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Temperature-sensitive mutants of *Physarum polycephalum* – expression of mutations in amoebae and plasmodia

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SUMMARY

Over 100 temperature-sensitive mutants of *mt*-h (apogamic) strains of *Physarum polycephalum* were isolated either by testing clones of mutagenized amoebae (ATS mutants) or by the more laborious method of testing plasmodia derived from such clones (PTS mutants). When amoebae and plasmodia of each mutant were tested for growth temperature-sensitivity on different media (to give optimum growth of each phase), only 21% of 73 ATS mutants and 32% of 31 PTS mutants appeared to be temperature-sensitive in both phases, suggesting that the majority of mutants are phase-specific, as concluded from several similar studies by previous authors. When the mutants were tested on a third medium which allows growth of both amoebae and plasmodia, many of the mutants no longer had a temperature-sensitive phenotype in either phase. Among the remainder, 51% of ATS mutants and 67% of PTS mutants were temperature-sensitive in both phases. It was suggested that certain media have a remedial effect on some temperature-sensitive mutants so that the phenotype is apparently normal. Thus, the proportion of phase-specific mutants may be over-estimated if tests of temperature-sensitivity are done on the different media commonly used for culture of amoebae and plasmodia respectively. It was concluded that the most efficient procedure for isolation of temperature-sensitive mutants expressed in plasmodia is to screen clones of amoebae on a medium resembling as closely as possible that which is to be used for testing plasmodia.

1. INTRODUCTION

Several authors have reported isolation of temperature-sensitive growth mutants in the slime mould *Physarum polycephalum* (Haugli & Dove, 1972; Gorman & Dove, 1974; Gingold *et al.* 1976; Wheals, Grant & Jockusch, 1976; Del Castillo, Oustrin & Wright, 1978; Sudbery, Haugli & Haugli, 1978; Laffler *et al.* 1979). The ultimate aim of all these studies has been to identify mutations causing temperature-sensitive lesions of the nuclear-division cycle and to analyse their effects in the multinucleate, syncytial plasmodium. The naturally synchronous mitosis (Howard, 1932) and DNA synthesis (Braun, Mittermayer & Rusch, 1965) of the plasmodium offer an attractive experimental system for investigating the control of these processes. The initial isolation of mutants, however, can clearly be accomplished much more quickly and easily in the alternative growth phase of the life-cycle, the haploid uninucleate amoebae. If a strain in which plasmodia develop

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within clones of amoebae is used (Wheals, 1970; Cooke & Dee, 1974), the expression of the mutations in haploid plasmodia derived from mutant amoebae can then be investigated.

Although screening plasmodia directly for mutants is much more laborious than screening amoebae, this method has been advocated by most authors who have isolated temperature-sensitive growth mutants in *P. polycephalum* on the grounds that few of the mutations that they have identified have been expressed in both phases (Gingold *et al.* 1976; Wheals *et al.* 1976; Sudbery *et al.* 1978; Laffler *et al.* 1979). However, these conclusions have been based on the results of growth tests carried out on the different media commonly employed for culture of amoebae and plasmodia. Since it is known that, in other micro-organisms, the expression of temperature-sensitivity in a mutant may often be modified by altered culture conditions (Russell, 1972; Bilsky & Armstrong, 1973), we have investigated the expression of a sample of temperature-sensitive mutants using the same medium for amoebae and plasmodia.

2. MATERIALS AND METHODS

(i) *Media*

SDM (semi-defined agar medium) was prepared by mixing equal volumes of liquid SDM (Dee & Poulter, 1970) and molten 3% water agar immediately before pouring plates. The main constituents of SDM are glucose, peptone, a vitamin mix and haematin.

DSDMB (dilute semi-defined medium agar with bacteria) was prepared by spreading 0·2 ml of a suspension of live *Escherichia coli* over the surface of a plate of DSDM agar (62·5 ml liquid SDM per litre molten 2% water agar).

LIAB (liver infusion agar with bacteria) was prepared by spreading 0·2 ml of a suspension of live *E. coli* over the surface of a plate of LIA (1 g Oxoid liver infusion powder per litre 2% water agar).

(ii) *Strains*

Parent strains for mutant isolation were either CLd (Cooke & Dee, 1975); genotype *mt*-h *npfC*$^-$ *fus*A2 (Anderson, 1979) or LU909; genotype *mt*-h *npf*A1 *fus*A2 *whi*-1 (Anderson, 1977). Both CLd and LU909 amoebae form haploid plasmodia in clones at 22 °C because they carry allele *mt*-h of the mating-type locus.

