Immunity, Vol. 8, 135–141, February, 1998, Copyright ©1998 by Cell Press

Nonsense Surveillance in Lymphocytes? Minireview

Shulin Li and Miles F. Wilkinson*
Department of Immunology
The University of Texas
M.D. Anderson Cancer Center
Houston, Texas 77030

The White Rabbit put on his spectacles. "Where shall I begin, please your Majesty?" he asked. "Begin at the beginning," the King said, very gravely, "and go on till you come to the end; then stop."—Lewis Carroll

Programmed Gene Rearrangements: Good News and Bad News

The immunoglobulin (Ig) and T cell receptor (TCR) genes are unique among vertebrate genes in that they require programmed rearrangement for functional expression (Kronenberg et al., 1986). The V, (D), and J elements that constitute the 5′ half of these genes are juxtaposed in different combinations during lymphocyte development to generate a diverse set of receptors that recognize different antigens. An important mechanism to increase the diversity of Ig and TCR molecules is the introduction of non–template-directed nucleotides ("N" nucleotides) at the junctions of the rearranging V, (D), and J elements by terminal transferase (Figure 1). Additional diversity comes from the transfer of nucleotides from the complementary strand to the coding strand ("P" nucleotides) as a result of endonucleolytic cleavage of the hairpins generated at an intermediate step of recombination (Figure 1).

Good news and bad news comes from the variability in the number of nucleotides at the junctions of V, (D), and J elements. The good news is the increased receptor repertoire. The bad news is that two of three rearrangement events change the reading frame, generating downstream premature termination codons (PTCs) (Figure 1). Such nonproductively rearranged genes, as well as those that acquire PTCs as a result of somatic mutations during affinity maturation, cannot encode functional receptors. Because there are two copies of the Ig and TCR genes in each lymphocyte, there are at least two opportunities to generate a functionally rearranged receptor gene that does not contain a PTC. If the first rearrangement is nonproductive and the second is successful, the resulting lymphocyte will contain one functional gene and one nonfunctional gene. Because nonproductive first rearrangements are common (they occur two of three times), a large number of functional B and T lymphocytes in the peripheral compartments of normal individuals contain both good (in-frame) and bad (PTC-bearing) receptor genes. If the truncated proteins encoded by PTC-bearing Ig and TCR genes were translated, they could pose a threat to the development and function of B and T lymphocytes (discussed below). Is there a mechanism that inhibits the expression of such potentially deleterious truncated proteins?

Several investigators have observed that the steady-state levels of mRNA from Ig and TCR genes harboring PTCs are dramatically lower than those of productively rearranged Ig and TCR genes (Baumann et al., 1985; Jäck et al., 1989; Connor et al., 1994; Lozano et al., 1994; Carter et al., 1995, 1996; Aoufouchi et al., 1996; Li et al., 1997). This down-regulatory response occurs not only in cultured lymphoid cells but is also a normal physiological response in vivo. Sequence analysis of TCRβ pre-mRNA and mature transcripts from fetal and adult thymus indicated that PTC-bearing TCRβ genes are actively transcribed but that mature mRNA from these genes does not accumulate (Carter et al., 1995). This posttranscriptional response is interesting not only because of its potential physiologic value but also because of the enigmatic mechanism that appears to be responsible for it. The signal that initiates the down-regulatory response, a PTC, appears not to cause mRNA destabilization in the cytoplasm, as was expected because codons are only known to be read by the cytoplasmic translational machinery. Instead, many lines of evidence suggest that PTCs decrease the levels of Ig and TCR by a posttranscriptional mechanism involving the nucleus. In this minireview, we will focus on this paradoxical nuclear effect of PTCs and how this perplexing finding may alter our perception of the rules governing gene expression. We will also discuss the biological importance of this down-regulatory mechanism, including its potential role as a surveillance system in lymphocytes.

The Paradox

Transcripts encoding Ig μ heavy and κ light chains in B cells and the TCRβ chain in T cells are down-regulated as much as 100-fold if they contain PTCs (Baumann et al., 1985; Jäck et al., 1989; Connor et al., 1994; Carter et al., 1995). Several lines of evidence suggest collectively that this down-regulation is mediated by a nuclear posttranscriptional mechanism, not a cytoplasmic mechanism. Subcellular fractionation studies demonstrated that PTCs decrease the levels of mature (fully spliced) Ig and TCR transcripts in the nuclear compartment (Lozano et al., 1994; Aoufouchi et al., 1996; Carter et al., 1996; Li et al., 1997). This decrease in nuclear RNA levels does not result from a blockade in Ig or TCR transcription, as indicated by nuclear run-on analysis and sequence analysis of pre-mRNAs derived by the reverse transcriptase–polymerase chain reaction (RT-PCR) (Jäck et al., 1989; Qian et al., 1993a; Carter et al., 1995). The amount of decrease measured in fractionated nuclei is comparable to that in the cytoplasmic fraction, consistent with the notion that the RNA is decayed in the nucleus, resulting in a decrease of RNA levels in the cytoplasm (Li et al. 1997). That the decay of the transcripts is occurring in the nucleus, not the cytoplasm, is further supported by the finding that PTCs do not appreciably affect the cytoplasmic half-lives of Ig and TCR transcripts (Lozano et al., 1994; Carter et al.,

*To whom correspondence should be addressed (e-mail: mwilkins@notes.mdacc.tmc.edu).

