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Evidence for a new kind of regulatory gene controlling expression of genes for morphogenesis during the cell cycle in *Ustilago violacea*

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SUMMARY

The first part of the paper provides strong supportive evidence for the previous findings (Cummins & Day, 1973; Day & Cummins, 1973) that the two alleles of the mating-type locus of the basidiomycete *Ustilago violacea* have different periods of inducibility during a cell cycle, and that the cell cycle characteristics of each allele are maintained in freshly isolated diploids. This difference in temporal properties of the alleles appears to be the basis of the dominance of allele a_2 as it is inducible during a phase of the cell cycle when allele a_1 is non-inducible. During G_1 both alleles appear to be inducible and apparently 'neutralize' each other so that the cell cannot mate.

The second part of the paper provides evidence for a unique genetic control mechanism. The evidence suggests that the period of cell cycle inducibility of a locus governing a morphogenetic pathway may be regulated by a separate control gene the *cc* locus, with two known alleles cc^{str} (a stringent or restricted period of inducibility) and cc^{rel} (a relaxed or non-restricted period of inducibility). This hypothesis stems from analysis of a diploid that was $a_1.cc^{str}/a_2.cc^{rel}$ and showed dominance of allele a_2 during the S and G_2 phases when freshly isolated, but which became incapable of mating after a period of subculturing. Analysis of haploids derived from this diploid strain showed that both mating-type alleles were functional but that it was now homozygous for cc^{str}, i.e. of genotype $a_1.cc^{str}/a_2.cc^{str}$. Thus the temporal and functional aspects of the mating type alleles are determined by different loci. It is postulated that cell cycle control loci may be widespread and serve to regulate the action of genes concerned with morphogenesis in relation to other cell cycle events.

1. INTRODUCTION

In recent years increased attention has been paid to the temporal control of gene activity during the cell cycle. While some genes appear to be transcribed continuously throughout the cell cycle, most genes appear to have limited periods of activity at certain stages of the cycle (see Mitchison, 1971). Two main hypotheses to account for such periodic synthesis of enzymes have been developed – the 'oscillatory repression' model (Masters & Pardee, 1965; Goodwin, 1966; Donachie

& Masters, 1969) and the 'sequential transcription' model (Halvorson, Carter & Tauro, 1971).

We have reported elsewhere preliminary observations on the activity of the alleles of the mating-type locus of *Ustilago violacea* that are difficult to explain on the basis of either of the above models (Cummins & Day, 1973; Day & Cummins, 1973). These observations are that the alleles of this locus are under *different* cell cycle controls, allele a_1 being active (i.e. inducible) only in G_1 (a stringent control) while allele a_2 is active during the whole cell cycle (a relaxed control). Furthermore these cell cycle controls appear to be allele specific so that each allele is regulated by its individual control system in heterozygous diploids. In view of the potential importance of these conclusions to the understanding of the nature of cell cycle controls, the first part of this paper provides confirmatory evidence using different experimental approaches from those reported previously. In the second part of this paper, a mutant cell cycle control system is described and we postulate the existence of a new locus (cell cycle control locus – *cc* locus) that regulates the time during the cell cycle that sexual morphogenesis may be induced.

Conjugation in this species involves at least five developmental steps leading to the formation of a tube or bridge, the copulatory organelle, between two cells of opposite mating type (Poon, Martin & Day, 1974). These developmental steps are under the control of the two mating-type alleles designated a_1 and a_2. Assembly of this organelle occurs *after* transcription and translation of 'sex message' (mating-specific messenger RNA) from both partners (Day & Cummins, 1974; Cummins & Day, 1974*a*) and we have postulated that at least some of these gene products may be exchanged between the cells through fimbrial connexions between the intact cells prior to the formation of the conjugation tube (Day & Poon, 1975).

2. MATERIALS AND METHODS

(i) *Stocks and culture conditions*

The stocks used in this study were the same auxotrophic stocks, 1.C2 and 2.716 described in Day & Cummins (1974). Homozygous and heterozygous diploids derived from these haploid stocks were obtained by the method of Day (1972*a*). The culture conditions are described in Day & Cummins (1974). The production and analysis of sexual diploid brandspores from diploid sporidia was carried out by the method described in Day & Jones (1969).

(ii) *Methods of synchronization*

Although the method of Williamson & Scopes (1962) has been used with this organism (Day & Jones, 1972) it is time-consuming and three more satisfactory methods have been developed.

(*a*) *Stationary phase outgrowth.* A culture obtained by inoculating 1 ml of late log or stationary phase cells into 50 ml complete medium reaches a stationary phase in about 3–4 days. Cells taken from such a culture on the 4th or 5th day are 90% unbudded and grow out synchronously when inoculated at about 10^6 cells/ml into

fresh complete medium. Diploid cells seem to be more synchronous in their outgrowth than haploid cells. The cell cycle under these conditions is typically about 5 h in duration.

(*b*) *Sucrose gradient top fractions*. About 2 ml of a log phase population of cells were pipetted carefully on top of 50 ml of a sucrose gradient. The gradient was centrifuged at 3000 rev/min for 5–7 min, after which time the cells were suspended over most of the gradient. The top fraction (5–10 ml) was drawn off and used to inoculate a 50 ml flask of fresh complete medium. The cells in this fraction are about 85–90 % unbudded and grow out synchronously in complete medium with a cycle time of 6–8 h.

