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CURRENT RESEARCH PROJECTS

J. A. HOUGHTON

Respiratory deficient mutants of A. nidulans.

PRELIMINARY NOTES

G. HOLT AND K. D. MACDONALD

Influence of copper on spore colour in Aspergillus nidulans and Penicillium chrysogenum and its availability from different media.

In the absence of copper the normally black spores of Aspergillus niger are brown or yellow (Roberg 1928). As far as we are aware it has only been recently shown that copper also affects conidial colour in Aspergillus nidulans. Agnihotri (1967) reported that in the absence of copper green spores become yellow green. We have found a similar effect, independently, in A. nidulans and Penicillium chrysogenum. When insufficient copper is available the normally green conidia of the wild types of both species are yellow and usually indistinguishable from yellow spored mutants isolated after ultraviolet irradiation. This note is written to stress the influence that some medium constituents may have on the availability of copper.

When grown on the Minimal Medium of Pontecorvo et al. (1953), solidified with 1.5% w/v Davis New Zealand agar, wild type strains of both species had green conidia. However, when a change was made to Oxoid No.3 agar (at the same 1.5% level), the wild type strains produced yellow conidia. Auxanography with salts of various metals showed that $CuSO_4 . 5H_2O$ restored green conidial pigmentation. Oxoid lonagar No. 2, which is a highly purified preparation also restored normal green conidiation when it replaced Oxoid No.3.

A chemical analysis of the copper content of the agars and the media prepared with each was carried out (ref. 2). The results were as follows:

Agar	Copper content µg/gm.	
	Agar Powder	Whole Minimal Medium
Davis	0.47	0.13
Oxoid No. 2	0.35	0.16
Oxoid No. 3	0.31	0.13

The copper in the agars accounts for about 5% of the copper in the media so that only relatively large differences in copper levels of agar would be expected to influence the copper levels of the media. There is little variation in the copper content of the media. Nevertheless, it was

found necessary to add $CuSO_4.5H_2O$ (Analar) at a level of 2.5 µg/gm. of MM to ensure green sporulation when Oxoid No. 3 agar is used.

A possibility is that Oxoid No. 3 agar contains one or more chelating agents which bind copper making insufficient available for the green pigment to develop. The addition of copper could be expected to ultimately saturate such chelators and then allow sufficient for green sporulation to occur. It is clear that a copper assay based on a chemical determination does not necessarily indicate the amount available to an organism. In crossing experiments involving different spore colour mutants, obviously, sufficient copper should be present and available to allow full development of spore colour, otherwise misleading results could be obtained.

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Z. GAJEWSKI & J. LITWINSKA

Methionine mutants of Aspergillus nidulans and their suppressors.

Twenty-four methionine mutants, all induced by UV treatment, were obtained. All of them /together with two already known mutants meth 1 and meth 2/ were tested for growth requirements and complementation in heterocaryons and diploids. The third known mutant meth 3 was unavailable. All mutants do not respond to cysteine and all respond to methionine or homocysteine except for two /meth 2 and mutant 10 /locus meth D/ which do not grow on homocysteine. Complementation tests proved that the mutants fall in seven separate complementation groups called meth 1, meth 2 and meth A to E. They probably represent seven separate loci with full complementation among them and no complementation within each group.

To meth 1 locus in chromosome IV belong 8 mutants /no. 18, 20, 21, 22, 23, 24, 27 & 35/. No new mutants allelic to meth 2 chr. III/ were found. Four mutants /no. 17, 32 33 & 34/ belong to locus called meth A mapped in chr. II between loci Acr1 and w 3.9 map units from w. Nine mutants /no. 4, 8, 14, 15, 25, 26, 28, 36 & 38/ of the locus called meth B were located in chr VI 10.8 map units from nic 10. One mutant no. 29 /locus meth C/ is probably in the left arm of the chr.I. Mutant No. 10 /locus meth D/ is in chr. III 7.7 map units from arg 2 and 11.2 map units from meth 2. Mutants no. 31 /locus meth E/ is not yet mapped.

Eight crosses between different mutants of locus meth B gave recombination values ranging from 1.8×10^{-5} to 9×10^{-3} of prototroph recombinants showing that they map on relatively short chromosome segment.

Spontaneous reversations of 6 mutants from different loci were scored and their frequencies varied from 0.02×10^{-6} to 130×10^{-6}. The revertants have been preliminary divided into six morphological groups designed r1 to r6 respectively. The frequencies and morphological spectra of revertants vary greatly for different loci and sites.

All revertant types that were tested in appropriate crosses proved to be of the suppressor type. Two types of suppressors r2 and r3 were more fully analyzed. They were fully or partly recessive and unlinked to their methionine loci. Suppressor r2 is morphologically more or less as the wild type and was found to be largely unspecific, acting on all loci and mutants that grow on homocysteine and/or methionine. It does not suppress meth 2 and meth D mutants which do not respond to homocysteine. Complementation tests with r2 suppressors obtained from different loci were negative indicating allelism.

