombinant reassociated the mutation with any wild *Bβ* allele.

Bβ2(1) f x *Bβ2* ---→ 5 *Bβ2(1)* and 1 *Bβ2 f* in a sample of 267,

Bβ2(1) f x *Bβ1,4*, or *6* ---→ 7 *Bβ2(1)* and 0 *Bβ1 f*, *Bβ4 f*, or *Bβ6 f* in 1139,

Bβ2 f x *Bβ6* ---→ 1 *Bβ2* and 0 *Bβ6 f* in 279.

The *f* mutation defines a gene or region of *Bβ* required for nuclear-acceptor function and is separable from the mutated site(s) that specifies the *Bβ2(1)* phenotype. The absence of recombinants of *f* with wild *Bβ* alleles other than *Bβ2* suggests that *f* may be allele-specific as well as locus-specific.

DISCUSSION

The regulation of sexual morphogenesis in *Schizophyllum* has been described as an eukaryotic regulatory system with the genes of the *A* and *B* incompatibility factors serving as master switching genes or the *regulatory component*, and many genes scattered throughout the genome, recognizable in impairment as "modifier" mutations, comprising the *regulated component* (2). The results detailed above add substantial evidence for the regulatory role of one of the master switching genes, *Bβ*.

The several types of *Bβ* mutations are interpreted as genic changes that alter various bits of information normally provided by wild alleles to control the events of morphogenesis. These alterations reveal the gene to be complex, and they demonstrate four controlling functions:

(*a*) *Specificity*—The first described and essential attribute of each wild-type *Bβ* allele is its ability to recognize self versus nonself: each *Bβ* allele alone results in *B-off* phenotype and interacts with other wild *B* alleles to elicit the entire B-sequence and the *B-on* phenotype.

In contrast to this, the primary mutation *Bβ2(1)* is *B-always-on* and interacts with all wild *Bβ* alleles. The primary mutant allele thus lacks specificity. At the other extreme, the secondary mutation *Bβ2(1–8)* is *B-always-off* and incapable of interacting with any wild *Bβ* alleles to turn *B-on;* it also lacks specificity.

The other secondary mutations possess different degrees of specificity. *Bβ2(1–0)* has regained the specificity of the progenitor allele *Bβ2*. The specificities of *Bβ2(1–1)* and *Bβ2-(1–3)* are different from that of the progenitor *Bβ2*, but whether they are the same or different is uncertain. That they will not interact may be dependent either on shared specificity or on the mutual loss of another function (see below). Mutation *Bβ2(1–6)* does not interact with *Bβ4* and might appear to have acquired the specificity of that allele; the alternate view that it has instead lost that part of its own function or specificity required to interact with *Bβ4* would appear the more likely in view of the next four classes, *Bβ2(1–9)*, *Bβ2-(1–4)*, *Bβ2(1–5)*, and *Bβ2(1–2)*. This series of mutations exhibits a progressive loss of ability to interact with specific wild alleles in the order *Bβ4* - - - - *Bβ'2*, as listed in Fig. 2.

(*b* and *c*) *Nuclear migration, acceptor- and donor-function*—The second and third functions of the *Bβ* gene relate to the exchange of nuclei in mating, this exchange being reciprocal between wild mates. Inspection of Fig. 2 clearly shows the mutations to affect both of these functions.

Limited competence to accept nuclei is characteristic of the primary mutant *Bβ2(1)*. It is uncertain, however, whether this is a direct attribute of the mutation or an indirect effect imposed by the morphology of the mutant. The state of continuous nuclear migration within the mutant strain probably blocks the efficient migration of nuclei from a mate. One observation shows that the *Bβ2(1)* mutation does not directly prohibit acceptance of nuclei in matings: Three modifier mutations are known (C. A. Raper, unpublished) that suppress the *B-on* morphology of *Bβ2(1)* and permit reciprocal nuclear migration in mating but leave its lack of specificity unchanged.

The secondary mutants all have *B-off* morphology, and the direct effects of the mutations on nuclear migration can be observed. A site specific for acceptor-function in nuclear migration and expressed only in *Bβ*-dependent interactions is apparent in the mutation designated *Bβ-f*, which blocks acceptor-function. It behaves as an integral part of the *Bβ* gene, is separable by crossover from the part(s) of the gene that determine(s) specificity and *B-on*ness, and probably accounts for the most common type of secondary *Bβ* mutation, *Bβ2(1-1)*. This site may also be affected in the several other secondary mutants that are unable to accept nuclei in *Bβ*-dependent interactions. *Bα* must also have such a site specific to it, although there is no mutative evidence of it.