(*c*) *Sucrose gradient centrifugation in a reorienting zonal centrifuge*. This proved to be the best method for obtaining both good synchrony and high yield of synchronized cells. A Sorvall SZ-14 zonal rotor was loaded with 1200 ml of a 15–50 % sucrose gradient while it was spinning at 2500 rev/min. Fifty ml of log phase cells were added to the top of the gradient and it was allowed to continue spinning for 7·5 min. At this time the rotor was decelerated slowly over a period of at least 10 min. The gradient was collected in about 50 fractions of 24 ml. The cells were found to be distributed throughout the gradient, with the heavier large-budded G_2 and mitotic cells near the bottom of the gradient, the small-budded S phase cells in the middle, and the unbudded G_1 cells near the top. The percentage budding in different samples across the gradient ranged from 5 to 10 % at the top to 90–95 % at the bottom. After collection of the samples, the sucrose was diluted with water and the cells harvested by centrifugation. The cells were washed twice, adjusted in concentration, and mixed with an equal number of cells of the tester mating-type. The mixed cells were then centrifuged, and the pellet was resuspended in a droplet of water and spread on water agar to permit conjugation (Day & Cummins, 1974). Tester cells were generally 4-day-old stationary phase cultures of haploid cells of opposite mating-type.

(iii) *DNA measurements*

The methods for the determination of the time of DNA synthesis by following the uptake of labelled adenine are described in Cummins & Day (1974*a*).

Estimates of the DNA content/10^8 cells in log and stationary phase cultures were carried out as described previously (Day, 1972*a*) except that the assay of Burton (1956) was used.

3. RESULTS

(i) *The cell cycle in* Ustilago violacea

The uptake of labelled adenine into DNA was followed during the synchronous growth of top fraction cultures of a_1 and a_2 haploids as well as a_1a_1, a_1a_2 and a_2a_2 diploids. The percentages of budded cells and cells in mitosis were followed in aliquots stained with acridine orange and examined under a fluorescence microscope (Poon & Day, 1974). Fig. 1 shows a typical result for the a_1 strain. The duration of the cell cycle varied between 5 and 8 h in different experiments, this being almost entirely due to variations in the G_1 period from 1·5 to 4·5 h. The S period

commenced at about the time of bud initiation and had at a constant duration of about 2 h. The length of the G_2 period is difficult to estimate as the onset of prophase cannot be ascertained easily, but the total duration of G_2 and mitosis was about 1 h. There was a post-mitotic G_1 period of approximately 30 min before cytokinesis was completed and the bud released.

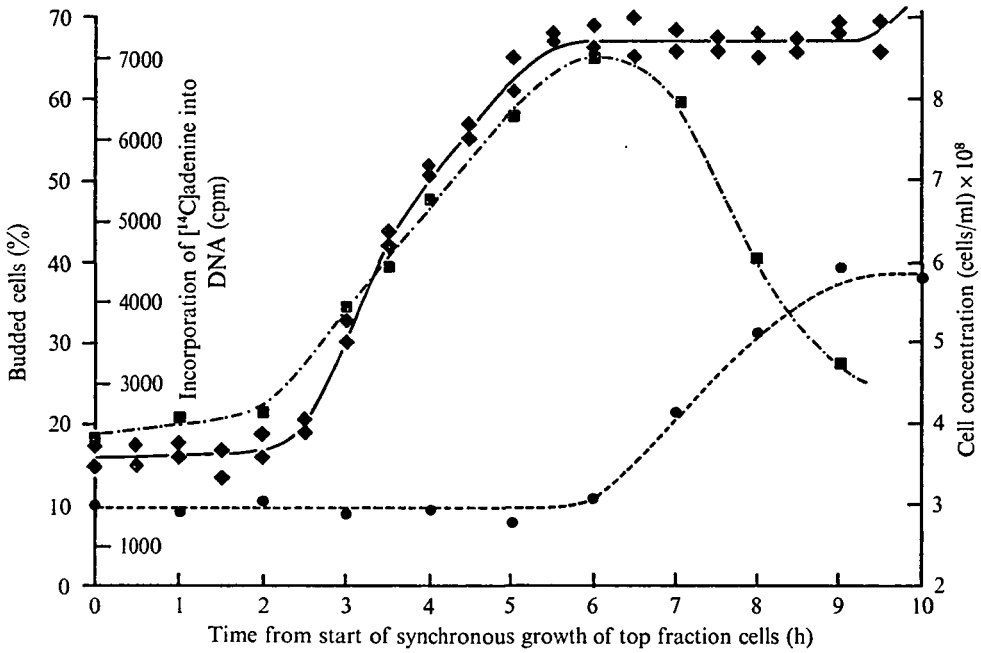

Fig. 1. The cell cycle in *Ustilago violacea*. Cells of an a_1 culture synchronized by the top fraction method were allowed to grow in complete medium and samples were taken at regular intervals to determine the cell number (●), the percentage of cells with buds (■) and the uptake of ^{14}C-labelled adenine into the DNA (◆).

The stage of the cell cycle at which stationary phase cells are arrested is critical to the arguments developed below and we have therefore examined this carefully. Chemical determination of DNA levels demonstrates that log phase cells have on average 20–30 % more DNA than stationary phase cells (Table 1).

