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# Activation of the *met* Proto-oncogene in a Human Cell Line *

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The *met* oncogene was identified in the MNNG-HOS cell line, derived by extensive treatment of a human osteosarcoma cell line (HOS) with *N*-methyl-*N*-*N'*-nitrosoguanidine (MNNG) [1]. DNA from MNNG-HOS cells was used to transform NIH/3T3 mouse fibroblast cells, and the transforming gene (*met*) was isolated from one of these NIH/3T3 transformants [2] (Fig. 1 a).

The activated *met* allele in MNNG-HOS cells is rearranged with sequences from another gene (Fig. 1). This second gene appears to provide the promoter for the activated *met* oncogene and has been named *tpr* for "translocated promoter region" [3]. The *tpr* locus has been mapped by somatic cell hybridization analysis to human chromosome 1 [3], whereas *met* mapped to chromosome 7 [2, 4]. Therefore, the *met* oncogene has been activated by a DNA rearrangement involving portions of chromosomes 1 and 7.

We examined the expression of the *met* and *tpr* sequences in HOS, MNNG-HOS, and transformed NIH/3T3 cells. As shown

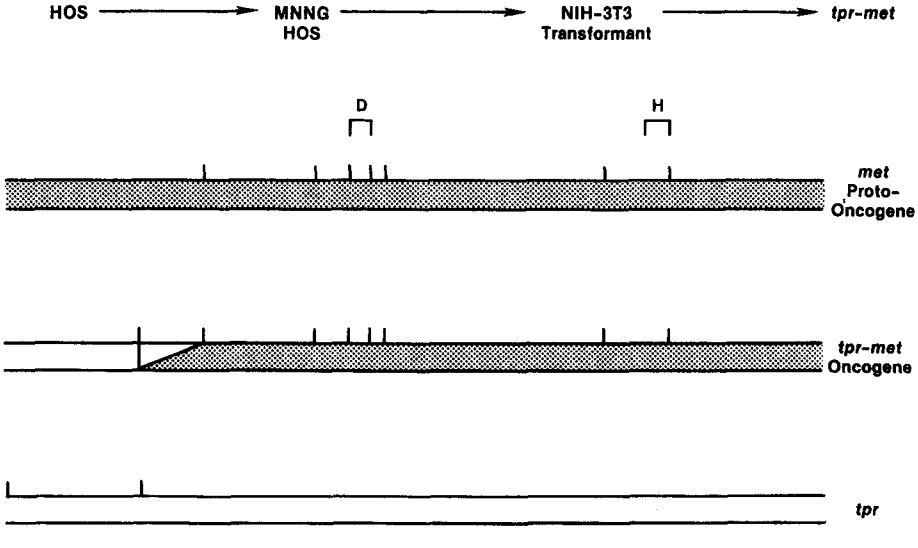


**Fig. 1.** Diagram of the *met* oncogene rearrangement. HOS cells treated with MNNG gave rise to MNNG-HOS cells. DNA from MNNG-HOS cells is capable of morphologically transforming mouse NIH/3T3 cells, and the *tpr-met* gene was isolated from a transformant

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**Table 1.** *met* and *tpr* mRNA species

| Probe | Size of mRNA species[a] (kb) in | | |
|---|---|---|---|
| | HOS cells | MNNG-HOS cells | NIH/3T3 transformants |
| *met* | 9.0, 7.0, 6.0 | 9.0, 7.0, 6.0 | – |
| *tpr-met* | – | 5.0 | 5.0 |
| *tpr* | 10.0 | 10.0 | – |

[a] mRNA species were determined by RNA blot hybridization, using *met* and *tpr* DNA as probes.

in Table 1, analysis with a *tpr* probe revealed an mRNA species of 10 kb in both HOS and MNNG-HOS cells. HOS and MNNG-HOS cells expressed *met* mRNAs of 9, 7 and 6 kb. However, analysis with probes from the 3′ end of the *met* gene and the 5′ end of *tpr* detected a hybrid 5.0-kb *tpr-met* transcript in MNNG-HOS cells [3] (Table 1), which is also found in NIH/3T3 cells transformed by MNNG-HOS DNA. We conclude that the gene rearrangement that activated the *met* locus results in the appearance of a hybrid mRNA transcript.

To determine the structure of the *met* gene product, we have begun to determine the nucleotide sequence of the coding regions. We have published the sequence of several *met* exons and demonstrated that *met* exhibits homology with the tyrosine kinase family of oncogenes and growth factor receptors [4]. We have recently isolated several *met* cDNA clones from a library prepared from A431 human squamous cell carcinoma cells. The longest clone obtained was 1.6 kb, and when the nucleotide sequence was translated, the sequence was found to contain a single, long, open reading frame. Figure 2 shows the *met* sequence in one-letter amino acid code and compares it with several other tyrosine kinase genes.

The *met* gene is most extensively homologous to the human insulin receptor gene [5] and the v-*abl* oncogene from Abelson murine leukemia virus [6]. The homology is mostly confined to the kinase domain of the proteins and reaches 50%–60% at the amino acid level. However, the carboxy terminus of *met* is significantly different from that of the other members of the family. Therefore, although *met* is homologous to the tyrosine kinases, it is not identical to any other known member of that gene family.

