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ERRATUM

Different Somatic and Germline *HPRT1* Mutations Promote Use of a Common, Cryptic Intron 1 Splice Site

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Lorel M. Colgin[1], Alden F.M. Hackmann and Raymond J. Monnat, Jr.*

University of Washington, Department of Pathology, Box 357705, Seattle, WA 98195-7705 USA; and [1]Children's Medical Research Institute, Westmead NSW, Australia.

**Correspondence to R.J.M., Jr. tel: 206.616.7392; fax: 206.543.3967; e-mail: monnat@u.washington.edu*

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Aberrant hypoxanthine phosphoribosyltransferase (HUGO-approved gene symbol HPRT1; MIM# 308000) RNA splicing promoted by splice site mutation or loss is a common mechanism for loss of the purine salvage enzyme HPRT1 from human cells. We report here two in vivo somatic HPRT1 mutations in human kidney tubular epi-thelial cells that disrupt HPRT1 intron 1 splicing and lead to the inclusion of intron 1 sequence in mature mRNA. Analysis of these mutations and of 14 additional HPRT1 intron 1 inclusion mutations provides an explanation for use of a common, cryptic intron 1 splice donor site by all 16 mutations.

KEY WORDS: HPRT1, mutation, somatic, kidney, mRNA splicing, intron inclusion

INTRODUCTION

Mutations that disrupt or alter mRNA splicing are an important cause of human genetic disease (Cooper and Mattox, 1997; Nakai and Sakamoto, 1994; Krawczak et al., 1992). Somatic mutations that disrupt or alter mRNA splicing are also common, and in some instances these mutations are disease-associated or causal (see, e.g., Luzatto et al., 1997; Qian and Germino, 1997). We have identified and characterized two *in vivo* somatic *HPRT1* gene (MIM# 308000) mutations in human kidney tubular epithelial cells that disrupt *HPRT1* intron 1 splicing and lead to the inclusion of intron 1 sequence in mRNA. These two "intron inclusion" mutations and 14 additional human *HPRT1* somatic and germline intron 1 inclusion mutations provide an unusual opportunity to examine splice site competition and the sequence determinants of splice site selection in a well-characterized human gene.

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MATERIALS AND METHODS

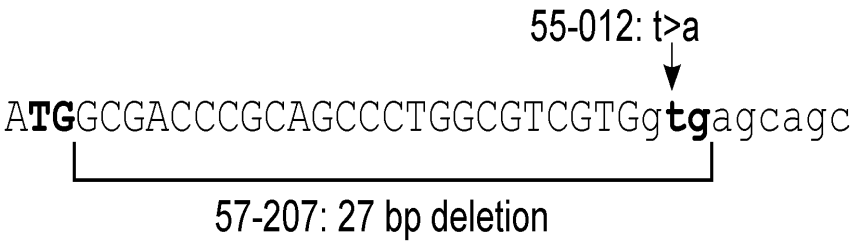
Both mutations were originally isolated by the direct cloning of pre-existing, thioguanine (TG)-resistant kidney cortical tubular epithelial cells from 34 year old (mutation 57-207) and 65 year old (mutation 55-012) males as previously described (Martin et al., 1996). The isolation of multiple TG-resistant clones from kidney donor 55 containing the 55-012 mutation suggests that this mutation had undergone clonal amplification *in vivo* prior to tissue sampling. RT-PCR analysis of the *HPRT1* open reading frame using primers that flanked the terminal exons 1 and 9 indicated that each mutant produced an *HPRT1* mRNA that had included a short segment of intron 1 sequence between exons 1 and 2 (Martin et al., 1996). Both mutations were further characterized by independent cDNA amplification and direct sequencing of both strands of PCR products (both mutants), and the sequencing of both strands of cloned mutant cDNA (57-207).