(iii) *Plasmodium formation*

To obtain plasmodia, amoebae were inoculated into a 0·1 ml suspension of live *E. coli* on a DSDM agar plate using a toothpick. Plasmodia usually appeared after 10–14 days incubation at 22 °C, and were then subcultured either to SDM agar or to DSDMB.

(iv) *Isolation of temperature-sensitive mutants*

Following UV or nitrosoguanidine mutagenesis of amoebae, mutants were isolated either by screening clones of amoebae or by screening plasmodia derived from clones of mutagenized amoebae. Amoebae were screened by transferring mutagenized clones to two plates of LIAB using toothpicks (25 clones/plate). One plate was then incubated at 22 °C and the other at 31 °C. Clones which grew normally or nearly so at the lower temperature but poorly or not at all at the higher, and which again gave this result when the tests were repeated, were designated ATS mutants. Plasmodia were screened by transferring blocks of agar ($\simeq$ 25 mm^2 plasmodial surface area) to fresh SDM agar plates which were incubated at 22 and 31 °C respectively; strains which repeatably showed normal growth at low temperature and poor or no growth at high temperature were designated PTS mutants. Most of the temperature-sensitive strains have also had their 'mutant' status confirmed by conventional genetic analysis or on the basis of inheritance of mutant phenotype through the apogamic life-cycle (Burland, 1978).

(v) *Growth tests of amoebae and plasmodia of mutant strains*

After initial isolation and re-testing of ATS and PTS mutants on LIAB and SDM respectively, plasmodia derived from the ATS clones and amoebae from the clones which had given rise to the PTS strains were tested for growth at 22 and 31 °C in the same way. Amoebae and plasmodia of all the mutant strains were also tested at both temperatures on DSDMB which supports good, though sub-optimal, growth of both phases of the life-cycle and is the medium regularly used for plasmodium formation in amoebal clones. Parent strains were included as controls. A mutant was scored as temperature-sensitive for growth on a particular medium if it grew distinctly less well than the parent strain at 31 °C but normally at 22 °C in duplicate tests. Growth was judged by visual inspection and was not measured rigorously; however, mutants could be classified into those that showed little or no growth at 31 °C ('non-leaky') and those whose growth was less reduced ('leaky'). If plasmodial morphology was distinctly abnormal this was also noted.

3. RESULTS AND DISCUSSION

(i) *Number of mutants isolated*

Seventy-five ATS mutants were obtained from a total of $3{\cdot}7 \times 10^4$ mutagenized amoebae screened; 26 were classified as non-leaky and 49 as leaky in initial tests on LIAB. Thirty-one PTS mutants were obtained by screening a total of $3{\cdot}5 \times 10^3$ plasmodia derived from clones of mutagenized amoebae; 14 non-leaky and 17 leaky in tests on SDM. The lower yield of ATS mutants among survivors of mutagenesis ($2{\cdot}0 \times 10^{-3}$) than of PTS mutants ($8{\cdot}9 \times 10^{-3}$) was probably due to the less severe mutagenic procedure used (Burland, 1978). On the basis of the mutagenic and isolation procedures used, it seemed likely that the mutants were all of separate origin, and complementation tests in plasmodial heterokaryons of a

sample of the mutants suggested that the majority were due to mutations in different genes (Burland, 1978).

(ii) *Growth tests of ATS and PTS mutants on LIAB and SDM at 31 °C*

Plasmodia derived from 73 ATS mutants were tested on SDM for temperature-sensitivity at 31 °C (Table 1, *a*); 14 (V, VI, VIII) showed temperature-sensitive growth and one (VII) abnormal morphology; thus only 15 (21 %) of the mutations detected in amoebae on LIAB were expressed in plasmodia on SDM. When amoebae of the 31 PTS mutants were tested for growth at 31 °C on LIAB (Table 2, *d*), only 10 clones (32 %) showed temperature-sensitivity (XV–XVI). These results

Table 1. *Growth of ATS mutants at 31 °C*

Plasmodia		Amoebae			
SDM (a)	DSDMB (b)	DSDMB (c)	LIAB (d)	Number of mutants	Class
+	+	+	−	31	I
+	−	+	−	3	II
+	+	−	−	15	III
+	−	−	−	9	IV
−	−	−	−	9	V
−	M	−	−	1	VI
M	M	−	−	1	VII
−	+	−	−	4	VIII
				73	

+ = Growth indistinguishable from wild-type. − = Growth reduced or absent. M = Abnormal morphology but growth not reduced.

