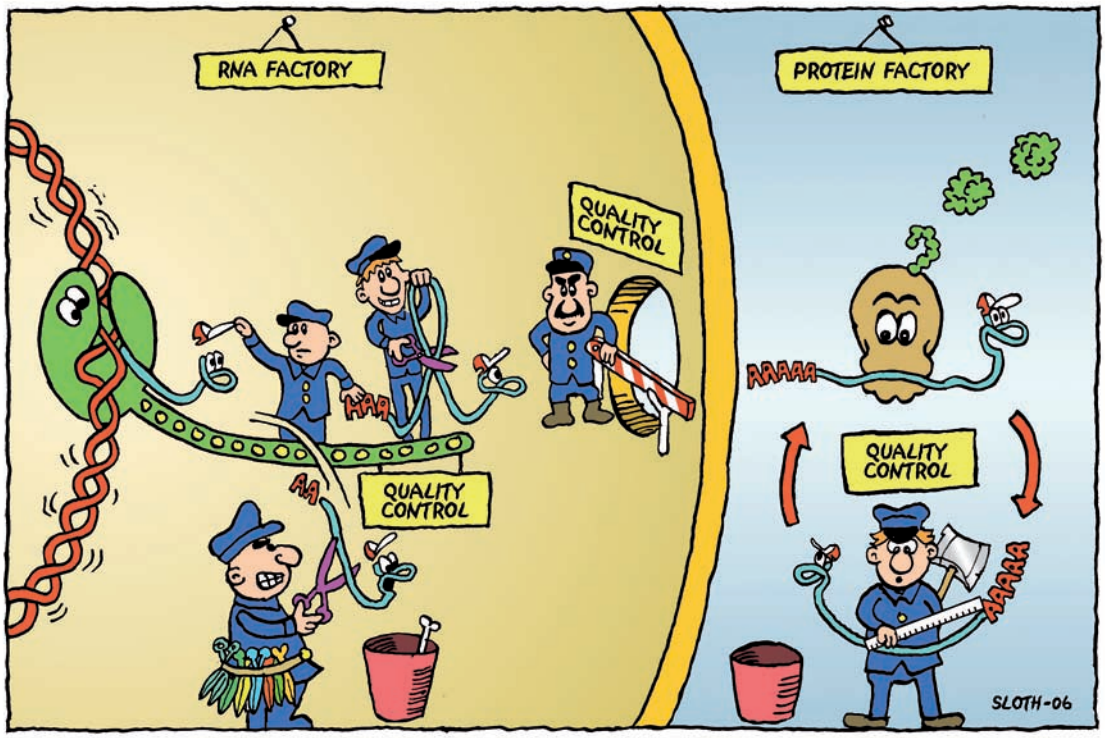

Quality Control of Gene Expression

Recognition of Nonsense mRNAs Prevents Synthesis of Truncated Proteins

To ensure accuracy of gene expression, cells have evolved quality control mechanisms to monitor the different steps of gene expression. One of the most intensively studied surveillance mechanisms is called nonsense-mediated mRNA decay (NMD), a process that recognizes and degrades transcripts containing a premature translation-termination codon (PTC). Thus, NMD prevents the cell from production of truncated proteins, which may act in a dominant-negative way. Several questions in NMD research have attracted much attention in recent years: How is a PTC recognized and discriminated from a physiological stop codon, and by which mechanism does a PTC trigger fast mRNA degradation? What is the role of NMD in the regulation of gene expression and what is the effect of NMD in genetic diseases?

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Various Processes Generate PTCs

PTCs can emerge from frameshift or nonsense mutations in the genome, as well as from errors in every step of gene expression, from the transcription of the nascent mRNA to its translation. The production of a correct mRNA requires a sequence of complicated biochemical processes in the nucleus: transcription, capping, splicing, 3' end formation, and the export to the cytoplasm (fig. 1A). The error rate during transcription is generally low and therefore only seldom a PTC is generated. In contrast, PTCs are frequently acquired by alternative splicing. It was estimated that in mammals, approximately one third of the alternatively spliced transcripts contain PTCs and are substrates for NMD [1]. This is a large fraction of the total mRNA population, given that about 74% of human multi exon genes are alternatively spliced. T cell receptor and immunoglobulin genes represent a special class of genes where PTCs are commonly acquired as a result of programmed V(D)J rearrangements during lymphocyte development [2].

