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Proapoptotic role of novel gene-expression factors

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Abstract The mechanisms that control cellular proliferation, as well as those related with programmed cell death or apoptosis, require precise regulation systems to prevent diseases such as cancer. Events related to cellular proliferation as well as those associated with apoptosis involve the regulation of gene expression carried out by three basic genetic expression regulation mechanisms: transcription, splicing of the primary transcript for mature mRNA formation, and RNA translation, a ribosomal machinery-dependent process for protein synthesis. While development of each one of these processes requires energy for recognition and assembly of a number of molecular complexes, it has been reported that an increased expression of several members of these protein complexes promotes apoptosis in distinct cell types. The question of how these factors interact with other proteins in order to incorporate themselves into the different transduction cascades and stimulate the development of programmed cell death, although nowadays actively studied, is still waiting for a clear-cut answer. This review focuses on the interactions established between different families of transcription, elongation, translation and splicing factors associated to the progression of apoptosis.

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Introduction

Apoptosis is an intrinsic programmed cell death mechanism important in cellular homeostasis, embryonary development, and during the appearance of a series of diseases such as cancer [1, 2]. The mechanism of apoptosis is divided mainly into the following four stages: the first comprises a series of stimuli received by cells that give rise the development of the process; the second involves transduction of signals that act directly on executor actors, i.e., specific proteases known as caspases [3]; third stage is composed of the action of these enzymes promoting cellular disassembly; and finally the fourth and last stage during which apoptotic bodies form and get absorbed through specific cell phagocytic processes [3].

There are two well recognised apoptotic pathways: the cytoplasmatic membrane death receptor pathway and the mitochondrial pathway. Although, both manage independent transduction cascades, they converge at the caspase activation point to accomplish cellular disassembly [4]. Despite the fact that both pathways have been well established, the different stimuli favouring their development have yet to be identified. Although several proapoptotic molecules have been described [5–8], recent developments address the proapoptotic role of an increased expression of several members of the transcription-factor family of proteins related to the translation of diverse proteins as well as to the onset of the splicing event [7, 9–11]. Studies conducted on the overexpression of such proteins suggest they can be integrated into the transduction pathway of apoptosis [7]. Therefore, in this review we emphasise the importance of these proteins as regulators of cell proliferation as well as programmed cell death.

Basal transcription factors

RNA polymerases active in eukaryote cells include RNA polymerase I, II and III (pol I, II, III), which transcribe DNA into rRNA, mRNA and tRNA respectively. During the transcription mechanism, in addition to polymerases, a series of proteins are required to participate in the initiation of the transcription process and denominated transcription factors. These factors recognise DNA cis sites as part of promoters or enhancers [12]. Ribosomal DNA transcription by RNA pol I is required for cell growth due to its role in the synthesis of ribosomal RNA (rRNA) [13], while transcription activity of RNA pol III allows cell cycle progression [14]. This review emphasises the key role of changes in the expression levels of transcription factors type II participating in mRNA synthesis, crucial for cell growth and programmed cell death.

In general, transcription factors that interact with RNA pol II are classified into three groups. The first group corresponds to general factors needed for RNA transcription of all promoters. Upon association with RNA pol II, they form a molecular complex located at the starting point of transcription constituting the basal transcription apparatus. The second group of factors, that correspond to DNA binding proteins, recognise sequences localised upstream of the initiation site. Finally, the third group comprises DNA binding proteins with a regulatory function. The latter are activated or synthesised by specific tissues at specific times, and sequences to which they bind in DNA are known as response elements [12]. Transcription factors that work with RNA pol II have been termed RNA pol II transcription factor X (TFIIX), where X corresponds to the letter that identifies the individual factor. These basal factors bind sequentially until they form the transcription initiation complex. TFIID corresponds to the first factor to bind a sequence upstream of the TATA promoter region and presents two TATA binding proteins (TBP) and TATA recognition proteins called TBP-associated factors (TAFs). After TFIID binding, they sequentially couple the TFIIA and TFIIB factors. During this process, TFIIF binds to RNA pol II and later associates to the transcription complex. Other transcription factors such as TFIIE, TFIIH and TFIIJ are necessary to allow RNA pol II to move along the entire promoter. The TFIIH transcription factor possesses ATPase, helicase and kinase activities, phosphorylating and activating RNA pol II [12]. In addition to the basal transcriptional factors group, other related families whose members favour transcription development, such as the E2F and STAT families, are described next.

E2F and STAT families

The E2F transcription group of factors corresponds to a series of proteins originally discovered in an adenovirus and demonstrated to interact with the E1A transforming protein, thus mediating the E2 viral promoter transcription activity [15]. E2F factors constitute a family of transcription proteins that participate in cell cycle regulation and in mammal cell apoptosis [16] (Table 1). The nucleotide sequence recognised by this factor in the E2 promoter corresponds to TTTCGCGC. Similar sequences have been recognised with other promoters whose functions are important during cell cycle (cyclins and other DNA synthesis-associated genes) [9]. The majority of E2F proteins heterodimerise with DRTF-1 DNA binding protein (DP) polypeptide family members to form active transcription factors [9, 17, 18]. E2F controls transcription of a gene group that codifies for cell cycle G1/S transition event-related function proteins that include the thymidine kinase, α polymerase DNA and dihydrofolate reductase [19]. Nevertheless, despite the fact that eight mammalian E2F family genes have been identified (E2F1–8), the majority of E2F proteins, with the exception of E2F7, heterodimerise with DP1 and DP2, each codified by a sole gene [20, 21].

E2F/DP complexes interconnect with proteins codified by three interrelated genes: *Rb*, *p107* and *p130* [20, 22] (Fig. 1). In general, E2F family members are selected according to their function as transcription-process activators (E2F1, E2F2 and E2F3a) or repressors (E2F3b, E2F4, E2F5, E2F6, E2F7 and E2F8) [20, 23, 24] (Fig. 1). From this list, E2F8, when overexpressed in mouse-embryo primary fibroblasts, decreases cellular proliferation [24]. It is known that E2F1–3 proteins localised in the nucleus principally bind to Rb. E2F-4 and E2F-5 do not present a nuclear localisation signal and preferentially bind to *p107/p130*. It has also been demonstrated that E2F6 suppresses transcription activity of different proteins that belong to the E2F family [16].

E2F proteins actively participate in the transcription process and interact with basal transcription factors such as TBP and the TFIIH transcription factor [25]. Similarly, they participate as mediators for carrying out the interaction between TFIID and TFIIA factors [26]. E2F also presents the capacity to unfold DNA, thus activating transcription [27]. Moreover, as several mechanisms are known where E2F proteins participate in transcription activation, it is necessary to bear in mind that different E2F proteins posses distinct effects on the cell cycle.This situation occurs with EF1-3 inducing the S phase in several cell types [28, 29].

