T Cell Receptor (TCR) Mini-Gene mRNA Expression Regulated by Nonsense Codons: A Nuclear-associated Translation-like Mechanism

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Summary

Premature termination codons (PTCs) are known to decrease mRNA levels. Here, we report our investigation of the mechanism for this downregulation using the TCR-β gene, which acquires PTCs as a result of programmed rearrangements that occur during normal thymic development. We found that a mini-gene version of this gene, which contains only three TCR-β exons, exhibited efficient downregulation in response to PTCs. This demonstrates that the full coding sequence is not necessary for appropriate regulation. Mutation of the translation start AUG and a downstream in-frame AUG that displayed similarity to the Kozak consensus sequence reversed the downregulatory response to PTCs. Thus, an AUG start codon is required to define the reading frame of a PTC. Specific suppressor tRNAs also reversed the downregulatory response, strongly implicating the involvement of a translation-like process. Remarkably, the addition of suppressor tRNAs or the inactivation of the start AUGs caused a dramatic rise in the levels of PTC-bearing transcripts in the nuclear fraction prepared by two independent methods. Collectively, our results provide evidence for a codon-based surveillance mechanism associated with the nucleus that downregulates aberrant transcripts encoding potentially toxic polypeptides from nonproductively rearranged genes.

Premature termination codons (PTCs) typically promote a reduction in mRNA levels (for reviews see references 1, 2). Because termination codon recognition requires cytoplasmic ribosomes, it was anticipated that PTCs would cause mRNA decay in the cytoplasm. One could envisage, for example, that premature termination of translation in the cytoplasm would leave the 3' portion of an mRNA susceptible to nucleases attack. Consistent with this cytoplasmic decay hypothesis, PTCs have been shown to decrease the half-lives of some mRNAs in the cytoplasmic compartment of mammalian cells (3–5), and indirect evidence suggests that the rapid decay of PTC-bearing transcripts in Saccharomyces cerevisiae occurs in the cytoplasm (6–8). Surprisingly, many other reports have provided evidence that PTCs do not appreciably affect cytoplasmic RNA half-life, but instead cause the degradation of mRNAs in the nuclear fraction of cells. Transcripts that have been reported to display nuclear downregulation in response to PTCs include those encoding dihydrofolate reductase (DHFR), triose phosphate isomerase (TPI), v-src, the mouse major urinary protein, and β-globin (9–13). Nuclear run-on analysis showed that the decrease in nuclear mRNA levels imposed by PTCs is not due to a decrease in the rate of gene transcription (9, 13, 14), thus implicating a nuclear posttranscriptional mechanism. The notion that nonsense codons regulate nuclear posttranscriptional events is further supported by the observations that PTCs depress the splicing of transcripts from the minute virus of mice (MVM) (15) and increase the accumulation of alternatively spliced fibrillin mRNAs that have skipped the offending PTC (16).

The ability of nonsense codons to regulate nuclear RNA metabolism is a paradox, because the only known entity that can scan codons is a cytoplasmic ribosome. The TCR and Ig genes are attractive gene systems for studying this enigmatic nuclear response to PTCs. Before their functional expression, TCR and Ig genes undergo programmed DNA rearrangements that permit them to encode a diverse number of receptors (17, 18). Part of this diversity is provided by the imprecise joining of the V, D, and J segments; further diversity is engendered by random nucleotide additions at the junctions of these gene segments by terminal transferase. The consequence of these processes is that approximately two-thirds of the rearranged gene segments are not in the proper translational reading frame, which results in the generation of PTCs. It has been shown that nonpro-

1Abbreviations used in this paper: BHK, baby hamster kidney cells; DHFR, dihydrofolate reductase; HBS, Hepes-buffered saline; MVM, minute virus of mice; nt, nucleotides; oligo, oligonucleotide; PTC, premature termination codon; TPI, triose phosphate isomerase.
ductively rearranged (PTC-bearing) Ig heavy and light chain genes are underexpressed compared with their productively rearranged counterparts (19–22). The downregulation of Ig transcripts has been reported to occur posttranscriptionally (20) in the nucleus of B cells in vivo (22) and B cell nuclear extracts in vitro (23).

