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Chromosomal locations of genes that control major RNA-degrading activities in common wheat (*Triticum aestivum* L.)

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Abstract Seventeen RNA-degrading enzymes of common wheat, with apparent molecular masses from 42.2 kDa to 16.3 kDa, were observed by the RNA-SDS-PAGE assay. To determine their chromosome locations, all chromosome arms of common wheat except 4BS were assayed in their null condition by using a set of ditelosomic or nulli-tetrasomic lines of the cultivar Chinese Spring. Our results showed that only one chromosome location each was identified for the 22.8-kDa and the 21.2-kDa enzymes, as well as for the 21.6 kDa enzyme, and they are on chromosome arms 2AS and 2DS, respectively. Loci controlling the 20.1 kDa activity were on chromosome arms 2AL, 4BS, 4DS and 6BS. The locus or loci coding for the gene(s) of the 42.2-kDa, 40.9-kDa and 39.2-kDa enzymes were probably located on chromosome arm 5AS, and their expression, in agreement with most other RNA-degrading enzyme activities were stimulated when chromosome arm 5AL was missing, indicating a inhibiting locus on 5AL. Our data suggested that the 31.9-kDa, 30.6-kDa and 29.6-kDa enzymes were possibly products of a common precursor which might be coded by a gene(s) on chromosome arm 6BS, and that the processing is co-regulated by loci on chromosome arms 2BS, 3DS, 6AL, 6BL and 7BS. The remaining of the enzyme activities were consistently found in all of the lines tested, and thus are presumably encoded by multiple loci. The only other possibility is that, their loci may be on chromosome arm 4BS which we have not assayed in its null condition.

Key words Ribonuclease · Gene mapping · Biochemical genetics · Aneuploid

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Introduction

Altered levels of common wheat (*Triticum aestivum* L.) RNA-degrading activities were previously reported to be associated with plant response to water deficit (Yi and Todd 1979), senescence and aging (Sodek and Wright 1969; Blank and McKeon 1989, 1991), seed germination (Vold and Sypherd 1968; Okamoto et al. 1980), and pathogen infection (Rohringer et al. 1961; Chakravorty et al. 1974; Barna et al. 1989). However, the lack of information on the genetic and physical locations of the loci for these enzymes is a major limitation to understanding, manipulating and isolating the respective genes. Our previous study reported 15 RNA-degrading enzymes, with apparent molecular masses ranging from 16.3 to 40.1 kDa, in common wheat (Yen and Baenziger 1993).

The use of null mutants of genes for RNA-degrading enzymes has been a powerful approach to address the contribution of individual bacterial RNA-degrading enzymes to mRNA-degrading pathways (Deutscher 1993), and it also should be useful in the study of plant RNA-degrading enzymes (Green 1994). In common wheat, aneuploids, such as ditelosomic and nulli-tetrasomics, are available for almost all chromosome arms, and they are the equivalent to null mutants of the loci on the missing chromosome arm. In the present study, we have used a set of ditelosomic or nullisomic-tetrasomic (when the respective ditelosomics are not available) lines to find the chromosome arms that contain the loci controlling the activities of, or coding for, major RNA-degrading enzymes in common wheat.

Materials and methods

The plant materials used in this study were all of the 36 available ditelosomics (designated as DtXXX, where XXX represents the chromosome arm; i.e., Dt1AL=ditelosomic line for chromosome arm 1AL) and nulli-tetrasomics N2AT2B, N2BT2A, N5AT5B, N5BT5D and N5DT5A (here, N and T stand for nullisomic and tetrasomic status, respectively; i.e., N2AT2B is the line in which one extra pair of chromosome 2B has replaced a pair of chromosome 2A) of the com-

