

# Involvement of BCL2 family members in the regulation of human oocyte and early embryo survival and death: gene expression and beyond

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## Abstract

In women, up to 99.9% of the oocyte stockpile formed during fetal life is decimated by apoptosis. Apoptotic features are also detected in human preimplantation embryos both *in vivo* and *in vitro*. Despite the important consequences of cell death processes to oocyte competence and early embryonic development, little is known about its genetic and molecular control. B cell lymphoma-2 (BCL2) family proteins are major regulators of cell death and survival. Here, we present a literature review on BCL2 family expression and protein distribution in human and animal oocytes and early embryos. Most of the studies focused on the expression of two antagonistic members: the founding and survival family member BCL2 and its proapoptotic homolog BAX. However, recent transcriptomic analyses have identified novel candidate genes related to oocyte and/or early embryonic viability (such as *BCL2L10*) or commitment to apoptosis (e.g. *BIK*). Interestingly, some BCL2 proteins appear to be differentially distributed at the subcellular level during oocyte maturation and early embryonic development, a process probably linked to the functional compartmentalization of the ooplasm and blastomere. Assessment of BCL2 family involvement in regulating the survival of human oocytes and embryos may be of particular value for diagnosis and assisted reproductive technology. We suggest that implications of not only aberrant gene expression but also abnormal subcellular protein redistribution should be established in pathological conditions resulting in infertility.

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## Introduction

In humans, it has been estimated that more than two-thirds of the female germ cells present in the fetal ovary degenerate by apoptosis soon after their formation. During postnatal life, the oocyte stockpile continues to be depleted until exhaustion at the moment of menopause (for reviews, see Morita & Tilly (1999) and Haouzi & Hamamah (2009)). Moreover, if the few ovulated oocytes (300–400 during a woman's reproductive life) are not fertilized on time, they will succumb to apoptotic death (Morita & Tilly 1999). Apoptosis was also suggested to be involved in some cases of early embryonic arrest and/or fragmentation (Jurisicova & Acton 2004, Haouzi & Hamamah 2009). Indeed, classical apoptotic features have been observed in

human and other mammalian preimplantation embryos both *in vivo* and *in vitro* (Jurisicova *et al.* 1996, 2003, Levy *et al.* 1998a). Although the apoptotic process seems to be of crucial importance for oocyte survival and early embryonic development, little is known about its genetic control in these tissues.

B cell lymphoma-2 (BCL2) family proteins are key regulators of the apoptotic process. They are characterized by the presence of one or more conserved domains called BH (for 'BCL2 homology') domains (BH1–4). In humans, 21 members of this family have been identified (Aouacheria *et al.* 2005) and classified into three subgroups: i) multidomain antiapoptotic (e.g. BCL2, BCL2L1L (BCL-XL), and BCL2L10), ii) multidomain proapoptotic (e.g. BAX, BAK, and BOK) and iii) BH3-only proapoptotic members (e.g. BID, BAD,

and BIM) (for review, see Youle & Strasser (2008)). These proteins exert their function at the mitochondrial level by regulating the permeability of the outer mitochondrial membrane. Incidentally, most of the BCL2 family proteins possess a transmembrane (TM) C-terminal domain, allowing their anchorage to mitochondrial membranes as well as to the membranes of other cellular organelles, such as the endoplasmic reticulum (ER) and nuclear envelope. Several members of the BCL2 family have been found to be expressed in mammalian oocytes and early embryos (Exley *et al.* 1999, Hartley *et al.* 2002, Jurisicova *et al.* 2003, Metcalfe *et al.* 2004, Guillemin *et al.* 2009), and some recent reports provide a global view of transcript levels of BCL2 family members (Hamatani *et al.* 2004, Assou *et al.* 2006, 2010, Wood *et al.* 2007, Pan *et al.* 2008, Grondahl *et al.* 2010). Although useful and informative, these expression data are not sufficient to understand the regulatory functions of the associated proteins. On the one hand, it has been demonstrated that protein–protein interactions and post-translational modifications, such as phosphorylation, cleavage and conformational changes, can influence the subcellular localization and/or the function of BCL2 proteins (Youle & Strasser 2008). On the other hand, as the oocyte and, to a lesser extent, embryonic blastomeres are among the biggest cells in the body, this situation probably implies a specific design and use of the apoptotic machinery associated with other tissue-specific peculiarities. Prominent among these are major structural changes occurring in the cytoplasm during oocyte maturation and early embryonic cleavages, leading to a functional compartmentalization of the cell volume. Recent studies have provided evidence that some BCL2 family proteins could be redistributed to different subcellular compartments during these processes (Antczak & Van Blerkom 1999, Metcalfe *et al.* 2004, Perez *et al.* 2005, Guillemin *et al.* 2009).

The aim of this review is to provide an update of BCL2 family expression and subcellular distribution in the oocyte and early embryo. Indeed, past studies focused on BCL2 and BAX expression, but recent transcriptomic analyses have revealed a dynamic expression profile with other BCL2 homologs being expressed differentially during oogenesis and early embryogenesis. The subcellular localization of BCL2 family proteins may also be relevant for their function in cells as large as the oocyte and embryonic blastomeres and are discussed in that respect.

## The oocyte

### Role of BCL2 family members in fetal oocytes

Myeloid cell leukemia-1 (MCL1, MCL1L (long)) is a BCL2 family member that blocks programmed cell death. A selective increase in *MCL1* mRNA expression was detected in the developing human ovary between

the 14th and 18th weeks of gestation (Hartley *et al.* 2002). Notably, the increase of *MCL1* transcripts was accompanied by a concomitant change in the ovarian histological distribution of the MCL1 protein from small germ cells at the periphery of the ovary at 14–16 weeks of gestation to the largest germ cells in the medullar region by 17–18 weeks, as well as in oocytes of newly formed primordial follicles at 21 weeks. The proapoptotic short splice variant MCL1S was also detected as a minor form. This result suggests that the MCL1L/MCL1S ratio could be imbalanced in fetal germ cells undergoing apoptosis. These findings indicate that MCL1 may play important roles in regulating female germ cell survival during fetal life, particularly at the critical step of oocyte association with somatic cells to form primordial follicles.