We recently found that TCR-β transcripts are also subject to nonsense-mediated downregulation. Sequence analysis of pre-mRNA and mature mRNAs from PCR products amplified from mouse thymus demonstrated that out-of-frame TCR-β genes (bearing PTCs) are actively transcribed but that specifically spliced transcripts derived from these genes do not accumulate (24). To study the mechanism for this downregulatory response, we performed transfection studies in cultured cell lines (24, 25). These studies showed that frameshift or nonsense mutations that generate PTCs cause a dramatic decrease in nuclear TCR-β mRNA levels. Intron deletion and addition studies demonstrated that a PTC must be followed by at least one functional (i.e., spliceable) intron to trigger this downregulatory response. This is consistent with a nuclear-based mechanism.

During the course of our studies we observed that the downregulation of TCR-β transcripts by PTCs could be reversed by incubation with any of several different protein synthesis inhibitors that possessed different mechanisms of action: anisomycin, cycloheximide, emetine, pactamycin, puromycin, and poliovirus (24, 25). This observation implicated the involvement of a ribosome or an unstable protein in the nuclear downregulatory response to PTCs. In the present investigation, we further explored the possibility that the downregulation of PTC-bearing TCR-β transcripts involves a ribosome-based process. We found that inactivation of initiator AUGs or expression of suppressor tRNAs caused an increase in the levels of PTC-bearing mRNAs in the nucleus, providing evidence that a translation-like surveillance mechanism exits that acts in the nuclear fraction of mammalian cells.

Materials and Methods

Plasmid Construction. Construct A is identical to pAC/1FΔ, prepared as described (24). Site-directed mutagenesis was used to introduce a missense (UAC) or a nonsense (UAA) codon at amino acid position 50 in the VDJ exon of construct A to generate constructs B and C, respectively, using the oligonucleotides (oligo) previously described (24). Construct D was made by mutating the start AUG in construct A to an AUC using the sense oligo MDA-154 (5′-GTCTCAGGATCTGCTTCCAGAC-3′) with the mutated portion underlined. Construct E was made by mutating the AUG at nt position 6 of exon 2 in construct A to a GUU using the sense oligo MDA-155 (5′-CAGAACAAGCTGGAGGCTG-3′) with the underlined portion mutated. Construct F was generated by replacing exon 2 (AUG) in construct D with the AUG-variant form of exon 2 in construct E. Constructs G–I were made in an identical manner to constructs D–F, respectively, except that the parental vector used for site-directed mutagenesis was construct C. Construct J contained a UAG nonsense codon at amino acid position 50 in the VDJ exon as a result of mutagenesis of construct A directed by the antisense oligo MDA 247 (5′-GTCAAGCAGATATGCTAATGATTTGT-3′ with the underlined portion mutated). The three Su+ T RNA constructs (pUC C3Som, pUC C3Soc, and pUC C3Sop) contain the TrNA genes cloned into the BamHI site of a pUC C-based plasmid.

Cell Culture and Transfection. Human T lymphoblastoid cell lines (HELs) were grown in DMEM containing 10% fetal bovine serum. For transfection, the cells (3 × 10^6 per 10-cm plate) were washed twice with Heps-buffered saline (HBS; 140 mM NaCl, 0.75 mM NaHPO₄, 25 mM Hepes pH 7.05), resuspended in 250 μl DMEM without serum, incubated at room temperature with 8 μg plasmid DNA (unless other amounts are specified in figure legends) for 10 min, electroporated at 250 V using an Electroporator II (Invitrogen, Inc.), and then incubated with 1 ml DMEM with 10% fetal bovine serum for 10 min in the cuvette before seeding on a 10-cm plate. For transient transfections, RNA levels were analyzed at least 2 d in culture. All transient transfections were repeated at least twice. For stable transfections, the cells electroporated with a given plasmid DNA sample were seeded onto three plates to generate three independent cell lines. Starting 1 d after transfection, stably transfected cells were selected by incubating with the antibiotic G418 (800 μg/ml).

