Genet. Res., Camb. (1973), **22**, *pp.* 169–179
With 1 *text-figure*
Printed in Great Britain

Definition of the blue mutant phenotype and its genetic basis in *Saccharomyces cerevisiae*

By I. de G. MITCHELL and E. A. BEVAN
Department of Plant Biology and Microbiology, Queen Mary College, London University, London, E.1

(*Received* 16 *April* 1973)

SUMMARY

Mutant colonies of yeast are described which varied in colour from blue to green and in intensity of colour when grown on medium containing methylene blue. Blue mutant colonies contained more dead cells, decolorized redox dyes more slowly, had a higher respiratory quotient on glucose containing medium, and absorbed more methylene blue dye than did wild-type. The blue colony phenotype was induced both spontaneously and at high frequency by ultraviolet light and ethylmethane-sulphonate in both haploid and diploid strains. Many of the light-coloured colonies isolated following mutagenic treatment reverted to wild-type when subcultured but most dark-coloured colonies remained stable. Stable blue mutants were shown to arise by mutation of many separate nuclear genes in haploids and were often caused by recessive lethals in diploids.

1. INTRODUCTION

Mutant strains of yeast whose colonies were blue instead of white on medium containing 0·003% methylene blue were first observed by Costello (1965), who attributed the phenotype to a diaphorase mutation because the blue mutant cells decolorized methylene blue more slowly than those of wild-type. Blue variants were observed to be induced at high frequency with acridine, ethylmethanesulphonate (EMS) and ultraviolet light (UV) in both diploid and haploid strains of yeast. Although this suggested cytoplasmic inheritance preliminary investigations showed only nuclear gene control of the blue phenotype.

The present work describes experiments undertaken to investigate alternative explanations for the blue phenotype, namely a high dead-cell content (Lindegren, 1949) and/or increased dye uptake, and to resolve the uncertainty about its inheritance.

2. MATERIALS AND METHODS

(i) *Strains*

The strains used in the experiments were wild-type N1 and K1 obtained from the Oxford Stock Collection, a neutral petite ac_1, a red adenine auxotroph $ad_{2\cdot1}$, and blue mutant strains B3, B5, B8, B15, B17–B19, B21–B23, B28, B29, B32 and B33 which were derived from wild-type N1 using the mutagens EMS and UV.

(ii) *Media and growth conditions*

Yeast complete agar medium (Cox & Bevan, 1962) was used; 0·003 % methylene blue was added for observation of the blue phenotype. Incubation temperature was 28 °C.

Sporulation was carried out on acetate medium (Fowell, 1952) at 24 °C.

(iii) *Measurement of stainable and viable cells*

The percentage of cells which stained in methylene blue was determined by direct observation of 500–1000 cells after staining in 0·01 % methylene blue dye dissolved in saline.

The percentage of cells able to form colonies was found by plating $2–4 \times 10^3$ cells on to yeast complete agar medium and observing the number of colonies which were formed.

(iv) *Pedigree analyses*

Buds were removed from individual parent cells as they were formed using a Singer micromanipulator (Barer & Saunders-Singer, 1948) with a microloop made on a de Fonbrunne microforge and the open-plate method adapted for yeast (Bevan & Woods, 1963). The buds or the colonies arising from them were observed after 3 days growth on complete medium containing methylene blue.

(v) *Measurement of redox dye reduction*

Dichlorophenol-indophenol (DCPIP) was used as the redox dye since this is not autoxidizable. The colour change at pH 4·8 was from orange to yellow. Reduction rate was given as μg DCPIP reduced per 10^8 cells per min and was measured colorimetrically (on an EEL colorimeter with an ORCI green filter) in 10 ml of reaction mixture pH 4·8 which contained 0·863 % K_2HPO_4, 0·528 % citric acid, 2×10^8 yeast cells and 100 μg DCPIP. The rate was calculated from the time taken to go from 20 to 50 μg reduced DCPIP.