Experiments utilizing the synchronous outgrowth of stationary phase cells show clearly that a round of DNA synthesis occurs before the first mitotic division. This result has been obtained with haploid strains of both mating types, as well as with an a_1a_1 diploid (Fig. 3) and with an a_1a_2 diploid (Day & Cummins, 1973). These results therefore show that the stationary phase cells are arrested in the G_1 condition.

(ii) *Cell cycle control of mating in haploid strains*

Log phase populations of each mating type were synchronized separately by centrifuging in two sucrose gradients. The top fraction of each gradient consisting of predominantly G_1 cells, was collected, washed twice and divided into two fractions. One fraction of each population was stored at 4 °C as a suspension in

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Table 1. *The DNA content of sporidia of* Ustilago violacea *at different stages of growth*

Culture	Budding (%)	Phase	DNA ($\mu g/10^8$ cells)	Ratio DNA content to mean haploid value in S.P.
a_1 haploid	16·1	Early S.P.	4·90	1·02
	46·9	L.P.	6·08	1·27
a_2 haploid	4·3	S.P.	4·70	0·98
	44·9	L.P.	5·81	1·21
Diploid a_1a_1	< 1	S.P.	10·1	2·1
Diploid a_2a_2	< 1	S.P.	9·62	2·0
Diploid a_1a_2	< 1	S.P.	8·76	1·83

L.P. = log phase. S.P. = stationary phase.

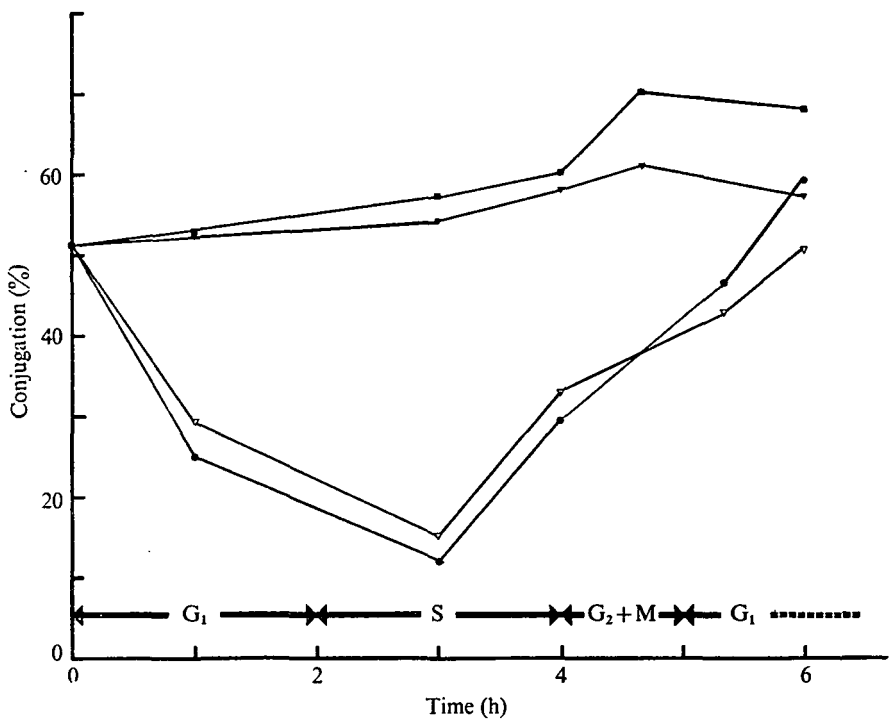

Fig. 2. Conjugation in 'out of phase' cultures of *U. violacea*. The percentage conjugation was compared in combinations involving top fraction synchronized cells held in the G_1 phase for the whole period of time, and cells which were passing through the cell cycle as indicated at the bottom of the figure. The combinations shown are: *a_1 cells in G_1 × a_2 cells in G_1* (▼); *G_1 a_1 cells × actively growing a_2 population* (■); *actively growing a_1 population × a_2 cells in G_1* (●); *actively growing mixture of a_1 and a_2 cells* (co-synchronous or 'in phase)' (▽).

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water, while the other fraction was inoculated into liquid complete medium and incubated at 20 °C. The fraction stored in water at 4 °C neither budded nor replicated its DNA during storage for over 10 h. However, the ability of such cold stored cells to mate remained constantly high over this period. The fraction of cells incubated in complete medium budded synchronously and passed through a somewhat extended cell cycle in 8 h. At intervals during their growth samples were mated with equal numbers of cells from either the synchronous population, or the cold-stored population of the opposite mating-type (Fig. 2).

Thus it was possible to compare the percentage conjugation in cultures mated in phase with those mated out of phase. An examination of Fig. 2 shows clearly that a_2 cells at any stage in their progress through the cell cycle mate well with cold-stored (G_1) a_1 cells. On the other hand, a_1 cells as they progress through S and G_2 lose the ability to mate with either cold-stored (G_1) a_2 cells or actively growing a_2 cells. The stringent nature of the control of the a_1 allele, and the relaxed control of a_2 are thus confirmed.

(iii) *Cell cycle controls in homozygous diploid strains*

Stationary phase cultures of a_1a_1 and a_2a_2 diploids were inoculated separately into flasks of liquid complete medium. Samples from the synchronous populations were taken at intervals and mated with log phase haploid cultures of the opposite mating type. Parallel cultures were samples to determine the degree of synchrony (percentage budding, and rate of incorporation of labelled adenine). The results presented in Fig. 3 show that the a_1a_1 diploid, like the a_1 haploid, has a stringent cell cycle control and mates only during G_1 while the a_2a_2 diploid, behaves similarly to the a_2 haploid and maintains its ability to mate throughout the cell cycle (a relaxed control).