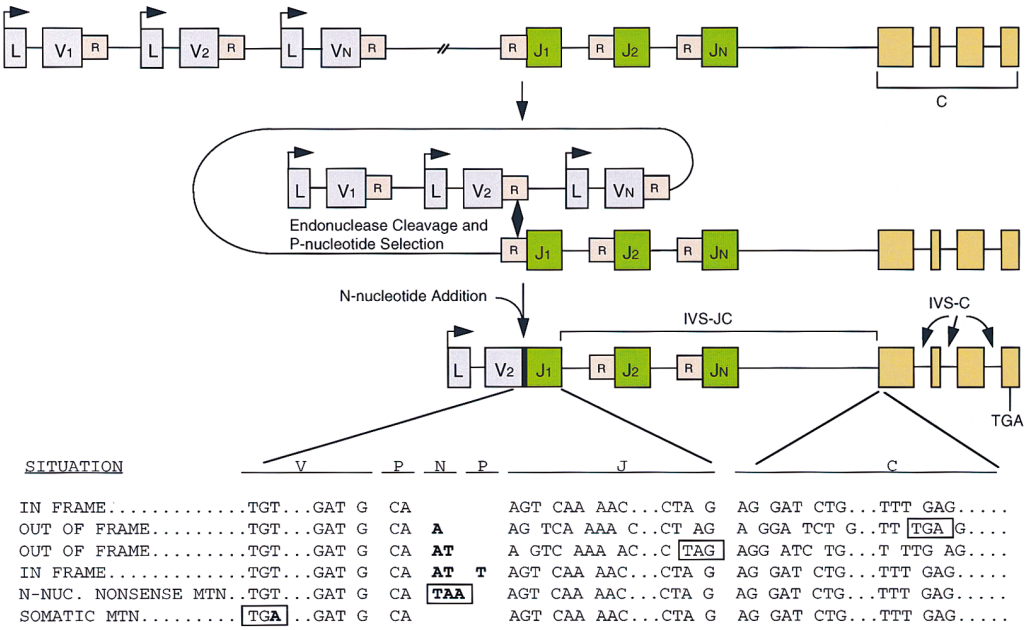

Figure 1. Ig and TCR Gene Rearrangements and the Generation of PTCs

A generic Ig/TCR gene is shown, including the V and J elements that undergo rearrangement, and the recombination signals (R) that mediates this event. The lower portion of the figure depicts PTCs being generated in the V, J, and C elements, as well as in the V-J junctional region. The V, J, and C sequences provided are from the mouse $V_{\beta 8.1}$, $J_{\beta 2.4}$, and Cβ2 gene elements in the TCRβ gene, respectively. The TCRβ gene also contains D elements (data not shown), which can be juxtaposed between the V and J elements in the rearrangement process but are not obligatory participants in this event (the TCRγ and Ig heavy chain genes also contain D elements).

1996). This apparent nuclear down-regulatory response is a paradox because nonsense codons are only known to be scanned by the cytoplasmic translational machinery.

The down-regulatory effect of PTCs is not restricted to Ig and TCR messages. PTCs have been shown to decrease the levels of most messages that have been evaluated in mammals, *Caenorhabditis elegans*, and *Saccharomyces cerevisiae*. Importantly, the nucleus has been implicated as the site of degradation for many mammalian transcripts, including those encoding dihydrofolate reductase, β-globin, NS-1 from the minute virus of mice (MVM); triosephosphate isomerase (TPI), v-src, mouse major urinary protein, and APRT (Urlaub et al., 1989; Baserga and Benz, 1992; Naeger et al., 1992; Cheng and Maquat, 1993; Belgrader and Maquat, 1994; Simpson and Stoltzfus, 1994; Kessler and Chasin, 1996). As with Ig and TCR mRNAs, the involvement of the nucleus is based on several lines of investigation but remains unproven (reviewed by Maquat, 1995).

If mammalian mRNAs are scanned for PTCs in the nucleus, these mRNAs could be unspliced, partially spliced, or fully spliced. There are two lines of evidence that the RNA template contains introns. First, in some instances PTCs appear to inhibit RNA splicing. Igκ genes containing PTCs generate elevated levels of precursor mRNA that have retained the leader intron (Lozano et al., 1994). That the levels of mature (fully spliced) Igκ mRNA are depressed by PTCs implies that RNA splicing is inhibited. More recently, an in vitro system that recapitulates this regulation was developed (Aoufouchi et al., 1996). Nuclear extracts from B-cell lines transcribe and splice transcripts from an Igκ gene template containing a complete open reading frame but do not splice transcripts from Igκ gene templates containing PTCs, as judged by RT-PCR. Another study showed that PTCs at several positions increase the levels of intron-bearing MVM mRNA (which encodes NS-1 protein) and decrease the levels of spliced MVM mRNA (which encodes NS-2 protein), as demonstrated by quantitative (Northern blot and RNase protection) analyses (Naeger et al., 1992). Importantly, the nonsense codons only affect the ratio of spliced to unspliced mRNA if they are in frame with the initiator AUG. The data imply that in-frame nonsense codons inhibit RNA splicing.

The second line of evidence that an intron-containing RNA is the template for a nonsense surveillance pathway is the finding that there must be introns downstream of a nonsense codon for it to trigger the down-regulatory response. Removal of the introns downstream of nonsense codons in the TCRβ gene reverses the down-regulatory response (Carter et al., 1996). As a complementary experiment, it was shown that the "normal" stop codon at the end of the TCRβ reading frame triggers the down-regulatory response if introns are introduced downstream of it (Carter et al., 1996). A single downstream intron is sufficient to engage the down-regulatory response (Carter et al., 1995; 1996). Based on these data, a two-signal rule was proposed in which a nonsense codon and a downstream intron are both required to trigger down-regulation (Figure 2).