(vi) *Measurement of respiratory quotients*

R.Q. were measured in Warburg manometers by the direct method (Umbreit, Burris & Stauffer, 1957); 10^7 to 10^8 cells per ml in saline adjusted to pH 6·5 were used with 4 % glucose and 2·5 % glycerol as substrates.

(vii) *Mutagenic treatments*

From an overnight culture of yeast, 10^6 cells were suspended in saline at pH 6·5 and 28 °C; 0·1 ml of EMS was added to this suspension in a standard container. The suspension was well shaken to form an emulsion. Alternatively the suspension was poured into an 11 cm diameter Petri dish and irradiated with UV at 254 mμ.

(viii) *Genetic crosses*

Occasionally single cell matings were carried out using a micromanipulator. More often a mass mating technique was used followed by streaking out the

mating mixture and separating out the mating figures by micromanipulation at the appropriate time. Diploids were separated from mating figures, grown up on complete agar medium at 28 °C and then spored on acetate medium for 4 days at 24 °C. Asci walls were dissolved in mushroom enzyme (Bevan & Costello, 1964) and tetrads of ascospores separated by micromanipulation.

3. RESULTS

(i) *Definition of the blue colony phenotype in haploid strains*

Colonies from different blue mutants varied from green to blue and from low to high intensity of colour. For convenience mutants were divided into light (L), medium (M) and dark (D) mutants. Colour depended very much on the medium used and on the growth conditions and it was therefore necessary to standardize both. We found that optimum colour differentiation between mutants occurred when colonies were grown for 4 days at 28 °C from single cells on unbuffered complete medium containing 0·003 % methylene blue and adjusted to pH 5·8.

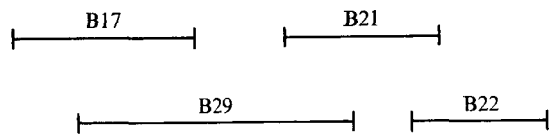

Fig. 1. Systems of overlapping mutants in the gene containing B17, B21, B22 and B29. It is theorized that no gene conversion can occur between mutants of overlapping 'influence'.

Table 1 shows that blue mutant colonies are characterized by their (*a*) content of a large proportion (up to 45 %) of cells staining in methylene blue and an even larger proportion (up to 92 %) of reproductively inviable cells, (*b*) slower reduction of DCPIP (redox dye) than wild-type, (*c*) almost double the R.Q. of wild-type when grown on glucose and (*d*) greater absorption of methylene blue dye from the medium than either wild-type or the equivalent number of dead (heat-killed) cells. Of these characteristics only the increased uptake of methylene blue from the medium correlated with the observed intensity of the colony colour.

The results of pedigree analyses shown in Table 2 show that cells from blue mutant strains produced at irregular intervals reproductively inviable buds (as opposed to the original cell having a short life of division). By direct microscopic observation of the separated buds on methylene blue agar medium we found that a bud never took up methylene blue immediately at its formation. Inviable buds appeared as blue cells only after remaining for 24 h on methylene blue medium.

(ii) *Production of blue mutant strains*

By comparison with the frequency of mutation to red colony phenotype (ad_1 and ad_2 loci), mutation frequency of haploid wild-type N1 to blue mutant pheno-

https://doi.org/10.1017/S0016672300012957 Published online by Cambridge University Press

Table 1. *Morphological and physiological characteristics of selected blue mutants*