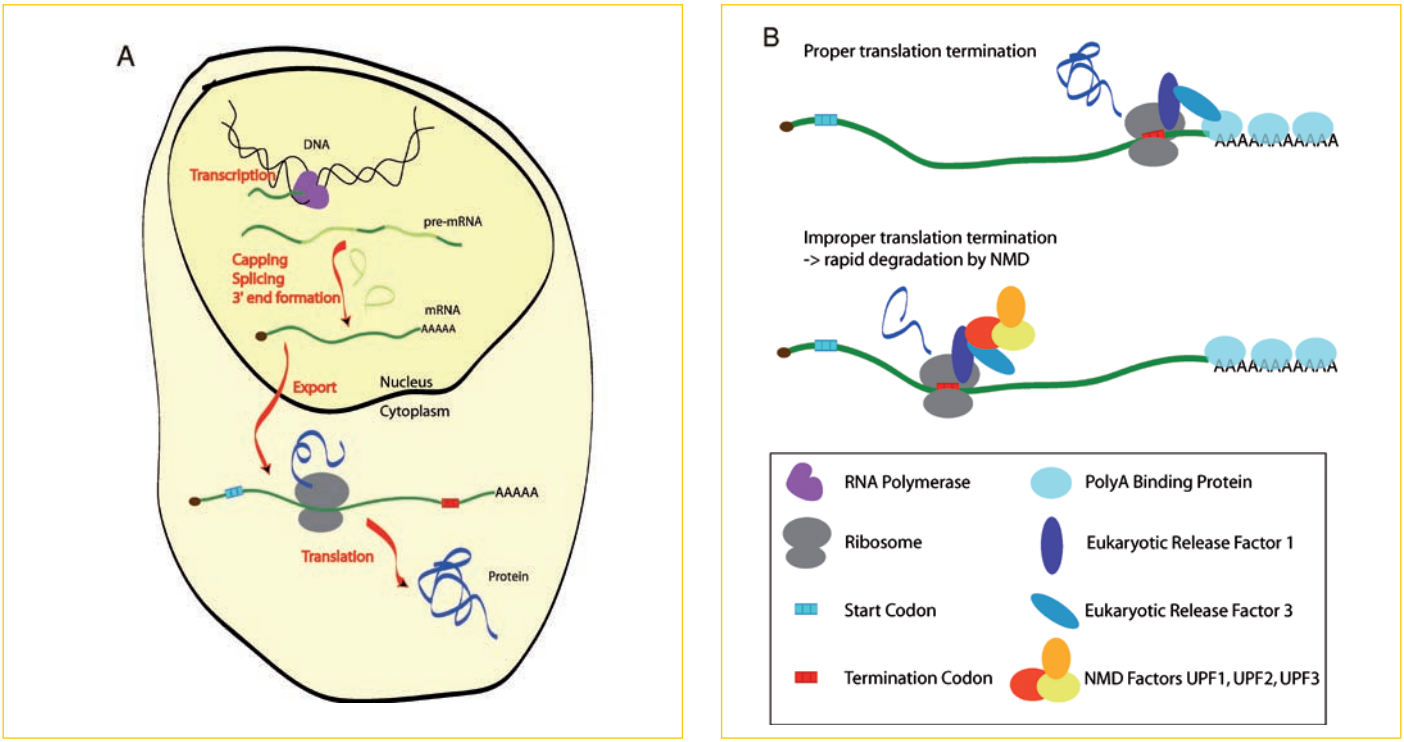

Fig. 1: (A) The general pathway of gene expression. DNA is transcribed into pre-mRNA by RNA polymerase. The pre-mRNA maturates in the cell nucleus: the 5' end is modified (capping), introns are removed by splicing, and the 3' end is cleaved and polyadenylated. The mature mRNA is then exported to the cytoplasm, where it is translated into protein by ribosomes.
(B) Proper versus improper translation termination. When a ribosome stalls at a termination codon, the eukaryotic release factor 1 and 3 bind instead of an aminoacetylated tRNA. Proper/efficient translation termination requires the interaction of the terminating ribosome with the polyA-binding protein. In contrast, our NMD model postulates that failure of the polyA-binding protein to interact with the terminating ribosome is a hallmark of improper/inefficient translation termination and leads to the recruitment of the UPF1-3 factors and to subsequent rapid degradation of the mRNA.

The Mechanism of NMD

NMD is active in all eukaryotes investigated so far, and the three core factors UPF1, UPF2 and UPF3 are conserved from yeast to human. NMD in higher eukaryotes involves additional factors that are not present in yeast. According to the prevailing model for mammalian NMD, a termination codon is identified as premature by its position relative to the last exon-exon junction. This model postulates that transcripts harboring a termination codon more than 50–55 nucleotides upstream of the last exon-exon junction will be subjected to NMD [3]. But recent results suggest that this model needs revision, because a fair number of PTC-containing transcripts violate this rule. In one of those cases that our laboratory investigated, we found that the distance between the termination codon and the poly(A) tail is an important parameter for triggering NMD in human cells, which fits well with data from yeast studies [4, 5]. Therefore, we propose an evolutionary conserved mechanism for PTC recognition, by which a termination codon is recognized as premature, if the ribosome stalls at the termination codon where it fails to interact with factors bound to the poly(A) tail that are necessary for proper translation termination. According to this revised NMD model, the binding of the NMD factors to the mRNA and its subsequent degradation would be the consequence of improper/inefficient translation termination (fig. 1B).