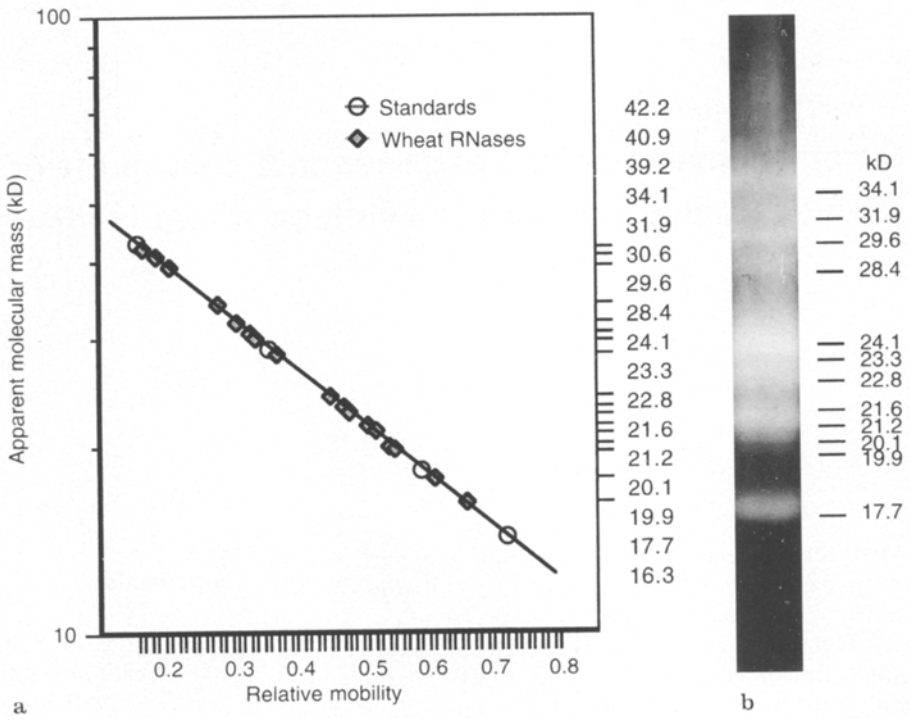

Fig. 1 **a** Relative mobility and apparent molecular mass of individual RNA-degrading enzyme activities observed in this study; **b** Gel image of the RNA-degrading assay of common wheat (*T. aestivum* L.) cultivar Chinese Spring. It should be noted that some wheat RNA-degrading activities in **a** only become visible when a particular chromosome arm(s) is missing. Hence, the number of wheat RNA-degrading activities in **a** and **b** do not match

mon wheat cultivar Chinese Spring (CS). These aneuploids represent all the chromosome arms except 4BS, which carries a gene(s) controlling fertility so that any aneuploid that lacks 4BS will be sterile and thus unavailable for the present study. All the materials were checked for their correctness with the C-banding technique and kindly provided by Dr. A. J. Lukaszewski of University of California-Riverside.

For the RNA-SDS-PAGE assay, a clear band on the stained gel results from negative background-staining of the RNA molecules cast in the separating gel and represents the in situ digestion of RNA molecules by the electrophoretically separated RNA-degrading enzymes in the crude extracts tested. The assay procedures reported by Yen and Green (1991) and Yen and Baenziger (1993) were modified as described below. Separating gels [13.69% (w/v) acrylamide, 0.38% (w/v) N′,N′-methylene-bis-acrylamide, 0.11 M TRIS (pH 9.0)] were cast with 2.62 mg/ml of high-molecular-weight *Torulopsis utili* RNA (from the Sigma Chemical Company, St. Louis, Mo., USA). Two sources of SDS were used: Lot 82H-0708 from Sigma, and Lots 71229 and 84111 from USB (Cleveland, Ohio, USA).

The protein content in the plant extractions was determined according to the method of Bradford (1976). Equal amounts of protein were loaded for each lane. To insure the repeatability of our results, multiple gels were loaded with crude plant extract samples from: (1) the same extractions (simply repeating the gel assay procedure), (2) different extractions of the same plants (avoiding tissue-specific results and sampling errors), and (3) extractions from different plants of the same genotype (avoiding single plant-specific errors). Estimates of apparent molecular mass were calculated with the Gel Fragment Sizer v1.4 computer program written by Don Gilbert of Indiana University, and based on the mobilities of the enzyme activity bands relative to those of the pre-stained protein markers as specified by the supplier (Bethesda Research Laboratories) in the absence of reducing agents. In the RNA-SDS-PAGE gel assay, a dramatic increase or decrease of the band density will indicate that the missing chromosome or chromosome arm carries a locus, or loci, that control the activity of the respective RNA-degrading enzyme. The results were summarized based on gels from separate experiments.

Results and discussion

Seventeen RNA-degrading enzyme activity bands of common wheat were observed by the RNA-SDS-PAGE assay (Fig. 1). These bands, with apparent molecular masses ranging from 42.2 kDa to 16.3 kDa, corresponded to the existence of individual RNA-degrading enzymes. The increased polymerized-acrylamide concentration in the separating gel in this study greatly improved separation of the RNA-degrading enzyme activity bands compared to our previous study (Yen and Baenziger 1993). The visualization of the activity bands further benefitted from the use of a mixture (1:1) of SDS from Sigma and USB in the running buffer, because SDS from different sources are known to have quite different effects on the re-naturing of the electrophoretically separated RNA-degrading enzymes (Blank et al. 1982). Figure 1 shows their relative mobilities in the gel and their estimated apparent molecular masses.