(iv) *Cell cycle controls in the heterozygous diploid*

Previous observations of log phase heterozygous diploids synchronized in the zonal rotor demonstrated that they mate only in S and G_2 and are inactive during G_1 and stationary phase (Day & Cummins, 1973). Further evidence supporting the conclusion that a_1a_2 cells are inactive in G_1 was obtained by the use of co-synchronous mixed populations of a_1 and a_1a_2. Such cells did not mate at any time during their progress round the cell cycle, although an asynchronous mixed population mated with high frequency. Such a result would be expected if a_1 cells mate only in G_1 and a_1a_2 cells only in S or G_2. Finally light-microscopic observations of the mating cell show that most of the cells of an a_1a_2 random population that initiate conjugation are budded and therefore have commenced DNA replication.

(v) *Mutant cell cycle controls defining the* cc *locus*

One fresh isolate of an a_1a_2 diploid originally mated with a_1 partners during its log phase of growth. After about 4 transfers over a period of 6–8 weeks, very few cells ($< 5\%$) could mate with the haploid a_1 partner. This neutral culture appeared

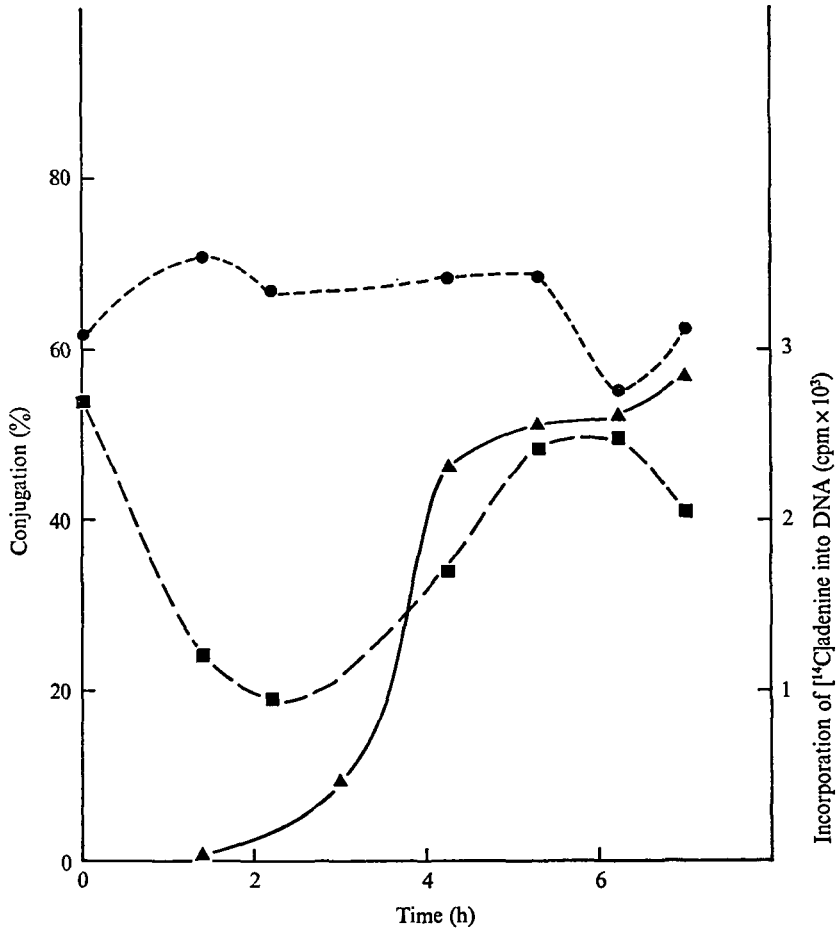

Fig. 3. The effect of the cell cycle on conjugation in diploids of *U. violacea* homozygous for mating type. The percentage conjugation with cells of the opposite mating-type was followed during the synchronous outgrowth from stationary phase of a_1a_1 (■) and a_2a_2 (●) diploids. The rate of incorporation of [^{14}C]adenine into DNA in the a_1a_1 diploid is shown in the remaining curve (▲). Mitosis was observed to take place in the a_1a_1 culture at about 5·5–6 h.

to be still diploid by other criteria such as cell size, colony morphology and solopathogenicity (i.e. capability as a pure culture to produce diploid sexual spores in a host plant) (Day & Jones, 1968). To discover whether this neutral strain was truly sterile, i.e. had lost function at one or both of its alleles, or whether it had an altered cell cycle control system, it was inoculated into a host plant to produce the sexual brandspores. The haploid segregrants produced after a meiotic division in these brandspores were collected and analysed firstly for mating-type and secondly for cell cycle control properties.

It was found that all haploid sporidia derived from these neutral diploid strains were capable of mating and that both mating types were equally represented. Thus the neutral diploid was heterozygous for two fully functional mating type alleles.

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Cultures of each of two randomly chosen cultures from both the a_1 and a_2 haploid segregants of the neutral diploid were synchronized in a sucrose gradient in the zonal rotor and the different fractions were tested for mating ability with appropriate standard cultures of the opposite mating-type (Fig. 4).

