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Review

Disassembly of the Dying: Mechanisms and Functions

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The disassembly of an apoptotic cell into subcellular fragments, termed apoptotic bodies (ApoBDs), is a hallmark of apoptosis. Although the generation of ApoBDs is generally understood as being stochastic, it is becoming increasingly clear that ApoBD formation is a highly regulated process involving distinct morphological steps and molecular factors. Functionally, ApoBDs could facilitate the efficient clearance of apoptotic material by surrounding phagocytes as well as mediate the transfer of biomolecules including microRNAs and proteins between cells to aid in intercellular communications. Therefore, the formation of ApoBDs is an important process downstream from apoptotic cell death. We discuss here the mechanisms and functions of apoptotic cell disassembly.

Trends

Apoptotic cell disassembly is a highly complex process regulated by a series of well-coordinated morphological steps including apoptotic membrane blebbing, apoptotic protrusion formation, and fragmentation.

Plasma-membrane blebbing is not the sole process required for apoptotic body (ApoBD) formation, but membrane protrusions including microtubule spikes, apoptopodia, and beaded apoptopodia may act in concert to aid the generation of ApoBDs.

The mechanism of how apoptotic cells disassembly can determine the quantity and quality (size and contents) of ApoBDs.

ApoBDs can aid intercellular communication by transporting DNA, microRNAs, proteins, and infectious agents between cells.

Several clinically approved drugs can target the apoptotic cell disassembly process and may represent novel therapeutics to treat diseases associated with cell death.

Cell Disassembly as a Key Downstream Process of Apoptotic Cell Death

Billions of cells undergo apoptosis (a form of programmed cell death) daily as part of physiological homeostasis [1]. At later stages of apoptosis, some cell types can generate subcellular (1–5 µm) membrane-bound extracellular vesicles termed **apoptotic bodies** (ApoBDs, see Glossary) [1–3]. ApoBDs are the largest type of extracellular vesicle compared to microparticles (50–1000 nm) and exosomes (30–100 nm) [2,4–6] (Box 1). The formation of ApoBDs involves a series of morphological changes through a process termed apoptotic cell disassembly [1] (Figure 1). It has been well documented that a key mediator of apoptotic cell disassembly is plasma-membrane **blebbing**, a process controlled by **actomyosin contraction** [7]. The subsequent separation of plasma-membrane blebs to generate discrete ApoBDs is dependent on the formation of thin membrane protrusions [1,8,9]. Although the importance of apoptosis and the prompt removal of **apoptotic cells** in normal physiological and disease settings have been extensively studied [10,11], the function of apoptotic cell disassembly (i.e., the intermediate step between apoptosis and cell removal) is not fully defined. Nevertheless, the disassembly of apoptotic cells can facilitate efficient cell clearance [12] and mediate the transport of biomolecules between cells to aid in intercellular communication [13,14].

Because most investigators focus on the level of cell death rather than on the cell disassembly process, and because the clearance of apoptotic material by phagocytes is extremely rapid, *in vivo* evidence of ApoBD formation is limited. Nonetheless, several studies have observed the ability of apoptotic cells to disassemble into ApoBDs under *in vivo* settings, including the generation of thymocyte-derived ApoBDs [9,15] and the formation and subsequent removal of epithelial cell-derived ApoBDs in the basal epithelium [16] (Table 1). These studies support the concept that ApoBD formation can occur *in vivo* under physiological conditions and is not simply an *in vitro* phenomenon when neighbouring phagocytes and tissue architecture are absent. We review here the current mechanistic insights into the complex steps of apoptotic cell disassembly, and the significance of this process in physiological and pathological settings.

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Box 1. Comparison of the Three Major Classes of Membrane-Bound Extracellular Vesicles

Apoptotic Bodies (ApoBDs)

ApoBDs (1–5 μm) are the largest type of vesicle in the extracellular vesicle family. Their formation is regulated by the morphological steps of apoptotic cell disassembly, controlled by protein kinases such as ROCK1 [20] and MLCK [7], the membrane channel PANX1, and vesicular transport [1]. The formation of ApoBDs can promote efficient removal of cell debris by surrounding phagocytes. In addition, ApoBDs can harbour biomolecules including microRNA [13] and DNA [14] to regulate intercellular communication. Currently, the only known surface marker of ApoBDs is PtdSer [1].

