MOUSE FIBROBLASTS A9 ARE DEFICIENT IN HPRT AND APRT

To the Editor: A hypoxanthine phosphoribosyl transferase (HPRT)-deficient derivative of mouse L cells, A9, has been used extensively by many investigators in cell hybridization studies. Genes have been assigned to specific human chromosomes, and various aspects of cell regulation and gene expression have been considered. It follows, therefore, that the enzymatic and metabolic constitution of A9 cells is of importance in interpreting such experimental results. Recently we found that HPRT-deficient A9 cells also are deficient in adenine phosphoribosyl transferase (APRT) [1]. This observation was buried in the Materials and Methods section of an extensive paper, and we have been encouraged to bring it to the attention of investigators using A9 cells for studies in somatic cell genetics. Moreover, the absence in A9 cells of both enzymes required for the "purine scavanger pathway" may provide important advantages for studies on purine biosynthesis and interconversions.

The A9 cells are derivatives of L cells (NCTC clone 929 L) selected for their resistance to 8-azaguanine (3 μg/ml) by Dr. John Littlefield, who kindly supplied us with both A9 and the parent L cell lines [2]. During radioautographic studies on the failure of incorporation of ^{3}H-hypoxanthine into HPRT-deficient A9 cells, some cultures were incubated with ^{3}H-adenine as a control of cell viability, metabolic activity, and availability of the cofactor phosphoribosyl pyrophosphate (PRPP), since the APRT enzyme carries out an analogous reaction. Unexpectedly, A9 cells failed to incorporate either ^{3}H-adenine or ^{3}H-hypoxanthine into cellular material. The possibility that a single mutation resulted in a loss of HPRT and APRT activities was excluded when the parent L cells from which the mutant A9 cell was derived failed to incorporate ^{3}H-adenine into cellular material, although, as expected, it incorporated normal amounts of ^{3}H-hypoxanthine.

Activity of HPRT and APRT was assayed on supernates of cells disrupted by freezing and thawing using either ^{14}C-8-hypoxanthine or ^{14}C-8-adenine as substrate by the method of Rubin et al. [3]. Specific activities are expressed as nmoles AMP or IMP formed per minute per milligram protein. The A9 cells had less than 0.05 specific activity for both HPRT and APRT. The parent L cell had specific activities for HPRT of 11.9 and for APRT less than 0.05. Three other derivatives of L cells had specific activities for HPRT of 7.1–3.5 and for APRT of 6.6–4.5.

The A9 cell line, frequently used for cell hybridization, is deficient in both HPRT and APRT. Evidently the parent L cell line from which A9 was derived had previously undergone a spontaneous mutation to APRT deficiency. The double enzyme deficiency in A9 cells should be recognized by investigators who use this line for studies on somatic cell genetics.

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