Table 2. *Growth of PTS mutants at 31 °C*

Plasmodia		Amoebae			
SDM (a)	DSDMB (b)	DSDMB (c)	LIAB (d)	Number of mutants	Class
−	+	+	+	7	XI
−	−	+	+	6	XII
−	−	−	+	7	XIII
−	M	−	+	1	XIV
−	−	−	−	8	XV
−	−	+	−	2	XVI
				31	

See Table 1 for symbols.

agree quite well with those of most previous investigators using LIAB and SDM (or similar media) who found that between 10 and 20 % of growth temperature-sensitive mutants isolated in amoebae or plasmodia showed temperature sensitivity in the other phase (Gingold *et al.* 1976; Wheals *et al.* 1976; Sudbery *et al.* 1978; Laffler *et al.* 1979). Del Castillo, Oustrin & Wright (1978), however, reported

that 65 (73%) of 89 mutants isolated as temperature-sensitive in amoebae on LIAB were also mutant as plasmodia on SDM.

(iii) *Growth tests of amoebae and plasmodia of ATS and PTS mutants on DSDMB at 31 °C*

When amoebae of the ATS mutants were tested on DSDMB at 31 °C, the temperature-sensitive phenotype was lost by 34 of the 73 clones (Table 1, *c*; classes I, II). The majority of these mutants were leaky on LIAB. The most likely explanation of this result is that DSDMB had a remedial effect on the phenotype; there is no reason to suppose that a change in gene expression occurred at the transcriptional level. Although the amoebae were grown on a bacterial lawn on both media, it is known that they can also absorb nutrients from the underlying agar and that DSDMB contains several vitamins required by amoebae (McCullough & Dee, 1976), as well as differing in many other ways from LIAB. Whatever the reason for the lack of mutant expression in the amoebae, it is not surprising that the plasmodia derived from 31 of these 34 strains also failed to show temperature sensitivity on DSDMB. The 3 strains (class II) which were temperature-sensitive as plasmodia on DSDMB were unexpected. All 3 were leaky mutants on DSDMB and it is possible that they were misclassified, since criteria for temperature sensitivity were subjective and growth which was indistinguishable from wild-type may in fact have been slightly reduced, i.e. they may belong to class IV. It is also possible, however, that a particular medium has a remedial effect on a mutant in only one of the two growth phases because of differences between amoebae and plasmodia; for example in their surface:volume ratio, in the abundance of slime or in permeability.

Among the 39 ATS mutants which retained amoebal temperature sensitivity on DSDMB (Table 1, III–VIII), 20 (51%) showed a temperature-sensitive phenotype for growth or morphology in plasmodia on DSDMB (IV–VII). Only 11 of these 20 plasmodia were temperature-sensitive on SDM, presumably because of remedial effects of this medium; SDM differs from DSDMB in containing all the constituents of liquid SDM at approximately 10 × higher concentration and in the absence of bacteria. The 4 mutants in class VIII are unexpected; they may perhaps be explained by the same possibilities as those suggested for class II above.

When the 31 PTS mutants were tested on DSDMB at 31 °C, 7 failed to show a temperature-sensitive phenotype and none of these mutants was temperature sensitive as amoebae on either DSDMB or LIAB. Again, we consider that this is most probably due to remedial effects of the media; in these mutants it is notable that wild-type growth was in every case observed in the presence of bacteria. Among the remaining 24 strains which retained plasmodial temperature sensitivity on DSDMB (XII–XVI), 16 (67%) were temperature-sensitive as amoebae on DSDMB (XIII–XV). Only 8 of these 16 clones (XV) had mutant phenotype as amoebae on LIAB. The 2 strains in class XVI resemble those in class II (Table 1) and the same comments apply; since both were leaky mutants as plasmodia on SDM and DSDMB, it is probable that they should be included in class XV.

Sudbery *et al.* (1978) found that enrichment and pre-screening for amoebal temperature-sensitive mutants prior to screening for mutant plasmodia greatly increased the yield of PTS mutants (by at least 50-fold). However, only 18% of the mutant clones isolated were temperature-sensitive as amoebae in subsequent growth tests, although the same amoebal medium was used as for pre-screening. It was concluded that many of the mutants may have shown very weak expression in amoebae. Both these results and our own suggest that apparently normal growth at the restrictive temperature under certain conditions may sometimes conceal a mutant phenotype.