The notion that functional introns serve as a second signal for PTC-mediated down-regulation is also supported by other studies. Analysis of numerous mammalian genes has shown that stop codons only cause down-regulation if they are present in internal exons (i.e., if there is at least one intron downstream of the nonsense codon) (Urlaub et al., 1989; Connor et al., 1994; Hall and Thein, 1994; Maquat, 1995; Kessler and

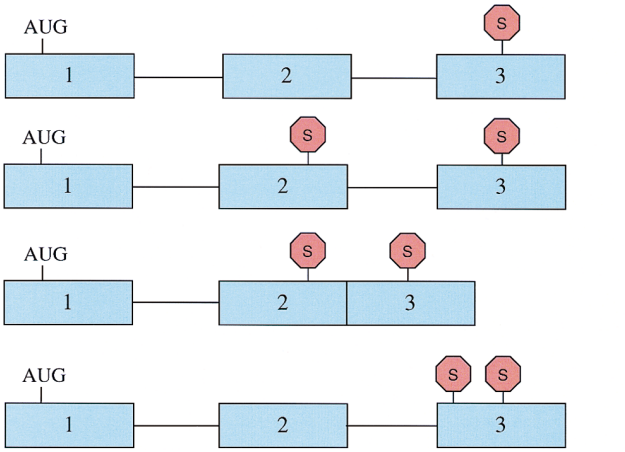

Figure 2. The Two-Signal Rule

Two signals are necessary to trigger a decrease in mRNA levels: a stop codon (designated as a stop sign), and at least one downstream intron. Experimental support for this rule comes from studies of TCR, Ig, and other transcripts in mammalian cells. A downstream exonic element, rather than an intron, is the second signal for PTC-mediated RNA decay in *S. cerevisiae* (Jacobson and Peltz, 1996).

Chasin, 1996). In contrast, normal stop codons are in the terminal exon of most vertebrate genes (Hawkins, 1988). Experimental support comes from analysis of the *TPI* gene; deletion of the final *TPI* intron partially reverses the down-regulation caused by a nonsense codon in the upstream exon (Cheng and Maquat, 1993).

If the RNA template for the nonsense surveillance pathway were completely unspliced (a primary transcription product), it is hard to imagine how the frame of nonsense codon could be recognized with respect to the initiator AUG (if these two signals are in separate exons). A more likely scenario is that the template for the scanning mechanism is a partially spliced mRNA from which at least the introns before the PTC have been excised. To test whether the RNA template is partially spliced before PTC recognition, investigators have examined the down-regulatory effect of PTCs that span two exons and thus presumably can be read only after RNA splicing. This was first reported for an Ig μ heavy chain gene containing a UG at the 3′ terminus of the C_{m3} exon and an A in the 5′ terminus of the C_{m4} exon, such that a UGA termination codon is generated only after RNA splicing. This "split" nonsense codon strongly down-regulates transcripts encoding the membrane-bound form (μM) (by about 100-fold), demonstrating that nonsense codons can be recognized after RNA splicing (Connor et al., 1994). In accordance with the two-signal rule (Figure 2), transcripts encoding the secreted form (μS), which has no introns after the split PTC, are not down-regulated. Similar experiments with the TCRβ and *TPI* genes demonstrated that either a UAG or a UGA nonsense codon split by an intron can cause down-regulation (Carter et al., 1996; Zhang and Maquat, 1996). In the case of the TCRβ gene it was shown that removal of the three introns downstream of the split UAG ameliorated down-regulation, which strongly supports the two-signal rule (Carter et al., 1996). The available data support the notion that the template for PTC scanning is a partially spliced transcript. However, as discussed in the section "Models" (below), an alternative viewpoint has also been put forward.

The Role of Translation

The ability of PTCs to reduce the abundance of transcripts in the nuclear fraction of cells is a paradox because the only known entity that can scan codons is the cytoplasmic ribosome. Do ribosomes direct PTC-mediated down-regulation? This question has been addressed by using translation inhibitors. Protein synthesis inhibitors with different mechanisms of action all increase the levels of TCRβ transcripts harboring PTCs to the levels of in-frame TCRβ transcripts lacking PTCs (Qian et al., 1993a, 1993b; Carter et al., 1995). Similar results have been demonstrated for β-globin, α-L-iduronidase, and MSH2 transcripts (Menon and Neufeld, 1994; Carter et al., 1995; Andreutti-Zaugg et al., 1997). One interpretation of these data is that the translational apparatus is responsible for scanning mRNAs and that disrupting this process with translation inhibitors blocks the surveillance mechanism, allowing transcripts harboring PTCs to accumulate at high levels. However, an alternative interpretation is that translation inhibitors merely deplete cells of one or more unstable proteins involved in PTC-mediated down-regulation. In that case, PTC-bearing transcripts might not be scanned by the translational machinery; they could be scanned by a novel macromolecule instead.