On the other hand, the pocket family of proteins considered as cell cycle regulators is composed of pRB, *p107* and *p130*. These proteins function during the G1-S transition and are associated with regulation of target genes that respond to E2F transcription factors [20] (Fig. 2).

There is evidence that E2F transcription factors are targets for hypophosphorylated Rb, fulfilling its tumour suppressor function by inhibiting E2F and blocking its cell-cycle transcription and progression activator func-

Table 1 Gene expression factors

Expression factors	Cell localisation	Functions in cell cycle	Binding ligand	Proapoptotic function	References
Transcription factors					
E2F family					
E2F1, E2F2, E2F3a	Nucleus	Activators of cell cycle	Rb	E2F1, E2F2, E2F3a, E2F3b	[23, 24, 32, 34, 39, 43–47]
E2F3b, E2F4, E2F5, E2F6, E2F7		Repressors of cell cycle	p107/p130	E2F4, E2F5	[16, 23, 24, 48, 49]
E2F8		Repressor of cell cycle			[24]
STAT family					
Stat 1–4, 5A, 5B, 6	Cytoplasm	Transcription factors 1, Stat 3 and Stat 5 function as cell cycle activators. Cell transformation, inhibition of apoptosis		Stat 1	[12]
Other transcription factors					
TP53 tumour suppressor gene belongs to a family of highly conserved genes that contains at least two other members, P63 and TP73	Nucleus	Growth arrest, maintenance of genome integrity, cellular senescence, DNA repair, inhibition of cell cycle and apoptosis	TP53 participates in transactivation of several proapoptotic target genes as members of bcl-2 family. Activation of extrinsic apoptosis pathway in response to DNA damage	Proapoptotic function	[12, 35, 36]
Rb	Nucleus	Inhibition of cell cycle and apoptosis	E2F1, E2F2, E2F3a, E2F3b	Established proapoptotic function	[12, 20]
p107	Nucleus	Inhibition of cell cycle and apoptosis	E2F4, E2F5	Established proapoptotic function	[12, 20]
p130	Nucleus	Inhibition of cell cycle and apoptosis	E2F4, E2F5	Established proapoptotic function	[12, 20]
Translational factors					
eIF-1A, eIF-3, eIF-4A, eIF-4E, eIF-4F, eIF-4G, eIF-5A	Cytoplasm	Translation initiation factors, cell proliferation, human oncogenes eIF4E, eEF1A2	Assembly complex in the ribosome for initiation of translation		[10, 11, 79–81]
EF-1, EF-2, eEF-1A, eEF-1B	Cytoplasm	Translation elongation factors	Elongation mechanisms of translation	eEF-1A	[82, 83]
eRF-1	Cytoplasm	Translation terminus factors	Terminus mechanism of translation		[85]
Splicing factors					
UAP56, KIAA0801, U5-100, U5-200, KIAA0577, hPrp16, Hrh1, mDEAH9, Prp8	Cytoplasm	Splicing of primary RNA to produce mRNA	Formation of spliceosome complex	Fragment of Prp8	[7, 110]

tion [9, 30, 31] (Fig. 1). Notwithstanding this, in 1998 Kowalik et al. demonstrated that activation of apoptosis carried out through overexpression of factor E2F1 can be due to the fact that this protein acts as a specific signal inducing the apoptotic mechanism and affecting the accumulation of the tumour suppressor protein p53 [32] (Fig. 2). Despite the fact that E2F1 participation in apoptosis induction was thought to be p53-independent, it has been demonstrated that the E2F1 protein promotes p53 phosphorylation [33, 34].

Interestingly, the p53 protein gene was the first gene that, on being expressed, acts as a tumour suppressor. Mutations of this gene have been found in between 50 and 55% of all types of human cancer [12, 35, 36] (Table 1). p53 protein-generated translational pathways can be activated by different stimuli, such as DNA damage and aberrant cell growth signalling [12]. Between these stimuli, environmental pollutants and even medical treatments promote a dose response increase of p53 [37, 38]. p53 target genes are related to cell cycle inhi-

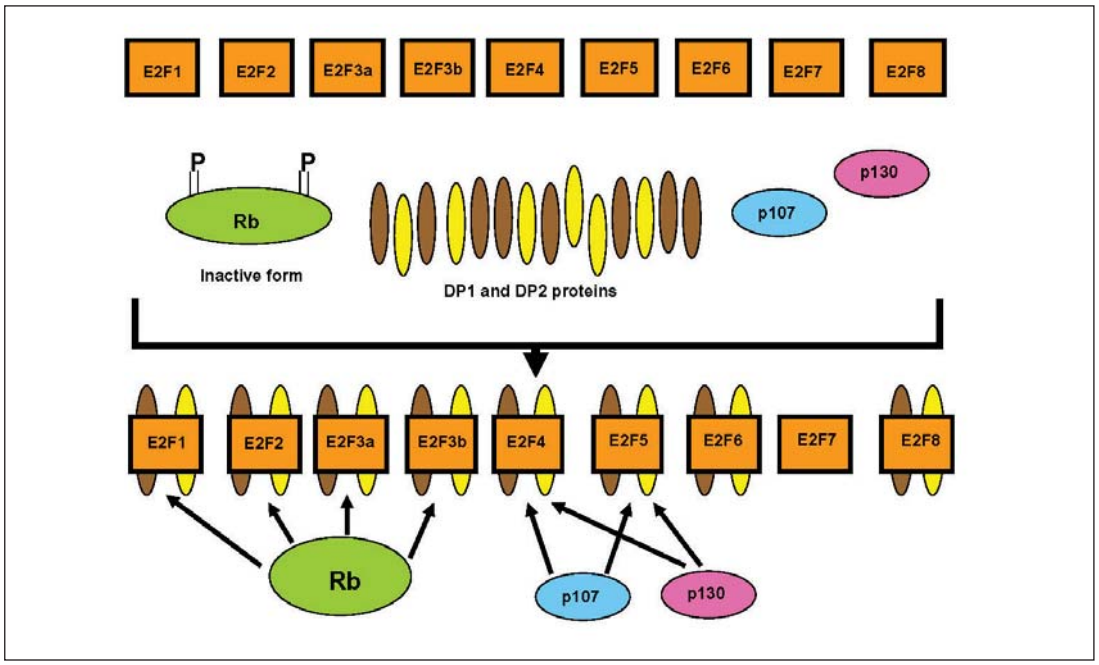

Fig. 1 Molecular interaction between E2F family members and RB, *p107* and *p130* proteins. While E2F proteins heterodimerise with DP1 and DP2 proteins, the E2F7 protein homodimerise. When hypophosphorylated Rb binds to transcription factors, cell cycle progression is arrested. In contrast, the phosphorylated Rb protein releases transcription factors and promotes initiation of the cell cycle.

bition, DNA repair and apoptosis associated to the inhibition of blood vessel formation through transcription regulation of thrombospondin (TSP1) [12].