The Role of NMD in Gene Expression

NMD not only rids the cell of mRNAs coding for truncated proteins, but also regulates the expression of ~10% of the transcriptome in yeast, *Drosophila* and human cells [5]. Among the transcripts subjected to NMD in human cells, several groups of genes coding for functionally related proteins were found, including translation factors and ribosomal proteins [1]. These observations suggest that NMD contributes to control whole metabolic pathways by regulating the expression of probably only few mRNAs. NMD is not essential for yeast under laboratory conditions, but the knockout of the NMD factor UPF1 in mouse embryos is lethal, suggesting that NMD might be essential in mammals [6]. Additional to the function in NMD, some NMD factors have been reported to function also in telomere maintenance, transcription and translation regulation, RNA interference, cell proliferation, cell cycle control, cellular transport and organization, and metabolism. NMD may also control noncoding RNAs, which are especially important in higher eukarotes, where a high percentage of the genome is transcribed into noncoding RNAs. These examples demonstrate that in addition to its role as a quality control system, NMD also contributes to the regulation of gene expression.

NMD in Human Diseases

It is estimated that up to 30% of all alleles contributing to genetic diseases in humans harbour a PTC [7]. In these diseases, NMD modulates the phenotype in a beneficial or harmful way. In the beneficial cases, NMD prevents the accumulation of truncated proteins that would otherwise act in a dominant-negative way and disturb cell functions. However, NMD can also cause more severe clinical symptoms by inhibiting the synthesis of truncated proteins that, if expressed, would still be functional. Therefore, the exact phenotype of a disease often depends on the NMD efficiency, which in turn is mainly determined by the location of the PTC, and on the properties of the truncated protein (table 1).