Strain	Colony phenotype	% cells which stained blue with M.B.*	% survival col growing / cells plated × 100	Rate of reduction DCPIP† μg per 10^8 cells per min	Respiratory quotient: Glycerol substrate	Respiratory quotient: Glucose substrate	μg M.B. extracted from 10^8 cells
Wild-type K1	White	13·0	—	4·5	—	—	—
Wild-type N1	White	4·7	93·8	3·8	1·11	7·60	0·5
Petite ac_1	White	6·0	—	3·0	—	—	—
$ad_{2\cdot1}$	Red	10·0	75·0	3·0	—	—	—
Heat-killed cells	—	100·0	—	0·0	—	—	3·3
B3	Light coloured	44·2	8·1	2·5	1·15	5·32	—
B15	Medium coloured	31·9	31·5	2·5	—	—	2·4
B19	Medium coloured	45·3	41·7	2·0	—	—	—
B32	Medium coloured	45·4	23·4	1·8	—	—	—
B22	Medium-dark coloured	35·8	40·8	1·8	0·93	5·08	3·8
B23	Medium-dark coloured	37·6	45·1	2·6	—	—	4·0
B28	Medium-dark coloured	36·4	47·0	2·5	0·99	4·67	2·3
B18	Dark coloured	43·6	44·8	1·6	0·94	4·88	4·3
B29	Dark coloured	30·9	44·5	2·0	—	—	4·3
B33	Dark coloured	70·7	7·8	2·7	1·02	5·00	4·6

* Methylene Blue. † Dichlorophenolindophenol.

type is very high both spontaneously and by induction using EMS and UV (Table 3). Furthermore, the frequency of mutation of the diploid resulting from the cross N1a × N1α to blue colony phenotype is of a similar order of magnitude as the haploid frequency.

Table 2. *Pedigree analysis of strains observed after 72 h incubation at 28 °C*

	Cell or colony phenotype from buds (1–11) arising from parent cell O											
Strain	0	1	2	3	4	5	6	7	8	9	10	11
Wild-type	bc	–	.	.	.	.	.	.	.	.	.	.
	W	W	W	W	W	W	W	W	W	W	.	.
N1	W	W	W	W	bc	W	W	W	W	W	W	.
	W	W	W	W	W	W	W	W	W	W	.	.
	W	W	W	W	W	W	W	W	W	W	.	.
	W	W	W	W	W	W	W	W	W	W	.	.
B3	B	B	B	B	B	B	B	B	.	.	.	.
	bc	–	.	.	.	.	.	.	.	.	.	.
	B	B	B	B	B	B	B	B	.	.	.	.
	B	B	B	B	B	B	B	B	B	.	.	.
	bc	bc	bc	–	.	.	.	.	.	.	.	.
	bc	–	.	.	.	.	.	.	.	.	.	.
B18	bc	–	.	.	.	.	.	.	.	.	.	.
	B	B	B	B	B	B	B	B	B	B	B	.
	bc	.	.	.	.	.	.	.	.	.	.	.
	B	B	B	2bc	2bc	B	B	B	B	2bc	B	.
	B	B	B	B	B	B	B	B	B	B	B	B
	B	B	B	B	B	B	B	B	B	B	B	.
B22	B	B	bc	B	B	B	B	.	.	.	.	.
	B	B	B	B	B	B	B	B	.	.	.	.
	B	B	B	B	B	.	.	.	.	.	.	.
	bc	–	.	.	.	.	.	.	.	.	.	.
	bc	–	.	.	.	.	.	.	.	.	.	.
	bc	–	.	.	.	.	.	.	.	.	.	.
B28	bc	–	.	.	.	.	.	.	.	.	.	.
	bc	–	.	.	.	.	.	.	.	.	.	.
	bc	–	.	.	.	.	.	.	.	.	.	.
	bc	–	.	.	.	.	.	.	.	.	.	.
	B	B	B	bc	B	3bc	B	B	.	.	.	.
	B	B	B	B	B	B	B	B	.	.	.	.

W = white phenotype colony; B = blue phenotype colony; bc = blue cell; – = end of cell line.

(iii) *Stability of the blue mutant strains and their behaviour when crossed with wild-type*

Because sectored mutant colonies are often unstable (Lindegren, Hwang, Oshima & Lindegren, 1965; Nasim, 1967) only whole-colony mutants were selected for further analysis.