The *Bcl2l1* gene (also known as *Bcl-X*) also generates long and short transcripts that encode two proteins with pro-survival and proapoptotic activity, respectively. Female mice harboring a hypomorphic allele of *Bcl2l1* gene were born with a severely reduced number of primordial and primary follicles due to extensive germ cell apoptosis during fetal life (Rucker *et al.* 2000; Table 1). These data suggest that the long isoform of BCL2L1 (BCL2L1L) may be a crucial determinant of female germ cell survival during fetal life. Consistent with this protective role, mouse primordial germ cells transfected with a BCL2L1L expression vector showed an increased survival rate when cultured *in vitro* (Watanabe *et al.* 1997).

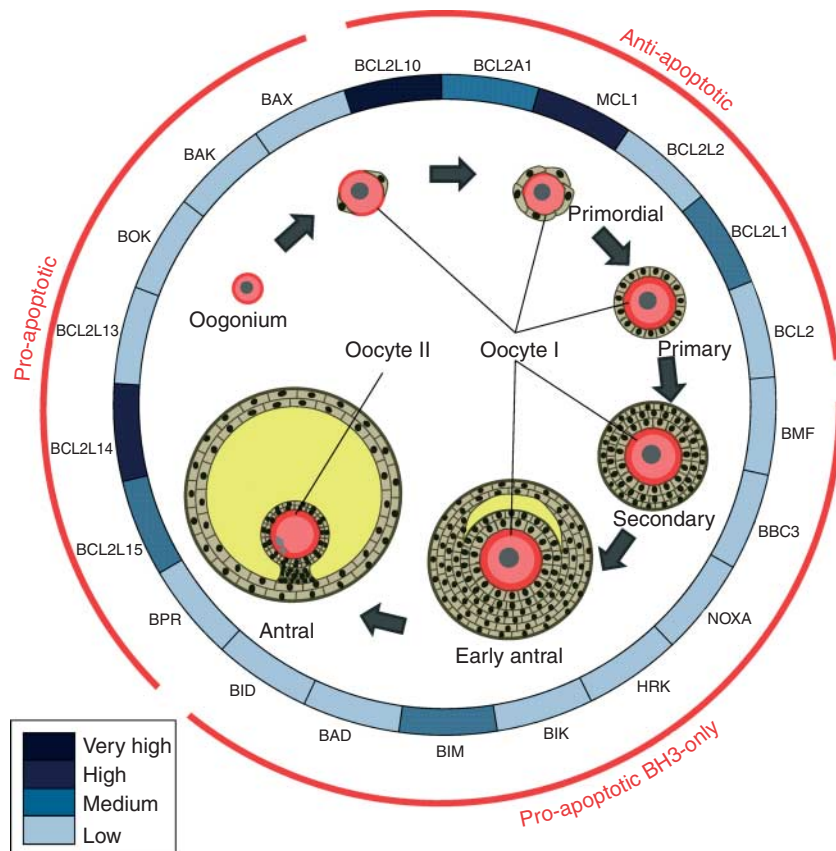
The proapoptotic factor BAX is expressed in human (Hartley *et al.* 2002) and mouse fetal germ cells (Felici *et al.* 1999). The complete deletion of *Bax* alleles from *Bcl2l1* hypomorphic mice has been reported to increase female germ cell numbers to the levels observed in wild-type animals (Rucker *et al.* 2000; Table 1), suggesting that the BCL2L1L/BAX ratio is critical in determining the fate of mouse fetal germ cells. This observation was also confirmed *in vitro*, since annexin V-positive mouse fetal germ cells showed a significantly reduced ratio of BCL2L1L/BAX transcripts compared with annexin V-negative germ cells (Lobascio *et al.* 2007).

### Role of BCL2 family members in adult oocytes

MCL1 mRNA and protein are expressed at high levels in adult human oocytes (Hartley *et al.* 2002, Jurisicova *et al.* 2003, Wood *et al.* 2007, Grondahl *et al.* 2010; Fig. 1). Interestingly, *MCL1* mRNA abundance was positively correlated with oocyte quality in cows (Melka *et al.* 2009). These findings suggest that MCL1 could play important roles in controlling oocyte survival in the adult. Since knocking out *Mcl1* causes early lethality in mice (Rinkenberger *et al.* 2000), a conditional knockout in the female germ line would be of great interest for a better understanding of MCL1-specific functions in female reproduction.

**Table 1** Effects of the knockout/knockdown/inhibition of B cell lymphoma-2 (BCL2) family genes on folliculogenesis and embryonic development. Adapted from Boumela *et al.* (2009) with permission from Elsevier. (Boumela I, Guillemin Y, Guérin JF & Aouacheria A 2009 The Bcl-2 family pathway in gametes and preimplantation embryos. *Gynécologie Obstétrique & Fertilité* 37 720–732. © 2009 Elsevier).