On the contrary, the suppressor r3 obtained from mutant 28/ locus meth B/ is specific, acting only on some sites of the locus meth B and being ineffective on mutants from other methionine loci tested. Suppressors of the type r3 are different from wild type in respect of the rate and type of growth Their mycelium accumulate brown pigment diffusing into the medium. Preliminary tests for allelism of different r3 suppressors show complementation/all suppressors were fully recessive/ and point to non-allelism and the possibility of many different suppressor loci, within this group. Mapping of r2 locus and at least of one of r3 loci are now in progress. To elucidate the mode of action of different methionine loci and their suppressors enzyme studies are planned.

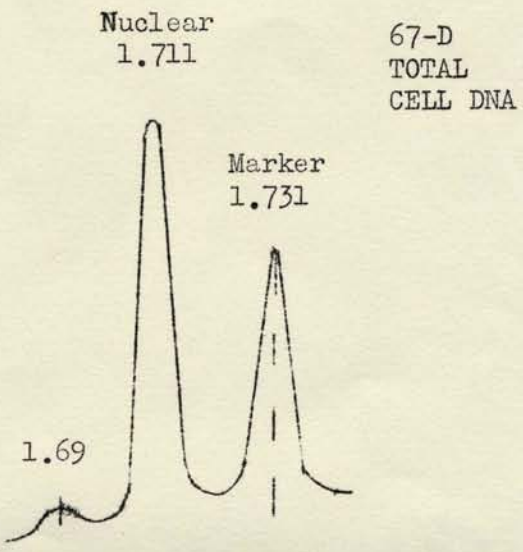
G. PONTECORVO

DNA of Aspergillus nidulans.

"Spoolable" total cell DNA has been obtained only by guarding drastically against degradation in the short time between cell lysis and inactivation of DNAse. After trying a number of variations on Marmur's technique (J. Mol. Biol., 1961, 6: 108), to which reference is made for the details of solutions and treatments, the following has given consistently good results.

Two 2 l. Erlenmeyer siliconized flasks, each with 750 ml. MM + 30 µg. biotin + 0.6 g. Difco Casamino Acids, are inoculated with 2 ml. 1/1000 Tween 80 conidial suspension of strain bi1 from slants 3 weeks to 2 months old. Incubation is in a New Brunswick gyrotory shaker, speed 11, at 36° - 37°C. After 20 - 22 hours theyield of mycelium, drained on Buchner filter, is about 25g. per flask. The

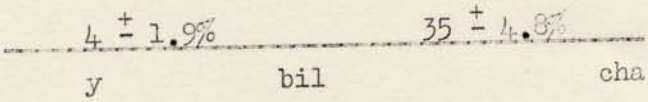
mycelium is harvested through a coarse-mesh cloth; it is twice re-suspended in 500 ml. water and strained; twice re-suspended in 500 ml. cold standard saline citrate and strained; then re-suspended in 500 ml. cold saline versene pH8 and drained on Buchner filter, weighed, and kept frozen at -60°C until required. The frozen mycelium is ground to a fine powder in a pre-frozen mortar, changing mortar repeatedly in order to keep the temperature well below freezing point. (An alternative is to lyophilize the pre-frozen mycelium and grind the dry material at room temperature.) The fine powder, frozen or dried, is dropped in 100 ml. saline versene pH8, pre-heated at 80C, mixed thoroughly and kept at 65° for 10 mins. After cooling, the steps of Marmur's technique are followed. The first EtOH precipitation does not usually give spoolable material with A. nidulans. Only after repeated deproteinizations and reduction of the volume to less than 10 ml. is good spooling obtained. At the end of the preparation the isopropanol step - preferably repeated two or three times - is essential to remove material, with density of about 1.67, which contributes to the OD_{257} and which is not DNA. A tracing of a CsCl equilibrium gradient centrifugation in the analytical ultracentrifuge is given below. It shows two peaks: the higher at 1.71 is nuclear DNA; the lower at 1.69 is presumed to be mitochondrial DNA. Other work, still in progress, shows the second peak enriched and the higher peak eliminated in preparations in which the mitochondria have been freed of nuclear material. The density of the Aspergillus nidulans nuclear DNA from several preparations is estimated as 1.711 - 1.712, corresponding to a G + C ratio of 50%. The density of "mitochondrial" DNA is estimated, less accurately, as 1.69, corresponding to a G + C ratio of about 32%. The best yields of total cell DNA are some 50 µg. per g. of frozen cells, and some 700 µg. per g. of lyophilized cells; the yield depends very much on the thoroughness of grinding.



B. W. BAINBRIDGE

Meiotic linkage in translocation (I - VIII)

Linkage has been detected between markers in linkage groups IR and VIII R in a cross heterozygous for T(I - VIII). This was first detected in a sample of 254 colonies which showed a recombination of 36 ± 3% between y and cha (spore colour markers). A three point cross including bi1 showed the following map:-



This data supports the suggestion by Käfer that the translocation (I - VIII) involves the transfer of chromosomal material from linkage group VIII to linkage group I R.

Reference: Käfer, E. (1965) Genetics, 52, 217.