Several studies have shown that E2F1, E2F2 and E2F4 present specific structural properties, and induce apoptosis in astrocytoma cells [39]. While the E2F1 protein presents structural domains like cyclin A and the pRb binding domain, E2F4 does not possess a cyclin A binding domain and preferentially binds to p107 and p130 [40]. Employing transgenic megakaryocytes, it has

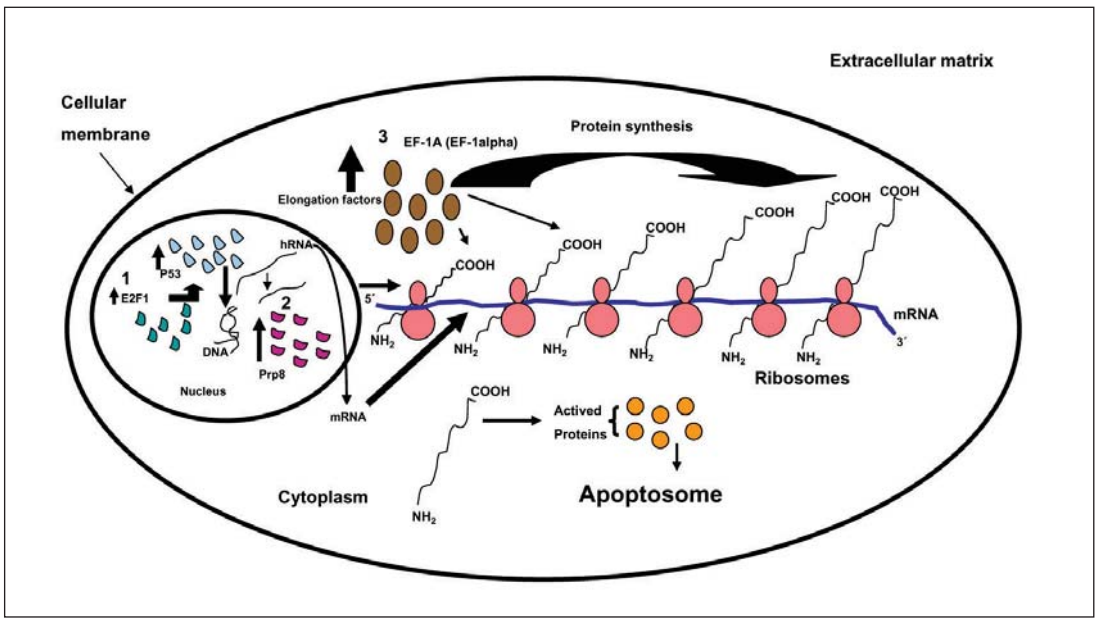

Fig. 2 The increased expression of E2F1, *Prp8* and EF-1A (EF-1alpha) factors as a mechanism to induce apoptosis. (1) transcription, (2) splicing and (3) translation are crucial mechanisms in the regulation of cell physiology and directly related with the progression of the cell cycle and programmed cell death.

been shown that overexpression of the E2F1 transcription factor increases apoptosis [41], while knockout mice for E2F1 slow down the onset of this process [42]. Therefore, it is important to mention the functional variability of diverse E2F family members, for example, E2F1 induces apoptosis in several cell types [32, 34, 43–47] and E2F3 participates in cell proliferation [48, 49], while E2F4 and E2F5 intervene in differentiation and development mechanisms, respectively [50–52].

On the other hand, novel E2F family members have been studied recently; for example, E2F3b codifying for an mRNA unique transcript of the E2F3 locus that presents structural differences with the member now denominated E2F3a [53, 54]. It is known that E2F3a corresponds to a transcription activator, while E2F3b functions as a transcription repressor [55]. E2F6 as well as E2F7 can also be transcription repressors and their primary RNA presents alternative splicing; therefore, they produce several isoforms whose function has yet to be determined [56, 57]. Additionally, the antagonistic behaviour of E2F, another family member, has been questioned. While increased EF2-1 levels induce cell apoptosis, augmented EF2–4 levels provoke apoptosis inhibition in different cell types [58]. Leone and colleagues in 2001 demonstrated that Myc overexpression induced cell death after culture without growth factors, and corresponds to an E2F functional protein-dependent process [59]. Moreover, in 2002 Wells and colleagues found that E2F1-specific target genes possess different functions if compared with target genes for the remainder of the E2F family [60]. Several examples of these target genes include the thioether S-methyltransferase, the hydroxysteroid sulphotransferase and the carboxylesterase, which when altered provoke different responses [60]. In addition, it has been found that the E2F1 protein regulates BH3 proapoptotic protein expression such as PUMA, Noxa, Bim and Hrk/DP5, by means of a direct transcriptional mechanism, demonstrating the connection between E2F and the apoptotic machinery [61]. Adenoviral vector-associated E2F-1 overexpression suppresses *in vitro* and *in vivo* growth of head and neck squamous carcinoma cell lines Tu-138 and Tu-167 through induction of apoptosis [62, 63].

Members of the E2F family show different individual functions in cells, the most generalised being at the S-phase through transcription regulation of genes [64, 65]. It is speculated that the E2F-RB complex can possess a direct function on DNA replication mechanisms [66, 67]. Moreover, it is known that many genes whose function is primordial in mitotic process development correspond to E2F1 transcription-factor target genes [65]. Other E2F protein-regulated genes include those involved in DNA repair mechanisms [68]. Another extremely important characteristic contributing to cellular homeostasis by the E2F family corresponds to the induction into programmed cell death by means of the activation of some of its members after DNA damage or through overexpression of E2F1 [47, 67, 69] (Fig. 2). Apoptosis induction properties of E2F2 and E2F3 can be achieved through p53-dependent as well as independent apoptotic pathways, where in the latter another family member such as p73 must participate [70, 71].