RESULTS AND DISCUSSION

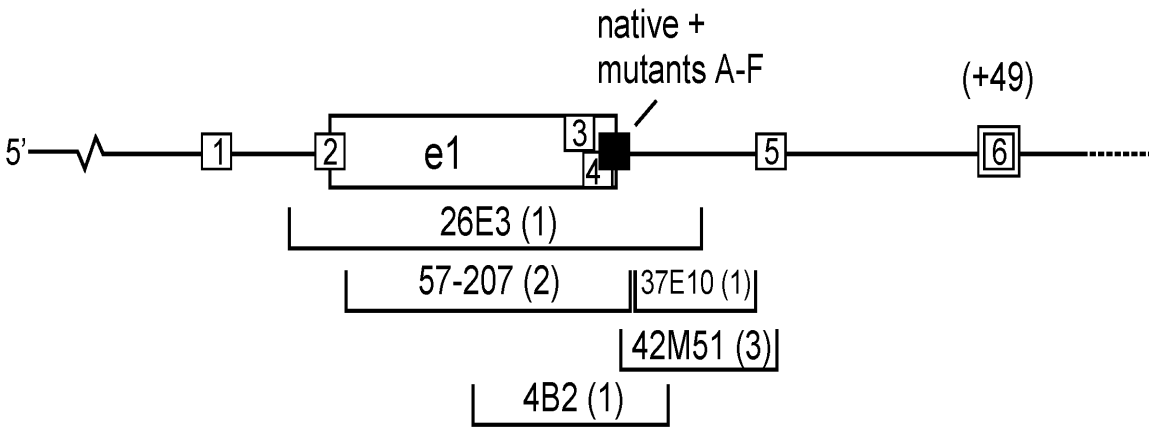
One mutant (55-012) contained a T>A transversion in the conserved GT dinucleotide of the splice donor site with inclusion of the first 49 bp of intron 1 in the mutant cDNA. Mutant 57-207 contained a 27 bp deletion that eliminated all of exon 1 except the ATG start codon, and extended 3 nucleotides into the intron 1 splice donor site. The subsequent 46 nucleotides of intron 1 were included in the cDNA of this mutant (Figure panel A). No other splice product or additional mutation in the *HPRT1* open reading frame was identified in either mutant, and the *HPRT1* open reading frame sequence of control (TG-sensitive) clones from each donor was normal (additional data not shown). Both of the intron 1 inclusion mutants shown in Figure panel A generate frameshifts that lead to translation termination in exon 2. Fourteen additional mutations have been reported that alter human *HPRT1* exon 1 splicing with the inclusion of intron 1 sequence (Figure panels B and C left). These include one mutation identified in a Lesch-Nyhan patient (Marcus et al., 1993), six spontaneous somatic mutations identified in TG-resistant T-cell clones (Steingrimsdottir et al., 1992; Burkhart-Schultz and Jones, 1997; O'Neill et al., 1998; J.P. O'Neill, pers. communication), and seven mutations induced in peripheral blood T-cells or cell lines *in vitro* by BPDE (Andersson et al., 1992), deoxyadenosine (Mattano et al., 1990), ionizing radiation (Rigaud et al., 1995), malathion (Pluth et al., 1998), trimethylpsoralen (Guillouf et al., 1993) or UV (Steingrimsdottir et al., 1992). The spontaneous mutations were equally divided between deletions and base substitutions, whereas all of the induced mutations were base substitutions.

The processing of primary transcripts to generate mature mRNAs is well understood in outline, with the selection of splice sites depending in part on how closely a given potential site resembles a splice consensus sequence (Berget, 1995; Reed, 1996; Cooper and Mattox, 1997; O'Neill et al., 1998). The extent of sequence similarity between individual and consensus splice sites has been quantified in the form of a splice site "score" (Shapiro and Senapathy, 1987; Senapathy et al., 1990). An analysis of the splice site scores for the splice sites shown in the Figure is instructive. The native intron 1 donor splice site ranks 6th out of the 8 *HPRT1* intron donor sites (splice site score 81.4). Each of the six mutant donor sites has a substantially lower splice site score (64.1 - 68.8) that is, nonetheless, higher than 5 of the 6 cryptic donor splice sites in the first 250 bases of the human *HPRT1* mRNA (Figure panel C, right: cryptic 1-5). The remaining alternative donor splice site, cryptic 6/+49 with a splice site score of 69.1, is located 49 bp downstream of the native splice donor site. This cryptic donor site, cryptic 6/+49, is used by all 5 deletions that eliminate part or all of the native intron 1 splice donor site and 1 or more of the remaining 5 alternative splice donors in the 5' end of the human *HPRT1* mRNA (Figure panel B). The mRNAs generated from each of these deletions includes the retained portion of the 49 bases of intron 1 between the deletion 3' endpoint and the cryptic 6/+49 donor site. The cryptic 6/+49 site is also used by six different base substitution mutations that disrupt the native intron 1 splice donor site. Each of these base substitutions leads to the inclusion of 49 bases of intron 1 into the *HPRT1* mRNA (Figure panel C, left).

