Molec. gen. Genet. 138, 81—84 (1975)

Short Communication

Distribution of Mutant Sites in the *ftr* Cistron Depends upon the Medium Used for Selection

David Moore and Magdalene S. Devadatham

Department of Botany, The University, Manchester

Received February 26, 1975

Summary. Mutants belonging to the *ftr* cistron have all been selected for resistance to inhibitory sugar analogues on media which completely inhibited growth of wild type spores. The position in the allele map occupied by the mutants obtained, however, was found to depend on both the identity of the analogue and the identity of any non-inhibitory carbon source present in the selection medium. Interpretation of the data in terms of the differential function of different regions of the gene product is discussed.

The hexose analogues 2-deoxy-D-glucose (deGlc), D-glucosamine (GA), and L-sorbose all inhibit extension growth of *Coprinus lagopus* hyphae (Moore and Stewart, 1972). Sorbose is the least effective (50% inhibition of growth rate caused by 0.8 mM), GA intermediate (50% inhibition caused by 0.3 mM) and deGlc the most inhibitory (0.04 mM for 50% inhibition). The degree of inhibition was found to depend also on the identity and concentration of normal carbon sources present in the medium. Acetate and fructose were virtually ineffective in reversing inhibition, while the effects of all three analogues were readily reversed by addition of glucose to the medium. Mutants have been obtained which are resistant to inhibition by deGlc (Moore and Stewart, 1971) or by selection for resistance to GA or sorbose (Moore, 1973a). All mutants so far obtained have proved to be alleles of a single cistron which is called *ftr*. The cistron appears to control a function involved in sugar transport; mutants being defective in the accumulation of fructose, glucose, sorbose and deGlc (Moore, 1973a). In consequence *ftr* alleles have a physiologically pleiotropic phenotype; they are cross-resistant to inhibition by any of the three analogues (no matter which one was used in their selection) and their growth with fructose as sole carbon source is restricted to a thin, barely visible mycelium. A fine-structure gene map of deGlc-selected alleles has been prepared and a degree of correlation between map position and physiological phenotype was recognised (Moore, 1972).

The *ftr* cistron offers the unusual advantage of selection in two directions: forward towards sugar analogue resistance and in the reverse direction from mutant inability to make good growth on fructose-media towards wild type ability to grow well on fructose. It is thus feasible to attempt the selection of alleles under different conditions and then determine the position of each allele in the cistron in order to see whether different selection conditions favour the identification of mutants localised in different parts of the cistron. Any such

localisation might be correlated with polypeptide function. The *ftr* system is also unusual in offering a wide variety of selection procedures. Alleles selected on four contrasting media have been mapped in the work reported here. Spontaneous mutants selected for resistance to sorbose or to GA have been described before (Moore, 1973a). Strains resistant to deGlc obtained after treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine were selected in two ways, either on medium containing fructose + deGlc or on medium containing sodium acetate + deGlc (Moore and Stewart, 1971). The contrasting mutational origin (spontaneous or nitrosoguanidine-induced) has no influence on the distribution of the alleles within the cistron (Moore, 1972).

The basis of the existing allele map is provided by alleles selected for resistance to deGlc, most of them having been selected on fructose + deGlc media. The distributions of sorbose-selected, glucosamine-selected and acetate + deGlc selected mutants were established by intercrossing these new alleles with reference alleles of known position in the existing allele map. Use was made of 12 reference alleles spaced out over the full length of the map, and each new allele was crossed with between 3 and 7 of the reference alleles. The intention was to establish the general distribution of each class of mutant, consequently the smallest number of crosses needed to place a new allele within about 5 units of a reference allele were undertaken (the whole map extends over about 80 units). In the event the majority of the new alleles can be positioned far more closely than this, even after allowance is made for the variability of recombination frequencies in this cistron (Moore, 1973b). A total of 41 alleles selected on fructose + deGlc, 32 selected on acetate + deGlc, 17 selected for resistance to glucosamine and 18 selected for resistance to sorbose have been mapped. The distributions of these four types of mutants can be summarized in the form shown in Fig. 1.

The distribution of alleles selected for resistance to sorbose is quite distinctive; mutations in just one small segment of the cistron are preferentially isolated when sorbose is used as the selective agent. Considerable localisation is also evident in the distribution of alleles selected on acetate + deGlc. The most scattered distributions are obtained with alleles selected either for resistance to deGlc in the presence of fructose, or for resistance to glucosamine.

