Fungal Genetics Reports

Resistance to hydroxyurea in Aspergillus nidulans by gene mutation and by putative gene amplification.

J. A. Roper

D. J. Scanlon

Recommended Citation

Resistance to hydroxyurea in Aspergillus nidulans by gene mutation and by putative gene amplification.

Abstract

Resistance to hydroxyurea in Aspergillus nidulans by gene mutation and by putative gene amplification.

Roper, J.A. and D.J. Scanlon

Resistance to hydroxyurea in

Aspergillus nidulans by gene

mutation and by putative gene

amplification

Colonies of pabaA6 yA2 adE20 were grown on complete medium (CM) with 40 mM hydroxyurea (HU), a concentration which reduced the linear growth rate to about 15% of the CM control. After 10 days incubation, 30 stunted, poorly-conidiated colonies had produced 45 vigorous, HU-resistant sectors. Of these, 28 gave stable cultures which were wild type in growth rate and morphology. The remaining 17 were all unstable on vegetative growth; in this, in their colony morphology, growth rate, and in their behavior in analysis, they showed the characteristics of known duplication strains, such as those selected on the basis of a leaky, rate-limiting mutation (Sexton and Roper 1984 J. Gen. Microbiol. 130:583-595). Four resistant strains, HU-R1, 2, 3, and 4 were analyzed. Each was combined in diploid with a translocation-free master strain (MS); the known markers in the resistant haploids were on chromosome I, and the MS carried markers on the remaining 7 chromosomes (wA3; galA1; pyroA4; facA303; sB3; nicB8 riboB2). Two strains, HU-Rl and HU-R2, were also crossed to the MS. Mitotic and meiotic segregants were tested for resistance on CM with 40 mM HU, a concentration suitable for distinguishing high and intermediate resistance from the substantially lower, but variable, resistance of stock strains.

Meiotic and mitotic analysis of HU-R1, a stable isolate, showed that its resistance arose from mutation in a single gene. The meiotic segregation was 80 resistant : 74 sensitive, with free recombination between the resistance gene and all other segregating markers. The diploid, HU-R1//MS, had a standard, low degree of spontaneous mitotic instability on CM. On average, each 7-day colony gave only one mitotic segregant with a conidial color (dark green, yellow or white) different from the paler green parent, over which these segregant sectors showed no growth advantage. Haploidization analysis located the gene for resistance to chromosome V; there were 10 resistant fac^+ and 20 sensitive fac^- haploids, with markers on all other chromosomes segregating independently.