A



B



C

intron 1 native	GTGgtgagc	81.4	consensus	CAGgt$^{a}_{g}$agt	
mutant A (4)	GTG**t**tgagc	64.1	cryptic 1	**GCA**gt**c**ag**c**	61.8
B (5-7)	GTG**a**tgagc	64.1	2	**TCC**gt**t**a**tg**	47.2
C (2,8,9)	GTGg**a**gagc	64.1	3	**GGC**gt**cgtg**	36.6
D (3)	GTGgtga**a**c	67.6	4	**GTC**gtgag**c**	43.8
E (3)	GTGgtga**t**c	67.6	5	C**C**Ggt**tcag**	53.6
F (10)	GT**A**gtgagc	68.8	6/+49	CAGgtg**gcg**	69.1

Figure: Human *HPRT1* intron 1 inclusion mutations. A. Mutation 55-012 is a T>A substitution at nucleotide 1705 (IVS1+2T>A). Mutation 57-207 is a 27 bp deletion that includes exon and intron 1 sequence (1680-1706del). Flanking bold **TG** and **tg** nucleotides indicate regions of break-point uncertainty. Exon 1 sequence is shown in upper case, and intron 1 sequence in lower case. Nucleotide numbering is after Edwards et al., 1990 (accession no. M26434) **B.** The 5' end of *HPRT1* mRNA and deletion mutations. Exon 1 is shown as a rectangle (e1) flanked by 5' untranslated or intron 1 sequence lines. The locations of native intron 1 and cryptic donor splice sites are indicated respectively by filled (◎) or open numbered (**”**) boxes. The cryptic 6/+49 site used by all 16 intron 1 inclusion mutations is shown as a double open box. The extent of each deletion is indicated below the sequence by brackets that include deletion references in parentheses. **C.** Native, consensus, mutant and cryptic human *HPRT1* intron 1 donor sites. The native and 6 mutant donor sites are shown in the left panel, with each mutant followed by reference(s) and a splice site score. The last 3 nucleotides of exon 1 are shown in upper case. The right panel shows the consensus primate and six cryptic sites in the *HPRT1* exon 1 region and their splice site scores. Base substitution differences between native or consensus sites are shown in bold.

Figure references: 1: Burkhart-Schultz, 1997; 2: Martin, 1996 and this paper; 3: Steingrimsdottir, 1992; 4: Mattano, 1990; 5: Andersson, 1992 ; 6: Marcus, 1993; 7: Rigaud, 1995; 8: Guillouf, 1993; 9: Pluth, 1998; 10: O'Neill, 1998 and pers. communication.

We observed an excellent rank-order correlation between the splice site scores of *HPRT1* native, mutant and cryptic splice donor sites and splice site utilization by 16 independent human *HPRT1* mutations (Figure). This was surprising, as splice site selection appears in many instances to be determined by factors in addition to site score (Berget, 1995; Reed, 1996; Cooper and Mattox, 1997; O'Neill et al., 1998). The correlation we observed may in part reflect our having examined a terminal exon donor site: terminal exons lack flanking acceptor and donor sites, and thus may place proportionately more reliance on the remaining splice site during splice site selection (Berget, 1995; Reed, 1996). Donor sites are also shorter and more clearly defined than acceptor sites, and thus may be more revealing in this type of analysis. A final point is that the relationship between splice site scoring and site selection may be more readily revealed in the context of local splice site competition, as opposed to simple splice disruption, analyses. The roles of splice site sequence and exon position on splice site utilization could be further explored in at least two ways: intron inclusion mutations in other loci could be re-examined from the standpoint of local splice site competition as shown above; and splice site selection or competition experiments could be performed using substrates that allow direct comparisons of terminal and internal exon splice site sequence preferences (see, e.g., Tian and Kole, 1995; Bouck et al., 1998).

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