Table 4 shows that many blue colony variants reverted to wild-type when subcultured, especially those which were light coloured. Twenty-seven out of

thirty-one of the fertile stable mutants when crossed with wild-type N1 gave a diploid with a wild-type phenotype which on sporing produced two blue to two white spore colonies from each tetrad. Initially only five tetrads were analysed per diploid, but the results were confirmed with 14 of the mutants for which

Table 3. *Induction of blue mutation using ultraviolet light and ethanemethanesulphonate*

Strain used	Induction method	Survival (%)	Light coloured mutants (%)	Dark coloured mutants (%)	Blue mutants as sectored colonies (%)	Red colony adenine requiring mutants (%)
Haploid N1	Spontaneous	99	1·2	0·35	39	0
Diploid N1a × N1α	Spontaneous	102	0·9	0·25	42	0
Haploid N1	EMS 3 h	25	19·0	10·0	66	0·3
Diploid N1a × N1α	EMS 3 h	62	16·0	4·5	90	0
Haploid N1	UV 1 min	24	24·0	3·0	70	0·15
Diploid N1a × N1α	UV 1 min	71	8·0	4·0	76	0

Table 4. *Stability of blue mutants and their behaviour when crossed with wild-type strains*

Mutant colony colour	Total no. of mutants isolated	No. of revertants after subculturing	Analysis of stable mutants crossed with wild-type: Total no. Infertile	Total no. Fertile	Diploid phenotype	No. giving 2B:2W ratio	No. giving 4W:0B ratio	No. giving 1W:3B ratio
Light	20	12	3	5	All white	4	1	0
Dark	44	5	13	26	All white	23	0	3

B = Blue colony phenotype: W = White colony phenotype.

20 tetrads per diploid were analysed. Amongst the latter there were the occasional (about 1 in 14) aberrant spore colony ratios. There were also some incomplete tetrads and the white spore colonies were, on average, 2% more viable than the blue ones.

(iv) *Crossing blue mutants* inter se

Reciprocal crosses were carried out between 14 mutants which had aggregated 2 white to 2 blue spore colonies when crossed with wild-type. For each mating three independently isolated diploids were analysed and the complementation matrix, shown in Table 5, was drawn up. Ten tetrads were analysed for each cross. As reciprocal crosses were carried out 20 tetrads were examined per pair of mutant strains crossed *inter se* except where infertility prevented this number of analyses.

Diploids formed from non-complementing mutants had very low fertility.

https://doi.org/10.1017/S0016672300012957 Published online by Cambridge University Press

No spores at all were formed in four of them whilst in the rest tetrads were very infrequent. When such tetrads were analysed, exclusively blue spore colonies were found except in the diploids derived from the crosses B17 × B21, B17 × B22 and B22 × B29, where a significant number of white spore colonies arose (Table 6).

Table 5. *Complementation results between fourteen blue mutant strains*

Mutant colour	*a* mating-type strain	α strain B3	B5	B9	B15	B17	B18	B19	B21	B22	B23	B28	B29	B32	B33
L	B3	−	+	+	+	+	+	+	+	+	+	+	+	+	+
M	B5	+	−	+	+	+	+	+	+	+	+	+	+	+	+
M	B9	+	+	−	+	+	+	+	+	+	+	+	+	+	+
M	B15	+	+	+	−	+	+	+	+	+	+	+	+	+	+
D	B17	+	+	+	+	−	+	+	−	−	+	+	−	+	+
D	B18	+	+	+	+	+	−	+	+	+	+	+	+	+	+
M	B19	+	+	+	+	+	+	−	+	+	+	+	+	+	+
D	B21	+	+	+	+	−	+	+	−	−	+	+	−	+	+
M–D	B22	+	+	+	+	−	+	+	−	−	+	+	−	+	+
M–D	B23	+	+	+	+	+	+	+	+	+	−	+	+	+	+
M–D	B28	+	+	+	+	+	+	+	+	+	+	−	+	+	+
D	B29	+	+	+	+	−	+	+	−	−	+	+	−	+	+
M	B32	+	+	+	+	+	+	+	+	+	+	+	+	−	+
D	B33	+	+	+	+	+	+	+	+	+	+	+	+	+	−

L = Light coloured colony; M = Medium coloured colony; D = Dark coloured colony; + = white diploid; − = blue diploid.