Member	Female fertility	Embryonic development	References	Proposed functions
Anti-apoptotic BCL2	Reduction in the number of primordial follicles	–	Ratts <i>et al.</i> (1995)	Primordial follicle survival
BCL2L1 (BCLX)	–	Lethal at embryonic D14	Motoyama <i>et al.</i> (1995) Rucker <i>et al.</i> (2000)	Germ cell and oocyte survival Primordial follicle survival
BCL2L1 hypomorph	Severe reduction in the number of primordial follicles	–	Riedlinger <i>et al.</i> (2002)	Redundant during post-natal life
Conditional KO of BCL2L1 in follicle components (post-natal)	Did not alter the number of oocytes	–	Print <i>et al.</i> (1998) and Ross <i>et al.</i> (1998)	–
BCL2L2 (BCL-W)	Fertile	–	Rinkenberger <i>et al.</i> (2000)	Germ cell and oocyte survival Early embryo implantation and survival
MCL1	–	Early embryonic lethality due to implantation defects	–	–
A1-A	Fertile	–	Hamasaki <i>et al.</i> (1998)	–
BCL2L10 (Diva/Boo)	Fertile	–	Russell <i>et al.</i> (2002)	–
BCL2L10 knockdown by RNAi in cultured mouse oocytes	Maturation arrest at the MI stage Abnormal spindle and chromosome organization	–	Yoon <i>et al.</i> (2009)	Regulation of nuclear oocyte maturation
BCL2L10 inactivation by micro-injection of specific antibodies in cultured human oocytes	Enhanced oocyte degeneration <i>in vitro</i>	–	Guillemin <i>et al.</i> (2009)	Oocyte survival
Pro-apoptotic BAX	Increase in the number of primordial follicles, elimination of the mouse equivalent of menopause	More resistant to the PAH-induced loss <i>in vivo</i> More resistant to glucose-induced apoptosis <i>in vitro</i>	Knudson <i>et al.</i> (1995), Moley <i>et al.</i> (1998), Perez <i>et al.</i> (1999, 2007), Chi <i>et al.</i> (2000) and Detmar <i>et al.</i> (2006) Lindsten <i>et al.</i> (2000)	Apoptotic degeneration of oocytes and early embryos
BAK	Fertile	–	–	Apoptotic degeneration of early embryos
BH3-only BAD	Fertile	–	Ranger <i>et al.</i> (2003)	–
BID	Fertile	–	Kaufmann <i>et al.</i> (2007)	–
BIM	Fertile	> 40% lethality before embryonic D10	Bouillet <i>et al.</i> (1999) and Coultas <i>et al.</i> (2005)	–
BIK	Fertile	–	Coultas <i>et al.</i> (2005)	–
HRK/DP5	Fertile	–	Coultas <i>et al.</i> (2007)	–
BBC3 (PUMA)	Fertile	–	Villunger <i>et al.</i> (2003)	Apoptotic degeneration of early embryos
NOXA	Fertile	–	Villunger <i>et al.</i> (2003)	–
DKO	–	–	–	–
BAX/BCL2	Reduction in the number of primordial follicles	–	Knudson & Korsmeyer (1997)	–
BAX/BCL2L1 hypomorph	Fertile	–	Rucker <i>et al.</i> (2000)	–



**Figure 1** Expression of BCL2 family members in adult human oocytes. The expression levels of the different members are relative, derived from the synthesis of several studies (Jurisicova *et al.* 2003, Guillemin *et al.* 2009; personal microarray, S Assou, J De Vos & S Hamamah, unpublished observations). The principle stages of human oogenesis/folliculogenesis are presented schematically.

The treatment of adult murine oocytes with the anti-cancer drug doxorubicin resulted in the down-regulation of BCL2L1L transcripts, accompanied by a concomitant switch in favor of its proapoptotic splicing variant BCL2L1S (BCL-XS; Jurisicova *et al.* 2006). Additionally, the microinjection of a recombinant BCL2L1L protein in oocytes was able to inhibit doxorubicine-mediated apoptosis, suggesting that the ratio of BCL2L1L/BCL2L1S may be critical for the oocyte to survive doxorubicin damage. However, although the deletion of the *Bcl2l1* gene within the follicle cellular components (including oocytes and somatic cells) during post-natal life resulted in reduced fertility, it did not alter the number of oocytes as observed in the fetal ovary (Riedlinger *et al.* 2002). This result suggests that other BCL2 family members may take over, at least partially, the function of BCL2L1L in promoting oocyte survival within the post-natal ovary.

One exciting candidate is BCL2L10. Indeed, a number of transcriptomic studies revealed that this antiapoptotic factor is abundantly and specifically expressed in adult human and mouse oocytes (Hamatani *et al.* 2004, Assou *et al.* 2006, 2010, Wood *et al.* 2007, Grondahl *et al.* 2010; Fig. 1). This observation is supported by the recent demonstration that BCL2L10 is the most highly expressed prosurvival factor of the BCL2 family members expressed in oocytes (Guillemin *et al.* 2009) and early

embryos (S Assou, J De Vos & S Hamamah, unpublished observations), showing a strikingly conserved expression pattern among vertebrates from fishes (Arnaud *et al.* 2006) to mammals. The human BCL2L10 protein appears to be a binding partner of proapoptotic BAX (Zhai *et al.* 2008, Guillemin *et al.* 2009), and microinjection of a monoclonal anti-BCL2L10 antibody promoted oocyte degeneration *in vitro* (Guillemin *et al.* 2009), suggesting a protective role to the oocyte and early embryo. Knockdown of mouse BCL2L10 (also known as DIVA/BOO) using RNA interference (RNAi) in cultured oocytes resulted in maturation arrest at the metaphase I (MI) stage, accompanied by abnormal spindle and chromosome organizations (Yoon *et al.* 2009). These findings indicate that, in addition to, or instead of, its antiapoptotic function, BCL2L10 may play other roles related to cell cycle control and oocyte maturation in mice. Notably, phylogenetic analyses indicate substantial divergence for human and murine BCL2L10, raising the possibility that these orthologous proteins may not be functionally and biologically equivalent (Aouacheria *et al.* 2005, Guillemin *et al.* 2009). In that respect, it is noteworthy that *Bcl2l10* knockout mice were fertile with no observable phenotype (Russell *et al.* 2002; Table 1). However, fertility of these mice has not been analyzed in adverse conditions (e.g. food deprivation) or over long term.