Microparticles (MPs)

MPs (50–1000 nm) are generated through budding or shedding from the plasma membrane under healthy and apoptotic conditions. The release of MPs is facilitated by spectrin, calpain, and Ca^{2+} stimulation [4], and can regulate processes including coagulation, inflammation, and cell activation [5]. Therefore, MPs are often implicated in the pathogenesis of particular disease settings including thrombosis and arthritis, and are often considered to be a biomarker for diseases such as atherosclerosis [5]. Characteristic markers of MPs include PtdSer exposure, integrins, VCAMP3, and CD40 [2].

Exosomes

These are the smallest (30–100 nm) and the best-characterised vesicle in the extracellular vesicle family. Exosome biogenesis is mediated by ESCRT, Rab, and SNARE proteins [2]. When observed by electron microscopy, exosomes have a distinct cup-shaped morphology. In addition, exosomes can be characterised based on enriched protein content including ALIX, TSG101, and CD9. Owing to their density, exosomes can be purified by various centrifugation techniques. Exosomes are involved in an array of biological processes including protein secretion, antigen presentation, and viral pathogenesis [6].

Molecular Mechanisms of Apoptotic Cell Disassembly

The dismantling of an apoptotic cell into ApoBDs has been thought to be a stochastic process. However, recent studies suggest that the generation of ApoBDs is controlled by several well-coordinated morphological steps. The apoptotic cell disassembly process can be divided into three sequential steps governed by distinct morphological changes [1] (Figure 1). Step 1 describes the formation of plasma-membrane blebs on the cell surface. Particular cell types can then generate thin membrane protrusions (Step 2) including **microtubule spikes** (Step 2a), **apoptopodia** (Step 2b), and **beaded apoptopodia** (Step 2c). Lastly, the fragmentation process (Step 3) leads to the generation of individual ApoBDs.

Step 1. Apoptotic Membrane Blebbing

In healthy cells, plasma-membrane blebbing plays a key role in directed cell migration [17]. Plasma-membrane blebbing is also a morphological hallmark of apoptosis *in vitro* and *in vivo* [7,15,18]. Apoptotic membrane blebbing (Step 1) constitutes the dynamic formation and retraction of plasma-membrane blebs at the cell surface during the early stages of apoptosis and is regulated by a series of processes (Figure 2). Hydrostatic pressure within the dying cell can facilitate the movement of intracellular fluids into membrane blebs and enable bleb inflation [19]. Simultaneously, actomyosin contraction and microtubule assembly regulate cytoskeletal dynamics to aid in the cyclic extension of blebs at the cell surface [8,20]. Notably, apoptotic blebs are distinct from necrotic blebs, which are generally larger, independent of actomyosin contraction, and are generated after membrane permeabilisation [21].

Apoptotic membrane blebbing is regulated by several protein kinases, in particular the Rho-associated protein kinase 1 (ROCK1) [22]. During apoptosis, active caspase 3 proteolytically cleaves ROCK1 and triggers kinase activation by releasing its autoinhibitory C-terminal domain [20,22]. In turn, caspase-activated ROCK1 phosphorylates myosin light chain (MLC) of myosin II and promotes actomyosin contraction to facilitate membrane blebbing [20]. The importance of ROCK1 activation in apoptotic membrane blebbing has been demonstrated in a variety of cell types including fibroblasts [12,23], epithelial cells [24], and T cells [20]. In addition to ROCK1,

Glossary

Actomyosin contraction: a cellular process that describes the generation of contractile force though the interaction between filamentous actin and myosin II.

Apoptotic body (ApoBD): a subcellular (1–5 μm diameter) extracellular vesicle generated from an apoptotic cell at the final stages of apoptotic cell disassembly.

Apoptotic cell: a cell that has begun apoptosis but has not undergone apoptotic cell disassembly.

Apoptopodia: string-like membrane protrusions found on apoptotic cells.

Beaded apoptopodia: beads-on-a-string-like membrane protrusions found on apoptotic cells.

Blebbing: a cellular process that describes the formation and retraction of plasma-membrane bulges at the cell periphery.