RNA Analysis. Total cellular RNA was isolated as described (26). Nuclear and cytoplasmic RNA was isolated by two different methods. Method 1 relies on incubation in citric acid to lyse cells and release intact nuclei (George Barker, Ph.D. thesis, 1992; details kindly provided by K. Beeman, The Johns Hopkins University, Baltimore, MD). In this method, trypsinized cells from a 10-cm plate were washed twice in HBS, resuspended in 1 ml 25 mM citric acid, and then placed in a dounce homogenizer. After homogenization, cell lysates were judged by microscopy, typically, complete cell lysis required 5–10 strokes of a tight-fitting pestle. The released nuclei were pelleted by centrifugation at 3,000 rpm for 2 min in a refrigerated microcentrifuge. The supernatant (cytosol) was combined with two or more volumes of denaturing guanidinium isothiocyanate buffer; cytoplasmic RNA was then prepared from this cytoplasmic lysate by ultracentrifugation over a cesium chloride cushion as described for total cellular RNA (26). The nuclear pellet was resuspended in 1 ml 0.25 M sucrose/25 mM citric acid, dounced three times with a dounce homogenizer, underlaid below 2 ml 0.88 M sucrose, 25 mM citric acid, and centrifuged at 3,000 rpm for 6 min. The supernatant was discarded, the nuclear pellet was resuspended in 1 ml 0.88 M sucrose in RB buffer (10 mM Tris pH 7.4, 10 mM NaCl, 3 mM MgCl₂), and the underlay step was repeated below 1 ml 0.88 M sucrose in RB buffer. After centrifugation, the supernatant was discarded and the pellet was resuspended in guanidinium isothiocyanate buffer, and nuclear RNA was prepared from the nuclear lysate by ultracentrifugation over a cesium chloride cushion, as described for total cellular RNA (26). In method 2, the nuclear and cytoplasmic fractions were obtained by cell lysis in the detergent NP-40, as described (25). Cytoplasmic RNA was purified from the cytosol by the means described for method 1. Nuclear RNA was prepared from the nuclei after washing the nuclei with the stringent detergent sodium deoxycholate, as described (25).

Unless otherwise specified, 10 μg of RNA was electrophoresed, blotted, and hybridized as previously described (27). TCR-β mRNA levels were assessed by hybridization with a 0.3-kb DNA fragment corresponding to TCR-β exon 2 from the mini-gene (V_{β}21) fragment kindly provided by Ed Palmer, Basel Institute for Immunology, Basel, Switzerland). For stably transfected cells, the relative amount of RNA loaded per lane was assessed by hybridization with the housekeeping gene CHO-A (28). For transiently transfected cells, the neomycin gene probe was used to normalize for transfection efficiency. The relative levels of TCR-β tran-
scripts was calculated by values obtained from phosphoimage analysis of TCR-β and neomycin mRNA amounts.

**Results**

Transcripts from a M initi gene construct A re Downregulated in Response to PTCs. To simplify the study of nonsense-mediated regulation, we chose to generate mini-gene TCR-β constructs that contained the 5′ portion of the third exon (Cβ2.1) fused to the 3′ end of the sixth exon (Cβ2.4). These constructs contained three TCR-β exons a Vαβ1 leader exon (exon 1), a rearranged Vαβ3Dβ2β2.3 gene segment (exon 2), and the fused Cβ2.2/β2.4 exons (exon 3) (Figs. 1–4). The mini-gene constructs are driven by the β-actin promoter which permits expression in HeLa cells. HeLa cells were chosen as recipient cells for transfection because they are more efficiently transfected by electroporation than are the T cell lines we have tested. We have demonstrated before that HeLa cells, like T cells, efficiently downregulate mRNA transcribed from transfected full-length, PTC-bearing TCR-β genes (24, 25).

To determine whether the mini-genes exhibited the same downregulatory response to nonsense codons that we had observed for the full-length gene, total cellular RNA was prepared from HeLa cells transiently transfected with three versions of the mini-gene differing only at codon position 50 in exon 2. Construct A had the normal UAU sequence at this position and thus possessed an open reading frame extending to the stop codon in the final exon (see schematic diagram in Fig. 4A). Construct B contained a UAC missense codon at this position and thus also had a complete open reading frame. In contrast, construct C possessed a UAA nonsense codon at codon position 50 which truncated the open reading frame (see Fig. 4A). Northern blot analysis of mature TCR-β mRNA showed that constructs A and B were expressed at an equivalent level in transfected HeLa cells that was >15-fold higher than that of construct C (data not shown). Therefore, the TCR-β mini-gene system exhibited appropriate downregulation in response to the UAA PTC.

Mutation of Initiator AUGs reverses the Downregulatory Response. Because evidence suggests that PTCs regulate RNA metabolism in the nuclear compartment (9–16, 22, 23, 25, 32), the involvement of ribosomes has not been clear. We used our mini-gene system to ask whether nonsense-mediated regulation requires a translation-like process. To determine whether an initiator AUG is required to define a PTC, we performed AUG mutagenesis studies. In addition to the in-frame AUG in exon 1 that defines the TCR-β protein reading frame, we identified another in-frame AUG in exon 2 that contained surrounding sequences (GAACACAUGG) similar to the Kozak consensus sequence for efficient translation initiation (GCGCC-AUGG) (29). We mutationally inactivated either or both of these two AUGs and examined the effect on expression from the in-frame mini-gene. Inactivation of the normal start codon in exon 1 (construct D) caused a modest but reproducible two-fold decrease in mRNA levels relative to the control (construct A) (Fig. 1). Similarly, inactivation of the start AUG in exon 2 (construct E), or in both the first and second exon AUGs (construct F) exhibited a two- to threefold decrease in mRNA levels. We do not know the mechanism responsible for the modest reduction in expression from the in-frame AUG knockout constructs D–F.