Because tRNA molecules are absolutely required for translation, their role in PTC-mediated down-regulation has also been investigated. Suppressor tRNAs, which compete with the release factors that normally terminate translation, have been used to address this question. It was found that suppressor tRNA molecules partially reverse the down-regulation of TCRβ and *TPI* transcripts caused by nonsense codons, implicating tRNAs in the regulation (Belgrader et al., 1993; Li et al., 1997). The increased levels of PTC-bearing TCRβ transcripts induced by suppressor tRNAs occurred in the nuclear compartment (Li et al., 1997). Further evidence for the role of a translation-like process in PTC-mediated down-regulation was the observation that mutational inactivation of the initiator AUG that defines the TCRβ and Igκ reading frames reversed PTC-mediated down-regulation of transcripts in the nuclear compartment (Aoufouchi et al., 1996; Li et al., 1997). Thus, the available data suggest that the translational or related apparatus is responsible for down-regulating PTC-bearing transcripts in the nuclear fraction of mammalian cells. However, the location of this scanning apparatus is not known, nor is it known whether it actually engages in translation.

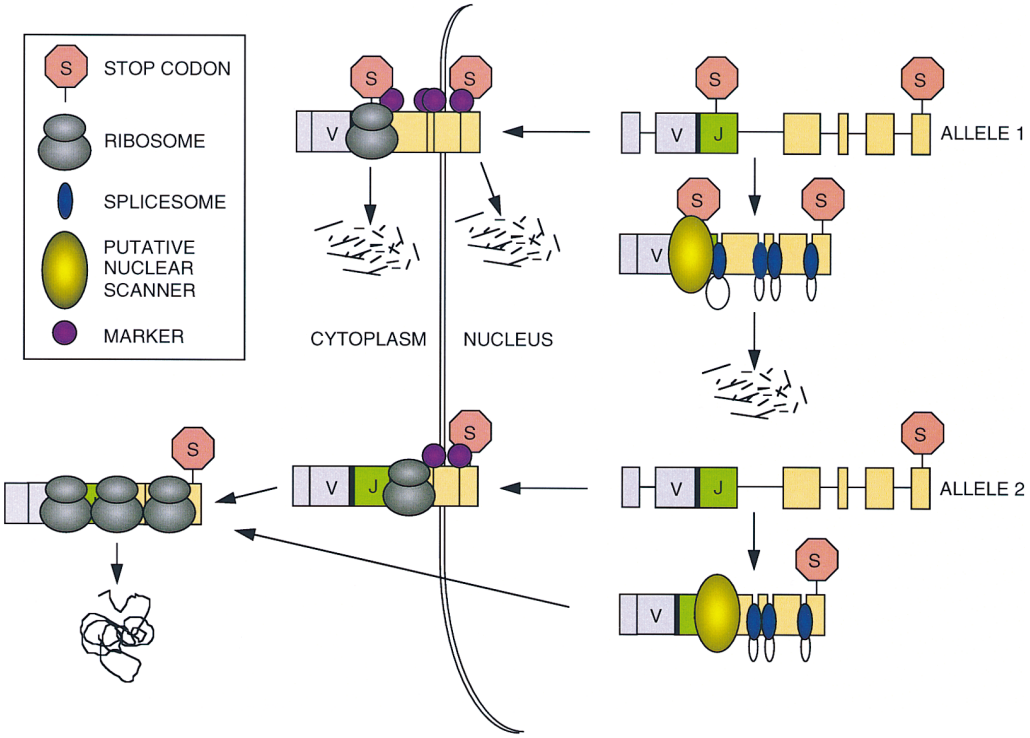

Figure 3. Two Models that Explain How Nonsense Codons Decrease mRNA Levels in the Nuclear Compartment of Mammalian Cells

In the marker model, "marks" are deposited on fully spliced mRNAs at the sites where introns resided in the pre-mRNA. The marked mRNAs are read by ribosomes as they exit the nucleus through the nuclear pores. The marks act as the second signal for PTC-mediated down-regulation (Figure 2), and therefore, if a traversing mRNA contains an in-frame nonsense codon followed by at least one mark (allele 1), it is targeted for degradation. mRNA decay occurs either in the nucleus (if the mRNA is still associated with the nuclear compartment) or in the cytoplasm (if the mRNA has fully exited the nuclear compartment). By contrast, if the first in-frame nonsense codon is in the final exon (allele 2), the typical case for a functional gene containing a complete open reading frame, the mRNA lacks a mark after the nonsense codon and therefore it is permitted export to the cytoplasm for translation. A related model has also been proposed in which introns with specific *cis* elements direct the introduction of the "mark" (Cheng et al., 1994).

The nuclear scanning model proposes that a nuclear scanner is responsible for PTC-mediated down-regulation in the nucleus proper. The nuclear scanner could be a ribosome, a modified ribosome, or a novel macromolecule. If this scanner meets a splicesome bound to an intron after a stop codon (allele 1), it triggers the decay of the transcript. If there are no introns after the first stop codon (allele 2), the decay mechanism fails to be engaged and the mRNA is exported to the cytoplasm for translation.