Colonies of HU-R2, 3 and 4 had a linear growth rate on CM less than that of wild type, and a modified surface morphology seen in various, known duplication strains. Their vegetative behavior suggested also that the proposed duplications determined HU resistance. Colonies of these strains gave clear-cut, faster-growing sectors. The majority of these sectors had wild type growth and were HU sensitive; they could be interpreted as arising by spontaneous, mitotic deletion of a duplicate chromosome segment which determined resistance and modified growth. A minority of sectors were still resistant, but they yielded second-order, sensitive sectors; this was consistent with the known stepwise loss of duplicate chromosomal material (Bainbridge and Roper 1966 J. Gen. Microbiol. 42:417-424). Diploids formed between HU-R2, 3, 4 and MS were of intermediate resistance. They were all extremely unstable on CM; each 7-day colony yielded between 5 and 14 large, faster-growing sectors, all of them HU-sensitive. In each case about 90% of the sectors were diploids with the standard, low degree of mitotic instability; the remainder were stable haploids with wild type morphology and growth rate. Such mitotic instability has been shown previously in diploids known to be heterozygous for a duplication (Case and Roper 1981 J. Gen. Microbiol. 124:9-16). Balanced haploids and diploids, which have lost the duplication by one or another process, overwhelm the diploid parent and any other unbalanced mitotic segregants. The haploids arise by the normal haploidization process, but with total selection against those slower growing haploids which have the duplication-bearing homologue; this allows location of the duplication to its chromosome. The diploids arise by mitotic crossing over between the chromosome arm bearing the duplication and its normal homologue. With appropriate segregation of chromatids, this gives a balanced, faster growing diploid. Suitable markers in the master strain allow location of the duplication to its chromosome arm. Figure 1. illustrates the processes, with HU-R2 as an example.
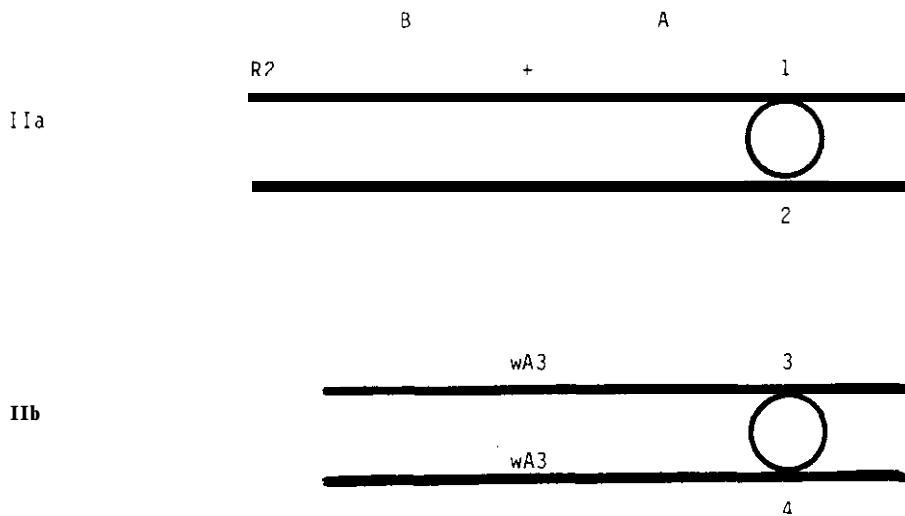
Figure 1.

The proposed chromosome II constitution of the HU-R2//MS diploid. In haploidization there is total selection against haploids with R2, on the IIa homologue. Balanced diploids are produced by mitotic crossing over between strands 2 and 3 and segregation together of 2 and 4. Crossing over in A produces a white diploid and in B a green diploid. R2 is a putative duplication, of an unknown segment, which determines HU resistance and slower growth. Arbitrarily it is shown in terminal location, like most of the spontaneous duplications studied by Sexton and Roper.

HU-R2//MS gave 99 white and 4 colored haploids, all HU sensitive. It was concluded that the resistance factor was in coupling with wA^+; the few colored (wA^+) haploids could be explained by spontaneous loss of the factor, before or during haploidization. Of 248 balanced, diploid sectors, 154 were white and 94 green; this showed location of the factor distal to the wA locus in IIL, HU-R2 was the only unstable strain analyzed meiotically. The segregation of 65 sensitive, normal colony size : 34 resistant, smaller colony, was typical of the meiotic segregation of a duplication; shortage of the duplication progeny results from spontaneous deletions of the duplicate segment and/or/ differential viability (Nga and Roper 1968 Genetics 58:193-209).

HU-R3 and HU-R4 gave results similar to each other in analysis. Mitotic haploidization of HU-R3//MS and HU-R4//MS showed in each a resistance factor linked to galA^+ (III). Balanced, diploid sectors were all HU sensitive and, in both cases, were some gal^+ and some gal^-; this indicated a IIIL location of the resistance factors, distal to the galA locus.

In earlier work, definitive evidence of duplication, and of the actual segment involved, was obtained by producing unbalanced haploids heterozygous for one or more genes in the duplicated segment. Such information is not yet available for HU-R2, 3 and 4. Nevertheless, there is substantial indirect evidence that duplications are responsible for HU resistance in these strains; possible candidates for duplications are the chromosome segment(s) carrying genes coding for the sub-units of ribonucleotide reductase, the enzyme which is inhibited by HU. There is intensive study of gene amplification in mammalian systems, in relation to drug resistance and to other clinically significant changes. These preliminary results suggest that amenable fungi might prove model systems for probing aspects of the amplification process. - - - Department of Genetics, The University, Sheffield S10 2TN, United Kingdom