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To the editor:

Not every polymorphism close to the AUG codon can be explained by invoking context effects on initiation of translation

The $-1C>T$ change in the annexin V gene, which reduces the risk of myocardial infarction,¹ cannot be explained as simply as your editorial note suggests.²

The demonstration by Gonzalez-Conejero et al¹ that $-1T$ increases the efficiency of translation might be correct (the fourth paragraph of this letter discusses some concerns about the in vitro translation assay), but if so, the effect would not be explicable by invoking the Kozak context rules. The rules predict the opposite of what was seen; that is, translation should be more efficient with C rather than T in position -1 , if that position scores at all.

GCCRCCaugG (R = purine) is the optimal context for recognition of the start codon in mammals. Within this motif, some positions are more important than others. Mutagenesis experiments with laboratory constructs showed that A or G in position -3 (3 nt upstream from the AUG codon, which is numbered $+1$ to $+3$) and G in position $+4$ make the strongest contributions.^{3,4} Only in the absence of $-3R$ and $+4G$ do mutations in positions -1 and -2 score strongly.³ Inasmuch as the annexin V start site conforms to the consensus motif in positions -3 and $+4$, the identity of the base in position -1 would be expected to affect translation only slightly, if at all.

The context rules, initially established by studying translation in cultured cells,^{3,5} can be replicated using in vitro translation systems, but the experiments must be designed carefully. Reaction conditions, such as the concentration of magnesium,^{4,6} can profoundly affect whether recognition of the AUG codon in vitro displays the same sensitivity to context as is seen in vivo. The coupled transcription/translation system used by many investigators, including Gonzalez-Conejero et al,¹ makes it difficult to adjust magnesium levels and to be sure that exactly the same amount of mRNA is generated from each allele. A small change in translational efficiency is believable when the reduction in initiation from the first AUG codon is accompanied by an increase in initiation from the next AUG downstream (eg, Figure 1 in Kozak⁷) or when an assay is used that directly monitors the initiation step.⁴ One cannot be as confident, however, about a small (1.4-fold) difference in translational efficiency based on measurement of proteins precipitable by trichloroacetic acid.¹

In addition to these practical complications in testing for context effects, there are theoretical limitations. Because secondary structure downstream from the AUG codon can compensate for a less than perfect context,⁸ not every mutation within the consensus motif will score.

In some human and mouse genes, a mutation or polymorphism close to the AUG codon, usually in position -3 or $+4$, has been shown to reduce translational efficiency, with pathological consequences.⁹ Only one previous example involves a change in position -1 : in a patient with ataxia with vitamin E deficiency, a $C>T$ mutation in the alpha-tocopherol transfer protein gene causes a 2-fold decrease in translation, measured in vivo.¹⁰ In addition to these pathologies linked to mutations in the consensus motif, there is a growing list of human diseases wherein translation of a critical regulatory gene is perturbed by restructuring the 5' UTR in ways that add or remove upstream AUG codons.^{9,11} Thus, the scanning mechanism for initiation of translation does provide a framework for understanding how some mutations cause disease.

It is possible that the demonstrated increase in plasma levels of annexin V protein associated with the $-1T$ allele¹ reflects an effect on mRNA stability or splicing. If followup studies rule out these alternative explanations and confirm that the $-1T$ allele indeed augments translation, the reason could conceivably involve an effect on mRNA secondary structure. I do not think this particular polymorphism can be explained by invoking conventional context effects on AUG codon recognition.

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Response:

Intricacies of initiator polymorphism studies

I appreciate Dr Kozak setting the record straight on technical pitfalls and interpretation of studies on initiation of transcription related to polymorphisms close to the initiator methionine. Her detailed critique should be borne in mind while considering the annexin V polymorphism study of Gonzalez-Conejero et al and related studies.

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To the editor:

Polymorphic expression of CD158k/p140/KIR3DL2 in Sézary patients

Sézary syndrome (SS) is a leukemic form of epidermotropic cutaneous T-cell lymphoma (CTCL) characterized by the rapid onset of a pruriginous erythroderma with diffuse adenopathies. Sézary cells are lymphocytes with a typical cerebriform nucleus and a CD4⁺CD45RO⁺ phenotype. No specific cell membrane receptor has been described until now. Recently, we reported that circulating and cutaneous Sézary cells express CD158k/p140-KIR3DL2.^{1,2} This transmembrane receptor is a member of the killer cell Ig-like receptors that inhibit natural killer (NK)-mediated lysis after interaction with HLA-A. In healthy individuals, KIR3DL2 is only detected on minor NKs and CD3⁺CD8⁺ subsets.³ Until now, 9 *KIR3DL2* alleles have been defined.⁴ We reported the restricted expression of the *KIR3DL2* 008 allele from 2/2 CTCL lines derived from Sézary patients, suggesting that expression of this allelic form could be associated to CTCL malignancies.¹ The role of KIR3DL2 on CTCL cells is still unknown. It could be possible that the *KIR3DL2* polymorphism might influence the recognition of HLA-I alleles. In order to determine whether Sézary cells express a specific *KIR3DL2* allelic form, we have determined the repertoire of *KIR3DL2* expression in 14 patients. Diagnosis of Sézary syndrome was based on clinical criteria (erythroderma, pruritus, palmoplantar keratoderma, diffuse adenopathies) and biologic criteria, including typical circulating Sézary cells (> 1000 cells/μL), histologic data (cutaneous epidermotropic T-cell lymphoma), and detection of an identical T-cell clone in the blood and in the skin by qualitative polychain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) γ.

Peripheral blood lymphocytes (PBLs) were isolated from heparinized venous blood samples by centrifugation over Ficoll-Hypaque (Eurobio, Paris, France). DNA was extracted using Dneasy tissue kit (Quiagen, Valencia, CA). Three micrograms DNA was PCR-amplified through 40 cycles, with 2 different

conditions depending on the *KIR3DL2*-specific primers used as described.³ Taq polymerase from Promega (Madison, WI), dNTP 10 mM each (Boehringer Mannheim, Roche Diagnostics, Meylan, France) and 25 mM Mg²⁺. The amplification products were analyzed on a 1% agarose gel.

The results indicate that in all patients *KIR3DL2* cDNA PCR amplification gave good bands. Ten of 14 patients expressed 2 different alleles, including 009/007 (1 patient), 002/005 (1 patient), 007/006 (1 patient), 002/007 (3 patients), 003/007 (3 patients), and 005/007 (1 patient), whereas 4 of 14 patients were homozygous including 009 (2 patients), 002 (1 patient), and 001 (1 patient) alleles. None of the patients was homozygous for *KIR3DL2* 008 found expressed in CTCL line.¹ These results demonstrate that there is no preferential allelic expression of KIR3DL2 associated with Sézary syndrome.

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