Models

The paradoxical ability of PTCs to affect nuclear events has inspired numerous models. One model proposes that PTC recognition takes place at the nuclear pore (Urlaub et al., 1989; Maquat, 1995). According to this cotranslational export model, nonsense codons are recognized by the cytoplasmic translational machinery as transcripts traverse the nuclear pore. When a PTC is recognized, the RNA is degraded while still associated with the nucleus. This model predicts that a nonsense codon just upstream of the last intron would not be recognized by the cytoplasmic translation machinery before the intron is spliced in the nucleus, and, therefore, because of the two-signal rule (Figure 2), the down-regulatory mechanism would fail to be engaged. In contrast to this prediction, TCRβ transcripts containing nonsense codons just 8 or 10 nucleotides upstream of the last intron are down-regulated (Carter et al., 1996). To explain this result, a marker model was proposed whereby a "mark" introduced after intron splicing is the second signal for PTC-mediated down-regulation (Figure 3). This mark could be an RNA-binding protein that remains bound to the exon after splicing (Blencowe et al., 1995) or a modification of the RNA itself. Because RNA splicing occurs at or near the site of transcription in the nucleus proper, it is likely that the mark would be introduced in the nucleus proper (Steinmetz, 1997). This implies that once the message reaches the nuclear pore for PTC scanning it has excised all of its introns. The notion of a mark (rather than a downstream intron per se) as a second signal for PTC-mediated down-regulation is attractive because it avoids the problem of the order of intron splicing; for example, introns are not necessarily removed in a 5′ to 3′ order, and therefore introns upstream of a PTC may not be spliced out (to permit PTC recognition) before the downstream introns are spliced (Kessler et al., 1993).

Although the marker model explains how transcripts can be degraded while still associated with the nucleus, it fails to explain how nonsense codons regulate RNA splicing. A model that better accommodates effects on RNA splicing is the nuclear scanning model, which proposes that PTCs are recognized not at the nuclear pore but in the nucleus proper (Urlaub et al., 1989). The principal advantage of such a nuclear scanning model is that the effects of PTCs on nuclear mRNA decay and

nuclear RNA splicing occur by a direct mechanism within the same cellular compartment as PTC recognition (Figure 3). Although such a nuclear scanning model is difficult to accept given that ribosomes are not known to function in the nucleus, the results from several studies support it.

First, PTCs cause a similar decrease in mRNA levels in highly purified nuclei (or even nuclear matrices) as they do in the cytoplasmic fraction (Belgrader et al., 1994; Kugler et al. 1995; Carter et al., 1996; Li et al., 1997). Although it is not possible to exclude the possibility that cytoplasmic contamination is responsible for this effect, in one study the nuclei were judged to be free of the double-membraned nuclear envelope and surrounding cytoplasm by electron microscopy (Belgrader et al., 1994). Second, nuclear extracts depleted of contaminating ribosomes (by ultracentrifugation) appear to be capable of selectively inhibiting the splicing of Igκ transcripts harboring PTCs (Aoufouchi et al., 1996). Third, the nature of the promoter dictates whether PTCs engage the down-regulatory response. PTCs trigger mRNA decay from constructs in which the β-globin gene is driven by the β-globin or cytomegalovirus immediate early promoters but not when it is driven by the thymidine kinase promoter (Enssle et al., 1993; Kugler et al., 1995). This remarkable finding may reflect the fact that many eukaryotic transcripts undergo RNA splicing while being synthesized in vivo (Steinmetz, 1997). Perhaps the polymerase II transcription complexes that form on different promoters differ in their ability to support RNA splicing or other posttranscriptional events that impact PTC-mediated down-regulation. Fourth, PTCs appear to regulate RNA splicing events. As described earlier, there is evidence that PTCs inhibit the splicing of MVM and at least some Igκ transcripts (Naeger et al., 1992; Lozano et al., 1994; Aoufouchi et al., 1996). In addition, nonsense codons may regulate splice-site selection. Numerous studies have demonstrated that some mutant PTC-bearing genes associated with genetic diseases express mRNAs that lack the exon containing the in-frame nonsense codon (reviewed by Maquat, 1995, 1996). The most compelling published evidence linking nonsense codons and alternative splicing is a study showing that PTCs in exon 51 of the fibrillin gene cause the accumulation of an alternatively spliced transcript that lacks this exon (Dietz et al., 1993). Transfection experiments demonstrated that only in-frame nonsense mutations, not missense mutations or out-of-frame nonsense mutations, are responsible for the increased accumulation of exon-skipped mRNA (Dietz and Kendzior, 1994).

Although the recognition of PTCs in the nucleus proper, as hypothesized in the nuclear scanning model, provides an explanation for the nuclear effects of PTCs, several questions remain unanswered. First, why do PTCs trigger different types of nuclear posttranscriptional events under different situations? One possibility is that the fate of individual mRNAs harboring PTCs would depend on their requisite collection of *cis* elements: some transcripts would be prevented from completing RNA splicing, others would undergo alternative RNA splicing, and still others would be rapidly destroyed by nuclear ribonucleases. Second, how can a nonsense codon regulate the use of an upstream splice site if codon scanning occurs in a 5′ to 3′ direction. This is an issue not only for exon skipping but also for splicing inhibition, because nonsense codons have been suggested to inhibit the splicing of upstream Igκ and MVM introns (Naeger et al., 1992; Lozano et al., 1994; Aoufouchi et al., 1996). Finally and perhaps most importantly, what is the entity that scans codon triplets in the nucleus proper? The available evidence suggests that tRNAs are involved, but it is not known what other molecules participate in PTC scanning (Belgrader et al., 1993; Li et al., 1997).

A Lymphoid-Specific or a Universal Mechanism?