Table 6. *Analysis of the diploids arising from crosses between non-complementing blue mutants B17, B21, B22 and B29* inter se

Cross	Diploid phenotype	No. of spores analysed	Inviable spores (%)	White spore colonies
B17 × B17	Blue	40	15	0
B17 × B21	Blue	80	6	33
B17 × B22	Blue	80	17	13
B17 × B29	Blue	80	8	0
B21 × B21	Blue	0	(No tetrads)	
B21 × B22	Blue	20	35	0
B21 × B29	Blue	80	12	0
B22 × B22	Blue	40	18	0
B22 × B29	Blue	80	37	18
B29 × B29	Blue	28	7	0

Most of the complementing blue mutants gave white colony diploids which on tetrad analyses gave 22–38 % white spore colonies and the rest blue spore colonies. A few crosses, B5 × B19, B15 × B19 and B15 crossed with each of B17, B21, B22 and B29 yielded diploids which gave only 14–18 % white spore colonies whilst in one diploid from the cross B9 × B23 blue spore colonies only were found.

There were many incomplete tetrads in all crosses involving blue mutants. Also certain mutants – for example, B3 and B32 – always gave a diploid with

a large number of inviable spores when they were used as parents in a cross. However, in such crosses there was not a disproportionate number of white spore colonies, indicating that the occurrence of lethality was independent of the spore colony colour character.

Table 7. *Tetrad analysis of blue diploids produced by mutation of a wild-type diploid*

Method of mutant production	No. of tetrads analysed	Colonies resulting from the 4 spores of the analysed tetrads					
		4–	3–1W	2–2W	1–3W	4W	Other tetrads 1B:2W:1–
Spontaneous	10	.	1	9	.	.	.
Spontaneous	10	.	2	8	2	.	.
UV	5	.	.	2	2	1	.
UV	5	.	3	2	.	.	.
UV	5	.	1	4	.	.	.
EMS	5	.	2	2	1	.	.
EMS	5	3	2	.	.	.	.
EMS	4	2	1	.	.	.	1B:3–
EMS	5	4	1	.	.	.	.
EMS	5	4	.	.	.	.	1B:3–
EMS	5	5	.	.	.	.	.
EMS	5	5	.	.	.	.	.

– = Inviable spore; W = white spore colony; B = blue spore colony.

(v) *Analyses of blue mutant diploids derived by mutation of the wild-type diploid N1a × N1α*

Twenty-three independently produced blue diploid variants were studied. Six light coloured ones reverted to wild-type on subculturing and five would not spore. The rest were spored and their tetrads were analysed (Table 7). In two there were no viable spores and in a further four only one or two spores survived out of 20 analysed. In the rest of the six mutants a variable number of white spore colonies were found after tetrad analysis, the most usual ratio being two viable to two dead spores with the viable spores giving, with three exceptions, only white colonies.

4. DISCUSSION

(i) *Definition of the blue phenotype*

We observed that the colour of blue mutants was approximately related to the capacity of their colonies to absorb methylene blue dye from the medium. This was not related to dead cell content because (*a*) the equivalent number of dead cells did not absorb as much dye as blue mutant colonies and (*b*) some of the lighter coloured mutants had the greatest number of dead cells. For example, light

coloured B3 had 92% inviable cells whereas dark coloured B29 had only 56% inviable cells.

Although blue mutants decolorized a redox dye more slowly than did wild-type per unit cell, this decreased rate could be accounted for by the presence of dead cells which stained with methylene blue (Lindegren, 1949). If the rate of redox dye reduction, given in Table 1, is recalculated per cell not staining with methylene blue, this rate turns out to be similar in blue mutants and wild-type. These observations clearly show that blue mutation was not a diaphorase (redox dye reducing enzyme) mutation in otherwise normal cells as was thought by Costello (1965). This conclusion was further emphasized by calculations from the results in Table 1 which showed that blue mutants contained 11–48% of partially moribund cells which did not stain with methylene blue but which were unable to produce visible colonies.