Thus, further analyses are needed to determine more definitively if BCL2L10 is dispensable or not for mouse fertility and whether it is involved in human female reproductive function.

The role of BCL2 in regulating oocyte survival has been largely studied since it is the founder of the family, and yet this member appears to be expressed at low levels in human (Guillemin *et al.* 2009; Fig. 1) and mouse oocytes (Exley *et al.* 1999). The ovaries of *Bcl2* knockout mice were shown to contain reduced numbers of primordial follicles (Ratts *et al.* 1995; Table 1). However, this loss is likely due to granulosa cell apoptosis, indirectly impacting follicle formation and oocyte survival. In support of this hypothesis, transgenic mice overexpressing BCL2 in their ovarian somatic cells (granulosa, theca, luteal and stroma cells) showed increased spontaneous ovulation rates and larger litter size when treated with a high dose of pregnant mare serum gonadotropin (Hsu *et al.* 1996). In contrast, the overexpression of BCL2 in growing oocytes during post-natal life did not change the number of follicles, the ovulation rate or the litter size, even though the proportion of atretic preantral follicles was reduced *in vivo* and the oocytes were more resistant to apoptosis induced by doxorubicin *in vitro* (Morita *et al.* 1999). Furthermore, when BCL2 was overexpressed in fetal oocytes, the mice were born with increased numbers of primordial follicles, but this surfeit was not maintained in the adult ovary (Flaws *et al.* 2001).

The proapoptotic member BAX appears to be consistently expressed in human (Jurisicova *et al.* 2003; Fig. 1) and mouse oocytes (Exley *et al.* 1999). Interestingly, the expression levels of its mRNA and protein were increased in oocytes of aged mice (Perez *et al.* 2005). In addition, immunocytochemical analyses showed that BAX protein was activated in human oocytes treated with microtubule-damaging drugs or after prolonged periods of culture (Guillemin *et al.* 2009). Besides, the microinjection of recombinant BAX protein into mouse oocytes was able to trigger apoptotic degeneration by itself (Morita *et al.* 2000). A particularly noteworthy finding is that *Bax*<sup>-/-</sup> mice were born with a threefold larger oocyte stockpile compared with wild-type controls (Perez *et al.* 1999; Table 1). This excess of oocytes was maintained in adult mice, leading to a delay in the onset of the 'mouse equivalent' of menopause. Moreover, oocytes of aged *Bax*<sup>-/-</sup> mice were developmentally competent since they were able to form viable embryos under IVF conditions (Perez *et al.* 1999). The fact that these mice did not become pregnant *in vivo* is likely due to extrinsic causes (endocrinological defects). Indeed, when young wild-type mice were grafted with the ovarian tissue of aged *Bax*<sup>-/-</sup> mice and then mated with fertile *Bax*<sup>+/-</sup> males, they gave birth to viable *Bax*<sup>-/-</sup> pups (these pups being necessarily derived from the *Bax*<sup>-/-</sup> ovarian graft; Perez *et al.* 2007).

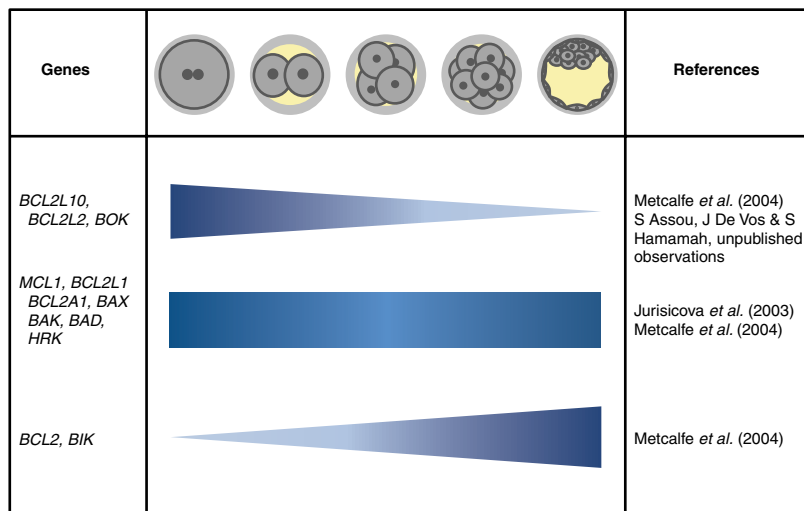
In addition to its role in developmental oocyte loss, many studies reported the involvement of the proapoptotic factor BAX in female germ cell death induced by exogenous stimuli. Indeed, *Bax*-deficient oocytes were more resistant to apoptosis induced by the anti-cancer drug doxorubicin, *in vivo* as well as *in vitro* (Perez *et al.* 1997). Polycyclic aromatic hydrocarbons (PAHs) are toxic chemicals found in air pollution and tobacco smoke, and are known for their adverse effects on female fertility (Mattison & Nightingale 1980). Exposure of female mice to PAHs resulted in BAX mRNA and protein accumulation in their oocytes prior to their elimination. Such loss was suppressed by *Bax* inactivation (Matikainen *et al.* 2001). PAHs act through activation of the aromatic hydrocarbon receptor (AHR), a member of the PER-ARNT-SIM family of transcription factors. The BAX promoter was found to contain two AHR response elements, which are required for PAH-induced BAX transcription (Matikainen *et al.* 2001). All these reports provide evidence for considering BAX as a major mediator of oocyte death.