Previous studies mapped the rearrangement of the *tpr-met* gene to a 3.4-kb *Eco*RI fragment [3]. To determine the nucleotide sequence of the breakpoint, we used *met* and *tpr* probes flanking the site of rearrangement to screen a $\lambda$ phage library of human placental DNA. The rearrangement was mapped to small fragments present in the placental *met* and *tpr* phage clones. These fragments and a portion of *tpr-met* were cloned into M13 vectors and the nucleotide sequence was determined.

Figure 3 shows a portion of a sequencing gel of the *tpr* and *tpr-met* genes surrounding the chromosomal breakpoint. The se-

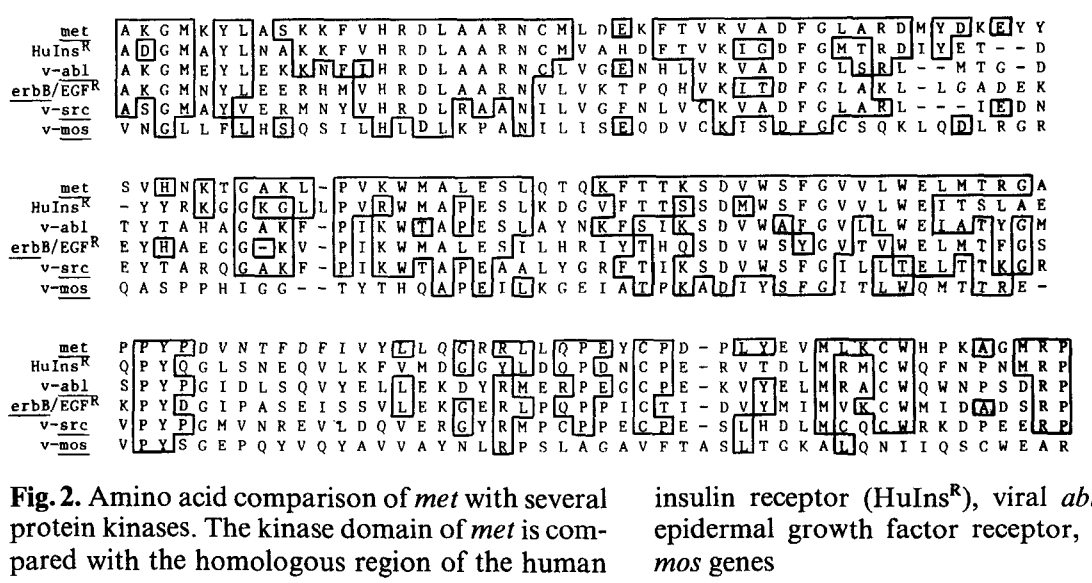


**Fig. 2.** Amino acid comparison of *met* with several protein kinases. The kinase domain of *met* is compared with the homologous region of the human insulin receptor (HuIns$^R$), viral *abl* gene, *erb*B/epidermal growth factor receptor, viral *src* and *mos* genes

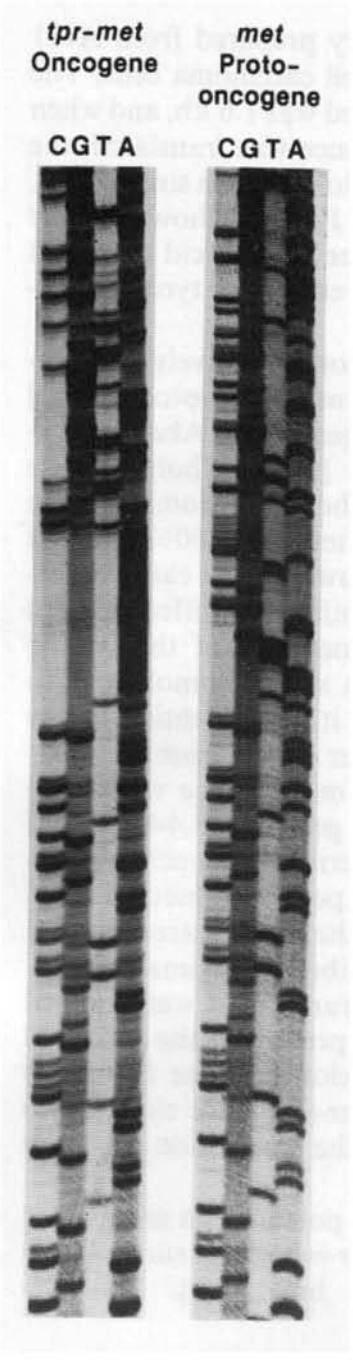


**Fig. 3.** Portion of a nucleotide-sequencing gel showing the *tpr-met* breakpoint. *C*, cytosine; *G*, guanine; *T* thymine; *A*, adenine