Cell body: the largest membrane-bound portion of an apoptotic cell generated at the final stages of apoptotic cell disassembly; often contains the majority of the nuclear and cytoplasmic contents.

Early-stage membrane blebbing: the formation of small membrane blebs at the cell periphery.

'Eat-me' signals: molecular factors exposed on the surface of dying cells that could trigger their uptake by phagocytes.

'Find-me' signals: molecular factors released from dying cells that could recruit phagocytes towards the site of cell death.

Late-stage apoptotic membrane blebbing: the formation of dynamic and large membrane blebs after early-stage blebbing. Blebs at this stage often contain nuclear materials.

Microtubule spikes: rigid and microtubule-rich membrane protrusions found on apoptotic cells.

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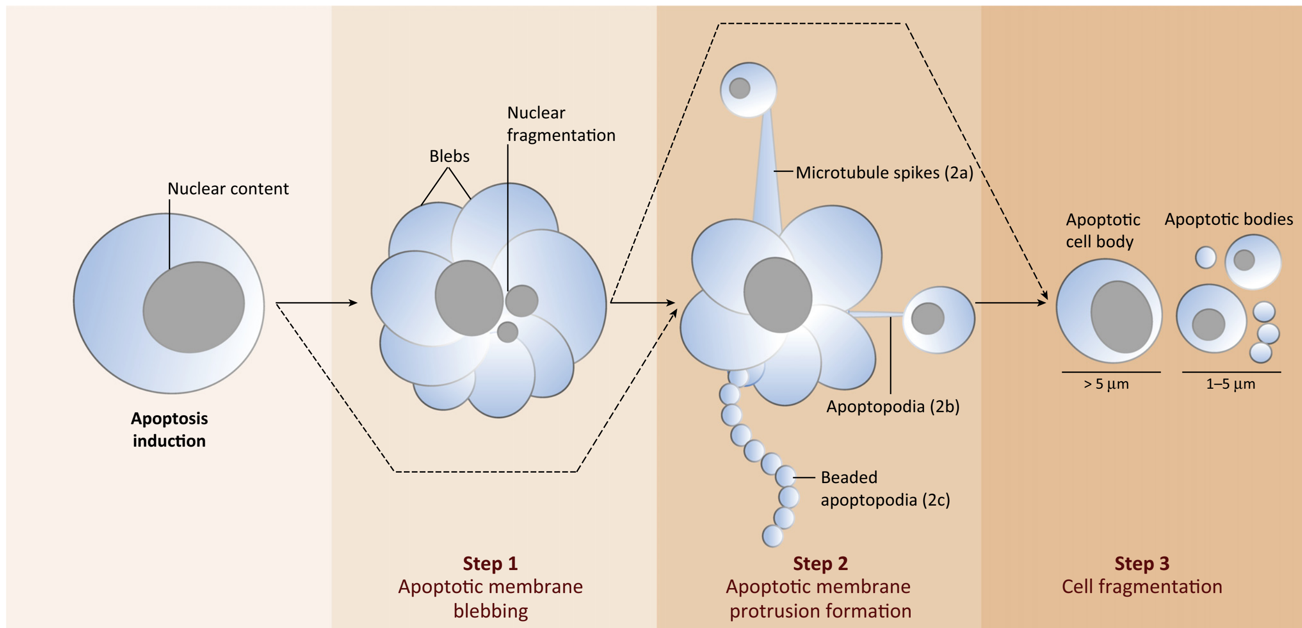

Figure 1. Morphological Stages of Apoptotic Cell Disassembly. The disassembly of an apoptotic cell can be divided into three sequential morphological steps. Step 1 describes the formation of balloon-like membrane structures at the cell surface, termed apoptotic membrane blebs. After the onset of blebbing, the apoptotic cell can generate different types of apoptotic membrane protrusions (Step 2), including microtubule spikes (Step 2a), apoptopodia (Step 2b), and beaded apoptopodia (Step 2c). At the later stages of cell death, the apoptotic cell and/or apoptotic membrane protrusions can undergo fragmentation to generate separate apoptotic bodies (small membrane-bound vesicles less than 5 µm in diameter) from the apoptotic cell body (Step 3). Dotted lines indicate that for some cell types Step 2 can occur in the absence of Step 1, and Step 2 is not a prerequisite for Step 3.