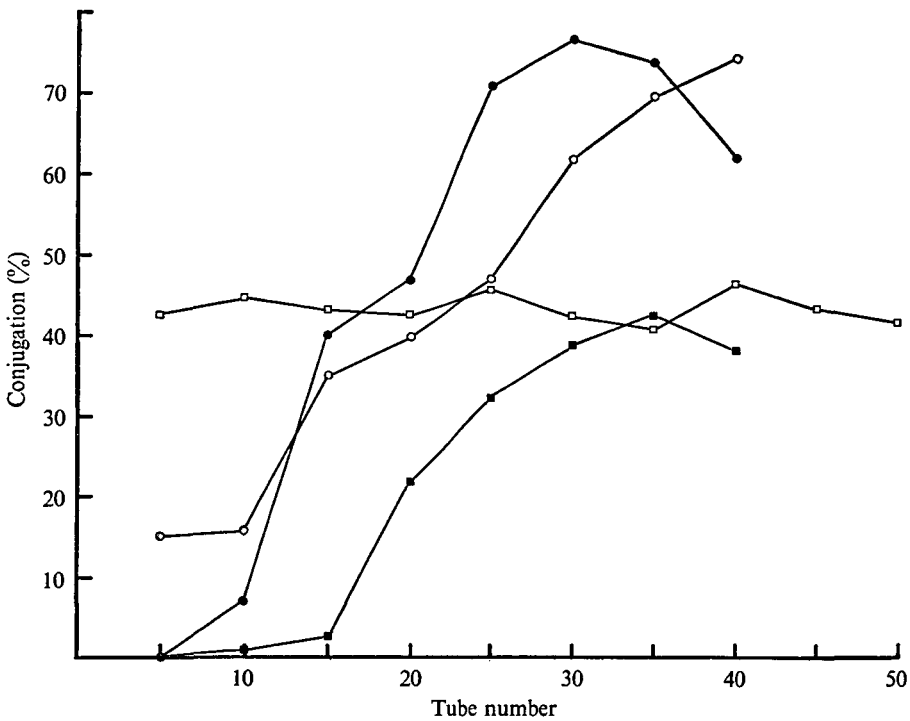

Fig. 4. The effect of the cell cycle on conjugation in wild-type haploid stocks and in haploids obtained following meiosis in a 'neutral' diploid. The following stocks were synchronized by zonal rotor centrifugation and the resulting fractions were tested for mating with cells of the opposite mating type. Stock 1.C2 (original haploid a_1) (○); stock 2.716 (original haploid a_2) (□); a_1 haploid derived from neutral diploid originally synthesized from 1.C2 × 2.716 (●); a_2 diploid derived from same neutral diploid (■).

Both a_1 cultures tested retained their stringent control and did not differ in any way from the standard wild type and auxotrophic stocks described earlier (Cummins & Day, 1973). However, both a_2 cultures mated only in the G_1 phase of the cell cycle, and therefore were now associated with a stringent control, quite different from the relaxed control of the original a_2 cultures. Microscopic observation of these two strains and four other randomly chosen a_2 strains derived from the neutral diploid confirms this conclusion, as it was found and that they only mated in the unbudded G_1 condition.

Thus it is clear that during the growth of a freshly isolated a_1a_2 diploid an event occurred which resulted in a change of the a_2 control system from relaxed to stringent. As a further confirmation of these conclusions diploid cultures were

resynthesized from the haploids derived from the sterile diploid, i.e. a_1 (stringent control) and a_2 (stringent control). As expected, these diploids were neutral (less than 5 % mating with a_1 haploids) even when freshly isolated.

(vi) *The frequency of the change to a 'neutral' condition in a_1a_2 diploids*

Sixteen fresh a_1a_2 diploids were isolated and incubated to stationary phase for six successive cycles in complete medium over a period of several weeks. Log phase samples from each subculture of the 16 separate cultures were tested for mating-type. Twelve of the cultures remained constant in their mating behaviour for the whole period, and retained their high mating ability while in the S and G_2 phases of the cell cycle. Three other cultures were found to be more or less neutral from the start of the experiment, while the remaining culture declined in mating activity from about 30 % in the first cycle of subculturing to 2 % by the last cycle.

Six old diploid cultures synthesized from 2 to 5 years ago and maintained as dried cultures in bottles containing silica gel were also tested for mating-type. Two of these were found to be neutral, the rest had normal S and G_2 mating activity. Finally, one fresh isolate of the a_1a_2 diploid was kept in continuous log phase for a period of 6 days (about 40 generations) in a New Brunswick Chemostat. Samples were tested for mating activity at different times during this period. The mating activity remained constantly high (about 70 %) for the duration of the experiment.