E2F1/DP1 induces activation of other essential and common components of the different apoptotic pathways, such as caspases, Apaf1, and several proapoptotic and antiapoptotic family members including bcl-2. It was demonstrated that E2F1 inhibits NFkappaβ-promoted cell survival signals. Likewise, it was found that DP proteins are necessary for activity regulation of several E2F family members essential for the complex to function [72] (Fig. 1). E2F transcription factor activity is deregulated during the development of several cancer types [73], and related to the presence of mutations of the Rb gene, which functions as a ligand [20]. Several reports support this affirmation, for example E2F3 overexpression in gall bladder cancer [74]. E2F1 overexpression employing a Sk-MEL-2 melanoma cell line affects the expression of a broad range of genes, including transcription factors, oncogenes and cell cycle regulation-associated genes, all apparently related to apoptosis [75].

The family of proteins known as STAT correspond to a group of cytoplasmatic proteins that participate in the normal cellular response to cytokines and growth factors including EGF and PDFG (Table 1). There are seven members identified in mammals so far (Stat 1, 2, 3, 4, 5A, 5B and 6) [12]. Activation of STAT proteins mediates the expression of genes that control diverse cell processes such as development, differentiation, proliferation, inflammation and apoptosis [12].

In normal cells, ligand-dependent STAT activation is a transitory process, differing from many tumour cell lines in which STAT proteins (1, 3 and 5) are activated or phosphorylated for prolonged periods. Stat 1 importantly functions in growth arrest, as well as in the promotion of apoptosis, which is why it was proposed as a tumour growth suppressor. Nonetheless, Stats 3 and 5 possess antagonistic functions in cell cycle promotion, cell transformation and inhibition of apoptosis [12]. Different oncoproteins such as Src and Ab1 activate STAT family members, as in the case of Stats 3 and 5 [12]. In addition to the protein groups already described, it is important to mention other protein groups with functions indirectly related to the activation of the apoptotic process.

Translational factors and protein synthesis

The mechanism of protein translation is carried out in the ribosomes from mRNA transcribed in the nucleus. Protein synthesis consists of three stages: initiation, elongation and termination, where the initiation stage is complex and consists of ribosome dissociation, tRNA

initiator and mRNA binding to the 40 S subunit [76-78]. This process requires different factors, initially termed as initiation factors (IFs), among which are found eukaryote protein-synthesis initiation factors (eIF) eIF-1A, eIF-3, eIF-4A, eIF-4E, eIF-4F, eIF-4G and eIF-5 [10, 79–81] (Table 1). Initiation factors associated to protein synthesis permit the 60 S subunit associations for complete ribosome reconstitution. Then, the second or elongation phase begins and requires, as did the initiation phase, factors known as elongation factors EF1 and EF2 [82]. Factors involved in amino acyl-tRNA incorporation-related elongation factors include eEF1A (formerly known as EF-1α) and eEF1B, while ribosome translocation is taken over by eEF2 [83]. eEF1A, presenting guanine nucleotide binding sites, interacts with amino acyl-tRNA and is transported to site A in the ribosome [84]. A liberation factor known as eRF-1 participates during the termination phase [85].

mRNAs possess different stabilities within a cell with a half-life that fluctuates approximately between 30 min and 15 h. mRNA stability is associated with nucleotide sequences present in the RNA within the 3' untranslated (3' UTR) region that promotes rapid deanylation of the 3' polyadenylated tail. The 5' extreme cap is eliminated sequentially, thus, mRNA degrades in the 5'–3' direction. The most labile mRNAs contain one or more AU-rich sequences in this region [86, 87]. However, approximately 3–5% of mRNAs are translated by a cap-independent mechanism containing an internal ribosome entry site (IRES) in 5'UTR [88, 89]. Many mRNAs that contain IRESs codify for proteins that participate in cell growth, proliferation, differentiation and apoptosis regulation processes [90]. Several studies have shown that overexpression of eIF-4E promotes a proliferation increase and the neoplastic transformation of specific tissues [91] (Table 1). It has been demonstrated that eIF-4E can be overexpressed in head and neck cancer cells [92], in HeLa cells [93], as well as colon, lung and breast cancers [92, 94]. It has been reported that among the functions carried out by this eIF-4E translational factor, an increased production of a series of proteins that promote cell growth, such as cyclin D1 and c-Myc, has been described [95, 96]. eIF-4E factor overexpression favours development of metastasis through the expression of proteins involved in this mechanism, such as type IV collagenase [97]. An increased eIF-4E expression also provokes apoptosis inhibition in cMyc-induced cells [98], as well as during cell transformation activation (Table 1). Both functions are dependent on post-translational modifications consisting of a chemical modification of a lysine, the hypusine amino acid [N epsilon-(4-amino-2-hydroxybutyl) lysine] protein precursor [99]. As eIF-4G overexpression causes malignant transformation in NIH3T3 cells, eIF4E and eEF1A2 factors were recognised as human oncogenes [100]. Nevertheless, an increased translational-factor expression is not necessarily related with neoplasia development, as this event is cell-type dependent [94]. Moreover, several reports show translation initiation factors as substrates for caspases. Such is the case with eIF2α, which is phosphorylated and cleft at the carboxy-terminal sequence [101].

On the other hand, GTP-dependent aminoacyl-tRNA binding to ribosomes as well as other functions related with cytoskeleton organisation depend on peptide elongation factors. Serum elimination induced apoptosis in mouse 3T3 fibroblast cells, a phenomenon that seems to be associated to a change in the level of EF-1α [102]. Other studies conducted using H_2O_2-induced apoptosis in rat embryo heart cells demonstrated that this elongation factor increases during the process, and promotes the apoptotic event [102, 103] (Fig. 2). EF-1A (EF-1α) overexpression in haematopoietic IL-3 cells also induces the apoptotic process [102, 103] (Table 1).

Splicing factors

The first transcripts produced by RNA pol II present intercalated sequences that interrupt exons known as introns. While the latter are eliminated by splicing, mature mRNA produced afterwards is transported into the cytoplasm for protein synthesis [104]. To perform the splicing process, the specific conformation of a molecular complex is required. This is composed of small nuclear RNAs, snRNAs U1, U2, U4/U6 and U5 (U, uridine-rich), which are associated with several proteins [105, 106]. The RNA-protein molecular association receives the name of ribonucleoproteic particles (snRNPs), or snurps for those smaller in size [105, 106]. The spliceosome is a molecular complex whose components include specific-function proteins divided mainly into two groups: snRNPs proteins, which are closely associated with snRNAs, and the non-snRNP splicing factors [107]. The former are subdivided into snRNPs and Sm proteins, which congregate around a U-rich RNA sequence [108, 109]. Among the splicing factors whose presence is indispensable for spliceosome assembly, Prp 8 has been considered an important promoting factor for splicing [110].