MLC kinase (MLCK) has been implicated in mediating apoptotic membrane blebbing [7]. However, the molecular mechanism responsible for MLCK activation during apoptosis is unclear. Furthermore, caspase-mediated cleavage of the serine/threonine LIM domain kinase 1 (LIMK1) is sufficient to induce LIMK1 activation and promote membrane blebbing in apoptotic human T cells and epithelial cells [25]. Because LIMK1 can inactivate cofilin, an actin-binding protein that facilitates actin depolymerisation, caspase-activated LIMK1 may support apoptotic

Table 1. Morphological Steps of Apoptotic Cell Disassembly Can Be Observed under *In Vivo* Conditions

Tissue	Cell type	Apoptotic stimulus	Step observed	Method of detection	Key findings	Refs
Skeletal muscle	Myoblast	Homeostatic	Step 1	Immunohistochemistry	Myoblasts undergo plasma-membrane blebbing	[18]
Cardiac tissue	Not specified	Homeostatic	Step 1/3	Immunohistochemistry	Autoantigens are distributed into apoptotic membrane blebs and ApoBDs	[76]
Thymus	Thymocyte	Irradiation	Step 1/3	Scanning electron microscopy	Thymocytes can generate an abundance of ApoBDs	[15]
Lymph node	T cell	Homeostatic	Step 3	Intravital two-photon imaging	Apoptotic T cells can disassemble into ApoBDs	[66]
Hair follicle epithelium	Basal epithelial cell	Laser ablation	Step 3	Intravital multiphoton microscopy	Apoptotic epithelial cell-derived ApoBDs are phagocytosed by surrounding viable epithelial cells	[16]
Thymus	Thymocyte	Dexamethasone	Step 3	Flow cytometry	PANX1 is a negative regulator of apoptotic thymocyte disassembly	[9]
Aorta	Not specified	Atherosclerosis	Step 3	Histology	ApoBDs may accumulate in atherosclerotic plaques	[82]