Lastly, it should be noted that other proapoptotic members such as BCL2L13 (BCL-RAMBO), BCL2L14 (BCL-G) and the BH3-only member BIM (BCL2L11) are also detected in human oocytes (Wood *et al.* 2007, Grondahl *et al.* 2010; Fig. 1). BH3-only proteins initiate apoptotic signaling by sensing cellular stresses. The fact that transcriptomic analyses did not detect the presence of other BH3-only members is intriguing. It is possible that expression of BH3-only members is induced in low-quality oocytes, and is thus not detected in these studies using good-quality ones. For example, one study detected the expression of BAD in oocytes obtained from old mice but not in those obtained from younger mice (Pan *et al.* 2008).

## The preimplantation embryo

### The expression profile of BCL2 family members during early embryonic development

During the early cleavage stages preceding embryonic genome activation (EGA), embryonic functions are largely controlled by maternally inherited mRNAs, proteins and other molecules. *BCL2L10* transcripts, which are the most abundant among the BCL2 family mRNAs in oocytes, were found to decrease gradually after fertilization and then become undetectable at the morula stage in rhesus monkeys, suggesting that this gene is exclusively maternal (Guillemin *et al.* 2009). This finding was confirmed in humans (S Assou, J De Vos & S Hamamah, unpublished observations) and mice (Yoon *et al.* 2009), where *BCL2L10* transcripts decreased rapidly by the eight- and two-cell stages (corresponding to EGA in these species), respectively, and disappeared thereafter, indicating that BCL2L10 could play important roles before and at the moment of



**Figure 2** The expression profile of BCL2 family members during human early embryonic development. Some BCL2 family members are exclusively maternal, expressed before the embryonic genome activation (EGA) and disappear rapidly during early development; others are both maternal and embryonic, expressed throughout early embryonic development, while few others are exclusively embryonic, expressed mainly after the EGA.

fertilization, during the oocyte-to-embryo transition as well as during the very early embryonic stages.

Interestingly, only *Mcl1* inactivation resulted in early embryonic lethality in mice (Rinkenberger *et al.* 2000; Table 1). However, apoptosis was not increased within the *Mcl1*<sup>-/-</sup> embryos. Further analyses showed that the mutant blastocysts were unable to implant, suggesting that MCL1 might be involved in cellular adhesion of the embryonic trophectoderm to the uterine epithelium. In support of this assumption, Boisvert-Adamo *et al.* (2009) reported that MCL1 knockdown using RNAi in melanoma cells induced anoikis, an apoptotic process induced by loss of cell adhesion or inappropriate cell adhesion to the extracellular matrix. Furthermore, MCL1 degradation was correlated with anoikis occurrence in the NIH3T3 cell line (Woods *et al.* 2007).

Like *BCL2L10*, *BCL2L2* and *BOK* mRNAs have been found to disappear rapidly after fertilization in human embryos (Metcalf *et al.* 2004, Li *et al.* 2006; Fig. 2). Other maternal BCL2 family members such as *MCL1*, *BCL2L1*, *BCL2A1* (*BFL1*), *BAX*, *BAK*, *BAD* and *HRK* are also expressed at the moment of EGA and maintained until the blastocyst stage (Jurisicova *et al.* 2003, Metcalf *et al.* 2004; Fig. 2), suggesting that these factors are needed throughout human early embryogenesis. It should be noted that *BOK*, *BAK* and *BAD* transcripts were not assessed in unfertilized oocytes but were detected from the pronucleus stage (Metcalf *et al.* 2004), suggesting a maternal origin. *BCL2* and *BIK* appear to be embryonic genes, their expression being mainly detected after the four- to eight-cell stages in human embryos (Metcalf *et al.* 2004; Fig. 2).

Microarray analysis revealed the up-regulation of two BH3-only members, *BIM* and *BIK*, in human eight-cell embryos (S Assou, J De Vos & S Hamamah, unpublished observations). A high expression of *BIK* was also reported in rhesus monkey embryos from the eight-cell stage until

the blastocyst stage (Guillemin *et al.* 2009). The induction of *BIM* and *BIK* expression at the critical stage of EGA might be highly significant and suggests that these sensor proteins could promote apoptosis in embryos that fail to activate their genome.

#### **Role of BCL2 family members in preimplantation embryo demise**

The proapoptotic member *BAX* was shown to be consistently expressed during preimplantation development in human (Jurisicova *et al.* 2003, Metcalf *et al.* 2004) and mouse (Jurisicova *et al.* 1998) embryos. Notably, no correlation between *BAX* mRNA levels and embryo fragmentation was observed (Jurisicova *et al.* 1998, Liu *et al.* 2000). This observation was as expected, since the *BAX* protein needs to be activated by conformational changes prior to its translocation to mitochondria to initiate actual cell death. On the other hand, the *BAX/BCL2* protein ratio was highly altered in fragmented bovine blastocysts (Yang & Rajamahendran 2002), suggesting that at least, in some cases, translational and post-translational control may be of critical importance. Incubation of murine blastocysts in media supplemented with the PAH agent 7,12-dimethylbenz(a)-anthracene resulted in increased levels of *BAX* mRNA and protein. Moreover, *Bax* knock-out embryos were more resistant to the PAH-induced embryonic loss *in vivo* (Detmar *et al.* 2006). Other studies reported that *BAX*-deficient blastocysts were more resistant to glucose-induced apoptosis *in vitro* (Moley *et al.* 1998, Chi *et al.* 2000). Conversely, wild-type embryos cultured in medium containing a high concentration of glucose or recovered *in vivo* from hyperglycemic diabetic mice were more sensitive to apoptosis and showed elevated levels of *BAX* mRNA and protein (Moley *et al.* 1998, Shen *et al.* 2009). These data appear to designate *BAX*,

once again, as a central mediator of cell death in the mammalian embryo. The transcription of the BAX homolog BAK and the BH3-only member HRK appears to be up-regulated in fragmenting embryos at the four-cell stage (Jurisicova *et al.* 2003, Metcalfe *et al.* 2004), suggesting a potential role for these proapoptotic members in regulating embryonic death and fragmentation in a redundant manner to BAX or upstream of BAX, respectively. Furthermore, an alternative splicing of the *BCL2L1* gene in favor of the short proapoptotic isoform BCL2L1S was observed in a subset of human and mouse fragmented and/or dying embryos (Jurisicova *et al.* 1998, 2003, Perumalsamy *et al.* 2010), suggesting that subtle post-transcriptional modifications could be implemented to regulate embryonic fate. Accordingly, decreasing the BCL2L1L/BCL2L1S ratio in mouse embryos by using an antisense strategy that up-regulates BCL2L1S and concomitantly down-regulates BCL2L1L has severely affected embryonic developmental competence (Perumalsamy *et al.* 2010).

