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Nutritional conditional mutants of *Drosophila melanogaster*

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The existence of defined culture media for *Drosophila melanogaster* (Hinton, Noyes & Ellis, 1951; Burnett & Sang, 1963; Geer, 1965) should allow isolation of mutant strains in which survival is dependent upon chemically specific supplements to the 'normal' minimal growth medium. However, there is, as yet, no report of their isolation after mutagenic treatment although Hinton, Ellis & Noyes (1951) and Ellis (1959) have shown certain nucleic acid precursors to increase survivorship in two pre-existing strains. The determination of the 'requirement' was genetically complex in both cases and, apparently, conditional upon the pH of the medium (Ellis, 1959). Should strains with complete nutritional requirements exist, it might be expected that, in some cases, the molecular mechanisms underlying lethality and the response to supplement would be identical to the 'metabolic block' explanation which holds for many auxotrophic mutants of micro-organisms (Beadle & Tatum, 1941). We wish to report some progress in isolating nutritionally dependent strains using a direct application of conventional defined culture and mutagenesis techniques.

Sex-linked lethal mutations were produced by exposure of Oregon R wild-type males to the mutagen ethyl methane-sulphonate by the method of Lewis & Bacher (1968) and following Muller's procedure, as described by Spencer & Stern (1948), A mutation rate of 38 % of sex-linked recessive lethals was obtained in this test. a figure comparable with those published by other workers. No mutants were found in a small control. The second generation of the test, in which the absence of wild-type males from the progeny identifies a sex-linked lethal mutation, was grown on a defined medium of Geer (1965) containing fourteen amino acids and other essential nutrients, as well as RNA, which is a requirement in a medium of this particular composition.

Lethal mutants were then retested both on Geer's amino acid medium and a yeast–sucrose medium (similar to that used by Nash & Bell (1968), but without the addition of chloramphenicol), which we assume to contain a large number of additional components, some of which could, assuming current biochemical knowledge, become absolute requirements for the fly. One conditional mutant (*11523*) has so far been obtained by this technique; viable, fertile *11523* males of wild-type appearance are found in the yeast–sucrose cultures.

Non-mutants, as defined by the original test, were transferred to the defined casein medium of Burnett & Sang (1963) which contains more amino acid residues

than Geer's (1965) medium, but lacks RNA. Two strains (*1308* and *1625*) proved lethal under this condition.

The three conditional mutants were then retested for their growth capacities on the casein medium (with or without the addition of 4·0 mg/ml RNA) and the yeast–sucrose medium. The results are shown in Table 1.

All test and retest cultures were maintained in axenic conditions, either by sterilization of the eggs or by sterile transfer from established axenic cultures. Critical cultures were tested for the absence of foreign organisms by placing killed larvae or adults or food samples on nutrient agar plates.

Table 1. *Retests of the survival of nutritional conditional lethals on various media*

	Strain								
	1308			*1625*			*11523*		
Medium	−RNA*	+RNA*	Yeast	−RNA*	+RNA*	Yeast	−RNA*	+RNA*	Yeast
Genotype									
♀ *Basc*/+	49	47	78	26	7	14	9	62	35
♀ *Basc*/*Basc*	33	28	52	21	10	18	1	7	8
♂ +	0	9	44	0	8	18	0	0	8
♂ *Basc*	42	42	42	16	7	21	8	35	18

The segregations above were found in progeny of the cross *Basc*/+ ♀ × Basc ♂, which is a repeat of the Muller-5 test, generation 2. In the table the sign '+' indicates the chromosome which, although carrying a conditional mutant, is wild-type with respect to the markers in the *Basc* chromosome.

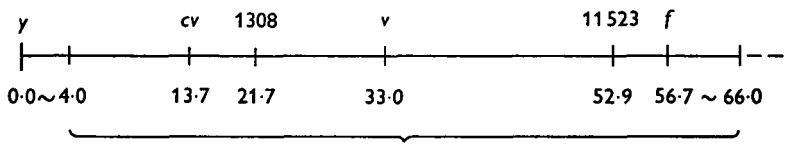
* Burnett & Sang's casein medium with or without 4·0 mg/ml RNA added.