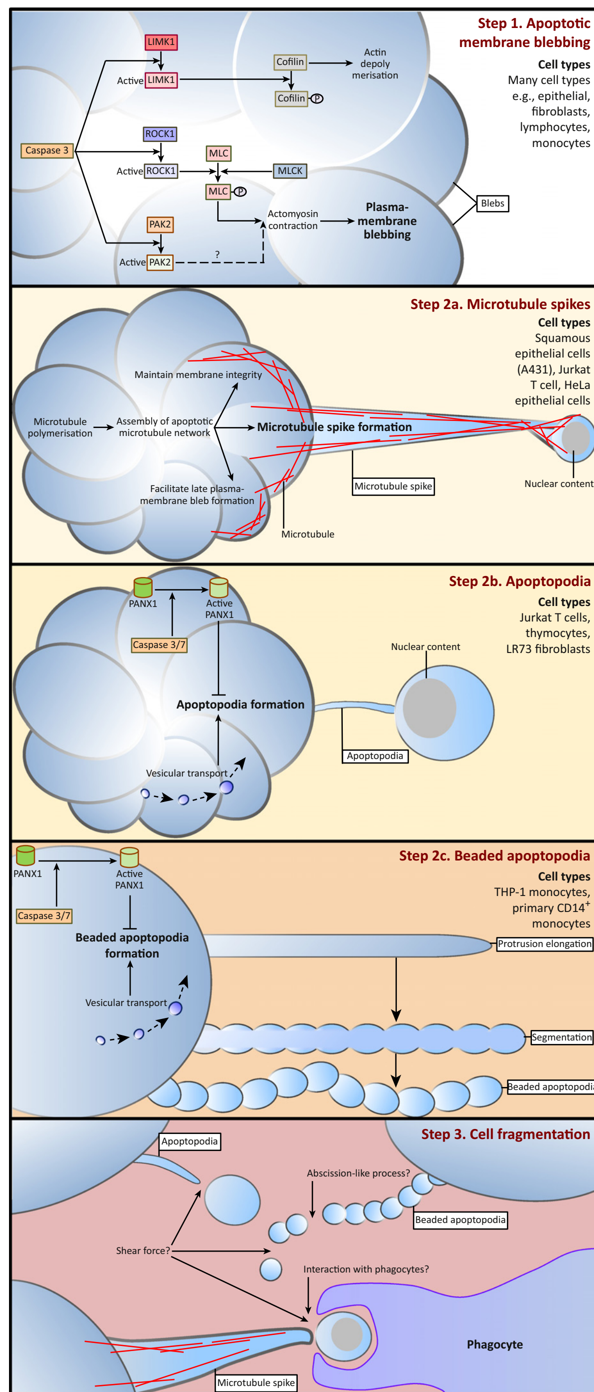

Figure 2. Molecular Mechanisms of Apoptotic Cell Disassembly. Apoptotic membrane blebbing (Step 1) is regulated by several protein kinases including ROCK1, LIMK1, MLCK, and PAK2. Assembly of microtubule network is required for the maintenance of plasma-membrane integrity, late-stage blebbing, and microtubule spike formation (Step 2a). The formation of apoptopodia (Step 2b) and beaded apoptopodia (Step 2c) is regulated by PANX1 channels and vesicular transport. Beaded apoptopodia are generated through an initial protrusion elongation phase, followed by a segmentation phase. Finally, apoptotic membrane protrusions can undergo fragmentation to generate individual apoptotic bodies (Step 3). Although currently undefined, apoptotic body release may be facilitated by shear force, an abscission-like process, or through interactions with neighbouring phagocytes.

membrane blebbing by promoting actin polymerisation through cofilin inhibition [26,27]. Lastly, caspase-mediated activation of p21-activated kinase (PAK2) has also been implicated in facilitating apoptotic membrane blebbing and consequently the formation of ApoBDs in human T cells [28]. It should be noted that the role of these kinases in apoptotic cell disassembly is based largely on pharmacological studies or the expression of dominant negative mutants in a limited number of cell types [22]. Therefore, further validation by genetic approaches using a diverse array of cell types is necessary to define the importance of ROCK1, MLCK, LIMK1, and PAK2 in apoptotic cell disassembly.

In addition to actomyosin contraction, microtubule assembly plays a prominent role in apoptotic cell disassembly by preventing premature membrane lysis and regulating membrane blebbing. During the early stages of apoptosis, the existing interphase microtubule network is dismantled, and densely packed microtubules reform against the plasma membrane, creating a 'cocoon'-like structure termed the apoptotic microtubule network (AMN) [29,30]. The formation of AMN aids in membrane stability and prevents membrane permeabilisation, thus avoiding the early onset of secondary necrosis [29]. This AMN-dependent membrane stability could be required for the morphological changes of apoptotic cell disassembly. The establishment of AMN during apoptosis may also act in concert with actin dynamics to regulate **late-stage apoptotic membrane blebbing** and bleb composition in apoptotic epithelial cells [8,31].

Step 2a. Apoptotic Membrane Protrusions: Microtubule Spikes

It has been assumed that blebbing alone is responsible for the formation of ApoBDs [12,31]. However, some cell types including neuronal and epithelial cells undergo dynamic apoptotic membrane blebbing in the absence of ApoBD formation *in vitro* [1]. Therefore, apoptotic membrane blebbing alone is not sufficient to mediate ApoBD formation for particular cell types, and additional processes may act in concert with and/or independently of blebbing to facilitate apoptotic cell disassembly. A type of rigid and microtubule-rich membrane protrusion, the microtubule spike, was identified on apoptotic human A431 epithelial cells that could aid in ApoBD formation in the presence or absence of membrane blebbing [8] (Figure 2). Microtubule spikes were observed at the periphery of A431 cells approximately 1 h after the onset of apoptotic membrane blebbing [8]. The formation of microtubule spikes was suggested to facilitate the separation of membrane blebs into individual ApoBDs, with individual or clusters of ApoBDs attaching to either the tip or along the length of the spike [8]. Inhibition of actomyosin contraction by the ROCK1 inhibitor Y-27632 markedly reduced apoptotic membrane blebbing but had minimal effects on ApoBD formation [8,31]. By contrast, inhibition of microtubule polymerisation by nocodazole impaired both apoptotic membrane blebbing and microtubule spike formation, resulting in a significant reduction in ApoBD formation [8,31]. These findings highlight that apoptotic membrane blebbing is not a prerequisite for ApoBD formation by particular cell types, and other processes such as microtubule spikes alone may be sufficient to drive apoptotic cell disassembly.