By elimination it is suggested that blue mutations may be caused by some change in cell wall or membrane structure which permits greater uptake of methylene blue dye than wild-type. Passow, Rothstein & Lowenstein (1959) showed that living wild-type yeast cells took up methylene blue as we found; however, our blue mutants absorb nine times as much dye as our wild-type. The production of dead cells at irregular intervals, and the increase in R.Q. when cells were grown on glucose suggested that the changes in membrane or wall structure which gave blue phenotype resulted in some metabolic imbalance and was semi-lethal.

(ii) *The genetic basis of blue mutation*

Many of the light coloured blue mutants were ephemeral and could probably be explained either by temporary change in gene function or by permanent damage to a proportion of genes showing genetic redundancy followed by selection for wild-type in vegetative segregation, or by damage to non-genetical material with extremely slow turnover. Such explanations would account for the similar frequency of spontaneous and mutagenic production of light blue colonies from both haploids and diploids.

Most dark coloured haploid mutants were stable and when crossed with wild-type behaved as if controlled by single recessive nuclear genes. The occasional aberrant ratio found in tetrads could be explained by the technical errors likely in tetrad analyses and by natural errors at meiosis (Lindegren, 1949; Winge & Roberts, 1954). The frequencies of reverse mutation, suppression (Gorini & Beckwith, 1966) or mitotic recombination and gene conversion (James & Lee-Whiting, 1955; Roman & Jacob, 1957; Parry, 1972; Mori & Nakai, 1972) are probably too low to have accounted for the frequency of aberrant tetrads.

When fourteen blue mutants were crossed *inter se*, each of eight complemented the other thirteen yielding white colony diploids which gave some white spore colonies. This showed that these blue mutants belonged to eight separate genes. Two of the 14 mutants, B9 and B23, complemented giving a white diploid, but this diploid yielded only blue spore colonies suggesting that these were mutants of the same or very closely linked genes. Four mutants, B17, B21, B22 and B29,

were non-complementary and probably therefore belonged to the same locus, in which case the occurrences of white spore colonies from diploids of crosses B17 × B21, B17 × B22 and B22 × B29 required explanation. Because there were no white spore colonies in the homozygous crosses (B17 × B17, B29 × B29, B22 × B22 and B21 × B21) it is unlikely that they arose by reverse mutation or by suppression (Gorini & Beckwith, 1966) in B17 × B21, B17 × B22 and B22 × B29 diploids (Table 6). Most probably mitotic recombination and/or gene conversion (James & Lee-Whiting, 1955; Roman & Jacob, 1957; Parry, 1972; Mori & Nakai, 1972) occurred between these non-identical mutants of the same locus. The lack of white spore colonies from diploids in B17 × B29, B21 × B22 and B21 × B29 might be explained if gene conversion could not occur between 'overlapping mutants' of the same locus as shown in Fig. 1. This data, however, is difficult to explain on the basis of the polaron models of recombination put forward by Whitehouse & Hastings (1965). Once a wild-type gene had arisen there would be strong selection for it in tetrad analysis because heterozygotes were observed to form tetrads much more readily than homozygotes.

The white to blue spore colony ratio showed that mutant genes of strains B5, B19, B15 and B(17, 21, 22, 29) were linked in that order. All the other blue genes segregated independently of each other as they yielded white diploids when crossed and between 22 % and 38 % white spore colonies when such diploids were analysed. The slight excess of white spore colonies over the theoretical 25 % could be accounted for by the greater viability of wild-type spores.

When stable blue mutant diploids were produced directly from a wild-type diploid it was found that recessive lethals could cause blue mutant phenotype. Deviations from the expected ratio of two wild-type viable spores to two inviable spores were frequent, but this was expected as similar results were reported by James & Werner (1966) using X-rays to produce recessive lethals in diploid yeast. The occasional blue spore colony amongst the wild-type (white) spore colonies could possibly have arisen from meiotic errors (Lindegren, 1949; Winge & Roberts, 1954).