In interpreting these data it is important to recognise that although the analogues differ in their ability to inhibit growth, all of the mutants were selected on media which completely inhibited growth of the wild type. Furthermore, mutation at any point in the cistron confers cross-resistance to all three analogues. Assuming that the *ftr* cistron specifies a permease protein, mutation must lead to resistance because the resultant alteration in polypeptide structure greatly diminishes the affinity of the permease for the inhibitory sugar. Differences in the distribution patterns of alleles selected under contrasting conditions must reflect variations in the affinity of the permease for the analogues. Although a defect in any part of the cistron is sufficient to affect sorbose transport, not all defects are able to so adversely affect transport as to confer selective advantage when sorbose is present in the medium. Consequently selection of mutants on a sorbose-containing medium tends to favour the identification of those changes which have occurred in positions that most seriously affect sorbose transport. In the *ftr* cistron these are clearly very highly localised, being almost entirely restricted

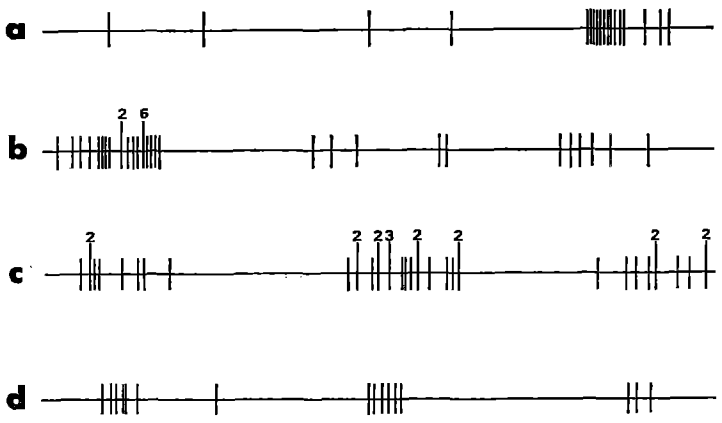

Fig. 1a—d. Distributions of alleles selected on different media in the *ftr* fine structure map. In each case the horizontal line represents the long axis of the chromosome while the vertical lines show the positions of the recombinable sites. Extended vertical lines indicate sites at which more than one allele maps; the numeral above the extended lines shows the number of independently isolated alleles which occupy that position. The positions of (a) mutants selected for resistance to sorbose, (b) mutants selected on a medium containing acetate + 2-deoxy-D-glucose, (c) mutants selected on a medium containing fructose + 2-deoxy-D-glucose, and (d) mutants selected for resistance to glucosamine are shown to approximate scale. No attempt has been made to intercross these alleles in all possible combinations so that the possibility exists that some of the sites indicated as being separate are in fact non-recombinable. Since each allele referred to is a totally independent mutation + selection event this would not alter our conclusions for it would make the localisation of the different types of mutants even more extreme than is indicated here

to a region at the right-hand end of the gene. Presumably this region of the cistron specifies the part of the polypeptide which interacts with the feature that most clearly characterises the sorbose molecule as distinct from, say, the glucosamine molecule. Similarly, when deGlc is the selection agent in a medium which contains only acetate as a companion carbon source alterations in a part of the polypeptide with affinity for a different aspect of hexose structure are preferentially identified. Interestingly, the presence of another hexose, as in the fructose + deGlc selection medium abolishes the selection specificity. Competition of the non-inhibitory hexose for the permease must so alter the relationship between permease and deGlc as to equalise selection pressure over the whole cistron. An amino group at the C-2 position (glucosamine) has a similar effect.

It is premature at this time to offer a too detailed interpretation of these genetic data. We see this analysis as contributing to an understanding of the molecular details of the hexose-permease interaction. However, this requires integration with a biochemical characterisation of sugar transport; these studies are presently under way. The data are of wider implication though, for they illustrate the subtle and precise way in which the selection process can influence the type of mutant obtained. There must be many instances where such influences are not so readily recognised, and perhaps not even suspected. In the absence

of data to the contrary it should not be assumed that any mutant isolation procedure has equal influence on all potentially mutant sites within a cistron.

References

Moore, D.: Genetic fine structure, site clustering and marker effect in the *ftr* cistron of *Coprinus*. Genet. Res. **19**, 281–303 (1972)

Moore, D.: Mutants of *Coprinus* selected for resistance to D-glucosamine and L-sorbose. Genet. Res. **22**, 205–209 (1973a)

Moore, D.: Variability of recombination frequencies in the *ftr* cistron of *Coprinus* and its influence on the identification of marker effect alleles. Genet. Res. **22**, 187–193 (1973b)

Moore, D., Stewart, G. R.: Mutants of *Coprinus lagopus* selected for resistance to 2-deoxy-D-glucose. Genet. Res. **18**, 341–352 (1971)

Moore, D., Stewart, G. R.: Effects of 2-deoxy-D-glucose, D-glucosamine, and L-sorbose on the growth of *Coprinus lagopus* hyphae. J. gen. Microbiol. **71**, 333–342 (1972)

Communicated by B. A. Bridges

Dr. David Moore
Mrs. Magdalene S. Devadatham
Department of Botany
The University
Manchester M13 9PL
England