Step 2b. Apoptotic Membrane Protrusions: Apoptopodia

In support of the notion that additional processes besides membrane blebbing are required for ApoBD formation, thin string-like membrane protrusions termed apoptopodia are generated during the progression of apoptosis (Figure 2). After dynamic membrane blebbing, apoptotic human Jurkat T cells, primary mouse thymocytes, and fibroblasts generate apoptopodia between membrane blebs (Step 2b). These membrane blebs then separate from the **cell body** to form distinct ApoBDs [9]. While it is unclear which cytoskeletal components are involved in apoptopodia formation, the caspase-activated pannexin 1 (PANX1) channel was identified as a key regulator of apoptopodia formation [9]. Before apoptotic membrane blebbing and exposure of the **'eat-me' signal** phosphatidylserine (PtdSer), PANX1 is activated by caspase 3 and 7, resulting in the release of **'find-me' signals** into the extracellular space [9,32–34].

Unexpectedly, pharmacological or genetic inhibition of PANX1 activity not only blocked the release of ‘find-me’ signals but also promoted the formation of apoptopodia and ApoBDs [9]. Thus, PANX1 is a negative regulator of apoptotic cell disassembly [9]. It is important to note that the ability of PANX1 to positively and negatively regulate the release of ‘find-me’ signals and ApoBD formation by apoptotic cells, respectively, raises an intriguing possibility that these two processes are inversely related with regards to controlling the efficiency of apoptotic cell clearance. For example, specific apoptotic cell types may have a higher level of caspase-activated PANX1, which favours the recruitment of macrophages rather than cell disassembly, which supports efficient cell clearance. Conversely, some apoptotic cell types may have a lower level of caspase-activated PANX1 to favour ApoBD formation, which would aid in their removal by neighbouring or distant phagocytes.

Although PANX1 has been implicated in a variety of processes including cell fusion [35], inflammasome activation [36], T cell activation [37], and pyroptosis [38], the molecular basis of how PANX1 controls apoptopodia formation is undefined. In addition to PANX1, vesicular transport was recently identified as a process that regulates apoptopodia formation and apoptotic cell disassembly [1], possibly by mediating the trafficking of additional membrane materials and/or molecular factors to aid in the generation of apoptopodia.

Step 2c. Apoptotic Membrane Protrusions: Beaded Apoptopodia

Another type of apoptopodia-like membrane structure was described recently in apoptotic human THP-1 monocytic cells and primary human monocytes, termed beaded apoptopodia [1] (Figure 2). After apoptosis induction, monocytes undergo subtle **early-stage membrane blebbing**, followed by the formation of thin membrane protrusions [1]. Through a segmentation-like event, these protrusions are transformed into a string of connecting membrane vesicles that are generally 1–3 μm in diameter. Finally, membrane vesicles are dissociated from the beaded apoptopodia and generate an abundance of ApoBDs [1]. It should be noted that the formation of ApoBDs via beaded apoptopodia appears to be highly efficient at forming vesicles. Instead of individual or a small number of ApoBDs being separated from the cell body via microtubule spikes or apoptopodia, fragmentation of a single strand of beaded apoptopodia can release many (approximately 10–20) ApoBDs simultaneously [1]. Therefore, the formation of beaded apoptopodia represents a unique mechanism of generating a large amount of ApoBDs rapidly [1]. Interestingly, beaded apoptopodia and ApoBD formation can also be observed in a small subset of monocytes that fail to undergo apoptotic membrane blebbing [1], further supporting the concept that membrane blebbing is not a prerequisite for apoptotic cell disassembly. The formation of beaded apoptopodia was also found to regulate selective sorting of cellular contents into ApoBDs. In contrast to ApoBDs generated by fibroblasts [39], epithelial cells [31], and thymocytes [40], ApoBDs generated from apoptotic THP-1 monocytes were devoid of nuclear contents [1].