R. J. THRELFALL

Genetic analysis of tolerance to pentachloronitrobenzene (PCNB)

When exposed to PCNB at 10 ppm or more in the medium or to the vapour produced by 1 mg. deposited in the lid of the Petri dish growth and sporulation of Aspergillus nidulans are reduced. Inhibited colonies give off fast growing sectors over 90% of which retain their tolerance on subculture in the absence of PCNB. These cultures show marked variation in their level of tolerance, growth rate and morphology in the presence or absence of the inhibitor. Three stable cultures isolated from auxotrophic strains have been studied and found to show enhanced resistance to other halogenated nitrobenzenes. PCNB tolerance has been tentatively ascribed to genes $PCNB_1$, $PCNB_2$ and $PCNB_3$. A diploid synthesised between y pro_1; Acr_1 $PCNB_1$ and Master Strain D (su ad_{20}y ad_{20}; Acr_1; $phen_2$; $pyro_4$; lys_5; s_3; nic_8; $ribo_2$) grew more quickly than a sensitive strain in the presence of PCNB although conidiation was reduced. In the presence of PCNB fast growing sectors with abundant buff or yellow conidia were produced. These were diploid and haploid respectively and about equally frequent. Of 57 haploids all save one had no requirement for phenylalanine. Crosses with strains marked on Chromosome III show $PCNB_1$ to be 15 map units from arg_2. $PCNB_1$ is now being mapped mitotically. In all possible crosses between strains carrying $PCNB_1$, $PCNB_2$ and $PCNB_3$ only tolerant progeny were produced so it is presumed that they are allelic.

In the presence of PCNB the DNA content of sensitive strains is

increased and glucose utilization reduced. PCNB has no such effect on tolerant strains. Present work seeks to explain why PCNB has these effects on the sensitive strain and how they are overcome in the tolerant mutant.

B. L. A. CARTER & A. T. BULL

Hetero eneity of the Aspergillus nidulans Tyrosinase

An initial difficulty in this work was the poor reproducibility of tyrosinase activity in cell-free mycelial extracts. The mechanically disrupted mycelia frequently darkened, a fact suggesting that phenolic or quinonoid products were being formed during disruption and were inhibiting the enzyme. However, the removal or prevented formation of these compounds did not permit enzyme recovery. The enzyme was eventually resolved by ion-exchange chromatography on DEAE-cellulose using an eluting system of 0.005 M tris-HCl buffer (pH 8.2) incorporating a continuous gradient of NaCl. The enzyme was very strongly absorbed onto the cellulose and was eluted only when the concentration gradient approached 1 M. Consequently, this single step procedure yields a tyrosinase preparation of considerable purity, the bulk of the proteins having been eluted at much lower molarities.

When the enzyme was (i) assayed in the presence of non-active fractions, the presence of an endogenous inhibitor (I) was revealed in the initial protein fractions; (ii) recycled on Sephadex G-200, two activity peaks (E^1 and E^2) were obtained. Furthermore, when the inhibitor was recycled on G-200, it was recovered together with a tyrosinase activity, E^3. Using calibrated Sephadex columns we have obtained molecular weights of the following order:- E^1(500,000), E^2, E^3 and I (125,000). The present data suggest that E^2 and E^3 represent a single tyrosinase component. The heterogeneity of the A. nidulans tyrosinase was confirmed by paper electrophoresis (0.05 M phosphate buffer, pH 6.1); two cathode-migrating components being distinguished. As yet we have not established whether the latter are referable to activities E^1 and E^2 obtained by gel filtration methods. Work is continuing on the kinetics of this problem and on the possible interrelationships of the tyrosinase components and the inhibitor.

The questions of control and physiological significance of tyrosinase in A. nidulans are also being investigated and our intial approach involves the analysis of steady state enzyme levels in carbon-limited chemostat cultures.

C. F. ROBINOW & C. E. CATEN

Mitosis in Aspergillus nidulans

Mitosis has been studied in living germ tubes by phase contrast microscopy in Helly-fixed preparations of the same material stained either directly with 1 : 60,000 acid fuchsin in 1% acetic or, after hydrolysis, with Giemsa solution. In some instances the same nucleus has been stained successively by these two methods. Recently we have found that, following hydrolysis, staining with 1% synthetic orcein (Allied Chemical and Dye Corp.) in 50% acetic is superior to Giemsa for the study of mitotic figures.

We have not been able to arrive at a precise understanding of how the distribution of sister sets of chromosomes to daughter nuclei is achieved. However, division involves a well defined sequence of stages which are seen with great regularity. Immediately before separation the chromosomes appear to be aligned in two straight chains parallel to each other and to the long axis of the hypha. We are puzzled to find that this arrangement is invariably resolved into daughter nuclei by a transverse break. Essentially the same behaviour has already been reported for Marasmius (Duncan and MacDonald 1965. Trans. Roy. Soc. Edin., LXVI, 20). At all stages in the division cycle haploid and diploid nuclei differ in size and depth of staining, but not in the number of separately visible elements.