Next we determined the effect of AUG inactivation on nonsense-mediated regulation. Mutation of the normal start codon in exon 1 (construct G) failed to significantly reverse the downregulatory effect of the PTC in this construct. Similarly, mutation of the putative start codon in exon 2 (construct H) had no measurable effect on mRNA expression. However, mutational inactivation of the AUGs in both exons 1 and 2 (construct I) strongly reversed the
downregulatory effect of the PTC, permitting expression to increase to at least 50% of that of a corresponding non-PTC-bearing construct (construct F). This set of constructs (A, C-I) was also transiently transfected into baby hamster kidney (BHK) cells and the same results were obtained as in HeLa cells (data not shown). We conclude that use of the start AUG in either exon 1 or exon 2 permits nonsense-mediated regulation, but when both AUGs are rendered nonfunctional the nonsense-mediated downregulatory pathway is at best only partially engaged.

Because of the evidence that PTCs exert their effect in the nuclear compartment of mammalian cells, we asked if inactivation of start AUGs permitted reexpression of PTC-bearing transcripts in the nucleus. Stably transfected cells were used to prepare nuclear RNA because of the large number of cells required. Fig. 2 shows data from highly purified nuclei isolated by the citric acid method (method 1 in Materials and Methods); comparable results were obtained using the NP-40/deoxycholate method (method 2; data not shown). We found that the AUG<sup>1</sup>PTC-bearing gene (construct C) accumulated a low amount of the 1.0-kb mature (fully spliced) transcript that was even lower in level than the 2.8 kb pre-mRNA transcript. In contrast, the double AUG knockout PTC-bearing gene (construct I) expressed much higher steady state levels of mature mRNA than pre-mRNA in the nuclear compartment. The ratio of mature mRNA to pre-mRNA for construct I was comparable to that for the control non-PTC-bearing gene (construct F).

Figure 2. Mutational inactivation of AUG start codons causes upregulation of PTC-bearing transcripts in the nuclear compartment. Northern blot analysis of nuclear and cytoplasmic RNA from HeLa cells stably transfected with constructs C, F, and I was isolated by method 1, as described in Materials and Methods. The data shown are from one of three independent transfected cell lines expressing each construct. Hybridization of the blot, previously hybridized with the TCR-β probe, with the CHO-A housekeeping gene [28] demonstrated equivalent loading of all lanes. Methylene blue staining [42] of the blots showed that the 32S and 45S rRNA precursors were present at high levels in the nuclear RNA and were absent in cytoplasmic RNA prepared at the same time (data not shown; see Wilkinson and MacLeod [40] and Wilkinson [43] for the use of 32S and 45S rRNA precursors as a measure of nuclear RNA enrichment).

Figure 3. Specific suppressor TRNAs reverse nonsense-mediated regulation. (A) Constructs A and C are described in the Fig. 1 legend. Construct J contains a UAG nonsense codon in place of the UAA nonsense in construct C but is otherwise identical. (B and C) Northern blot analysis of total cellular RNA isolated from HeLa cells co-transfected with the constructs (4 μg) shown in A, along with plasmids encoding UAA-, UAG-, and UGA-specific suppressor TRNAs (12 μg). As with Fig. 1, neomycin mRNA levels were used as a measure of transfection efficiency, and similar loading of RNA in all lanes was demonstrated by methylene blue staining of 18S and 28S rRNA (data not shown), performed as described [42].
We tested the specificity of the suppressor tRNAs on mRNA levels. The UAA-specific suppressor tRNA that upregulated expression from construct C had no significant effect on expression from an in-frame gene (construct A) (data not shown). To further investigate specificity, we analyzed the effect of UGA- and UAG-specific suppressor tRNAs on expression from the UAA-bearing gene (construct C). Little or no effect of these suppressor tRNAs on mRNA levels was observed (Fig. 3 B). We also tested the effects of suppressor tRNAs on a TCR-β construct containing a UAG PTC (construct J). Co-transfection with the UAG-specific suppressor tRNA plasmid upregulated expression from construct J by >10-fold (Fig. 3 C). In contrast, the UAA- and UGA-specific suppressor tRNAs failed to increase expression from construct J.