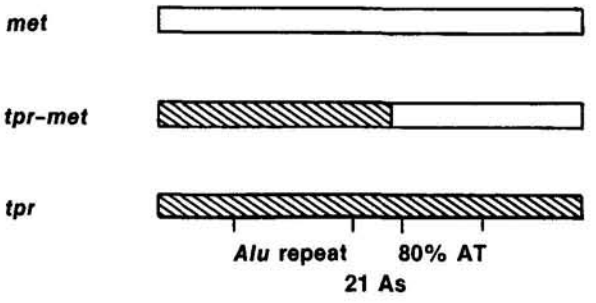


**Fig. 4.** Structure of the *tpr-met* breakpoint. Schematic of the rearrangement showing the position of the *Alu* repetitive element, 21 A residues, and the A-T-rich region flanking the breakpoint

quences of the *met* proto-oncogene and the *tpr-met* oncogene are identical through a stretch of 21 A residues. When the *tpr* sequence was used to search the NIH nucleotide data base, the sequence was shown to contain a member of the *Alu* family of highly repetitive sequences. These repeats are often followed by poly A-rich stretches, and the one in *tpr* is localized just upstream from the 21 A residues at the breakpoint. Therefore, the DNA rearrangement leading to the activation of *met* is located within this A-T rich region at the end of an *Alu* repeat.

Figure 4 is a schematic diagram of the *met* breakpoint region. The *met* and *tpr-met* genes are identical beyond the breakpoint for 600 residues, except for a single point mutation (not shown). Beyond the rearrangement site, on the *tpr* gene, is a stretch of 120 residues that are 80% A-T rich. Although chromosomal rearrangements have been observed in a wide variety of malignant cells [7], to our knowledge, this is the first time a chromosomal breakpoint has been sequenced in a nonhematopoietic cell. The nucleotide sequences of several other breakpoint sites have been determined and include rearranged c-*myc*, immunoglobulin heavy and light chain genes [8, 9], *bcll* [10], and T-cell receptor genes [11]; however, many of these rearrangements involve immunoglobulin switch-region sequences [8, 10] and are probably restricted to lymphoid cells. Our determination of the *tpr-met* chromosomal rearrangement also represents the first sequencing of a breakpoint isolated from a chemically transformed human cell.

The presence of an A-rich tract at the breakpoint suggests that these sequences may have played a role in the rearrangement. The rearrangement appears to be illegitimate because it occurred in a region with only four homologous nucleotides between *tpr* and *met* (Fig. 4a). MNNG is clastogenic [12] and may have created a free end in either *met* or *tpr*, which would promote rearrangement. Alternatively, the A-T-rich region of *tpr* may have contributed to the rearrangement. These regions may exist in a single-stranded structure and may be more suscep-

tible to cleavage. Chromosomal regions with increased lability (fragile sites) have been described previously and have been proposed as playing a role in chromosomal rearrangement [13]. In fact, many fragile sites are induced by agents that interfere with thymidine metabolism [12]. It will be interesting to see whether *tpr* maps to any of the fragile sites located on chromosome 1.

The MNNG-HOS cell line used in our study arose after 7 days of MNNG treatment to HOS cells [1]. We cannot be sure that MNNG participated in the *tpr-met* rearrangement, but it is an interesting possibility. The principal action of MNNG on double-stranded DNA is the methylation of the N-7 position of guanine [14]. A pair of G residues is located in *tpr* just 3′ to the breakpoint (not shown). MNNG is also capable of methylating adenine [14]; thus, MNNG-induced modification of residue(s) at the breakpoint may have contributed to the rearrangement. Besides the breakpoint, the only other change detected between *tpr-met* and placental DNA is a G-to-T transition. This alteration could be a polymorphic difference between individuals, could have arisen during transfection or cloning, or could have occurred as a consequence of MNNG treatment. At any rate, it is clear that massive mutation of the *tpr-met* gene did not occur in MNNG-HOS cells.

The expression of a truncated tyrosine kinase domain appears to be a common activation mechanism for this family of oncogenes. In addition to *tpr-met,* rearranged *bcr-abl* and *trk* genes have been isolated from human tumor DNA [15, 16]. Furthermore, retroviral insertion within the chicken epidermal growth factor receptor results in the expression of a truncated (*erb*B) kinase activity [17]. We are currently investigating the role, if any, of the *tpr* sequences in the transforming ability of the *tpr-met* oncogene.

The human *met* oncogene is located on a portion of chromosome 7 associated with nonrandom deletion in secondary acute nonlymphocytic leukemia patients [18]. Although we have not found any direct evidence of the fact that *met* plays a role in acute nonlymphocytic leukemia, an interesting outcome of that work is the discovery that *met* is very tightly linked to the gene for cystic fibrosis [19]. Cystic fibrosis is a recessive genetic disorder characterized by abnormal exocrine gland function. Cells from patients with cystic fibrosis show abnormal regulation of chloride ion transport and impaired secretion in response to $\beta$-adrenergic agents (inducers of adenylate cyclase). Although it may seem unlikely that *met* is involved in this pathway, several proto-oncogenes appear to be involved in signal transmission [20]. Recent evidence suggests that chloride channels can be phosphorylated by a tyrosine kinase [21] and that tyrosine kinases can cooperate with adenylate cyclase to modulate gene expression [22]. Clearly, the tyrosine gene family plays important roles in the cellular control of growth, differentiation, and metabolism.

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