4. DISCUSSION

The cell cycle of *U. violacea* consists of a G_1 phase of variable duration (minimum time 1·5 h) followed by an S phase of 2 h which begins at the time of bud initiation. The G_2 period is probably less than 30 min in duration and is followed by a mitotic division occupying approximately 45 min, depending on estimates of when prophase begins (Poon & Day, 1974), and a post mitotic G_1 phase of up to 30 min before cytokinesis separates mother and daughter cells. During stationary phase the cells are clearly arrested in G_1 as the DNA content is lower than in log phase cell and there is a round of DNA replication before they enter their first mitotic division during outgrowth in fresh medium. It is interesting to note therefore that the cell cycle of *U. violacea* is much more similar to that of *Saccharomyces cerevisiae* (Williamson & Scopes, 1960) than that of another species of *Ustilago*, *U. maydis*, where the stationary phase cells appear to be arrested in G_2 (Esposito & Holliday, 1964). The observations on the cell-cycle control of mating in haploids, homozygous diploids and heterozygous diploids provide firm experimental support for the hypothesis put forward previously by Cummins & Day (1973) and Day & Cummins (1973) that:

(i) the alleles of the mating-type locus in wild haploid stocks of *U. violacea* are under *different* cell-cycle controls, allele a_1 being restricted in expression to the G_1 phase (a stringent control) while allele a_2 is capable of mating at any phase of the cell cycle (a relaxed control);

(ii) that these cell-cycle controls are allele specific and operate independently

even in heterozygous cells. The cell cycle controls may be *cis* dominant as they only affect the mating type allele to which they are coupled;

(iii) that during G_1 in a heterozygous cell both alleles are active and in some way 'cancel out' so that the cell is incapable of mating (i.e. neutral);

(iv) that the dominance of allele a_2 in a heterozygous diploid is a direct result of the relaxed control of allele a_2 as this allele has a longer period of activity than allele a_1. Taken with point (iii) this explains why heterozygous diploids are neutral in G_1 and mate with a_1 cells during the rest of the cell cycle (see Fig. 5).

The observations reported here on the appearance of a neutral diploid and the cell cycle analysis of the component mating type alleles are important in two ways. Firstly, they can be fully explained on the basis of this hypothesis and therefore support and amplify it. Secondly, they provide the first evidence for the existence of cell cycle regulating genes which control the *temporal* expression of other genes. These points will be dealt with in turn below.

The hypothesis outlined above states that freshly isolated diploid a_1a_2 cells are neutral in G_1 because both mating type alleles are inducible simultaneously, and that the cells mate during the rest of the cell cycle because only one allele (a_2) is inducible. Thus a completely neutral diploid can be explained on the basis of this hypothesis by postulating that changes occur to the cell cycle control systems operating on one or both alleles such that both alleles are under identical controls. This could happen if the a_1 system changed from stringent to relaxed, or if the a_2 system changed from relaxed to stringent (Fig. 5). Analysis of haploids derived from the neutral diploid confirm this prediction and specifically show that a change occurred from relaxed to stringent control of allele a_2. Other preliminary work indicates that there may be different changes occurring in other diploids that have become completely neutral. Consequently, screening for neutral diploids appears to provide a useful selection procedure for the isolation of mating type mutants with altered cell cycle control properties.

The original relaxed control of allele a_2 and the new stringent form are stable in many generations of culturing of haploid strains and are therefore inherited. It is clear that the temporal and functional aspects of the mating type allele are separable. It is suggested therefore that there is a cell cycle control locus with at least two alleles, cc^{str} (cell cycle stringent) and cc^{rel} (cell cycle relaxed) which act to limit the temporal expression of the mating type gene.

In wild-type haploids cc^{str} is coupled with a_1 and cc^{rel} with a_2. The stability of this coupling in meiotic recombination and the *cis* dominance of the control system suggest that the loci may be closely linked. In the one neutral diploid examined in detail so far the cells became homozygous for cc^{str} while retaining heterozygosity for the mating type alleles. The events producing this change in most of the cells of a population of the a_1a_2 diploid are unknown and intriguing in view of the apparent stability of the *cc* alleles in haploids and in diploids undergoing meiosis. Our results suggest strongly that the event which causes loss of mating ability is a relatively rare genetic event with some selective value rather than non-genetic physiological change. Thus most (12 of 16) of the fresh diploid isolates that we have studied