We next determined if suppressor tRNAs act by regulating nuclear RNA metabolism. Highly purified nuclear RNA was prepared from cells transfected with construct C in the presence or absence of the SuRNA UAA plasmid. As shown in Fig. 4, the UAA-specific suppressor tRNA caused an upregulation (fivefold) of mature mRNA (1.0-kb) levels in purified nuclei. In contrast, this suppressor tRNA had no measurable effect on expression from a construct lacking a PTC but otherwise identical to construct C (construct A). The suppressor tRNA also had no effect on the levels of TCR-β pre-mRNA from either construct A or C. Cytoplasmic RNA prepared in parallel with the nuclear RNA displayed a pattern of mature mRNA expression similar to nuclear RNA. Collectively, the results suggest that suppressor tRNAs induce an increase in the nuclear levels of mature mRNA, and this, in turn, is reflected in the cytoplasmic compartment.

**Discussion**

A unique feature of T- and B-lymphocytes is the programmed rearrangements of the TCR and Ig genes necessary to elicit a selective immune response against foreign antigens. A consequence of these rearrangement events is the generation of a high proportion of out-of-frame TCR and Ig genes that possess PTCs. Several reports have demonstrated that such PTC-bearing genes give rise to transcripts that are expressed at exceedingly low levels, as compared with their in-frame counterparts (19–25). In this paper, we demonstrate that a TCR-β mini-gene recapitulates this downregulatory response to PTCs. This TCR-β mini-gene provides a simple system to study nonsense-mediated regulation. Our results with the mini-gene demonstrate that neither the full-length protein product of the TCR-β gene nor the δ-acting elements in the interior of the TCR-β gene are required for appropriate regulation. More importantly, we were able to use this mini-gene system to ask whether nonsense-mediated downregulation depends upon a translation-like process.

We found that mutational inactivation of the start AUG and a downstream in-frame AUG in the TCR-β mini-gene specifically reversed the downregulatory response to PTCs in the nuclear fraction of mammalian cells (Figs. 1 and 2). To our knowledge, this is the first formal demonstration that inactivation of a start AUG triggers the increased accumulation of a transcript in the nucleus in vivo. Our results are consistent with those of Aoufouchi et al. (23) who made similar observations using nuclear extracts in an in vitro transcription-RNA splicing system. They found that the inhibition of Igκ mRNA splicing by PTCs is reversed by mutational inactivation of the start AUG (23). Although we do not know whether TCR transcripts are also decreased in levels as a result of inhibited-splicing, regulation of TCR gene expression is clearly intron-dependent, based on our previous observation that at least one intron is required downstream of a nonsense codon to trigger downregulation (25). In contrast, the v-src gene, which has also been shown to depend on a start AUG to display decreased expression in response to PTCs in vivo (11), does not contain introns and thus must engage the downregulatory response by an intron-independent mechanism.

Suppressor tRNAs reversed the downregulation of TCR-β transcripts in response to PTCs (Figs. 3 and 4), thus strongly implicating a tRNA-dependent translation-
like process in nonsense-mediated regulation. We tested suppressor tRNAs specific for all three nonsense codons (UAA, UAG, and UGA) and demonstrated that only suppressor tRNAs with the appropriate cognate anticodon sequence could reverse the downregulatory response. Our results confirm and extend the findings of the Maquat laboratory, which showed that a UAG-specific suppressor tRNA partially reversed the downregulation of TPI transcripts bearing a UAG PTC (30). Because PTCs downregulated TPI transcript levels by only three- to fivefold, and since the reversal mediated by the UAG suppressor tRNA was only partial, Belgrader et al. (30) observed only a modest twofold increase in transcript levels in response to the suppressor tRNA. We also found that suppressor tRNAs elicited only a partial reversal of regulation but because TCR-β transcripts were so strongly downregulated by PTCs, we observed a more dramatic induction of transcript levels by suppressor tRNAs, in some cases, greater than 10-fold (Fig. 3). We demonstrated for the first time that suppressor tRNAs exert their action on nonsense codon-bearing mRNA in the nuclear compartment (Fig. 4). We also demonstrated that this is a selective effect on fully spliced mRNAs; pre-mRNA levels were not measurably affected by suppressor tRNAs, as assessed by quantitative northern blot analysis. This latter result suggests that suppressor tRNAs affect either gene transcription or pre-mRNA stability. Because we did not observe a decrease in cytoplasmic TCR-β levels in response to suppressor tRNAs, it is also unlikely that suppressor tRNAs act by inhibiting nuclear-to-cytoplasmic transport. Thus, the simplest explanation for our results is that suppressor tRNAs increase the nuclear stability of PTC-bearing, fully spliced transcripts. However, it is also possible that suppressor tRNAs reverse an RNA splicing block imposed by PTCs on TCR-β transcripts. Relief of RNA splicing inhibition would normally be expected to cause a measurable increase in pre-mRNA levels (a result that we did not observe), but this would not occur if the rate of nuclear RNA degradation was higher than the rate of RNA splicing.