(iv) *Implications of the results for programmes of mutant isolation*

Only 21% of the temperature-sensitive mutants isolated as amoebae on LIAB showed mutant phenotype in plasmodia on SDM. However, of the 39 strains which were detectable as mutant amoebae on DSDMB, 51% were mutant as plasmodia on DSDMB and 38% (classes V–VIII) as plasmodia on SDM. Thus, if the aim is to isolate mutants that can be studied in plasmodia on SDM, it will be more efficient to screen amoebae on DSDMB than on LIAB. Even better results would probably be achieved if amoebae could be screened on SDM. Strains of amoebae which can grow on liquid SDM are now available (McCullough, Dee & Foxon, 1978), but since they fail to grow on agar media in the absence of bacteria and cannot at present be cloned in axenic media, feasible screening methods have not yet been devised. One mutant strain, originally isolated as a temperature-sensitive plasmodium on SDM, which failed to show mutant phenotype in amoebae on LIAB (Gingold *et al.* 1976) has been tested for its expression in amoebae in liquid SDM and shown to be temperature-sensitive under these conditions (McCullough *et al.* 1978).

The results of testing both ATS and PTS mutants clearly indicate that a considerable proportion (perhaps 50%) of plasmodial mutants will be missed if screening is done only in amoebae even on DSDMB; however, we consider that the saving in time and materials achieved by this method, in comparison with screening plasmodia, more than compensates for this disadvantage except in circumstances where the genetic control of a process is likely to be different in the two phases. In searching for nuclear-division cycle mutants, it will be necessary to examine a large number of temperature-sensitive growth mutants before any with the required characteristics are discovered. Since it seems likely that many of the genes controlling nuclear division and mitosis are functional in both amoebae and plasmodia, we consider that the initial stages of mutant isolation can at present be most efficiently performed in amoebae, but that care should be taken to ensure that the culture conditions used for amoebae and plasmodia are as similar as possible.

(v) *Gene expression in amoebae and plasmodia*

The small proportion of temperature-sensitive mutants reported with mutant phenotype in both amoebae and plasmodia has been taken as evidence that these

two growth phases of *P. polycephalum* use different 'sets of genes' (Wheals *et al.* 1976). This conclusion was reinforced (Dee, 1975) by repeated failures to culture amoebae on even complex axenic media until many years after plasmodia were successfully grown on simple defined media (Goodman, 1972). Recent studies, however, have shown that amoebae and plasmodia have several specific nutritional requirements in common (McCullough & Dee, 1976) and amoebae can grow in SDM as a result of mutations in only a small number of genes (McCullough *et al.* 1978). The present investigation suggests that when amoebae and plasmodia are cultured on the same medium, they use many of the same genes. From the tests on DSDMB it was estimated that approximately 51 % of the mutations expressed in amoebae were also expressed in plasmodia and approximately 67 % of plasmodial mutations were expressed in amoebae. Each mutation presumably identifies a gene whose product is essential for survival, growth or normal morphology on this medium. The results provide *minimum* estimates of the proportion of such genes functional in both phases since, while the presence of a temperature-sensitive phenotype certainly indicates the presence of a mutant gene product, an apparently normal phenotype is not conclusive evidence of the absence of such a product. It is probable that the mutants in classes II, VIII and XVI should be added to the estimate of genes expressed in both amoebae and plasmodia (see 3(iii)).

The figures estimated have little bearing on the proportion of the *total* genome that is functional in each phase; for example, genes required for encystment or flagellation of amoebae (Mir *et al.* 1979) or for sporulation of plasmodia would not necessarily be detected among the mutants isolated. The results are of some relevance to studies of the amoebal–plasmodial transition, however. This developmental phase has recently been subjected to intensive genetic analysis (Adler & Holt, 1977: Davidow & Holt, 1977; Anderson, 1979) and can be induced in cultures with good synchrony (Youngman *et al.* 1977) but its usefulness as an experimental system will now depend on our ability to identify the products of genes regulated during the transition. The genes with which we are likely to be concerned are those which differ in their expression in amoebae and plasmodia grown in the same medium. The mutants in classes III and XII indicate the presence of genes of this type and the studies reported suggest that they may be less abundant, relative to the genes functional in both growth phases, than was originally supposed.

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REFERENCES

Adler, P. N. & Holt, C. E. (1977). Mutations increasing asexual plasmodium formation in *Physarum polycephalum*. *Genetics* **87**, 401–420.

Anderson, R. W. (1977). A plasmodial colour mutation in the Myxomycete *Physarum polycephalum*. *Genetical Research* **30**, 301–306.

Anderson, R. W. (1979). Complementation of amoebal–plasmodial transition mutants in *Physarum polycephalum*. *Genetics*. (In the Press.)

