The effect of the recombination-I gene on histidine-5

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Abstract

Effect of rec-1 on hist-5

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The recombination-1 gene described by Jessop and Catche.[1965] Hereditary: 20: 237 controls the frequency of recombination between pairs of histidine-1 alleles in such a way that recombination is favored by alleles bearing the dominant allele rec-1+ in one or both parents. The recessive allele rec-1 functions only when it is homozygous for a non-allelic marker. The recombination-1 gene does not control recombination at the amino acid cistron (Cotcheside 1966). Australian J. Biol. Sci., 19: 1039 indicating that its effect is locus specific. Since rec-1 is linked to the hist-5 region of linkage group V, its effect on the hist-5 gene in linkage group IV could be easily tested.

Initial tests measured recombination between the hist-5 alleles K553 and K512. The hist-5 (K553); rec-1+ was isolated from the wild type Em a, rec-1+ and crossed with a K512 A isolate of unknown rec-1 constitution. The recombinants in the progeny were of the type Em A, rec-1+, and ten K553 a and one of K512 a rec-1- stocks were isolated from the progeny of these crosses. Each of these ten K553 a isolates was crossed with each of the ten K512 A isolates, and the frequency of histidine prototrophic recombinants in the progeny was estimated. The probability that at least one of these 100 crosses is homozygous for rec-1- is 0.9%. If both parental stocks crossed to Em A, rec-1+ and rec-1- higher if one or both were rec-1-. Recombination frequencies in the 100 crosses ranged from 5.4/105 to 12.7/105. It can be confidently assumed that recombination-1 does not control recombination between K553 and K512 or that if it does then its effect is only very slight.

Exactly parallel tests were made to detect the effect of rec-1 differences on recombination between the hist-5 alleles K548 and K268 and also between K540 and K268. In no case was the frequency of recombinants in the crosses more than double that of the cross bearing rec-1+ in at least one parent. Thus, the probability that rec-1 controls recombination at the hist-5 locus is very small.

Matile, Ph. Porosity of the cell wall of N. crassa as related to the secretion of proteolytic enzymes in N. crassa. Hyphe of N. crassa secrete two acid proteases into culture media which contain protein as the only nitrogen source (Matite 1965, Z. Zellforsh. 65: 884). These enzymes are localized in intracellularly in small sacs, protease a proteinaceous source are transferred to the outside of the plasmalemma (Matite et al. 1965 Z. Zellforsh. 68: 205). This process leads to the occurrence of free extracellular proteases which subsequently cross the cell wall and appear in the culture medium. Thus, the size of the secreted enzyme molecules must be the same order of magnitude as the dimensions of the pores of the cell wall. In contrast, another secretion product, aminopeptidase, has never been observed outside the cells. This enzyme is localized somewhere between the plasmalemma and the cell wall (Matite 1964 Naturwissenschaften 51: 489). Travithick and Metzenberg (1966 J. Bacteriol. 92: 1010) have described a process of molecular sieving in Neurospora cell walls with respect to secreted invertase; this finding suggests the existence of a similar mechanism resulting in the retention of secreted aminopeptidase molecules. In order to prove the validity of the above hypothesis, the approximate molecular size of both secreted proteases and aminopeptidase has been determined, using the gel filtration method of Whiteaker (1963 Anal. Chem. 35: 1950).

For isolation of cell walls, 50 g of wet mycelium (strain chib-1 (34486), FGSC #485) were mixed with an equal volume of glass beads (0.45 mm) and a small volume of 1% NaCl. The cells were broken by the action of a vibration mixer (1 hour). The resulting suspension, containing less than 1% of intact cells, was diluted with 1% NaCl and the glass beads were allowed to settle. Centrifugation for 15 min at 100 g yielded a white pellet which contained the cell walls. This pellet was subjected to washings in 1% NaCl until the supernatant was perfectly transparent. After 20 washings, only clean fragments of cell walls could be observed in the phase contrast microscope.

Gel filtration with Sephadex-150 was carried out in a 15 x 300 mm column. Columns of isolated cell walls were completely incompossible for the solvent: in order to obtain a homogeneous working column (11 x 65 mm), a suspension consisting of isolated cell walls and glass beads (0.18 mm) was added in small portions under continuous gentle stirring. In NaCl in 0.1 M Tris-HCl buffer pH 7.2 was used for the elution of both Sephadex and cell wall columns. Blue dextran (Pharmacia Uppsala) was used for the determination of void volumes. Bovine serum albumin (Collochem. A-grade), cytochrome c and tyrosine (Fluka) served as reference substances. The proteolytic enzymes subjected to gel filtration were contained in a culture filtrate (acid proteases, pH optima 4.2 and 6.4) or in a high speed supernatant of a cell-free extract (aminopeptidase).

Both acid proteases were eluted from Sephadex-150 column at a Ve/Vo value of 2.9, the aminopeptidase at Ve/Vo = 2.0. The corresponding molecular weights are co. 22,000 and 83,000. Most probably these values don't represent true molecular weights, since it has not been established that the secreted proteolytic enzymes are pure proteins. It may be assumed that the aminopeptidase is a glycoprotein like invertase (Metzenberg 1963 Arch. Biochem. Biophys. 100: 503) and other extracellular enzymes of Neurospora and yeast (Lampe 1965 Symp. Soc. Gen. Micembol. 15: 115). However, the Ve/Vo values point to a considerably different size of protease and aminopeptidase molecules. This finding supports the hypothesis that the smaller protease molecules can, while the larger ones of aminopeptidase cannot, penetrate the pores of the Neurospora cell wall.

However, it appears from a comparison of the molecular sizes of invertase (which is partially released into the medium) and of aminopeptidase (which is not released) that the situation is more complex. Invertase molecules should be smaller than...