How is a translation-like mechanism able to downregulate transcripts in the nuclear fraction of mammalian cells? One possibility is that cytoplasmic ribosomes adjacent to the nuclear pore may direct the decay of PTC-bearing mRNAs during their transit from the nucleus. Such a translocation-translational or co-translational export model (2, 9) is consistent with evidence that mRNAs exit the nuclear pore with 5' to 3' directionality (see reference 2 and references therein). However, because RNA splicing appears to occur in the nucleoplasm proper, and not at the nuclear pore (31), this model is not easily reconciled with evidence that nonsense codons modulate RNA splicing (15, 16, 22, 23). Further evidence against this model is the finding that an intron must be downstream of a nonsense codon to trigger the downregulation of TCR-β transcripts, and that this intron will still engage the downregulatory response even when it is so close to the nonsense codon that it must be spliced before the entry of the nonsense codon into the cytoplasm (25). Still, while these lines of evidence argue against nuclear pore-associated ribosomes explaining all effects of nonsense codons, it remains an attractive hypothesis that ribosomes spatially situated on the cytoplasmic side of the nuclear envelope mediate some aspects of nonsense-mediated regulation.

A second possibility originally proposed by Urlaub et al. (9) is that a nuclear factor exists that scans and directs the decay of PTC-bearing mRNAs in the nucleus. This nuclear scanning model provides a simple explanation for why PTC-bearing mRNAs are degraded in the nuclear compartment. Nuclear recognition also explains why nonsense-mediated regulation is intron-dependent (10, 25, 32) and how nonsense codons could regulate nuclear RNA splicing (15, 16, 22, 23).

What macromolecular factor could mediate nuclear surveillance? The evidence provided in this report, as well as those of others (11, 23, 30) supports the possibility that it is a ribosome. Also consistent with this notion is the observation that factors required for translation, including eIF-4E, accumulate in the nucleus (33). The site for nuclear ribosome-some scanning could be the nucleolus, where complete or nearly complete 40S and 60S ribosomal subunits reside (34). Some mRNAs are known to accumulate in nucleoli under some circumstances, consistent with the notion that the nucleolus is a site for mRNA trafficking (35–38). However, the low abundance of most poly(A)+ mRNAs species in mammalian nucleoli (39), and lack of evidence that RNA splicing occurs in nucleoli, casts some doubt on the notion that nucleoli could be a site for nuclear surveillance of aberrant mRNAs.

Another possibility is that classical ribosomes do not mediate nuclear surveillance; instead a modified ribosome or an entire novel entity is involved. Recently, Aoufouchi et al. reported that the ribosomal inhibitors puromycin and cycloheximide failed to block the ability of B cell nuclear extracts to discriminate between PTC-bearing and non-PTC-bearing Ig transcripts (23). Because neither puromycin nor cycloheximide act on the 40S ribosomal subunit, their data suggested the involvement of either the 40S subunit or a novel factor. In contrast to their results, we found that protein synthesis inhibitors that possess different mechanisms of action, including cycloheximide, puromycin, anisomycin, pactamycin, emetine, and poliovirus, all reversed the downregulation of PTC-bearing TCR-β transcripts in intact cells (24, 25, 27, 40, 41). Because these inhibitors act directly on ribosomal subunits or associated factors, one interpretation of our finding is that the recognition of PTCs by a ribosome or a modified ribosome is blocked by incubation with the inhibitors. Another interpretation is that addition of the inhibitors blocked the translation of one or more unstable proteins that are part of a nuclear scanner or that are involved in RNA decay.

Nonsense-mediated regulation could act as a surveillance mechanism to downregulate the expression of PTC-bearing mRNAs encoding truncated polypeptides that possess dominant negative properties. Because TCR and Ig genes commonly gain PTCs during normal lymphoid ontogeny, these may be important targets for this putative surveillance
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