Acid fuchsin has revealed the presence of an intranuclear fibre apparatus similar to that found in yeast (Robinow and Marak 1966. J. Cell Biol., 29: 129). Duplicate staining has established that the elongation of the fibre is closely related to the movement of the chromosomes. The fibre in dividing diploid nuclei is considerably thicker than that in haploids.

For electron microscopy, nuclei seen under phase contrast to be entering division were fixed with Kellenberger's osmium-calcium solution. It was found that the nucleus remains enclosed in an envelope until late anaphase. The fibre consists of microtubules traversing the nucleus between dense plaques attached to the nuclear envelope.

LORNA J. LILLY

An Investigation into the Mutagenic action of Aflatoxin

Aflatoxin (the collective name for a group of the metabolic products of Aspergillus flavus) is a possible hazard to man when Aspergillus flavus grows on stored food. Aflatoxin is known to be a potent liver carcinogen in some mammals. It has been shown to induce chromosome

aberrations in Vicia faba and human leucocytes (Lilly, Nature 207, 1965). It is known (Rees, private communication) that aflatoxin forms a complex (not alkylation) with guanine and adenine and probably blocks both the major and minor grooves of DNA, where it inhibits both DNA and DNA dependent RNA synthesis. It therefore seemed likely to be a mutagen.

Experiments to test mutagenicity by back mutation at two loci in Aspergillus nidulans gave the following results:

	CONTROLS			AFLATOXIN		
LOCUS	VIABLE SPORES PLATED	REVER-TANTS	REVERTANTS PER 10^6 VIABLE SPORES	VIABLE SPORES PLATED	REVER-TANTS	REVERTANTS PER 10^6 VIABLE SPORES
ad_8	482.3×10^6	3	1 in 160.7×10^6	323.7×10^6	2	1 in 161.8×10^6
	329.4×10^6	1	1 in 329.4×10^6	335.5×10^6	0	0 in 335.5×10^6
ad_{14}	403×10^6	0	0 in 403×10^6	399.7×10^6	1	1 in 399.7×10^6
	454.9×10^6	0	0 in 454.9×10^6	545.1×10^6	0	0 in 545.1×10^6

(Spores treated for 3 hrs in 0.2 mg./cc. Aflatoxin)

There is no evidence of an increase in mutation at the "ad_8" locus and probably not a significant increase at the "ad_{14}" locus with aflatoxin treatment.

It is possible that Aspergillus nidulans is sufficiently closely related to Aspergillus flavus to be unaffected by aflatoxin. (Though a recent paper by Lillehoj & Ciegler & Hall, Experientia 23, suggests that the growth of Aspergillus flavus itself is reduced on certain medium containing aflatoxin). A survey by Burmeister & Hesseltine, Applied Microbiology 14, 1966, showed that a wide range of fungi (34 genera) are not sensitive to aflatoxin.

Tests for mutagenicity are being made in phage and Drosophila. Initial experiments have shown aflatoxin is highly toxic to Drosophila in concentrations down to about one tenth of that used to break Vicia chromosomes.

A. UPSHALL & J. H. CROFT

Following from the reports (1) (2) (3) that treatment of haploid strains of Aspergillus nidulans with acridines results in the recovery of a very high frequency of unstable variants, it has now been demonstrated

that these variants are produced, also with high frequencies, after the similar treatment of diploid strains. As in the case of one of the variants already described (2) a number of these variants have been shown to be of an aneuploid nature.

After treatment with various acridines of a diploid strain marked on all linkage groups, a large number of unstable variants were recovered. These had the general properties of the aneuploids described by Käfer (4), and a total of 30 independently isolated variants were fully analysed by sector analysis, and classified as follows:-

Hyperhaploids, n + 1: Disomic linkage group III (1 variant), V (4), VI (3) and VII (4);
n + 2: Disomic linkage groups III and VI (1 variant);
n + 4: Disomic linkage groups II, V, VII and VIII (1 variant).
Hyperdiploids, 2n + 1: Trisomic linkage groups I (7 variants), IV (1), V (7) and VIII (1).

All variants in which the aneuploidy involved the same linkage group were of a similar morphological appearance and different from those involving a different linkage group. The four hyperhaploids involving linkage group V were also very similar to the seven hyperdiploids involving that same group. In all cases all markers within a linkage group were recovered only in parental combinations.

Treatment with p-fluoro-phenylalanine has also been shown to produce similar unstable colonies. In a second diploid marked on linkage groups I, II and IV a hyperhaploid (n + 1), disomic for group II has been analysed.

References:-

(1) C. Ball (1964). A.N.L. 5, 13 - 14.
(2) C. Ball & J.A. Roper (1966). Genet. Res. 7, 207 - 221.
(3) J.H. Croft (1966). A.N.L. 7, 7 - 8.
(4) E. Käfer (1961). Genetics 46, 1581 - 1609.

I. R. BARACHO

Perithecium size and hybridization in Aspergillus nidulans

Seven strains of A. nidulans were crossed in all possible combinations (21 crosses) and from each cross, a number of 4 to 18 perithecia were isolated, measured and analysed, giving a total number of 246 perithecia analysed; of these perithecia, 106 were selfed, 105 were hybrid and 35 produced no viable ascospores.