Similar to apoptotic T cells, the disassembly of apoptotic monocytes via beaded apoptopodia is also regulated by caspase-activated PANX1 channels and vesicular transport [1]. However, it is worth noting that, although similar mechanisms appear to control both apoptopodia and beaded apoptopodia formation, the morphologies of these protrusions are substantially different. One possible reason for these differences could be the degree of apoptotic membrane blebbing during protrusion formation. As mentioned, beaded apoptopodia are generated after a subtle period or absence of membrane blebbing [1], whereas apoptotic T cells undergo dynamic membrane blebbing before and during apoptopodia formation [9]. Thus, the presence or absence of apoptotic membrane blebbing (Step 1) may determine whether the apoptotic cell will generate apoptopodia (Step 2b) or beaded apoptopodia (Step 2c), respectively. In support of this concept, inhibition of apoptotic T cell membrane blebbing during cell disassembly promoted the formation of beaded apoptopodia rather than of apoptopodia [1].

Step 3. Apoptotic Cell Fragmentation into ApoBDs

After the formation of apoptotic membrane blebs (Step 1) and/or protrusions (Step 2), the final stage of apoptotic cell disassembly is the disassociation of ApoBDs from the cell body or neighbouring ApoBDs (Step 3) (Figure 2). Although the mechanism controlling Step 3 is currently undefined, it may involve both cell-extrinsic and cell-intrinsic factors. One likely cell-extrinsic factor that could promote the separation of ApoBDs from apoptotic membrane protrusions is the presence of shear force in the extracellular environment. For example, following the separation of apoptotic membrane blebs by apoptopodia [9], shear force generated by the flow of surrounding culture media *in vitro* may break the connection between apoptopodia and ApoBDs. This phenomenon shares similarities with platelet formation where shear force in the vasculature can facilitate the fragmentation of long beaded strands of proplatelets generated by megakaryocytes [41,42]. Furthermore, interactions between cells undergoing apoptotic cell disassembly and neighbouring phagocytes (discussed further below) may generate the necessary physical force to disrupt apoptotic membrane protrusions and aid in the release of ApoBDs. The ability of neighbouring cells to exert force on apoptotic cells is well documented in the context of extruding apoptotic epithelial cells from the epithelium [43]. In addition to cell-extrinsic factors, the generation of ApoBDs through beaded apoptopodia may be intrinsically regulated via an abscission-like process that could 'pinch-off' individual ApoBDs from the protrusion, possibly resembling the cytokinetic abscission process during cell division. However, the molecular machinery that regulates the segmentation and fragmentation of beaded apoptopodia is yet to be defined.

The Role of Apoptotic Cell Disassembly in Cell Clearance and Intercellular Communication

The formation of ApoBDs has been proposed to mediate two key functions: (i) to aid in the efficient removal of apoptotic cells, and (ii) to carry biomolecules such as nucleic acids and proteins to facilitate intercellular communication. We describe here the functions of apoptotic cell disassembly and the importance of this process in normal physiological and pathological conditions.

Apoptotic Cell Disassembly in Inflammatory and Autoimmune Disease

If apoptotic cells are not rapidly removed by phagocytes, the dying cell can undergo secondary necrosis when the plasma membrane becomes permeabilised, leading to the release of proinflammatory intracellular contents and the triggering of unwanted inflammation [44]. These harmful consequences of impaired cell clearance are evident in the cardiovascular disease atherosclerosis, whereby an accumulation of uncleared post-apoptotic debris can promote chronic inflammation and the formation of necrotic plaques [45,46]. Furthermore, defects in apoptotic cell clearance have been linked to the onset and/or progression of autoimmune diseases such as systemic lupus erythematosus (SLE). For a subgroup of SLE patients, impaired cell clearance results in the accumulation of post-apoptotic debris that can promote inflammation and exposure to autoantigens [47–49]. Thus, because efficient apoptotic cell clearance is imperative to maintain tissue homeostasis, a series of molecular mechanisms have been described that could facilitate the prompt removal of apoptotic cells. First, 'find-me' signals such as ATP are released by apoptotic cells to recruit nearby macrophages to the site of cell death [33]. Following phagocyte recruitment, engulfment of apoptotic cells is triggered through the recognition of 'eat-me' signals, such as PtdSer, exposed on the surface of apoptotic cells [9,50–52].