### BCL2 family proteins subcellular localization

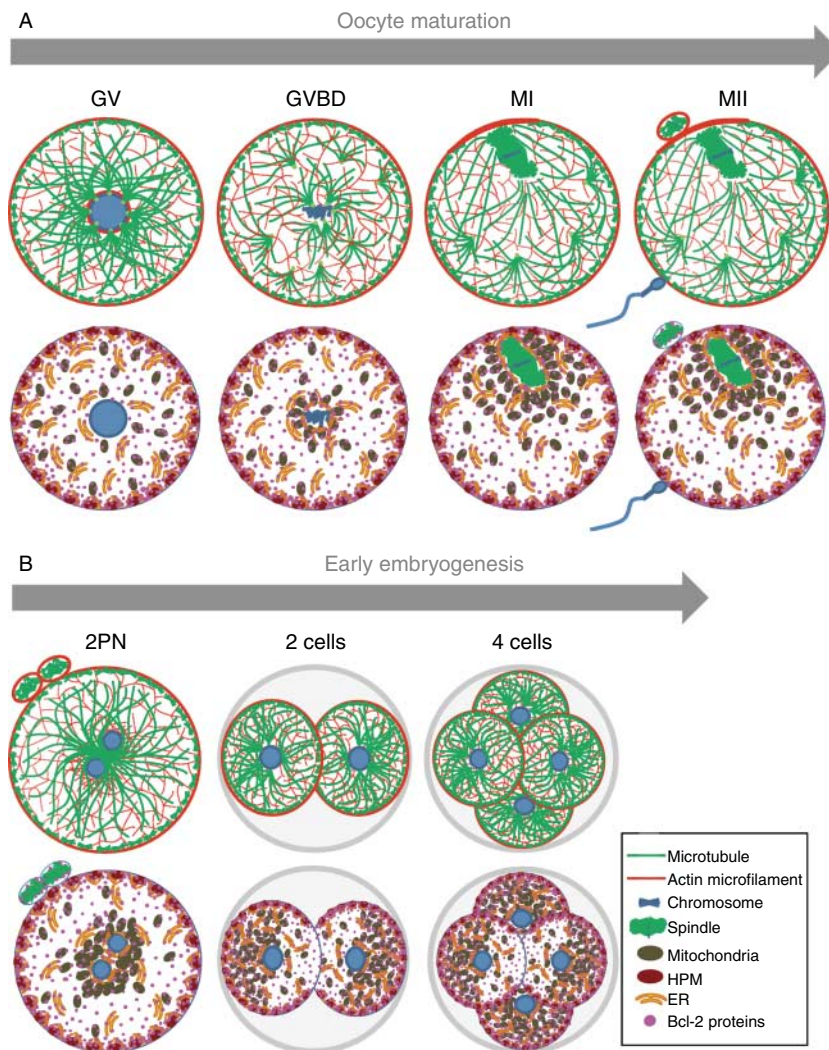
Only a few studies have analyzed the expression of the BCL2 family at the protein level. This shortcoming can be explained by the fact that immunoblotting is a highly material-consuming method, especially in the case of rare samples such as oocytes or preimplantation embryos. The subcellular localization of BCL2 proteins is also poorly studied. Nevertheless, it is well known that subcellular protein distribution is an important aspect, since the function of proteins may be localized to specific areas inside the cell or within cellular organelles. This is likely to be especially true in cells as large as the oocyte or embryonic blastomeres. Thus, in this section, we will present the few data available about BCL2 family proteins' subcellular localization during oocyte maturation and early embryonic development. Redistribution of BCL2 family proteins could be directed by the cytoskeleton network via protein-protein interactions or by binding to organelle membranes (e.g. mitochondria and ER) during the spatial reorganization of the cytoplasm. In this respect, we briefly describe the major cytoskeletal changes as well as mitochondria and ER redistribution during oocyte maturation and early embryonic development and compare these events with BCL2 proteins' localization.

#### *During oocyte maturation*

During oocyte maturation, drastic cytoskeletal and organelle reorganization take place in the ooplasm. In human germinal vesicle oocytes, microtubules and actin microfilaments form a reticular network that is particularly dense at the oocyte cortex and around the nucleus (Kim *et al.* 1998, Veselska & Janisch 2001, Combelles