Our group recently carried out the isolation, characterisation and expression in *Xenopus laevis* oocytes of an apoptosis-regulated protein (ARP2) from androgen-independent, lymph-node prostate cancer cells (LNCaP) [7]. *arp2* cDNA presents 1.3 kb and shows a 98% sequence homology with 18% of the complete sequence of Prp8 splicing factor [110]. mRNA expression of *arp2* in *Xenopus laevis* oocytes produces apoptosis-associated biochemical, morphologic and electrophysiologic changes [7, 111, 112]. The Prp8 cDNA splicing factor contains approximately 7 kb and the codified protein shows the function of snRNA U5 recognition and binds to RNA in the spliceosome [110]. Therefore, it was giv-

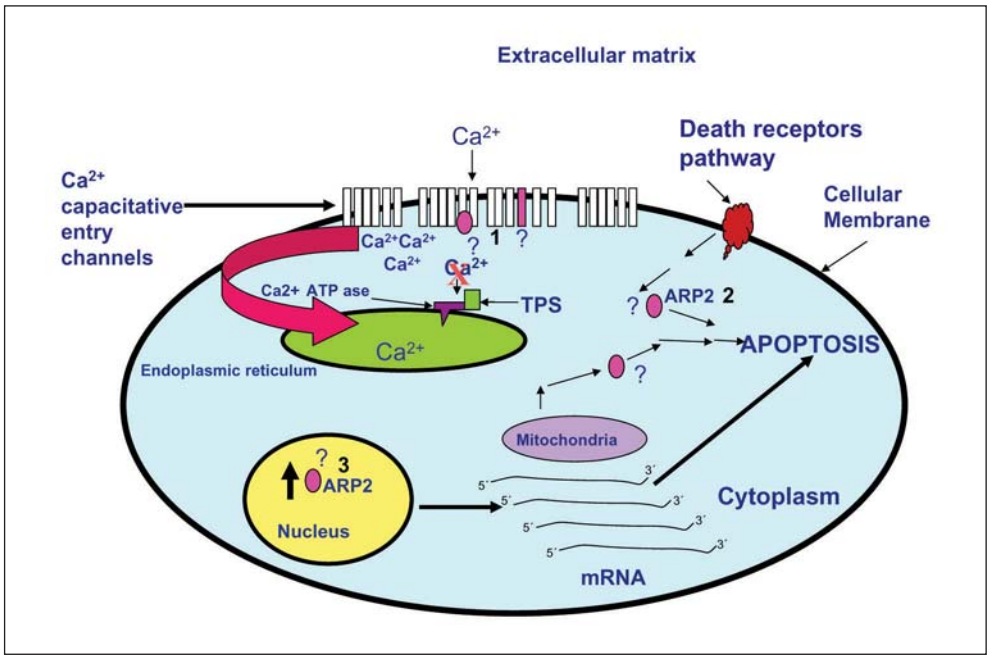

Fig. 3 Model for the possible localisation and function of ARP2 according to our reported data. (1) ARP2 directly related with Ca^{2+} movements through the plasma membrane, as a modulator protein of an intrinsic Ca^{2+} channel or as a Ca^{2+} channel itself. (2) ARP2 as a functional protein active in apoptotic transduction pathways. (3) ARP2 as a functional fragment of *Prp8*-cDNA that participates in splicing mechanisms of proteins related with the apoptotic machinery. Question marks define the fact that the different possible functions for ARP2 initially described and studied by us (7), are being further investigated in order to find out its specific role as a molecule already present and overexpressed at the initiation of apoptosis, or synthesized as an exclusive consequence of this process.

en a central role in RNA catalytic arrangements [108–110]. Interestingly, within the *arp2* cDNA nucleotide sequence, there are flanking sequences that correspond to the htrp3 capacitative calcium entry channel [113], which is why, during *arp2* mRNA expression, calcium entry currents were looked for and found. This report constitutes the first study demonstrating that arp2 mRNA expression in *Xenopus laevis* oocytes promotes the mechanism of apoptosis in these cells [7]. Notwithstanding this, due to *arp2* homology with Prp8 we consider that ARP2 corresponds to a proapoptotic molecule in itself. It is for this reason that we have proposed a functional model for ARP2 (Fig. 3). Recently Prp8 was shown to present specific segments important in spliceosome assembly [114], as well as specific ubiquitin binding domains similar to the ones found in other proteins related to ubiquitation and cell regulation [115]. These observations support our proposal that ARP2 might define a membrane-targeted structural domain with functions different to the ones fundamental in spliceosome assembly.

As genetic expression is a highly regulated cellular function that allows cells to carry out a coordinated and well controlled proliferation, mechanisms integrating this cellular function require inter- and intracellular signals promoting the activation of proteins that in turn give rise to transductional cascades. However, when there is DNA damage, cells send specific signals that are detected and consequently propagated to give notice that this is the decision-making moment for defining the cell's fate; that is, whether to repair the damage or to activate the programmed cell death pathway. This cell strategy defines an efficient way to avoid proliferation of physiologically non-viable cells, therefore impeding the development of diseases such as cancer. Although the main programmed cell death pathways have been established, multiple studies continue to find molecules that are involved in their modulation. The proapoptotic function proposed for several molecules, such as transcriptional, splicing and translation factors, can be associated with the fact they present specific structural domains that allow the association to specific molecules and function as tumour suppressor proteins. How these proteins integrate into signalling cascades is a process that remains under study. Further knowledge of the molecules such as the ones described here and the processes they are involved with, eventually will allow us to understand, and therefore propose control mechanisms to modulate, unchained cell proliferation.