Grouping the perithecia in two classes, according to his size (with more than 184 μ and with less than 184 μ in diameter), statistical analysis has revealed that the class with perithecia bigger than 184 μ contains a significant amount of hybrid perithecia, and the class with perithecia smaller than 184 μ contains a significant amount of selfed perithecia. The results have shown that, at least in some crosses, the search for hybrid perithecia can be facilitated since there is a correlation between size and hybrid or selfed state of perithecia.

I. PRASAD

Nuclear ratio in Aspergillus niger heterocaryons

Variation in the nuclear ratio in a heterocaryon between two auxotrophic mutants may be determined by the type of medium. Alteration in the amount of supplementation in the medium upon which a balanced heterocaryon grows, leads to the isolation of either parental component, and this sort of somatic flexibility may be directly proportional to the nuclear ratio in the heterocaryon. In the cytoplasm of a cell where dissimilar nuclei complement each other and provide just sufficient metabolic activities for it to grow on minimal medium, it is likely that nuclei maintain their rates of division to form a balanced hetercaryon. Changes in the supplementation of a medium by addition of growth factors above a certain limiting value may not alter the nuclear ratio appreciably. These possibilities were studied by using a heterocaryon between hist- and hypox- mutants, that grows nicely on minimal medium. The hetercaryon was inoculated in a modified Ryan tube having minimal medium supplemented with growth factor as set out below. After a period of 25 days incubation at 22°C spores were removed from the tube at 3 mm intervals from the point of inoculation and used to estimate the nuclear ratio by a plating technique.

The minimal medium was supplemented with Histidine and Hypoxanthine in ratios from 1:1 to 1:300. Provided that Hypoxanthine was > 0.001 mg/ml. there was no significant variation of nuclear ratio between hist- and hypox-nuclei. When the minimal medium was supplemented by a constant limiting amount of Hypoxanthine (.001 mg/ml) with different levels of Histidine, viz. 0.1, 0.2, 0.3 mg/ml. to give value of 100:1, 200:1, 300:1, then the nuclear ratio altered.

S.N.	Histidine per ml.	Hypoxanthine per ml.	Hist- conidia in %	Hypox- conidia in %
1	.1 mg.	.01 mg.	42.8	57.1
2	.2 mg.	.01 mg.	42.3	57.6
3	.3 mg.	.01 mg.	51.6	48.3
4	.1 mg	.001 mg.	36.6	63.3
5	.2 mg.	.001 mg.	40.5	59.4
6	.3 mg.	.001 mg.	50.8	49.1

This suggests that when the nuclear ratio is affected by a limiting

factor such as the amount of growth factor supplementation, the control may be exercised by the rates of division of the component nuclei.

B. M. FAULKNER

Spontaneous aneuploidy in haploid strains of A. nidulans

Unstable variants have been shown to arise spontaneously in a number of wild isolates of A. nidulans (Upshall, 1966). Similar unstable variants have arisen spontaneously in two haploid strains y; arg1 and Y paba1 (derivatives of Glasgow strains), and among the progenies of selfed and hybrid perithecia of the heterokaryon synthesized from them. Of 10,490 colonies obtained from hybrid ascospores 77 (0.734%) were unstable; having slow-growing central regions which produced faster-growing sectors. Seven of these unstable colonies (A1-A7) showed segregation of the Y/y and PABA1/paba1 markers. In the light of current data on the phenotypes of their segregant progeny, the following minimal genotypes have been assigned to them:

A1 : $\frac{\text{Y PABA1}}{\text{y paba1}}$; ARG 1

A2 : Y/y PABA1/? ; arg1

A3 : Y/y PABA1/paba1 ; ARG1

A4 : $\frac{\text{Y paba1}}{\text{y PABA1}}$; ARG1

A5 : $\frac{\text{Y paba1}}{\text{y PABA1}}$; ARG1

A6 : $\frac{\text{Y paba1}}{\text{y paba1}}$; ARG1

A7 : $\frac{\text{Y paba1}}{\text{y PABA1}}$; arg1

The conidial progeny of A3 contains between five and seven new unstable classes; all of which have a Y/y PABA1/? ; ARG1 minimal genotype. A4 and A5 are probably the same variant. They both produce ascospores. They have both segregated a more compact, unstable class which has a Y paba1 minimal genotype, and which does not produce ascospores.

Spontaneous hyperhaploidy appears to be the cause of these unstable variants. The breakdown of A2, A4 and A5 to give new unstable classes suggests either high polysomy of chromosome I alone, or polysomy involving chromosome I and some combination of chromosomes II - V, VII and VIII.

Work on these unstable variants is being extended using haploid strains with markers in all eight linkage groups.

Reference: Upshall, A. (1966). Nature 209, 1113 - 1115.

JAMES A. HOUGHTON

Respiratory-deficient Mutants of *Aspergillus nidulans*

Modifications of techniques developed in yeast for the isolation and characterisation of respiratory deficient (RD) mutants, have been found to be applicable to *A. nidulans*.

