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Transformation and reversion: Pitfalls imposed by heterokaryosis.

Selection for reversion of a mutant phenotype often involves the appearance of a revertant nucleus in the same (multinucleate) cell as an original nucleus, hereafter called parental. The same is true of transformants, which arise in multinucleate spheroplasts in the company of untransformed, parental nuclei. One implication of this is clear enough; namely, that the revertant will appear in greater numbers if it is dominant, especially if conidia are crowded on the selection plate. Another consequence emerged in our laboratory in the course of isolating spermidine-independent revertants of the spe-1 mutant after ultraviolet irradiation.

We irradiated and plated large numbers (ca. 1 x 10^6) of conidia of an ornithine decarboxylase-deficient spe-1 strain on Vogel's minimal medium. A very large number of revertants appeared, owing to the revertibility of this allele. We picked 26 of them to minimal medium, thereby maintaining selection. We then streaked the conidia on minimal medium and picked single conidial isolates for two serial generations, each time maintaining the isolates on minimal medium. The final isolates were plated on spermidine-containing medium as a test for the persistence of parental nuclei among the conidial population. Fourteen of the 26 cultures retained spe-1 nuclei.

This outcome was not wholly surprising, because selection on minimal medium is for prototrophic conidial colonies, not necessarily homokaryotic ones. Moreover, the ratios of heterokaryotic and homokaryotic (revertant) conidia might be expected to remain balanced in many cases, owing to the selection of a heterokaryotic conidium each time (nuclear ratios ranging from 1:2 to 2:l in bi- and trinucleate conidia). Nevertheless, more pure cultures might have been expected at this stage, and we therefore investigated the heterokaryotic cultures in detail.

The remarkable fact was that of the 14 impure cultures, 10 were impossible to purify, or yielded homokaryotic revertants that were exceedingly weak, even on supplemented medium. Conidia of many of the heterokaryons, when plated on such media, yielded distinctive germlings that failed to grow further. Thus the tendency to select heterokaryons had been enforced by the fact that both the spe-2 and the revertant homokaryons could not grow, or grow well, on minimal medium. The results suggested that reversion of the spe-1 mutation to partial or complete restoration of ornithine decarboxylase might be associated with the simultaneous loss of an indispensable function. Because the reversion event allowed the heterokaryon to grow we11 on minimal medium, the revertant homokaryon's weakness or lethality was not due to the incompleteness of the return to wild type catalytic function.

To determine whether the "lethal" event and the reversion event were at the same locus (a test for the location of the latter at the spe-1 locus came later), the standard rationale was applied: all isolates (heterokaryotic and homokaryotic) were mated to a spe-2 strain of the opposite mating type. All of the purified prototropic homokaryons, as expected, gave viable, prototrophic ascospores. However, so did all of the heterokaryotic cultures. This meant that the revertant nuclei of all heterokaryons contained two mutations, one the reversion to spe-1$^+$, the other a lethal or semilethal mutation elsewhere in the genome. The latter was lost during recombination in the cross. (Some of the distinctive germlings were seen among the progeny, assuring us that the lethals were bonafide, nuclear mutations.) In most, but not all cases, the two mutations were unlinked.

Of what interest is this story? There are two major points to be made. First, the ultraviolet irradiation used to induce the revertants was mild, calibrated for about 50% or less killing of wild type conidia. The appearance of a very large proportion of multiply mutant nuclei was wholly unexpected, and might be accounted for by peculiarities of the spe-1 phenotype. (It would not be unreasonable to find that a polyamine deficiency, hard to satisfy even in supplemented medium, might be unusually susceptible to DNA damage.) Nevertheless, to the extent that multiple mutation might be seen in reversion of other mutants, our experience underscores the need for a backcross to the mutant in question in order to shed the additional mutational events. If this is not done, pleiotropic effects might be falsely attributed to a reverse mutation. A more troubling consequence ensues in mating a heterokaryotic revertant to wild type to distinguish true reversions from intergenic suppressors: the mutant component of the heterokaryon will emerge among the progeny and will mislead one to the conclusion that reversion is due to a suppressor mutation.

The most important technical arena in which this problem might arise is transformation. Usually, a mutant is used as a recipient of DNA, and selection is then imposed for the positive phenotype. Owing to the apparently relaxed homology requirements for integration in Neurospora, there will be cases in which a plas-

mid or transforming DNA inserts the wild type gene into a resident, indispensable locus. (Alternatively, disruptive integration by a second DNA molecule could occur.) The nucleus in which this happens will be inviable as a homokaryon, but will contribute the selected function to a heterokaryotic transformant. The untransformed nucleus will be maintained in the transformant as the source of the indispensable function. As one tries to purify this strain, it will keep throwing off the untransformed mutant, and will appear to be an "unstable transformant". This might be interpreted as a plasmid that cannot be maintained, owing to poor replication or to excision. Crosses of the heterokaryotic transformant to wild type or the untransformed mutant will yield no transformants as ascospores in the cases in which transforming DNA is embedded in a disrupted, indispensable gene.

This is almost certainly a banal lesson to those familiar with the biology of Neurospora. However, those unfamiliar with the complications of heterokaryosis should not have to rediscover them first hand. Because this situation has no counterpart in transformation work in yeast, this description is offered to those who might rely unduly on yeast as a paradigm.

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