Beneficial Effects of NMD

β-thalassemias represent a well-documented example of a human disease where NMD is beneficial. Patients suffering from β-thalassemias have a nonsense

Table 1: Examples of genetic diseases where NMD modulates the phenotype (adapted from [8])

Gene name	NMD efficiency	Effect of Mutation / Phenotype
NMD beneficial		
β-globin	high	heterozygotes healthy; recessively inherited β-thalassemia major
	low	dominantly inherited β-thalassemia intermedia
Rhodopsin	high	heterozygotes have abnormalities on retinogram, but no clinical disease; recessively inherited blindness
	low	dominantly inherited blindness
Receptor tyrosine kinase -like orphan receptor 2	high	heterozygotes healthy; recessively inherited Robinow syndrome (orodental abnormalities, hypoplastic genitalia, multiple rib/vertebral anomalies)
	low	dominantly inherited brachydactyly type B (shortening of digits and metacarpals)
Cone-rod homeobox	high	mutation found in unaffected heterozygotes (no homozygotes found)
	low	dominantly inherited retinal disease
Coagulation factor X	high	heterozygotes healthy; recessively inherited bleeding tendency
	low	dominantly inherited bleeding tendency
Interferon γ receptor 1	high	heterozygotes healthy; recessively inherited susceptibility to mycobacterial infection
	low	dominantly inherited susceptibility to mycobacterial infection
von Willebrand factor	high	heterozygotes healthy; recessively inherited type 3 von Willebrand disease
	low	dominantly inherited type 2A disease
NMD detrimental		
Dystrophin	high	severe form of muscular dystrophy (Duchenne muscular dystrophy)
	low	milder form of muscular dystrophy (Becker muscular dystrophy)
CFTR (cystic fibrosis)	high	severe cystic fibrosis
	low	milder form of cystic fibrosis

mutation in the β-globin gene. In patients homozygote for the mutation, the amount of β-globin is insufficient and they develop a severe anaemia. In contrast, heterozygote carriers can synthesize enough β-globin from the functional allele and are asymptomatic in most cases. In these individuals, the nonsense transcript of the mutated allele is degraded by NMD, and almost no truncated β-globin protein is produced. A variant of β-thalassemia, where the PTC does not trigger degradation of the mutated mRNA efficiently, demonstrates the importance of NMD: the synthesized truncated β-globin forms toxic inclusion bodies in the cells, which leads to a severe phenotype even in heterozygotic individuals [8].

Harmful Effects of NMD

But there are also several diseases where NMD causes a more severe phenotype, like cystic fibrosis or muscular dystrophy. In these cases, the PTC-containing transcript encodes a truncated protein with some residual function, but NMD leads to a dramatic reduction of these protein levels and thereby worsens the disease phenotype. The severity of dystrophy correlates with the efficiency by which a PTC-containing mRNA is recognized and degraded. If located towards the 3' end of the gene, the transcript is a poor substrate for NMD and these mutations lead to a milder phenotype, called Becker muscular dystrophy. In the more severe form (Duchenne muscular dystrophy), the PTC is located further upstream in the gene, where it triggers more efficient NMD [7, 8].

Clinical Approaches

Different clinical approaches are currently under investigation to treat NMD-related diseases. In the cases where NMD is responsible for worse clinical symptoms, the strategy is to inhibit NMD by reducing the efficient translation termination with antibiotics or suppressor tRNAs, which lead to a read-through at termination codons [9]. Despite of the expected side effects of these approaches, several antibiotics are in clinical trials and a lot of effort is undertaken to find new substances to reduce their toxic side effects in humans. As an alternative, gene therapy approaches are used to repair nonsense mutations already in the genome [9]. And in cases where aberrant splicing produces nonsense mRNAs, gene therapy techniques that correct the splicing pattern are promising strategies.

Concluding Remarks

Until now, the molecular mechanism used by the cell to distinguish PTCs from "normal" termination codons is poorly understood und currently the focus of intensive research in several laboratories. Likewise, we are only beginning to understand the molecular details of the effector pathways leading to mRNA degradation and even less is known about the role of NMD in general regulation of gene expression. On the other hand, because of the documented role of NMD as an important modulator of the clinical manifestations of many genetic diseases, a better understanding of the molecular mechanisms underlying NMD is the key for developing techniques that allow regulation of NMD efficiency of specific transcripts and therewith more specific therapies for NMD related diseases.

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