Two RD mutants have been isolated following ultraviolet irradiation, by their inability to grow on a minimal medium containing either sodium acetate or lactate as sole carbon source. They also give negative Nadi reactions.

A useful diagnostic test for RD mutants employs an overlay of 2, 3, 5 - triphenyltetrazolium chloride (TTC) as a colour indicator. The overlay consists of 1.5% Bacto agar in 0.067M phosphate buffer at pH 7.0, 0.1% with respect to the TTC. The agar is made up in 15 ml. aliquots and autoclaved separately from the TTC solution to prevent reduction of the latter. The test agar is prepared by adding 5 ml. of TTC solution to an agar aliquot at 49°C.

Growth rate of the wild type is double that of the two RD mutants and with mixed platings the mutant colonies are overgrown by wild type. This problem is partly overcome by the addition to the medium of sodium desoxycholate. However, in the time required for the mutant colonies to develop, the wild type colonies are so heavily conidiated as to interfere with the test. This technique is, therefore, not suitable for selecting out RD colonies on a mixed plate following mutagenic treatment.

Mutant spores are plated at a density of 100 - 200 colonies per plate on minimal glucose medium containing 0.08% sodium desoxycholate. Small compact colonies with little conidiation are produced after 96 hours incubation at 37°C. Similar wild type colonies are produced after only 42 hours incubation.

To obtain a direct comparison between RD and wild type colonies, two similar sized semi-circles of medium, bearing wild type and mutant colonies respectively are brought together in a clean petri dish. The 20 ml. aliquot of TTC agar is gently poured over the plate and the plate is then incubated at 37°C. After two hours the wild type colonies are coloured red, whilst the RD colonies remain white.

Reference: Nagai, Yanagishima and Nagai (1961). Bact. Reviews Vol. 25 No. 4, 404 - 426.

R. F. ROSENBERGER & M. KESSEL

Synchrony of nuclear replication in individual hyphae

Clutterbuck & Roper (Genet. Res. Camb. 7, 185, 1966) have shown that when Aspergillus nidulans grows on glucose-nitrate agar mitosis in individual hyphal tips is synchronised. Although it seems to be widely accepted that nuclear replication in individual hyphae will always be synchronous, there is in fact, little data on this point. Our preliminary studies show that the nuclei in a single hypha replicate synchronously at fast but not at slow growth rates.

We have used conidiospores germinating in liquid cultures to determine the degree of synchrony. We took samples during the outgrowth of the germtubes, fixed and stained with acridine orange according to the method of Clutterbuck and examined the germinating spores under the microscope. If all the nuclei in a spore replicate in exact synchrony during germination only hyphae containing 2, 4, 8, 16 ..... nuclei will be seen. If the synchrony is not exact, hyphae containing other numbers of nuclei will appear and their frequency will be related to the degree of synchrony. If T = average nuclear doubling time, T + d and T - d are the longest and shortest nuclear division times respectively and n the number of generations since germination, the time S for all the nuclei in a hypha to divide = 2d (n-1). If germination is random, the proportion of hyphae with 2, 4, 8 ... (N_e) nuclei to those with other numbers (N_o) of nuclei will be $N_o/N_o+N_e = \exp\left[(S\ln 2)/T\right] - 1$.

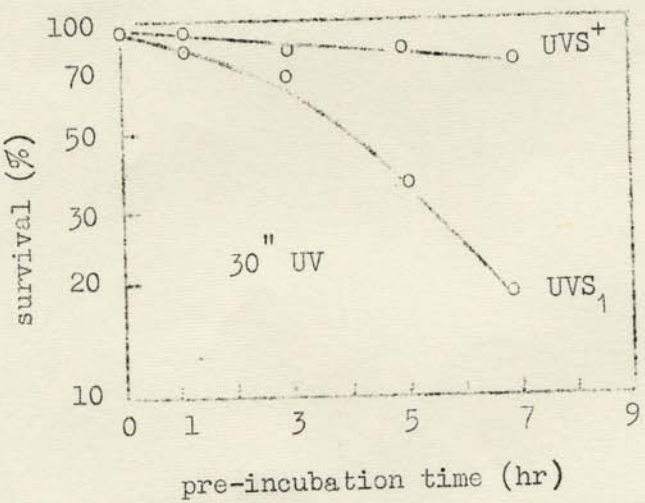
Our preliminary results show that during growth on glucose-nitrate medium (doubling time 1.6 h) nuclear replication was closely synchronised and 2d = 5% of the doubling time. With xylose as sole carbon source the doubling time was 9 h and 2d 58% of the doubling time; with histidine as sole nitrogen source doubling time = 7 h and 2d = 50% of the doubling time. These findings resemble those of Lark (Bact. Rev. 30, 3, 1966) with E. coli cells containing two chromosomes