Because Ig and TCR genes acquire PTCs at a high rate during normal development, it is possible that a unique mechanism has evolved to specifically down-regulate Ig and TCR transcripts harboring PTCs. Several observations support this possibility. First, the down-regulation of Ig and TCR transcripts in response to PTCs is robust (10-fold to 100-fold), whereas many other mammalian transcripts are more modestly down-regulated (e.g., *TPI*, MVM, and v-*src* transcripts are down-regulated by 3- to 4-fold in response to PTCs) (Baumann et al., 1985; Jäck et al., 1989; Naeger et al., 1992; Cheng and Maquat, 1993; Connor et al., 1994; Simpson and Stoltzfus, 1994; Carter et al., 1995, 1996). Second, PTC-mediated down-regulation is in some cases lymphoid-specific. T cells, but not HeLa (epithelial) cells, down-regulate RNA transcripts from a transfected TCRβ gene construct harboring a PTC in the penultimate exon (Carter et al., 1996). B-cell nuclear extracts, but not nuclear extracts from several other cell types, selectively decrease the levels of mature mRNA transcribed and spliced from PTC-bearing Igκ genes (at the concentration of nuclear extracts tested) (Aoufouchi et al., 1996). Third, there is some evidence for template specificity of PTC-mediated down-regulation. The accumulation of Igκ, but not β-globin mature transcripts, is decreased in response to PTCs when the transcripts are incubated with B-cell nuclear extracts (Aoufouchi et al., 1996). Fourth, the intron requirement for PTC-mediated down-regulation (Figure 2) differs in lymphoid and non-lymphoid genes. While a spliceable intron downstream of a PTC is required for down-regulation of the TCRβ gene, an intron downstream of a PTC in the *TPI* gene can be rendered unspliceable and still serve as a second signal for down-regulation (Cheng and Maquat, 1993; Carter et al., 1996). The nuclear down-regulation of v-*src* transcripts by PTCs is completely independent of introns (Simpson and Stoltzfus, 1994). Fifth, while a decrease in nuclear RNA stability accounts for the down-regulation of *TPI* transcripts harboring a PTC, inhibition of RNA splicing appears to be responsible for the down-regulation of PTC-bearing Igκ transcripts (Belgrader et al., 1994; Lozano et al., 1994; Aoufouchi et al., 1996).

A case can also be made for a single universal mechanism responsible for the down-regulatory effects of PTCs on different transcripts. The stronger down-regulation of Ig and TCR transcripts than that of many other mammalian transcripts may result from differences in *cis* elements within these transcripts rather than an intrinsic difference in the underlying mechanism for PTC-mediated down-regulation. The differences in cell-type specificity may result from quantitative differences in *trans*-acting factors present in different cell types. Clearly,

nonlymphoid cells contain factors capable of recognizing PTC-bearing transcripts from rearranging genes; transfected HeLa cells efficiently down-regulate the expression of TCRβ transcripts harboring PTCs at most positions (Carter et al., 1995, 1996; Li et al., 1997).

If such a universal mechanism exists, would it also operate in nonmammalian organisms? There has been a long standing debate concerning whether PTC-mediated RNA down-regulation in the well-studied organism *S. cerevisiae* involves the same basic mechanism as that in mammalian cells. The consensus view is that *S. cerevisiae* degrades PTC-bearing transcripts in the cytoplasm rather than in the nucleus (Jacobson and Peltz, 1996). Experimental support comes from studies performed with Upf2p, a protein necessary for PTC-mediated mRNA decay in yeast. A dominant-negative form of Upf2p interferes with PTC-mediated down-regulation when localized to the cytoplasm but not when localized to the nucleus (He and Jacobson, 1995). In addition, it has been shown that some higher eukaryotic transcripts that harbor PTCs are targeted for cytoplasmic decay, including Rous sarcoma virus *gag* and *Xenopus laevis XLPOU-60* (reviewed by Maquat, 1995). Rapid decay in both the cytoplasm and the nucleus appears to be the fate of PTC-bearing *aprt* transcripts (Kessler and Chasin, 1996). Importantly, the cytoplasmic instability of *aprt* mRNA harboring a PTC was revealed only when a tetracycline-regulated promoter was used to measure RNA half-life. The traditional approach of using the transcriptional inhibitor actinomycin D failed to show an effect of a PTC on *aprt* cytoplasmic RNA stability.

Some interpret these results as indicating that both cytoplasmic and nuclear surveillance mechanisms scan transcripts in mammalian organisms, whereas only the cytoplasmic mechanism exists in yeast. Others ("the universalists") have taken another stand; they argue that only a cytoplasmic mechanism exists in all eukaryotes. According to this view, the apparent "nuclear" effects in mammalian cells are attributed to either cytoplasmic contamination of nuclear preparations or a mechanism that operates at the nuclear pore. An attractive feature of this unifying cytoplasmic model is that it says that the cytoplasmic translational machinery is responsible for nonsense codon recognition, rather than a hypothetical nuclear scanner. However, this cytoplasmic model does not explain easily the effects of PTCs on RNA splicing in mammalian cells. Furthermore, even in yeast, because of technical difficulties in fractionating the yeast nucleus and the cytoplasm, it has not been established definitively whether PTC-mediated mRNA decay occurs in the cytoplasm. The observation that yeast transcripts harboring PTCs are on polysomes has been taken as evidence that PTC recognition occurs in the yeast cytoplasm, but instead these low-level polysome-associated transcripts may be those that have escaped decay by a surveillance mechanism that involves the nucleus (He et al., 1993). Recently, it was shown that a *S. cerevisiae* strain that exhibits a block in poly(A)$^+$ export as a result of a mutation in a nucleoporin gene is still able to rapidly degrade transcripts harboring PTCs, implying that PTC recognition may occur in the yeast nucleus (Arking et al. 1997). This notion is supported by in situ hybridization studies, which show that mutant *S. cerevisiae* that lack the PTC-mediated down-regulatory pathway exhibit an increase in the levels of PTC-bearing *lacZ* transcripts in both nucleus and the cytoplasm (Long et al., 1995). Importantly, the known *S. cerevisiae* proteins that dictate PTC-mediated RNA decay, Upf1p, Upf2p, and Upf3p, have each been linked with both the cytoplasm and the nucleus (He and Jacobson, 1995; Lee and Culbertson, 1995; Jacobson and Peltz, 1996; Atkin et al., 1997). Clearly, further studies are necessary to elucidate the precise location of PTC recognition in lower and higher eukaryotic organisms and whether a universal mechanism is responsible for the down-regulation of transcripts harboring PTCs.