BILSKY, A. Z. & ARMSTRONG, J. B. (1973). Osmotic reversal of temperature sensitivity in *Escherichia coli*. *Journal of Bacteriology* **113**, 76–81.

BRAUN, R., MITTERMAYER, C. & RUSCH, H. P. (1965). Sequential temporal replication of DNA in *Physarum polycephalum*. *Proceedings of the National Academy of Sciences* **53**, 924–931.

BURLAND, T. G. (1978). Temperature-sensitive mutants of *Physarum polycephalum*. Ph.D. Thesis, Leicester University.

COOKE, D. J. & DEE, J. (1974). Plasmodium formation without change in nuclear DNA content in *Physarum polycephalum*. *Genetical Research* **23**, 307–317.

DAVIDOW, L. S. & HOLT, C. E. (1977). Mutants with decreased differentiation to plasmodia in *Physarum polycephalum*. *Molecular and General Genetics* **155**, 291–300.

DEE, J. (1975). Slime moulds in biological research. *Science Progress, Oxford*, **62**, 523–542.

DEE, J. & POULTER, R. T. M. (1970). A gene conferring actidione resistance morphology on *Physarum polycephalum* plasmodia. *Genetical Research* **15**, 35–41.

DEL CASTILLO, L., OUSTRIN, M. L. & WRIGHT, M. (1978). Characterisation of thermosensitive mutants of *Physarum polycephalum*. *Molecular and General Genetics* **164**, 145–154.

GINGOLD, E. C., GRANT, W. D., WHEALS, A. E. & WREN, M. (1976). Temperature-sensitive mutants of the slime-mould *Physarum polycephalum*. II. Mutants of the plasmodial phase. *Molecular and General Genetics* **149**, 115–119.

GOODMAN, E. M. (1972). Axenic culture of myxamoebae of the Myxomycete *Physarum polycephalum*. *Journal of Bacteriology* **111**, 242–247.

GORMAN, J. A. & DOVE, W. F. (1974). A method of indirect mutant selection in *Physarum polycephalum* using the antibiotic Netropsin. *Molecular and General Genetics* **133**, 345–351.

HAUGLI, F. B. & DOVE, W. F. (1972). Mutagenesis and mutant selection in *Physarum polycephalum*. *Molecular and General Genetics* **118**, 109–124.

HOWARD, F. L. (1932). Nuclear division in plasmodia of *Physarum*. *Annals of Botany* **46**, 461–477.

LAFFLER, T. G., WILKINS, A., SELVIG, S., WARREN, N., KLEIN-SCHMIDT, A. & DOVE, W. F. (1979). Temperature-sensitive mutants of *Physarum polycephalum* affecting viability, growth and nuclear replication. *Journal of Bacteriology*. (In the Press.)

MCCULLOUGH, C. H. R. & DEE, J. (1976). Defined and semi-defined media for the growth of amoebae of *Physarum polycephalum*. *Journal of General Microbiology* **95**, 151–158.

MCCULLOUGH, C. H. R., DEE, J. & FOXON, J. L. (1978). Genetic factors determining the growth of *Physarum polycephalum* amoebae in axenic medium. *Journal of General Microbiology* **106**, 297–306.

MIR, L., DEL CASTILLO, L. & WRIGHT, M. (1979). Isolation of *Physarum* amoebal mutants defective in flagellation and associated morphogenetic processes. *FEMS Microbiology Letters* **5**, 43–46.

RUSSELL, R. R. B. (1972). Temperature-sensitive osmotic remedial mutants of *Escherichia coli*. *Journal of Bacteriology* **112**, 661–665.

SUDBERY, P., HAUGLI, K. & HAUGLI, F. (1978). Enrichment and screening of heat-sensitive mutants of *Physarum polycephalum*. *Genetical Research* **31**, 1–12.

WHEALS, A. E. (1970). A homothallic strain of the Myxomycete *Physarum polycephalum*. *Genetics* **66**, 623–633.

WHEALS, A. E., GRANT, W. D. & JOCKUSCH, B. M. (1976). Temperature-sensitive mutants of the slime mould *Physarum polycephalum*. I. Mutants of the amoebal phase. *Molecular and General Genetics* **149**, 111–114.

YOUNGMAN, P. J., ADLER, P. N., SHINNICK, T. M. & HOLT, C. E. (1977). An extracellular inducer of asexual plasmodium formation in *Physarum polycephalum*. *Proceedings of the National Academy of Sciences* **74**, 1120–1124.