G.J.O JANSEN

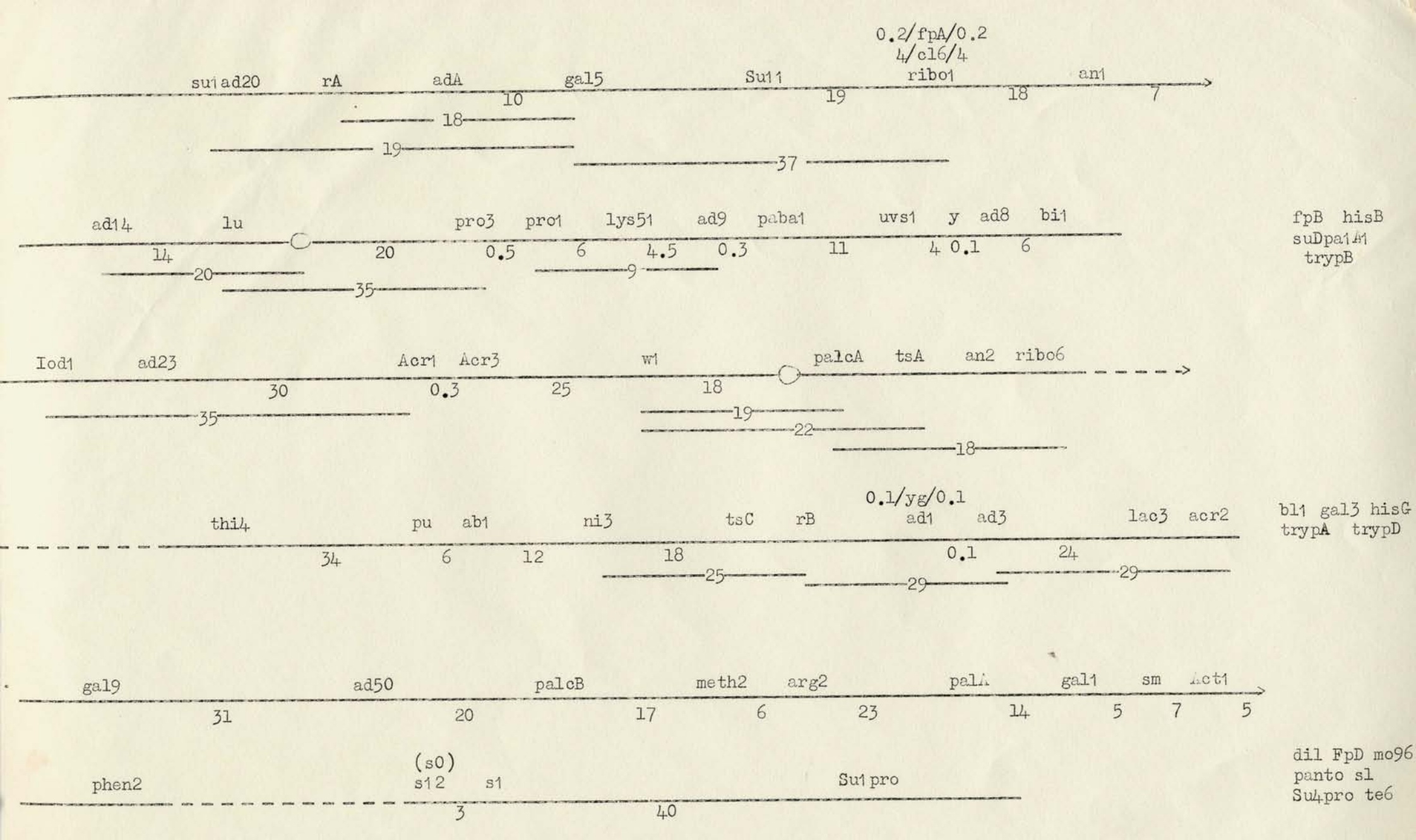
Some properties of the uvs_1 mutant of Aspergillus nidulans

UV-sensitive mutants of Aspergillus nidulans were isolated, one of which was designated uvs_1. One property of this mutant is that germinating uvs_1 conidia are more sensitive to the inactivating action of low UV doses than are germinating uvs^+ conidia. Representative data are given in the figure and were obtained in the following way Conidia of the strain $paba_{J8}$ uvs_1 bi_1 were plated in a layer of

supplemented minimal medium, which was poured on top of a basal layer of the same medium. The plates were incubated at 37°C. After 0, 1, 3,5 and 7 hours of pre-incubation sets of two plates were UV-irradiated for 30 sec, at room temperature, and at 120 cm from a Philips TUV 30W germicidal lamp. The irradiated plates were further incubated at 37°C until colonies could be counted. Control plates were not irradiated. Conidia of the strain $paba_{J8}$ uvs^{+} bi_{1} were subjected to similar treatments. The uvs_{1} mutation is a UV-induced chromosomal mutation and is recessive in diploids. It has no measurable influence on the frequency of meiotic and mitotic spontaneous intragenic recombination. The uvs_{1} locus lies on chromosome I, between the $paba_{1}$ and y locus; it gives recombination frequencies of about 11% with the $paba_{1}$ locus, and about 4% with the y locus.