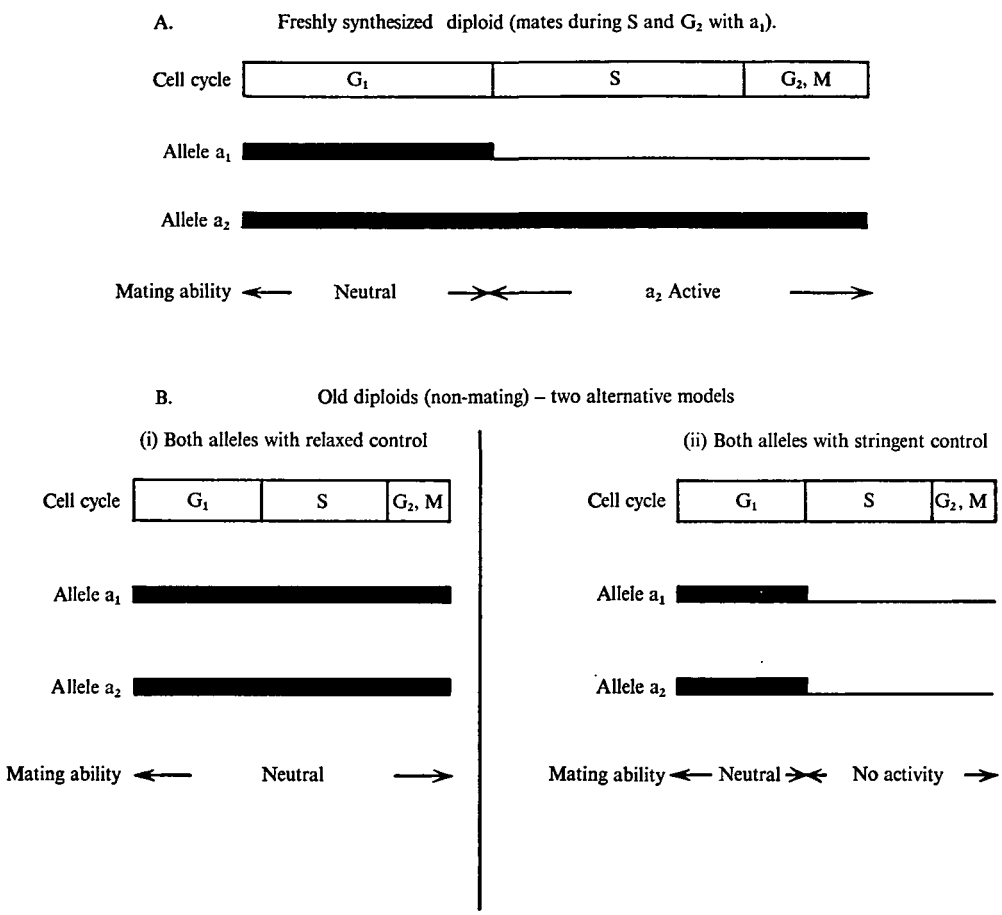

Fig. 5. Model of cell cycle control of mating in both freshly synthesized and old a_1a_2 diploids. Model assumes (1) period of inducibility of each allele same as corresponding haploid; (2) cell does not mate when both alleles active simultaneously.

retained their normal S and G_2 phase mating abilities for more than about 160 generations. However, 3 of 16 diploid colonies were more or less neutral as soon as they were tested. It may be possible that these neutral diploids were formed following fusion of haploid cells carrying mutated *cc* alleles, but this appears unlikely as we have found no evidence for such mutations in haploid strains. A more likely explanation is that a rare recombination event occurred in one of the first 20 divisions of the original diploid cell before sampling started, and that the new genotype was selected for. Only one culture lost its mating ability during the period of sampling. This culture appeared to have begun to decline in mating ability at the start of the experiment as only 30 % of the cells mated compared to about 50 % in most of the other cultures. Further genetic analyses of the changes occurring in these cells are being carried to determine the mechanism involved, but at present the most likely possibilities appear to be either mitotic crossing-over or gene conversion.

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Our results suggest that the event is more likely to occur in the initial divisions of a newly formed diploid cell than in its later growth.

It is important to stress that although the nature of the change is not understood, it is established that the cell cycle control system of a mating-type allele can be altered in a heritable manner, without the functional abilities of the allele being changed. This constitutes strong evidence for the existence of a specific locus concerned with the cell cycle control of the mating-type locus.

It is unclear at present if this is a unique system of whether other loci are also regulated by similar cell-cycle control genes. A widespread occurrence of *cc* controlled loci is quite consistent with current theories of genetic organization in eukaryotes, which suggest that each structural gene may have an extensive number of regulatory genes associated with it (Britten & Davidson, 1969; Crick, 1971; Beerman, 1972; Paul, 1972). Also it should be stressed that the concept of *cc* loci is not incompatible with the 'sequential transcription' or the 'oscillatory repression' models and could be superimposed on these systems. We are attempting to resolve some of these questions by screening diploids with neutral or altered mating activity for other kinds of *cc* alleles, and by examining the effects of these alleles on activity at genes other than the mating-type locus.

It has been suggested (Hereford & Hartwell, 1974) that the initiation of DNA replication in yeast signals a commitment to complete the cell cycle and excludes the replicating cells from mating activity. In smut, however, the exclusion of mating activity by the initiation of replication is restricted to one of the two mating types. The other mating type is not affected by DNA replication. The hybrid diploid is positively influenced to begin sexual morphogenesis during the initiation of DNA replication and in that regard may be comparable to the G_2 populations of animal cells (Gelfant, 1962). At any rate the present results with the mating system in smut tend to discourage generalizations about the role of DNA replication in regulating a commitment to the cell cycle or to morphogenesis in a variety of cell types. For a further discussion and comparison of the role of the cell cycle in smuts and yeasts see Cummins & Day (1974*b*).

The essential feature of the model for temporal allelic interaction is that individual alleles may have particular and distinct periods of inducibility or availability during the cell cycle, and therefore that the properties of a diploid will represent the interaction between these properties. In the particular situation of the mating type locus of *U. violacea* it appears that the alleles have a 'negative' effect when simultaneously active, i.e. the cell becomes neutral. It is clear, however, that in cases where the alleles have a positive 'additive' effect, the heterozygote may show overdominance as the total period of gene activity may be longer than in either homozygote. Such overdominance would provide a theoretical basis for cases of heterosis.

Furthermore, this novel kind of dominance generated by cell-cycle control systems may be relevant to considerations of the evolution of dominance. Most of those who have previously dealt with the evolution of dominance considered the quantity of gene product of prime importance and did not take into account the

temporal aspects of gene action. Fisher (1931) found it necessary to postulate systems of dominance modifiers to explain the establishment of a dominant allele and both Fisher (1931) and Haldane (1939) stressed the necessity of postulating modification of an allele to directly influence amount of gene product as a system of dominance is established. The observations provided in this communication suggest that cell-cycle controls influencing the most favourable time of gene action may function as an important new class of dominance modifiers in a manner that was not previously perceived by the early theoreticians. Temporal modification of allelic interaction may prove worthy of careful consideration by future evolutionary theorists.