*et al.* 2002; Fig. 3A). Mitochondria and ER are dispersed in the ooplasm but seem to accumulate preferentially in the cortical region (Motta *et al.* 2000, Liu *et al.* 2010; Fig. 3A). The dynamic redistribution of these organelles is more likely to be regulated by microtubular activity, as suggested in other animal oocytes (Van Blerkom & Bell 1986, Sun *et al.* 2001), but the involvement of microfilaments is not excluded (Baumann & Walz 2001, FitzHarris *et al.* 2007). During oocyte maturation, microtubules are progressively arranged into a symmetrical bipolar spindle and the cortical actin becomes asymmetrical, forming a thicker layer over the spindle (Kim *et al.* 1998, Combelles *et al.* 2002). Although the ooplasm of MI and MII oocytes seems to be devoid of microtubules, treatment with paclitaxel, a microtubule-polymerizing agent, revealed the presence of multiple asters in the ooplasm, particularly concentrated in the subcortical region (Battaglia *et al.* 1996, Combelles *et al.* 2002), suggesting that the unpolymerized pool of tubulin present in this area is in a very dynamic state. Battaglia *et al.* (1996) and others speculated that some of these cortical asters may play a role in mitochondrial relocation into regions requiring high ATP concentrations (e.g. spindle assembly, cortical granule exocytosis during fertilization). Consistent with this hypothesis, ultrastructural analyses revealed that, in this subcortical area, mitochondria form small clusters around ER cisternae (Van Blerkom 2004). These subplasmalemmal mitochondria are characterized by a high TM potential and were proposed to regulate key events during oocyte maturation, fertilization and early embryonic development (Van Blerkom 2009).

BCL2 family proteins have diverse intracellular locations: they can be soluble in the cytosol, attached to the cytoskeleton elements or associated with the membranes of mitochondria and ER (Youle & Strasser 2008). Thus, their localization could be affected by the major reorganization events occurring during oocyte maturation. The immunolocalization analyses of some BCL2 family proteins in human and mouse oocytes reveal a diffuse cytoplasmic distribution pattern. However, some subtle changes have been observed during oocyte maturation. For instance, BCL2L10 was shown to gradually accumulate at the cortical region of human and mouse oocytes, where it co-localized with peripheral mitochondria (Guillemin *et al.* 2009). Disruption of the microtubule network using drugs such as nocodazole (a microtubule depolymerizing agent) or paclitaxel (a microtubule polymerizing agent) was shown to alter BCL2L10 pericortical localization. Intriguingly, other BCL2 family proteins such as MCL1 (Hartley *et al.* 2002), BCL2L1L and BAX (Antczak & Van Blerkom 1999, Perez *et al.* 2005) were also found to concentrate in this subcortical region containing highly polarized mitochondria, raising the possibility of spatially specific regulatory functions (Fig. 3A).



**Figure 3** Intracellular reorganization during human oocyte maturation and early embryonic development. (A) Cytoskeleton (microtubule filaments and actin microfilaments, top panel), organelle (mitochondria and ER) and BCL2 protein (bottom panel) remodeling during oocyte maturation. (B) Cytoskeleton (microtubule filaments and actin microfilaments, top panel), organelle (mitochondria and ER) and BCL2 protein (bottom panel) remodeling during preimplantation embryogenesis (see text for references). HPM, high polarized mitochondria.

### During preimplantation development

The cytoskeleton remodeling and organelle trafficking observed during oocyte maturation resume at the moment of fertilization and continue during early cleavage stages. In humans, immediately after fertilization, a growing microtubule aster is formed in the perinuclear region and spreads throughout the cytoplasm (Fig. 3B). Actin microfilaments still form a cortical ring around the zygote but a dense network is also visible around the pronuclei (Van Blerkom *et al.* 2000, Branzini *et al.* 2007; Fig. 3B). In parallel, mitochondria and ER move and concentrate around the two pronuclei and become more diffuse after syngamy (Sousa *et al.* 1996, Van Blerkom *et al.* 2000; Fig. 3B). In the two-cell embryo, a dense microtubule network is arranged in the apical region of the blastomeres where mitochondria and ER accumulate (Sousa *et al.* 1996, Van Blerkom *et al.* 2000; Fig. 3B). During the four- and eight-cell stages, microtubules form a cytoplasmic network that appears to be less dense in basal regions where blastomeres are

opposed. Although actin microfilaments were detected in the cytoplasm, they still mostly organized into a cortical ring beneath the plasma membrane (Levy *et al.* 1998b). Both mitochondria and ER are still mainly localized to the apical region of blastomeres, correlating with microtubule organization (Sousa *et al.* 1996, Van Blerkom *et al.* 2000). Noteworthy, during embryonic cleavage stages, highly polarized mitochondria were shown to be inherited in each blastomere and to conserve their subplasmalemmal localization at the apical surface (Van Blerkom 2009).

Immunocytochemical analyses of BCL2, BCL2L1L, BCL2L2, MCL1, BAX, BAD, and HRK proteins showed a diffuse distribution in the cytoplasm of early human embryos (Antczak & Van Blerkom 1999, Jurisicova *et al.* 2003, Metcalfe *et al.* 2004). However, intriguingly, among these proteins, BCL2L1L, BCL2, BAX, and BAD appear to accumulate preferentially at the periphery of blastomeres, where the highly polarized mitochondria are located (Antczak & Van Blerkom 1999, Metcalfe *et al.* 2004; Fig. 3B). This observation is reminiscent of



what was described in the oocyte cortex, which could be indicative of a conserved functional localization of BCL2 family proteins. Nevertheless, co-immunolabeling studies are still needed to determine precisely whether this subplasmalemmal pool within the blastomeres colocalizes with highly polarized peripheral mitochondria. Curiously, the immunostaining of BAX protein was also detected in the nuclei of early-cleaving embryos (Metcalfe *et al.* 2004). BAX nuclear localization has been reported in several human cancer cells (Nishita *et al.* 1998, Huang *et al.* 2006). The biological significance of this fraction is still unclear but it seems to be correlated with apoptosis induction in some cases (Huang *et al.* 2006). A more intense staining of BAX and HRK proteins was observed within some blastomeres of the same fragmented embryo (Antczak & Van Blerkom 1999, Jurisicova *et al.* 2003). It was suggested that toxic proteins, such as these proapoptotic BCL2 family members, may concentrate within specific fragments before being eliminated to allow proper embryonic development (Antczak & Van Blerkom 1999, Jurisicova & Acton 2004):