Previously we biochemically characterized 15 RNA-degrading enzyme activity bands in common wheat with apparent molecular masses ranging from 40.1 kDa to 16.3 kDa (Yen and Baenziger 1993). The difference in the migration of the enzyme activity bands between the present and the previous report was due to the different gel concentrations of acrylamide used in the two experiments. The difference in the estimation of apparent molecular masses mainly resulted from the difference in the band migration and the use of different lots of the pre-stained protein standards (whose apparent molecular masses vary between lots according to the supplier) in the two studies. Although the

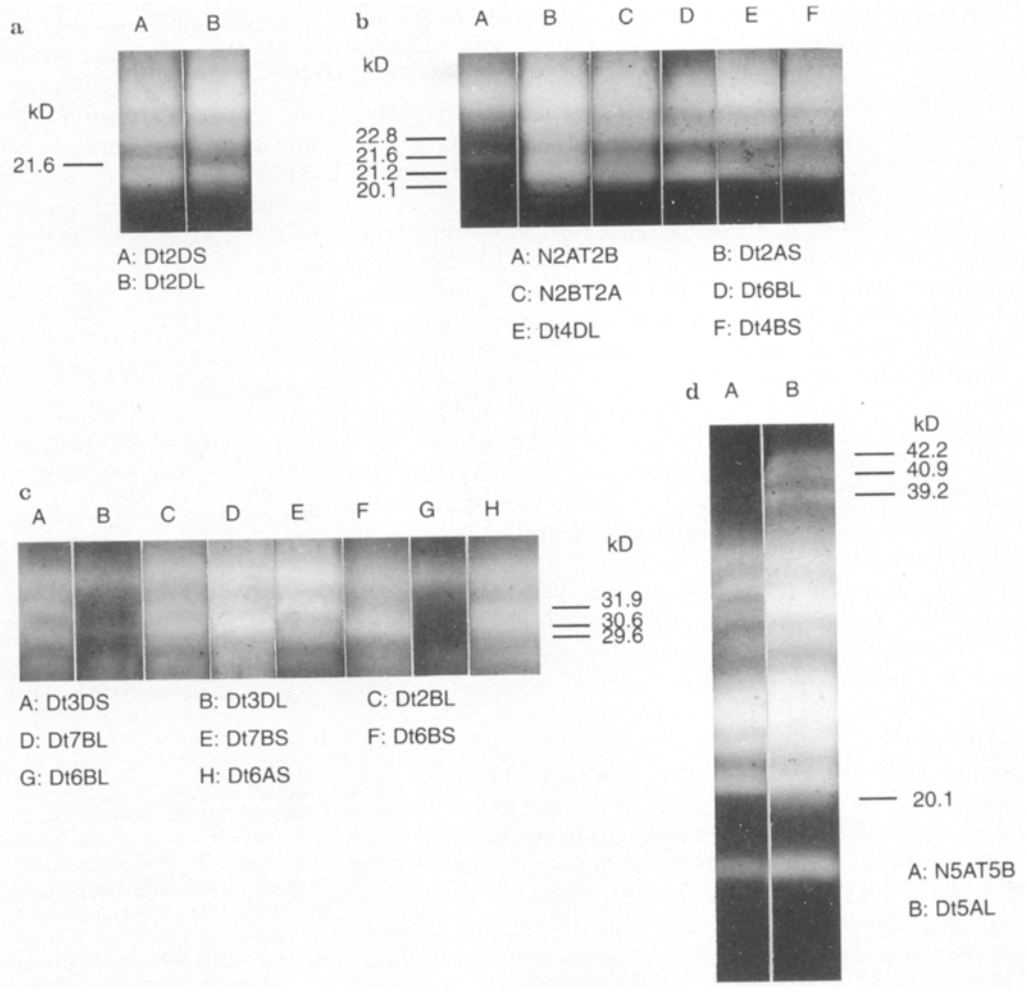

Fig. 2a–d Gel image of the RNA-SDS-PAGE assay of aneuploids of common wheat (*T. aestivum* L.) cultivar Chinese Spring. Each lane was loaded with 100 µg of proteins from the crude leaf extract. For the meanings of the abbreviations used in the figure see the Material and methods section

exact relationship between individual enzyme activities revealed in the present study and those reported in Yen and Baenziger (1993) is not known, the gel image of the activity bands suggests that the 42.2-kDa–28.4-kDa, the 24.2-kDa–19.9-kDa, and the 17.7-kDa–16.3-kDa activities detected in this study are related to the activities the 40.1-kDa–31.7-kDa, the 28.2-kDa–23.5-kDa, and the 18.8-kDa–16.3-kDa reported in Yen and Baenziger (1993), respectively.