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REFERENCES

Beerman, W. (1972). Chromosomes and genes. In *Developmental Studies on Giant Chromosomes* (ed. W. Beerman), pp. 1–31. Springer-Verlag.

Britten, R. & Davidson, E. (1969). Gene regulation for higher cells: a theory. *Science*, **165**, 349–357.

Burton, K. (1956). A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of DNA. *Biochemical Journal* **62**, 315–322.

Crick, F. (1971). A general model for the chromosomes of higher organisms. *Nature* **234**, 25–27.

Cummins, J. E. & Day, A. W. (1973). Cell cycle regulation of mating-type alleles in the smut fungus, *Ustilago violacea*. *Nature* **245**, 259–260,

Cummins, J. E. & Day, A. W. (1974*a*). Transcription and translation of the sex message in the smut fungus, *Ustilago violacea*. II. The effects of inhibitors. *Journal of Cell Science* **16**, 49–62.

Cummins, J. E. & Day, A. W. (1974*b*). The cell cycle regulation of sexual morphogenesis in a basidiomycete *Ustilago violacea*. In *Cell Cycle Controls* (ed. G. M. Padilla, I. L. Cameron and A. Zimmerman). New York: Academic Press.

Day, A. W. (1972). The isolation and identification of polyploid strains of *Ustilago violacea*. *Canadian Journal of Genetics and Cytology* **14**, 925–932.

Day, A. W. & Cummins, J. E. (1973). Temporal allelic interaction, a new kind of dominance. *Nature* **245**, 260–261.

Day, A. W. & Cummins, J. E. (1974). Transcription and translation of the sex message in the smut fungus, *Ustilago violacea*. I. The effect of ultraviolet light. *Journal of Cell Science* **14**, 451–460.

Day, A. W. & Jones, J. K. (1968). The production and characteristics of diploids of *Ustilago violacea*. *Genetical Research* **11**, 63–81.

Day, A. W. & Jones, J. K. (1969). Sexual and parasexual analysis of *Ustilago violacea*. *Genetical Research* **14**, 195–221.

Day, A. W. & Jones, J. K. (1972). Somatic nuclear division in the sporidia of *Ustilago violacea*. I. Acetic orcein staining. *Canadian Journal of Microbiology* **18**, 663–670.

Day, A. W. & Poon, N. H. (1975). Fungal fimbriae. II. Their role in conjugation in *Ustilago violacea*. *Canadian Journal of Microbiology* **21**, 547–557.

Donachie, W. D. & Masters, M. (1969). Temporal control of gene expression in bacteria. In *The Cell Cycle: Gene–Enzyme Interactions* (ed. G. M. Padilla, G. L. Whitson and I. L. Cameron), pp. 37–76. New York, London: Academic Press.

Esposito, R. & Holliday, R. (1964). The effect of 5-fluorodeoxyuridine on genetic replication and mitotic crossing over in synchronized cultures of *Ustilago maydis*. *Genetics* **50**, 1009–1017.

Fisher, R. A. (1931). The evolution of dominance. *American Naturalist* **68**, 370–374.

Gelfant, S. (1962). Initiation of mitosis in relation to the cell division cycle. *Experimental Cell Research* **26**, 395–402.

Goodwin, B. C. (1966). An entrainment model for timed enzyme synthesis in bacteria. *Nature* **209**, 479.

Haldane, J. B. (1939). Physiological and evolutionary theories of dominance. *American Naturalist* **68**, 24–53.

Halvorson, H. O., Carter, B. L. A. & Tauro, P. (1971). Synthesis of enzymes during the cell cycle. *Advances in Microbial Physiology* **6**, 47–74.

Hereford, L. & Hartwell, L. (1974). Sequential gene function in the initiation of *Saccharomyces cerevisiae* DNA synthesis. *Journal of Molecular Biology* **84**, 445–461.

Masters, M. & Pardee, A. B. (1965). Sequence of enzyme synthesis and gene replication during the cell cycle of *Bacillus subtilis*. *Proceedings of the National Academy of Sciences, U.S.A.* **54**, 64–70.

Mitchison, J. M. (1971). *The Biology of the Cell Cycle*. Cambridge University Press.

Paul, J. (1972). Organization of eukaryote chromosomes. *Nature* **238**, 444–447.

Poon, N. H. & Day, A. W. (1974). Somatic nuclear division in *Ustilago violacea*. II. Observations on living cells with phase contrast and fluorescence microscopy. *Canadian Journal of Microbiology* **20**, 739–746.

Poon, N. H., Martin, J. & Day, A. W. (1974). Conjugation in *Ustilago violacea*. I. Morphology. *Canadian Journal of Microbiology* **20**, 187–191.

Williamson, D. H. & Scopes, A. W. (1960). The behaviour of nucleic acids in synchronously dividing cultures of *Saccharomyces cerevisiae*. *Experimental Cell Research* **20**, 338–349.

Williamson, D. H. & Scopes, A. W. (1962). A rapid method for synchronising division in the yeast *Saccharomyces cerevisiae*. *Nature* **193**, 256–257.