- i) In this hypothesis (embryonic fragmentation used as a survival mechanism), the surviving embryos are those in which 'apoptotic' blastomeres are properly eliminated.
- ii) Extensive apoptosis in several blastomeres (in response to death stimuli) would result in early embryonic arrest (embryonic fragmentation as a death mechanism).
- iii) Absence of apoptosis would not necessarily be beneficial for embryonic development. Incidentally, it is well known that good morphological appearance does not guarantee a successful development. Besides, moderate fragmentation is not detrimental for embryonic development. We can hypothesize that fragmentation could be used as a survival/death switch during early embryonic development. Interestingly, mouse embryos lacking autophagy related 5 gene (*Atg5*, involved in autophagy, a form of programmed cell death characterized by the degradation of cellular components) were unable to develop beyond the four- to eight-cell stage (Tsukamoto *et al.* 2008), indicating that a basal level of autophagy is required to achieve preimplantation development. No similar evidence has yet been available for apoptosis, but it may represent an interesting clue for further investigations, especially since some BCL2 homologs such as BCL2, BCL2L1L, MCL1, BAD, and BIK have been found to be involved in the regulation of autophagy (Germain & Slack 2010).

Finally, subcellular redistribution of BCL2 proteins could be mediated by different mechanisms:

- i) Since several BCL2 proteins are initially attached or translocated to mitochondria and ER after apoptotic stimuli, it is very likely that they co-migrate with these organelles during the stage-specific remodeling of the ooplasm. It was observed that, in apoptotic cells, proapoptotic proteins BAX and the activated form of BID, tBID, translocate to mitochondria and move with them toward the perinuclear region (Li *et al.* 1998, Pucci *et al.* 2009). It has been suggested that this perinuclear aggregation facilitates apoptosis by concentrating mitochondrial apoptogenic molecules such as apoptosis inducing factor, endonuclease G and reactive oxygen species near to their targets in the nucleus (Aslan & Thomas 2009).
- ii) Some BCL2 proteins can interact with the microtubule and/or actin cytoskeleton (Table 2) and may also move along them. For example, in healthy cells, the BH3-only proteins BIM and BMF are sequestered to the microtubule and actin microfilament networks, respectively, via specific motor proteins (Puthalakath *et al.* 1999, 2001). Upon activation of apoptosis, they dissociate from the cytoskeleton and translocate into mitochondria. BCL2 protein was also found to interact with both microtubule and actin cytoskeletons in somatic cells (Porcelli *et al.* 2008, Ke *et al.* 2010). Furthermore, *in vitro* assays have demonstrated a direct interaction between several anti- and proapoptotic BCL2 family proteins and tubulin (Knipling & Wolff 2006; Table 2).
- iii) The movement of BCL2 family proteins may also be dependent upon interaction with binding partner proteins. The antiapoptotic members BCL2, BCL2L10, BCL2L1L, BCL2A1 and MCL1 can bind to translationally controlled tumor protein (TCTP; Zhang *et al.* 2002, Liu *et al.* 2005, Yang *et al.* 2005, Guillemin *et al.* 2009), a microtubule binding protein that could serve as a link for cytoskeleton-driven translocation. TCTP has been shown to be expressed in human and animal oocytes and early embryos (Tani *et al.*

**Table 2** Interaction of B cell lymphoma-2 (BCL2) family proteins with cytoskeleton components actin and tubulin.

Member	Actin	Tubulin	References
BCL2	+	+	Knipling & Wolff (2006) and Porcelli <i>et al.</i> (2008)
BCL2L1L		+	Knipling & Wolff (2006)
MCL1ES	+		Kim <i>et al.</i> (2009)
BAX peptide		+	Knipling & Wolff (2006)
BAK peptide		+	Knipling & Wolff (2006)
BID		+	Knipling & Wolff (2006)
BAD		+	Knipling & Wolff (2006)
BIM		+	Puthalakath <i>et al.</i> (1999)
BMF	+		Puthalakath <i>et al.</i> (2001)

2007, Ma *et al.* 2008, Guillemin *et al.* 2009). A co-localization between TCTP and BCL2L10 was observed around the meiotic spindle and in the pericortical cytoplasm (Guillemin *et al.* 2009), suggesting that TCTP could participate in the redistribution events of BCL2L10 during oocyte maturation.

## Conclusions/perspectives

The data presented in this review collectively support a prominent role of BCL2 family members in regulating oocyte and early embryo survival. In fact, several members of the family are expressed differentially during oocyte differentiation and early embryonic development. Among these members, the proapoptotic factor BAX has emerged as a major candidate. In fact, BAX presents a constitutive expression, suggesting that both oocytes and early embryos are under a permanent threat of death and that their survival depends on their ability to inhibit its proapoptotic activity. The antiapoptotic member BCL2L10 is expressed at high levels in oocytes and early cleaving embryos, suggesting that this member may represent a good candidate to antagonize BAX activity. It would be highly interesting to determine if the expression levels of these members (low expression of BAX and high expression of BCL2L10) could serve as biomarkers of oocyte quality and embryo developmental competence.

We would like to propose that the dynamic redistribution of certain BCL2 family proteins to specific subcellular compartments during oocyte maturation and early embryonic development could contribute to a functional compartmentalization of the apoptotic machinery, thus suggesting that changes in gene expression alone are not sufficient to predict apoptosis, as spatial localization may also play a significant role.

Finally, understanding BCL2 family functions and regulation is of great interest for understanding the molecular factors involved in oocyte and early embryo demise. Such knowledge could open new perspectives in assisted reproductive technologies, such as improving oocyte and embryo viability in culture and for selecting healthy embryos that are most likely to result in pregnancy.

## Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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