The genetic factors involved in the nutritional responses were mapped by recombination with the markers *yellow* (*y*), *cross-veinless* (*cv*), *vermilion* (*v*) *and forked* (*f*). Females heterozygous for the test and mutant chromosomes were mated to *y cv v f* males and the offspring reared under restrictive conditions (Burnett & Sang's (1963) casein medium). Male progeny with recombinant chromosomes carrying the conditional lethal factors should die under these circumstances, leaving a selected sample of males, the composition of which indicates the linkage relationships of the mutant factors.

Strain *1625* showed very low recombination rates (less than 5%), despite the theoretical map distance between *y* and *f* (56·7 map units). The strain was subsequently shown to contain an *X*-chromosome inversion which accounts for the reduced recombination frequencies. We designate the inversion In(1) 1625, 3F-20C, using the convention adopted by Lindsley & Grell (1968). The nucleolar-organizer (20C2) is included in the inversion. In view of the involvement of the nucleolus in ribosome synthesis (Ritossa & Spiegelman, 1965), we conjecture that the positioning of one break-point very close to the nucleolar-organizer may have caused the RNA requirement.

Strains 1308 and 11523 show more nearly normal crossover frequencies, as judged by the female progeny in the recombination test described above. About 40% of females carry recombinant chromosomes, compared with 50% in the control. The data for male progeny are shown in Table 2. These results yield the naïve estimates of map positions shown in (Fig. 1).

Calculation of the locations takes into account the observed inequalities in various reciprocal recombinant classes in the control. Certain other genetic factors may well disturb these estimates. Two examples can be cited: A factor close to forked, originating from the marker strain, may increase the viability of flies bearing the major factor associated with *1308* and a factor originating in the marked chromosome near to *cv* interacts strongly with another close to *11523* (perhaps *11523* itself) to reduce viability, even on permissive medium.



In (1) 1625 (from cytological data)

Fig. 1

As is noted in Table 2, between 2 and 5% of potentially 'lethal' classes of flies survive in the recombination test. This behaviour is characteristic of the conditional strains grown in crowded cultures on restrictive media, but not when they are grown in cultures with low population density (8–10 larvae/ml medium). We suggest that this may be due to cannibalism of dead larvae by surviving mutants.

Despite such problems the genetic factors responsible for the nutritional requirements of *1308* and *11523* appear primarily located within limited regions of the *X* chromosome. Recombination tests using other markers confirm that the estimates given above are approximately correct. There is still a chance that the regions are larger than would be expected of point mutations, but the evidence is suggestive that the factors are simple genetic alterations.

Of the three mutant strains, only *1625* (the inversion) displays morphologically abnormal traits when grown in permissive conditions. The RNA requirement associated with *1625* has been found to revert without concomitant loss either of the inversion or the morphological effects. Thus, there is no reason to suppose that the morphological effects are caused by the RNA requirement. They are probably position effects associated with the transposition of the tip of the *X* chromosome to the proximal heterochromatin.

Other than the nutritional requirements, only one of the remaining two mutant strains shows an additional phenotypic effect; homozygous *11523* females are sterile. Whether this is a pleiotropic effect of the conditional lethal mutant will be determined only when recombinant chromosomes are retested.

With respect to the nutritional requirements themselves we have had no success

Table 2. *Frequencies (in percentages) of non-recombinant and single recombinant phenotypes in the male progeny of females heterozygous for the* y cv v f X *chromosome and either a* 1308, 11523 *or* Or+ X *chromosome and reared on Burnett & Sang's casein medium without RNA*

Phenotype cross	Non-recombinants		Single recombinants						Multiple recom-binants	Sample size	No. of females in progeny
	ycvvf	+ + + +	*y* + + +	+ *cvvf*	*ycv* + +	+ + *vf*	*ycvv* +	+ + + *f*			
Or+	18·0	34·0	4·8	2·6	6·2	11·0	8·8	9·9	4·5	726	1036
1308	66·4	2·2*	0·2*	5·3	6·6	4·5	10·5	1·4	2·9	1188	3156
11523	68·5	2·4*	0·5*	4·8	0·3*	8·3	1·8	10·7	2·9	796	1914

* These classes of flies must be considered as escapes from lethality; numbers of such flies from the *11523* cross have been shown to carry a conditional lethal *X* chromosome.

in extensive attempts to further characterize the chemical nature of the factors required. Table 3 lists the chemicals and groups of chemicals which have been tested for stimulation of growth of *1308* and *11523*.