In summary, a possible explanation for the occurrence of the stable blue mutant phenotype could be that a partially lethal change in cell wall or membrane structure had arisen. The results of genetic crosses and complementation studies indicate that many gene loci can independently give rise to the blue phenotype. Thus we may conclude that the large number of genes accounts for the high frequency of induction of blue colony phenotype. The similar frequency of induction of stable blue mutants from haploid and diploid strains could be explained by the occurrence of recessive semi-lethals as well as 'blue genes' in the diploid which themselves cause the expression of a blue phenotype.

REFERENCES

Barer, R. & Saunders-Singer, A. E. (1948). A new single-control micromanipulator. *Quarterly Journal of Microscopical Sciences* **89**, 437–439.

Bevan, E. A. & Costello, W. P. (1964). The preparation and use of an enzyme which breaks open yeast asci. *Microbial Genetics Bulletin* **21**, 5.

Bevan, E. A. & Woods, R. A. (1963). *The Use of Yeast in Teaching Practical Genetics*, C. D. Darlington and A. D. Bradshaw. Oliver and Boyd.

Costello, W. P. (1965). *Genetic studies on mutants of specific loci in yeast*. D.Phil. Thesis, Oxford.

Cox, B. S. & Bevan, E. A. (1962). Aneuploidy in yeast. *New Phytologist* **61**, 342–355.

Fowell, R. R. (1952). Sodium acetate as a sporulation medium for yeast. *Nature* **170**, 578.

Gorini, L. & Beckwith, J. R. (1966). Suppression. *Annual Review of Microbiology*, pp. 401–422.

James, A. P. & Lee-Whiting, B. (1955). Radiation-induced genetic segregation in vegetative cells of diploid yeast. *Genetics* **40**, 826–831.

James, A. P. & Werner, M. M. (1966). Radiation-induced genetic mosaicism and lethal sectoring in yeast. *Mutation Research* **3**, 477–485.

Lindegren, C. C. (1949). *The Yeast Cell: Its Genetics and Cytology*. St Louis: Educational Pub. Inc.

Lindegren, G., Hwang, Y. L., Oshima, Y. & Lindegren, C. C. (1965). Genetical mutants induced by ethylmethanesulphonate in *Saccharomyces*. *Canadian Journal of Genetics Cytology* **7**, 491–499.

Mori, S. & Nakai, S. (1972). Induction and repair of gene conversion in ultraviolet sensitive mutants of yeast. *Mol. Gen. Genetics*. **117**, 187–196.

Nasim, A. (1967). The induction of replicative instability by mutagenesis in *Saccharomyces pombe*. *Mutation Research* **4**, 753–763.

Parry, J. M. (1972). Mitotic recombination in yeast. *Laboratory Practice* **21**, 417–419.

Passow, H., Rothstein, A. & Lowenstein, B. (1959). An all or nothing response in the release of potassium by yeast cells treated with methylene blue and other basic redox dyes. *J. gen. Physiol.* **43**, 97–107.

Prescott, D. M. & Mazia, D. (1954). The permeability of nucleated and enucleated fragments of *Amoeba proteus* to D_2O. *Experimental Cell Research* **6**, 117–126.

Roman, H. & Jacob, F. (1957). Effet de la lumier ultraviolette sur la recombonaison génétique entre alleles chez la levure. *Compte rendu des séances de la Société de biologie* **245**, 1032–1034.

Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1957). *Manometric Techniques*, rev. ed. Burgess.

Whitehouse, H. L. K. & Hastings, P. J. (1965). The analysis of genetic recombination on the polaron hybrid DNA model. *Genetic Research, Cambridge* **6**, 27–92.

Winge, O. & Roberts, C. (1954). Causes of deviations from 2:2 segregation in the tetrads of monohybrid yeasts. *C. R. Lab. Carlsberg Ser. Physiol.* **25**, 285–329.