Donor-function in nuclear migration may be shared by *Bα* and *Bβ*. The behavior of the mutant *B α3-β2(1-2)* suggests this: in *Bβ*-dependent interactions it acts as a donor in two cases and as an acceptor in none, whereas in *Bα*-dependent interactions it acts as an acceptor in all cases but fails as a donor in two cases. This behavior could be explained by the loss of *Bβ*-acceptor function, retention of *Bα*-acceptor function, and impairment of *Bα*- and *Bβ*-donor function. The latter may have a common basis in *Bα*-and *Bβ*-dependent interactions, as the segregation of donor-impairment with the *Bβ* mutant allele suggests the impaired site to lie close to or in *Bβ*. The mutant *B α3(2)-β2(1-8)* might represent a more extensive lesion including all of *Bβ* and extending into the *Bα* region. Like *B α3-β2(1-2)*, it has retained its *Bα*-acceptor function, but the impairment of donor-function is more severe and is again expressed differentially; the lack of recombination between this mutant and wild *B* factors suggests a large deletion.

(*d*) *Fusion of hook cell*—Hook-cell fusion occurs only as a corollary of nuclear migration, except in forced matings involving the primary *B-always-on* mutant. In the absence of nuclear migration, all forced matings involving *B-off* mutants result in no hook-cell fusion. In three cases, *Bβ2(1-9)*, *Bβ2-(1-5)*, and *Bβ2(1-7)*, there is nuclear migration into mutant or wild mates with no ensuing fusion of hook cells. Nuclear migration thus appears to be a prerequisite for hook-cell fusion but does not insure its subsequent occurrence.

The evidence presented here reveals these four functions to reside in separately mutable sites in the region of the *Bβ* gene. One of the functions, acceptance of nuclei, appears epistatic to another, hook-cell fusion, and is structurally separable from the specificity-function. A fourth function, donation of nuclei, has not been demonstrated to be separable from *Bβ* but may extend into the *Bα* region.

The nature of the alterations represented by *Bα3(2)* and *Bα3(3)* deserves brief comment. Although they cannot be rigorously located in *Bα*, they appear to be primary mutations and the first to be described in any of the four incompatibility genes that have not effected an *off* $\longrightarrow$ *on* change of phenotype. The change in the expression of the *Bα* gene is from *B-off* because of self-recognition to *B-off* due to partial or total loss of specificity and function. This type of mutant would not have been recovered in earlier selective systems, and their recovery here was incidental to their linkage to *Bβ*. The present selective system was based on the change *B-on* $\longrightarrow$ *B-off* due to *Bβ*-function, and the recovery of *B-always-off* mutants due to malfunction of *Bα* was fortuitous.

The simplest explanation for the loss of function of *Bα* in one mutant class, of *Bβ* in a second, and of both *Bα* and *Bβ* in a third would appear to be deletions. The entire *B* factor region would be missing in the latter class, perhaps with additional segments of varying lengths of contiguous chromosome. The *Bα–Bβ* interval may carry no indispensable genes, and x-ray-induced mutations would be expected to include gross chromosomal aberrations. The low frequency of *B-always-off* survivors in the progeny of some outcrosses and the apparent lack of recombination in these crosses could well result from deletions. Less extensive deletions than those indicated here have been postulated in the *Bα–Bβ* region as affecting the frequency of recombination in certain wild-type *B* factors (16).

The mode of action of the incompatibility genes, of which *Bβ* is the best known genetically, remains a matter of conjecture. An earlier repressor model—*Bα* and *Bβ* genes acting together to repress a constitutive B-sequence and *Bα* or *Bβ* alleles each interacting with other alleles of the same series to derepress the B-sequence (1)—now appears inadequate to rationalize the variety of effects of known *Bβ* alleles. Repression may be involved, but there is no evidence of it. A more recent hypothesis casts dimeric proteins as controlling agents but does not specify the manner of their action (17).

The array of *Bβ* mutations provides several hints that the overall action of the gene is positive rather than negative: (*a*) inactivating mutations should be more common than mutations to continuous function; the *B-off* secondary mutations are more frequent than the *B-always-on* primary mutations by a factor of a thousand times and thus more probably represent degrees of inactivation. (*b*) The *B-always-on* mutation is dominant in all respects to all wild *Bβ* alleles. (*c*) The *B-always-on* mutation is also dominant to the *B-always-off* mutation with respect to fusion of hook-cells. These facts are consistent with continuous function of the *B-always-on* mutation and the failure of any function of the *B-always-off* mutation, but not with the reverse. This reasoning suggests that the control exerted by *Bβ* is positive.

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