The Functional Role of Nonsense Surveillance

What is the functional role of PTC-mediated down-regulation? A likely possibility is that it increases the fidelity of gene expression by preventing the accumulation of aberrant mRNAs that, if translated, would produce toxic truncated proteins. PTCs at some positions in the myosin gene cause dominant-negative effects when the PTC-mediated down-regulatory pathway in *C. elegans* is inactivated by mutation of the *smg* genes (Pulak and Anderson, 1993). Such *smg*$^-$ mutant organisms also display various other phenotypic defects. Dominant thalassemias in humans are caused by 3′ PTCs in β-globin genes that escape PTC-mediated down-regulation but not by 5′ PTCs that do trigger down-regulation (Hall and Thein, 1994). Ig and TCR genes harboring PTCs encode truncated proteins composed of the variable (V[D]J) region and sometimes a small portion of the constant (C) region (Figure 1). If translated, such truncated proteins could be deleterious because they could act as dominant-negative mutants that inhibit the function of the wild-type Ig and TCR proteins (Herskowitz, 1987). For example, V peptides translated from PTC-bearing Ig light chain genes could interact with full-length Ig heavy chains and thereby inhibit their surface expression, secretion, and function. In addition, truncated Ig and TCR proteins could induce a stress response in the endoplasmic reticulum if they were expressed at sufficiently high levels or misfolded (Pahl and Baeuerle, 1997).

The nonsense surveillance system probably also protects against errors in other genes important for a functional immune system, such as those encoding cytokines, signal transduction molecules, and adhesion proteins. PTCs can be generated in these genes by several mechanisms: frameshift and point mutations, errors in transcription, and errors in RNA splicing (e.g., the use of cryptic splice sites). Another source of PTCs are pre-mRNAs, which typically contain in-frame nonsense codons in the introns. If pre-mRNAs were permitted to escape to the cytoplasm, they would translate potentially toxic proteins containing novel sequences encoded by the intronic sequences. The nonsense down-regulatory pathway in *S. cerevisiae* has been shown to prevent the accumulation of pre-mRNAs (He et al., 1993).

The Future

The Ig and TCR genes have been used as a paradigm for understanding many biological events, including DNA

recombination, cell-type–specific transcriptional regulation, and control of development. Here, we summarize evidence that these genes have unique properties that permit their use as a model system to study nonsense surveillance. The following are three broad issues for future study.

First, how does a cytoplasmic translation signal (a nonsense codon) regulate nuclear-associated events? In particular, how can nonsense codons regulate RNA splicing and nuclear RNA decay? Why are introns necessary for this pathway? Are nonsense codons recognized near the nuclear pore or in the nucleus proper? What is the entity that scans transcripts and recognizes nonsense codons? There is evidence for the involvement of tRNAs, but the role of ribosomes or translation is not known.

Second, are there different mechanisms for down-regulating Ig and TCR transcripts versus other transcripts? Because the Ig and TCR genes are unique in acquiring PTCs at an extremely high frequency (>50%) during normal lymphoid development, the existence of a unique mechanism would not be surprising. Some lines of evidence are consistent with this possibility, but mechanistic studies involving molecularly defined factors and in vitro systems are necessary to resolve this issue.

Finally, what is the physiological relevance of this down-regulatory pathway? If the pathway were inactivated, what would the consequences be? Would the truncated Ig and TCR proteins act as dominant-negative mutant proteins or induce a stress response in the endoplasmic reticulum? Would the increased accumulation of aberrant PTC-bearing transcripts encoding other immunologically relevant molecules (generated by mutation or infidelity of gene expression) exert toxic effects? Answering these questions should allow us to understand a potentially important surveillance mechanism and may alter our perception of the rules governing gene expression.

Acknowledgments

We would like to thank the following people for providing comments on this minireview: Thomas Cooper, Gilbert Cote, Maureen Goode, Hector Martinez-Valdez, Thomas Perrin, David Roth, Phillip Sharp, Marc Shulman, Ann-Bin Shyu, Ursula Storb, and Carrie Valentine.

Selected Readings

Andreutti-Zaugg, C., Scott, R.J., and Iggo, R. (1997). Cancer Res. *57*, 3288–3293.

Aoufouchi, S., Yélamos, J., and Milstein, C. (1996). Cell *85*, 415–422.

Arking, D.E., Perlick, H.A., and Dietz, H.C. (1997). Am. J. Hum. Genet. *61(4)*, 122.