GORDON L. DORN The Linkage map of Aspergillus nidulans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4/cl6/4
su1 ad20 rA adA gal5 Su11 ribo1 an1
10 19 18 7
18
19
37
ad14 lu pro3 pro1 lys51 ad9 paba1 uvs1 y ad8 bi1
14 20 0.5 6 4.5 0.3 11 4 0.1 6
20
35
9
fpB hisB
suDpa1A1
trypB
Iod1 ad23 Acr1 Acr3 w1 palcA tsA an2 ribo6
30 0.3 25 18
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18
0.1/yg/0.1
thi4 pu ab1 ni3 tsC rB ad1 ad3 lac3 acr2
34 6 12 18 0.1 24
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bl1 gal3 hisG
trypA trypD
gal9 ad50 palcB meth2 arg2 palA gal1 sm Act1
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3 40
dil FpD mo96
panto s1
Su4pro te6

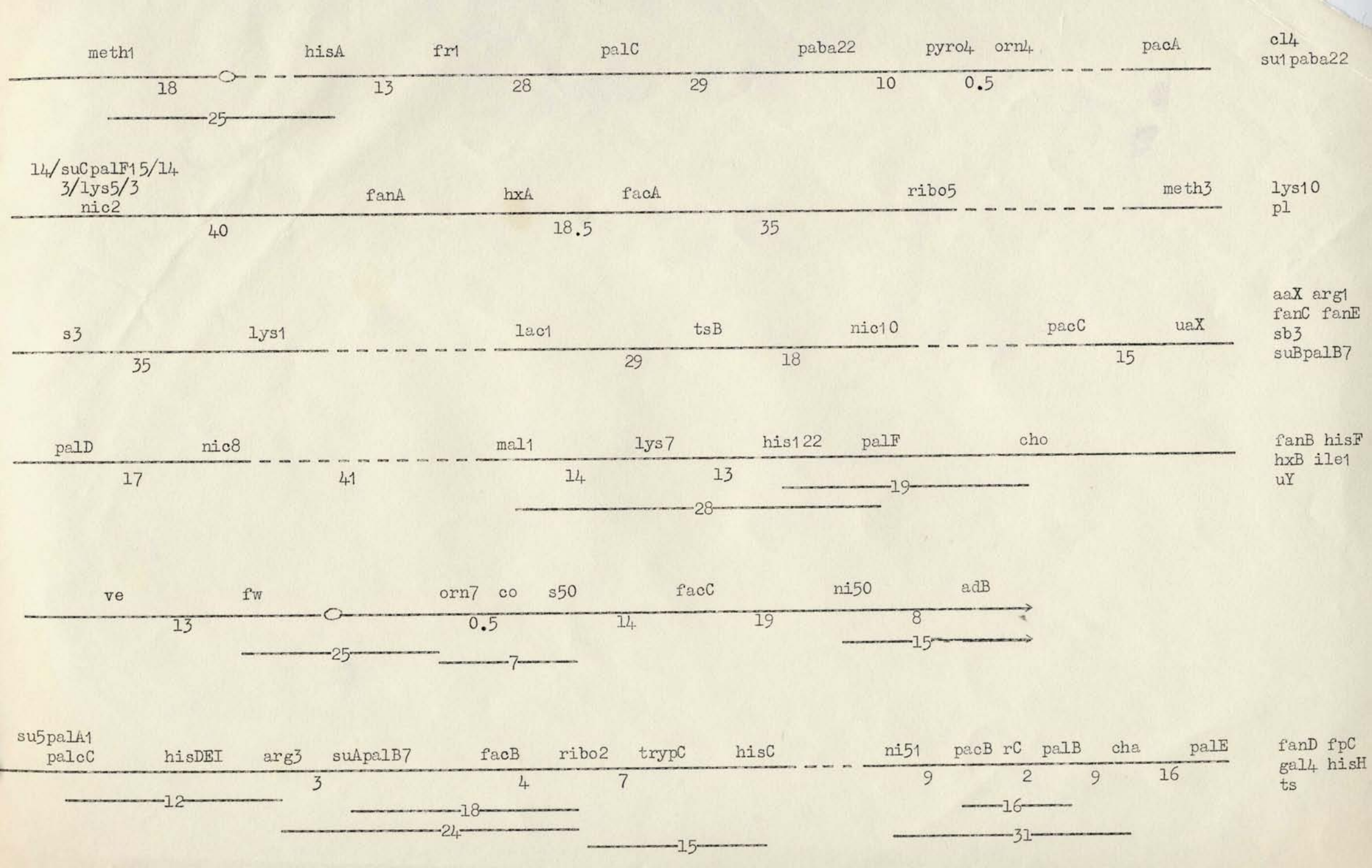
IV.

V.

VI.

VII

VIII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pyro4
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cl4
su1 paba22
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facA
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meth3
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aaX arg1
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suB palB7
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lac1
tsB
nic10
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mal1
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palF
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fanB hisF
hxB ile1
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orn7
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su5 palA1
palcC
hisDEI
arg3
suA palB7
facB
ribo2
trypC
hisC
ni51
pacB
rC
palB
cha
palE
fanD fpC
gal4 hisH
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