Table 3. *Tests for survival of nutritional conditional mutants on media supplemented with various chemicals*

	Or+ control			*1308*		
Supplemented	Larvae tested	Adults produced	% survival	Larvae tested	Adults produced	% survival
None	510	151	29·8	1105	0	0
RNA*	160	132	83·5	480	71	14·8
RNA†	325	204	62·8	455	85	16·7
RNA core	240	39	16·25	240	7	2·9
Guanosine-5′MP	240	70	29·2	320	3	0·9
Adenosine-5′MP	200	19	8·5	440	1	0·2
Xanthosine-5′MP	200	71	35·5	400	3	0·75
Inosine-5′MP	280	91	32·8	520	4	0·8

The following classes of additives failed to produce *1308* survivors: RNA bases;‡ ribonucleosides;‡ 2′-3′-P-ribonucleosides;‡ cytidine-5′MP; uridine-5′-MP; homopolymers poly-A, -G, -C, -U; ribose 5-phosphate; DNA.

Concentrations—nucleosides, nucleotides, homo-polynucleotides: 1·0 mg/ml; bases, ribose-5-phosphate: 0·5 mg/ml; RNA, DNA: 4·0 mg/ml.

The following additives failed to produce *11523* survivors (concentrations in parentheses): RNA (4·0 mg/ml); DNA (4·0 mg/ml); yeast extract (10·0 mg/ml); inositol (4·2 mg/100 ml); para-amino benzoic acid (0·2 mg/100 ml).

* Sigma Chemical type XI (97 % pure). † Nutritional Biochemical; Calbiochem; B.D.H.
‡ Singly and all four together.

The finding is not unexpected for *11523*. The range of components of the yeast medium is clearly large, and seeking the nutritional dependency is necessarily hit-or-miss. The only additional information on *11523* is the finding that relatively fewer *11523* individuals survive in crowded culture conditions, suggesting either that only small quantities of any critical substance are available in yeast–sucrose medium or that a large amount of the substance is required.

1308 has proved only slightly more revealing of its nature; the most pure samples of polymerized RNA available to us (Sigma Chemical Type XI) as well as a number of less pure commercially available samples support growth—as do purified ribosomal RNA, and ribosomal-core-RNA (a partially degraded ribosomal RNA derivative). No other RNA derivative convincingly supports growth. We assume that RNA is acting as a specific nutritional factor in supporting growth, but can only conjecture as to the manner in which it acts. The possibility that an unidentified contaminant common to our RNA samples is effective as a growth stimulant has not been ruled out. The loss in requirement, which Ellis (1959) found to characterize the two conditional strains of Hinton, Ellis & Noyes (1951) and Ellis (1959) when grown on medium at low pH, is not found.

From the results presented above it is clear that it is possible to produce nutritional conditional lethals. There is no good reason to suppose that many more

might not be found, since the three so far obtained were found amongst 604 chromosomes, or 227 recessive lethal chromosomes, upon which the tests have been carried to completion. Whether others will be obtained which are more easily analysed biochemically as true auxotrophs is an open question. There seems no reason to dismiss this possibility as yet, since tissue culture strains with auxotrophic characteristics are known (Eagle, Washington & Freidman, 1966; Price, Rotherham & Evans, 1967; Kao & Puck, 1968).

None the less, the alternative viewpoint, that the particular nature of a highly organized, multicellular animal such as *Drosophila* may reduce the chances of isolating critical mutants in biosynthetic pathways is worth consideration. It is well known that despite the theoretically large number of potentially mutable biosynthetic steps in higher plants, the only clearly defined auxotrophic mutants in the intensively studied crucifer, *Arabidopsis*, involve thiamine synthesis (Langridge, 1965).

SUMMARY

A small fraction of mutagen treated *X* chromosomes which would appear non-lethal in the Muller-5 test carried out on a yeast-containing medium act as lethals when tested on one or other of the defined *Drosophila* media. Such mutants have been termed 'nutritional conditionals'. The three mutants obtained in this way have been characterized genetically, but have not yet been shown to act as simple auxotrophs.

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