Of the 17 wheat enzyme activities, 11 were located to their carrier chromosome arms. The 21.6-kDa activity was observed in all lines except Dt2DL (lane B in Fig. 2a); therefore, we concluded that there is a locus on chromosome arm 2DS (which is missing from ditelosomic line Dt2DL) for the 21.6-kDa enzyme. As shown in Fig. 2b, when chromosome 2A was missing in the N2AT2B line, the 22.8-kDa, and 21.2-kDa activities became considerably weakened (lane A), while both activities were clearly visible in all the other lines assayed, where chromosome arm 2AS is present. Therefore, it was possible that chromosome arm 2AS contains a locus or loci for these two RNA-degrading activities. The 20.1-kDa enzyme activity could not be seen when lines N2AT2B, Dt4BS, Dt4DL and Dt6BL (Fig. 2b), and N5AT5B (Fig. 2d) were assayed. Therefore, loci controlling the 20.1-kDa enzyme's activity may be located on chromosome arms 2AS, 4BL, 4DS, 5AL and 6BS, and the normal function of the 20.1-kDa enzyme may require the coordination of all these loci. Since only one chromosome location was identified for each of the 22.8-kDa, 21.6-kDa, and 21.2-kDa enzymes (Fig. 2a, b), it is highly probable that these chromosomal locations each contain a gene locus (or loci) coding for the corresponding enzymes.

Unknown factors affected enzyme re-naturation of the 42.2-kDa, 40.9-kDa and 39.2-kDa activities which resulted in inconsistent visualization of these enzyme activity bands among assays in all of the lines except Dt5AL and N5AT5B. In every assay strong bands of these three activities were always observed when chromosome 5AS alone was absent in the Dt5AL line (lane B in Fig. 2d). However, these three RNA-degrading activities were never observed in the N5AT5B line (lane A in Fig. 2d) where two extra copies of chromosome 5B had been substituted for chromosome 5A. These results suggest that chromosome arm 5AS, which was missing in the Dt5AL line, contained a gene(s) which inhibits the expression of these three enzyme activities, and

the coding genes for these three enzymes may be located on chromosome arm 5AL, which was missing (together with 5AS) from the N5AT5B line. The inhibiting gene(s) may also have effects on the activities of other RNA-degrading enzymes since, as is evident in Fig. 2d, most of the wheat RNA-degrading enzymes increased their activity when chromosome 5AS was missing.

As shown in Fig. 2c, the absence of chromosome arms 3DS (lane B) and 6BS (lane G) coincided with the disappearance of the 31.9-kDa and the 29.6-kDa activities. Also, the absence of chromosome arms 6BL (lane F) and 7BS (lane D), and the absence of the chromosome arms 6AL (lane H) and 6BL (lane F), strongly weakened the 31.9-kDa and 29.6-kDa activities, respectively. In addition, the 30.6-kDa activity appeared when either chromosome arm 2BS (lane C), 3DS (lane G), 6BL (lane F), 6AL (lane H), or 7BS (lane D) was absent. The appearance of the 30.6-kDa activity was usually coincident with the absence of the 29.6-kDa activity (as in lines Dt6AS, Dt6BS and Dt7BL) or both the 31.9-kDa and the 29.6-kDa activities (as in line Dt3DL). However, the 31.9-kDa, 30.6-kDa and 29.6-kDa activities were all observed in line Dt2BL (lane C), in which the chromosome arm 2BS was missing, but none of them appeared in line Dt6BL (lane G), where the chromosome arm 6BS was missing. An explanation for these phenomena is that the 31.9 kDa, 30.6 kDa and 29.6 kDa bands were the result of the activities of three isozymes which are different products from a common precursor whose locus might be on chromosome arm 6BS.

The 34.1-kDa, 28.4-kDa, 24.1-kDa, 23.3-kDa and 17.7-kDa activities were always visible in all the aneuploid lines tested. This result suggests that these enzyme activities may be controlled, or encoded, by more than one locus whose phenotypic effects are independent from each other and which may be located on more than one chromosome arm. Alternatively, loci for these enzymes may be located on chromosome arm 4BS which we could not test in a null condition. However, the absence of chromosome arm 2AS and 6BS apparently weakened the 17.7-kDa activity (data not shown), indicating that these chromosome arms may also contain genes coding, or regulating, this enzyme. The bands of the 19.9-kDa and 16.3-kDa enzymes were too weak to assay for activity changes, and hence mapping them on their carrier chromosome arms was beyond the limit of this study.

Although loci of more than half of the assayed RNA-degrading activities were located to chromosome arms, the physiological and genetic functions of the corresponding RNA-degrading enzymes still remain unknown. A RNA-degrading enzyme may: (1) be involved in the decay of RNA molecules in general, or (2) participate in editing, or regulating, the half-life of particular mRNA molecules, and thus be involved in gene regulation. Further information in this regard may be obtained from the functional assay of RNA-degrading enzymes under various physiological or pathological conditions by using the aneuploid line(s) that carries (carry) the locus (or loci) of the tested enzyme(s).

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