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References

1. Kerr JF, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26:239–257
2. Thompson C (1995) Apoptosis in the pathogenesis and treatment of disease. Science 267:1456–1462
3. Vaux DL, Strasser A (1996) The molecular biology of apoptosis. Proc Natl Acad Sci U S A 93: 2239–2244
4. Guseva NV, Taghiyev AF, Rokhlin OW, Cohen MB (1998) Death receptor-induced cell death in prostate cancer. J Cell Biochem 91:70–99
5. Gutiérrez AA, Arias JM, García L et al (1999) Activation of a Ca2+-permeable cation channel by two different inducers of apoptosis in a human prostatic cancer cell line. J Physiol 517: 95–107
6. Tapia-Vieyra JV, Mas-Oliva J (2001) Apoptosis and cell death channels in prostate cancer. Arch Med Res 32:175–185
7. Tapia-Vieyra JV, Arellano RO, Mas-Oliva J (2005) ARP2 a novel protein involved in apoptosis of LNCaP cells shares a high degree homology with splicing factor Prp8. Mol Cell Biochem 269:189–201
8. Fadeel B, Orrenius S (2005) Apoptosis: a basic biological phenomenon with wide-ranging implications in human disease. J Intern Med 258: 479–517
9. Black AR, Azizkhan-Clifford J (1999) Regulation of E2F: a family of transcription factors involved in proliferation control. Gene 237:281–302
10. Raught B, Gingras AC, Sonenberg N (2000) Regulation of ribosomal recruitment in eukaryotes. In: Sonenberg N, Hershey JWB, Mathews MB (eds) Translational control of gene expression. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, pp 245–293
11. Thornton S, Anand N, Purcell D, Lee J (2003) Not just for housekeeping: protein initiation and elongation factors in cell growth and tumorigenesis. J Mol Med 81:536–548
12. Guasconi V, Yahi H, Ait-Si-Ali S (2002) Transcription factors. Atlas Genet Cytogenet Oncol Haematol
13. Russell J, Zomerdijk JC (2005) RNA-polymerase-I-directed rDNA transcription, life and works. Trends Biochem Sci 30:87–96
14. White RJ (2004) RNA polymerase III transcription and cancer. Oncogene 23:3208–3216
15. Kovesdi I, Reichel R, Nevins JR (1986) Identification of a cellular transcription factor involved in E1A trans-activation. Cell 45:219–228
16. Chae YC, Nakajima H, Illenye S et al (2000) Caspase-dependent apoptosis by ectopic expression of E2F-4. Oncogene 19:4713–4720
17. Chen Q, Hung FC, Fromm L, Overbeek PA (2000) Induction of cell cycle entry and cell death in postmitotic lens fiber cells by overexpression of E2F1 or E2F2. Invest Ophthalmol Vis Sci 41:4223–4231
18. Yamasaki L (1998) Growth regulation by the E2F and DP transcription factor families. Results Probl Cell Differ 22:199–227
19. Nevins JR (1992) E2F: a link between the Rb tumor suppressor protein and viral oncoproteins. Science 258:424–429
20. Cobrinik D (2005) Pocket proteins and cell cycle control. Oncogene 24:2796–2809
21. Ormondroyd E, de la Luna S, La Thangue NB (1995) A new member of the DP family, DP-3, with distinct protein products suggests a regulatory role for alternative splicing in the cell cycle transcription factor DRTF1/E2F. Oncogene 11: 1437–1446
22. Dimova DK, Dyson NJ (2005) The E2F transcriptional network: old acquaintances with new faces. Oncogene 24:2810–2826
23. Blais A, Dynlacht BD (2004) Hitting their targets; an emerging picture of E2F and cell cycle control. Curr Opin Genet Dev 14:527–532
24. Maiti B, Li J, de Bruin A et al (2005) Cloning and characterization of mouse E2F8, a novel mammalian E2F family member capable of blocking cellular proliferation. J Biol Chem 280:18211–18220
25. Emili A, Ingles CJ (1995) Promoter-dependent photocross-linking of the acidic transcriptional activator E2F-1 to the TATA-binding protein. J Biol Chem 270:13674–13680
26. Ross JF, Liu X, Dynlacht BD (1999) Mechanism of transcriptional repression of E2F by the retinoblastoma tumor suppressor protein. Mol Cell 3:195–205
27. Huber HE, Goodhart PJ, Huang PS (1994) Retinoblastoma protein reverses DNA bending by transcription factor E2F. J Biol Chem 269: 6999–7005
28. Lukas J, Bartkova J, Bartek J (1996) Convergence of mitogenic signalling cascades from diverse classes of receptors at the cyclin D-cyclin-dependent kinase-pRb-controlled G1 checkpoint. Mol Cell Biol 16:6917–6925
29. DeGregori J, Leone, G, Miron A et al (1997) Distinct roles for E2F proteins in cell growth control and apoptosis. Proc Natl Acad Sci USA 94:7245–7250
30. Cress WD, Johnson DG, Nevins JR (1993) A genetic analysis of the E2F1 gene distinguishes regulation by Rb, p107, and adenovirus E4. Mol Cell Biol 13:6314–6325
31. Muller H, Bracken AP, Vernell R et al (2001) E2Fs regulate the expression of genes involved in differentiation, development, proliferation, and apoptosis. Genes Dev 15:267–285