Atkin, A.L., Schenkman, L.R., Eastham, M., Dahlseid, J.N., Lelivelt, M.J., and Culbertson, M.R. (1997). J. Biol. Chem. *272*, 22163–22172.

Baserga, S.J., and Benz, E.J., Jr. (1992). Proc. Natl. Acad. Sci. USA *89*, 2935–2939.

Baumann, B., Potash, M.J., and Köhler, G. (1985). EMBO J. *4*, 351–359.

Belgrader, P., and Maquat, L.E. (1994). Mol. Cell. Biol. *14*, 6326–6336.

Belgrader, P., Cheng, J., and Maquat, L.E. (1993). Proc. Natl. Acad. Sci. USA *90*, 482–486.

Belgrader, P., Cheng, J., Zhou, X., Stephenson, L.S., and Maquat, L.E. (1994). Mol. Cell. Biol. *14*, 8219–8228.

Blencowe, B.J., Issner, R., Kim, J., McCaw, P., and Sharp, P.A. (1995). RNA *1*, 852–865.

Carter, M.S., Doskow, J., Morris, P., Li, S., Nhim, R.P., Sandstedt, S., and Wilkinson, M.F. (1995). J. Biol. Chem. *270*, 28995–29003.

Carter, M.S., Li, S., and Wilkinson, M.F. (1996). EMBO J. *15*, 5965–5975.

Cheng, J., and Maquat, L.E. (1993). Mol. Cell. Biol. *13*, 1892–1902.

Cheng, J., Belgrader, P., Zhou, X., and Maquat, L.E. (1994). Mol. Cell. Biol. *14*, 6317–6325.

Connor, A., Wiersma, E., and Shulman, M.J. (1994). J. Biol. Chem. *269*, 25178–25184.

Dietz, H.C., and Kendzior, R.J., Jr. (1994). Nature Genet. *8*, 183–188.

Dietz, H.C., Valle, D., Francomano, C.A., Kendzior, R.J., Jr., Pyeritz, R.E., and Cutting, G.R. (1993). Science *259*, 680–683.

Enssle, J., Kugler, W., Hentze, M.W., and Kulozik, A.E. (1993). Proc. Natl. Acad. Sci. USA. *90*, 10091–10095.

Hall, G.W., and Thein, S. (1994). Blood *83*, 2031–2037.

Hawkins, J.D. (1988). Nucleic Acids Res. *16*, 9893–9908.

He, F., and Jacobson, A. (1995). Genes Dev. *9*, 437–454.

He, F., Peltz, S.W., Donahue, J.L., Rosbash, M., and Jacobson, A. (1993). Proc. Natl. Acad. Sci. USA *90*, 7034–7038.

Herskowitz, I. (1987). Nature *329*, 219–222.

Jäck, H.-M., Berg, J., and Wabl, M. (1989). Eur. J. Immunol. *19*, 843–847.

Jacobson, A., and Peltz, S.W. (1996). Annu. Rev. Biochem. *65*, 693–739.

Kessler, O., and Chasin, L.A. (1996). Mol. Cell. Biol. *16*, 4426–4435.

Kessler, O., Jiang, Y., and Chasin, L.A. (1993). Mol. Cell. Biol. *13*, 6211–6222.

Kronenberg, M., Siu, G., Hood, L.E., and Shastri, N. (1986). Annu. Rev. Immunol. *4*, 529–591.

Kugler, W., Enssle, J., Hentze, M.W., and Kulozik, A.E. (1995). Nucleic Acids Res. *23*, 413–418.

Lee, B.-S., and Culbertson, M.R. (1995). Proc. Natl. Acad. Sci. USA. *92*, 10354–10358.

Li, S., Leonard, D., and Wilkinson, M.F. (1997). J. Exp. Med. *185*, 985–992.

Long, R.M., Elliott, D.J., Stutz, F., Rosbash, M., and Singer, R.H. (1995). RNA *1*, 1071–1078.

Lozano, F., Maertzdorf, B., Pannell, R., and Milstein, C. (1994). EMBO J. *13*, 4617–4622.

Maquat, L.E. (1995). RNA *1*, 453–465.

Maquat, L.E. (1996). Am. J. Hum. Genet. *59*, 279–286.

Menon, K.P., and Neufeld, E.F. (1994). Cell. Mol. Biol. *40*, 999–1005.

Naeger, L.K., Schoborg, R.V., Zhao, Q., Tullis, G.E., and Pintel, D.J. (1992). Genes Dev. *6*, 1107–1119.

Pahl, H.L., and Baeuerle, P.A. (1997). Trends Cell Biol. *7*, 50–55.

Pulak, R., and Anderson, P. (1993). Genes Dev. *7*, 1885–1897.

Qian, L., Theodor, L., Carter, M., Vu, M.N., Sasaki, A.W., and Wilkinson, M.F. (1993a). Mol. Cell. Biol. *13*, 1686–1696.

Qian, L., Vu, M.N., Carter, M.S., Doskow, J., and Wilkinson, M.F. (1993b). J. Immunol. *151*, 6801–6814.

Simpson, S.B., and Stoltzfus, C.M. (1994). Mol. Cell. Biol. *14*, 1835–1844.

Steinmetz, E.J. (1997). Cell *89*, 491–494.

Urlaub, G., Mitchell, P.J., Ciudad, C.J., and Chasin, L.A. (1989). Mol. Cell. Biol. *9*, 2868–2880.

Zhang, J., and Maquat, L.E. (1996). RNA *2*, 235–243.