32. Kowalik TF, DeGregory J, Leone G et al (1998) E2F1-specific induction of apoptosis and p53 accumulation, which is blocked by Mdm2. Cell Growth Differ 9:113–118
33. Rogoff HA, Pickering MT, Debatis ME et al (2002) E2F1 induces phosphorylation of p53 that is coincident with p53 accumulation and apoptosis. Mol Cell Biol 22:5308–5318
34. Qin XQ, Livingston DM, Kaelin WG Jr, Adams PD (1994) Deregulated transcription factor E2F-1 expression leads to S-phase entry and p53-mediated apoptosis. Proc Natl Acad Sci U S A 91: 10918–10922
35. Crighton D, Ryan KM (2004) Splicing DNA-damage responses to tumour cell death. Biochim Biophys Acta 1705:3–15
36. de Moura Gallo CV, Azevedo E Silva Mendonca G, de Moraes E et al (2005) TP53 mutations as biomarkers for cancer epidemiology in Latin America: current knowledge and perspectives. Mutat Res 589:192–207
37. Salazar AM, Ostrosky-Wegman P, Menéndez D et al (1997) Induction of p53 protein expression by sodium arsenite. Mutat Res 381:259–265
38. Menendez D, Bendesky A, Rojas E et al (2002) Role of P53 functionality in the genotoxicity of metronidazole and its hydroxy metabolite. Mutat Res 501:57–67
39. Dirks PB, Rutka JT, Hubbard SL et al (1998) The E2F-family proteins induce distinct cell cycle regulatory factors in p16-arrested, U343 astrocytoma cells. Oncogene 17:867–876
40. Dyson N (1998) The regulation of E2F by pRB-family proteins. Genes Dev 12:2245–2262
41. Guy CT, Zhou W, Kaufman S, Robinson MO (1996) E2F-1 blocks terminal differentiation and causes proliferation in transgenic megakaryocytes. Mol Cell Biol 16:685–693
42. Field SJ, Tsai FY, Kuo F et al (1996) E2F-functions in mice to promote apoptosis and suppress proliferation. Cell 85:549–561
43. Kowalik TF, DeGregory J, Schwarz JK, Nevins JR (1995) E2F1 overexpression in quiescent fibroblasts leads to induction of cellular DNA synthesis and apoptosis. J Virol 69:2491–2500
44. Shan B, Lee WH (1994) Deregulated expression of E2F1 induces S-phase entry and leads to apoptosis. Mol Cell Biol 14:8166–8173
45. Wu X, Levine AJ (1994) p53 and E2F-1 cooperate to mediate apoptosis. Proc Natl Acad Sci U S A 91:3602–3606
46. Hallstrom TC, Nevins JR (2003) Specificity in the activation and control of transcription factor E2F-dependent apoptosis. Proc Natl Acad Sci U S A 100:10848–10853
47. Rogoff HA, Pickering MT, Frame FM et al (2004) Apoptosis associated with deregulated E2F activity is dependent on E2F1 and Atm/Nbs1/Chk2. Mol Cell Biol 24:2968–2977
48. Humbert PO, Verona R, Trimarchi JM et al (2000) E2f3 is critical for normal cellular proliferation. Genes Dev 14:690–703
49. Leone G, DeGregori J, Yan Z et al (1998) E2F3 activity is regulated during the cell cycle and is required for the induction of S phase. Genes Dev 12:2120–2130
50. Rempel RE, Saenz-Robles MT, Storms R et al (2000) Loss of E2F4 activity leads to abnormal development of multiple cellular lineages. Mol Cell 6:293–306
51. Humbert PO, Rogers C, Ganiatsas S et al (2000) E2F4 is essential for normal erythrocyte maturation and neonatal viability. Mol Cell 6:281–291
52. Lindeman GJ, Dagnino L, Gaubatz S et al (1998) A specific, nonproliferative role for E2F-5 in choroid plexus function revealed by gene targeting. Genes Dev 12:1092–1098
53. He S, Cook BL, Deverman BE et al (2000) E2F is required to prevent inappropriate S-phase entry of mammalian cells. Mol Cell Biol 20:363–371
54. Leone G, Nuckolls F, Ishida S et al (2000) Identification of a novel E2F3 product suggest a mechanism for determining specificity of repression by Rb proteins. Mol Cell Biol 20:3626–3632
55. Aslanian A, Iaquinta PJ, Verona R, Lees JA (2004) Repression of the Arf tumor suppressor by E2F3 is required for normal cell cycle kinetics. Genes Dev 18:1413–1422
56. Dahme T, Wood J, Livingston DM, Gaubatz S (2002) Two different E2F6 proteins generated by alternative splicing and internal translation initiation. Eur J Biochem 269:5030–5036
57. Di Stefano L, Jensen MR, Helin K (2003) E2F7, a novel E2F featuring DP-independent repression of a subset of E2F-regulated genes. EMBO J 22:6289–6298
58. Crosby ME, Almasan A (2004) Opposing roles of E2Fs in cell proliferation and death. Cancer Biol Ther 3:1208–1211
59. Leone G, Sears R, Huang E et al (2001) Myc requires distinct E2F activities to induce S phase and apoptosis. Mol Cell 8:105–113
60. Wells J, Graveel CR, Bartley SM et al (2002) The identification of E2F1-specific target genes. Proc Natl Acad Sci U S A 99:3890–3895
61. Hershko T, Ginsberg D (2004) Up-regulation of Bcl-2 homology 3 (BH3)-only proteins by E2F1 mediates apoptosis. J Biol Chem 279:8627–8634
62. Lavia P, Jansen-Durr P (1999) E2F target genes and cell-cycle checkpoint control. Bioessays 21: 221–230
63. Liu TJ, Wang M, Breau RL et al (1999) Apoptosis induction by E2F-1 via adenoviral-mediat-

ed gene transfer results in growth suppression of head and neck squamous cell carcinoma cell lines. Cancer Gene Ther 6:163–171
64. Trimarchi JM, Lees JA (2002) Sibling rivalry in the E2F family. Nat Rev Mol Cell Biol 3:11–20
65. DeGregori J (2002) The genetics of the E2F family of transcription factors: shared functions and unique roles. Biochim Biophys Acta 1602: 131–150
66. Kennedy BK, Barbie DA, Classon M et al (2000) Nuclear organization of DNA replication in primary mammalian cells. Genes Dev 14: 2855–2868
67. Angus SP, Mayhew CN, Solomon DA et al (2004) RB reversible inhibits DNA replication via two temporally distinct mechanisms. Mol Cell Biol 24:5404–5420
68. Dimova DK, Stevaux O, Frolov MV, Dyson NJ (2003) Cell cycle-dependent and cell cycle-independent control of transcription by the Drosophila E2F/RB pathway. Genes Dev 17:2308– 2320
69. Du W, Xie JE, Dyson N (1996) Ectopic expression of dE2F and dDP induces cell proliferation and death in the Drosophila eye. EMBO J 15: 3684–3692
70. Ziebold U, Reza T, Caron A, Lees JA (2001) E2F3 contributes both to the inappropriate proliferation and to the apoptosis arising in Rb mutant embryos. Genes Dev 15:386–391
71. Stiewe T, Putzer BM (2000) Role of the p53-homologue p73 in E2F1-induced apoptosis. Nat Genet 26:391–392
72. Hitchens MR, Robbins PD (2003) The role of the transcription factor DP in apoptosis. Apoptosis 8:461–468
73. Ebihara Y, Miyamoto M, Shichinohe T et al (2004) Over-expression of E2F-1 in esophageal squamous cell carcinoma correlates with tumor progression. Dis Esophagus 17:150–154
74. Oeggerli M, Tomovska S, Schraml P et al (2004) E2F3 amplification and overexpression is associated with invasive tumor growth and rapid tumor cell proliferation in urinary bladder cancer. Oncogene 23:5616–5623
75. Jamshidi-Parsian A, Dong Y, Zheng X et al (2005) Gene expression profiling of E2F-1 induced apoptosis. Gene 344:67–77
76. Safer B (1989) Nomenclature of initiation, elongation and termination factors for translation in eukaryotes. Eur J Biochem 186:1–3
77. Hershey JWB (1989) Protein phosphorylation controls translation rates. J Biol Chem 264: 20823–20826
78. Caraglia M, Budillon A, Vitale G et al (2000) Modulation of molecular mechanisms involved in protein synthesis machinery as a new tool for the control of cell proliferation. Eur J Biochem 267:3919–3936
79. Gaspar NJ, Kinzy TG, Scherer BJ et al (1994) Translation initiation factor eIF-2. Cloning and expression of the human cDNA encoding the gamma-subunit. J Biol Chem 269:3415–3422
80. Price N, Proud C (1994) The guanine nucleotide-exchange factor, eIF-2B. Biochimie 76:748–760
81. Pain VM (1996) Initiation of protein synthesis in eukaryotic cells. Eur Biochem 236:747–771
82. Clark BFC, Thirup S, Kjeldgaard M, Nyborg J (1999) Structural information for explaining the molecular mechanism of protein biosynthesis. FEBS Lett 452:41–46
83. Browne GJ, Proud CG (2002) Regulation of peptide-chain elongation in mammalian cells. Eur J Biochem 269:5360–5368
84. Merrick WC, Nyborg J (2000) The protein biosynthesis elongation cycle. In: Sonnenberg N, Hershey JWB, Mathews MB (eds) Translational control of gene expression. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, pp 89–125
85. Frolova L, Le Goff X, Rasmussen HH et al (1994) A highly conserved eukaryotic protein family possessing properties of polypeptide chain release factor. Nature 372:701–703
86. Chen CY, You Y, Shyu AB (1992) Two cellular proteins bind specifically to a purine rich sequence necessary for the destabilization function of a c-fos protein-coding region determinant of mRNA instability. Mol Cell Biol 12:5748–5757
87. Chen CY, Chen TM, Shyu AB (1994) Interplay of two functionally and structurally distinct domains of the c-fos AU rich element specifies its mRNA-destabilizing function. Mol Cell Biol 14:416–426
88. Johannes G, Carter MS, Eisen MB et al (1999) Identification of eukaryotic mRNAs that are translated at reduced cap binding complex eIF4F concentrations using a cDNA microarray. Proc Natl Acad Sci U S A 96:13118–13123
89. Hellen, CU, Sarnow P (2001) Internal ribosome entry sites in eukaryotic mRNA molecules. Genes Dev 15:1593–1612
90. Holcik M, Sonenberg N (2005) Translational control in stress and apoptosis. Mol Cell Biol 6: 318–327
91. De Benedetti A, Harris AL (1999) eIF4E expression in tumors: is possible role in progression of malignancies. Int J Biochem Cell Biol 31:59–72
92. Sorrells DL Jr, Ghali GE, De Benedetti A et al (1999) Progressive amplification and overexpression of the eukaryotic initiation factor 4E gene in different zones of head and neck cancers. J Oral Maxillofac Surg 57:294–299
93. De Benedetti A, Rhoads RE (1990) Overexpression of eukaryotic protein synthesis initiation factor 4E in HeLa cells results in aberrant growth and morphology. Proc Natl Acad Sci U S A 87:8212–8216
94. Rosenwald IB (2004) The role of translation in neoplastic transformation from a pathologist's point of view. Oncogene 23:3230–3247
95. Rosenwald IB, Kaspar R, Rousseau D et al (1995) Eukaryotic translation initiation factor 4E regulates expression of cyclin D1 at transcriptional and post-transcriptional levels. J Biol Chem 270:21176–21180
96. Zimmer SG, DeBenedetti A, Graff JR (2000) Translational control of malignancy: the mRNA cap-binding protein, eIF-4E, as a central regulator of tumor formation, growth, invasion and metastasis. Anticancer Res 20:1343–1351
97. Graff JR, Zimmer SG (2003) Translational control and metastatic progression: enhanced activity of the mRNA cap-binding protein eIF-4E selectively enhances translation of metastasis-related mRNAs. Clin Exp Metastasis 20:265–273
98. Polunovsky VA, Gingras AC, Sonenberg N et al (2000) Translational control of the antiapoptotic function of Ras. J Biol Chem 275:24776–24780
99. Caraglia M, Tagliaferri P, Budillon A, Abbruzzese A (1999) Post-translational modifications of eukaryotic initiation factor-5A (eIF-5A) as a new target for anti-cancer therapy. Adv Exp Med Biol 472:187–198
100. Fukuchi-Shimogori T, Ishii I, Kashiwagi K et al (1997) Malignant transformation by overproduction of translation initiation factor eIF4G. Cancer Res 57:5041–5044
101. Hershey JW (1991) Translational control in mammalian cells. Annu Rev Biochem 60:717–755
102. Duttaroy A, Bourbeau D, Wang XL, Wang E (1998) Apoptosis rate can be accelerated or decelerated by overexpression or reduction of the level of elongation factor-1 alpha. Exp Cell Res 238:168–176
103. Talapatra S, Wagner JDO, Thompson CB (2002) Elongation factor-1 alpha is a selective regulator of growth factor withdrawal and ER stress-induced apoptosis. Cell Death Differ 9:856–861
104. Sharp PA (1994) Split genes and RNA splicing. Cell 77:805–815
105. Luhrmann R, Kastner B, Bach M (1990) Structure of spliceosomal snRNPs and their role in pre-mRNA splicing. Biochim Biophys Acta 1087:265–292
106. Will CL, Lurhmann R (1997) Protein functions in pre-mRNA splicing. Curr Opin Cell Biol 9: 320–328
107. Jiménez Garcia E, Tapia-Vieyra JV, Mas-Oliva J (2004) El espliceosoma: Corte y empalme del pre-ARNm. Revista de Educación Bioquímica 23:59–63
108. Staley JP, Guthrie C (1998) Mechanical devices of the spliceosome: motors, clocks, springs, and things. Cell 192:315–326
109. Kastner B, Bach M, Luhrmann R (1991) Electron microscopy of U4/U6 snRNP reveals a Y-shaped U4 and U6 RNA containing domain protruding from the U4 core RNP. J Cell Biol 112:1065–1072
110. Luo HR, Moreau GA, Levin N, Moore MJ (1999) The human Prp8 protein is a component of both U2 and U12 dependent spliceosomes. RNA 5:893–908
111. Bhuyan A, Varshney A, Mathew MK (2001) Resting membrane potential as a marker of apoptosis: studies of Xenopus laevis microinjected with cytochrome c. Cell Death Differ 8: 63–69
112. Braun T, Dar S, Vorobiov D et al (2003) Expression of Bcl-xs in Xenopus oocytes induces BH3-dependent and caspase-dependent cytochrome c release and apoptosis. Mol Cancer Res 1:186–194
113. Zhu X, Jiang M, Peyton M et al (1996) trp, a novel mammalian gene family essential for agonist-activated capacitative Ca2+ entry. Cell 85: 661–671
114. Turner IA, Norman CM, Churcher MJ, Newman AJ (2006) Dissection of Prp8 protein defines multiple interactions with crucial RNA sequences in the catalytic core of the spliceosome. RNA 12:375–386
115. Bellare P, Kutach AK, Rines AK et al (2006) Ubiquitin binding by a variant Jab 1/MPN domain in the essential pre-mRNA splicing factor Prp8p. RNA 12:292–302