In addition to these mechanisms, the morphological changes of apoptotic cell disassembly may aid in their efficient clearance. In particular, blockade of apoptotic Jurkat T cell membrane blebbing by inhibitors of actomyosin contraction markedly reduced their uptake by primary human monocyte-derived macrophages [53]. Although the precise mechanism of how

membrane blebbing promotes cell clearance is undefined [53], it is tempting to speculate that inhibition of apoptotic membrane blebbing could block the formation of ApoBDs and consequently reduce the efficiency of macrophage engulfment. In addition, the formation of microtubule spikes (Step 2a) has been proposed to aid in cell clearance by promoting interactions between apoptotic cells and phagocytes [8]. THP-1 monocytic phagocytes often interact with ApoBDs bound to the ends of microtubule spikes, suggesting that microtubule spikes 'present' ApoBDs to surrounding phagocytes [8]. Notably, blocking microtubule spike formation with nocodazole reduced the interactions between apoptotic A431 epithelial cells with THP-1 phagocytes, as well as the levels of apoptotic cell engulfment [8]. However, inhibition of microtubule polymerisation by nocodazole may also impair the phagocytic efficiency of THP-1 phagocytes [54,55]. Nevertheless, the formation of apoptotic membrane protrusions such as microtubule spikes, and possibly apoptopodia and beaded apoptopodia, represents a mechanism that could promote cell clearance by facilitating the initial interaction between apoptotic fragments and phagocytes.

It is well documented that a deficiency in apoptotic cell clearance machineries (e.g., Mertk, C1q) in mice can lead to the development of SLE-like diseases [49,56–58] and atherosclerosis [59]. Thus, mice lacking key positive and negative regulators of apoptotic cell disassembly, such as ROCK1 and PANX1, may be more or less susceptible to these pathological conditions, respectively. Although such phenotypes have yet to be reported for mice deficient in ROCK1 [60,61] or PANX1 [9,62,63], it should be noted that both ROCK1 and PANX1 can regulate other aspects of cell clearance in addition to ApoBD formation. For example, pharmacological inhibition of ROCK1 by Y-27632 promotes the phagocytic activity of fibroblasts, demonstrating ROCK1 as a negative regulator of phagocytosis [64]. Furthermore, PANX1 regulates phagocyte recruitment and inflammatory responses towards apoptotic cells by controlling the release of ATP during apoptosis [32,65]. Therefore, the function of ROCK1 and PANX1 in apoptotic cell clearance is complex and needs to be addressed cautiously. Despite the limited number of studies that examine the importance of apoptotic cell disassembly in cell clearance, it is evident that ApoBD formation and their removal can occur *in vivo* [15,16,66,67]. Thus, the disassembly of apoptotic cells may play a key role in maintaining homeostasis and preventing the development of diseases associated with impaired cell clearance.

Transport of Biomolecules through ApoBDs

Membrane-bound extracellular vesicles like exosomes and microparticles play an important role in intercellular communication by facilitating the transfer of biomolecules between cells [68,69]. For example, dendritic cell (DC)-derived exosomes regulate immunity by facilitating antigen trafficking [6,70]. Similarly, microparticles mediate the transport of microRNAs [71] and regulate vascular repair [72]. Likewise, ApoBDs can carry DNA, RNA, and proteins, suggesting that they can mediate intercellular communication through the transport of these biomolecules (Figure 3).

During apoptosis, membrane blebbing and protrusion formation facilitates the distribution of nuclear material into ApoBDs [1,8]. Subsequently, the transport of genomic DNA to neighbouring cells via ApoBDs enables horizontal gene transfer between different cell types [14,39]. For example, DNA packaged into lymphoma-derived ApoBDs was engulfed by surrounding fibroblasts, resulting in the integration of lymphoma-derived DNA into the fibroblast genome [14]. The functional significance of transporting DNA through ApoBDs was exemplified by the transport of oncogenes (h-Ras and c-Myc) via ApoBDs to recipient cells lacking p53, which promoted tumour formation *in vivo* [39]. In addition to DNA, ApoBDs derived from apoptotic endothelial cells have been shown to mediate the transfer of microRNA-126 to healthy endothelial cells *in vitro* and to induce the expression of chemokine CXCL12 by recipient endothelial cells [13]. In a mouse model of atherosclerosis, repetitive administration of microRNA-126-containing ApoBDs resulted in atheroprotective effects, possibly by inducing CXCL12 expression in luminal

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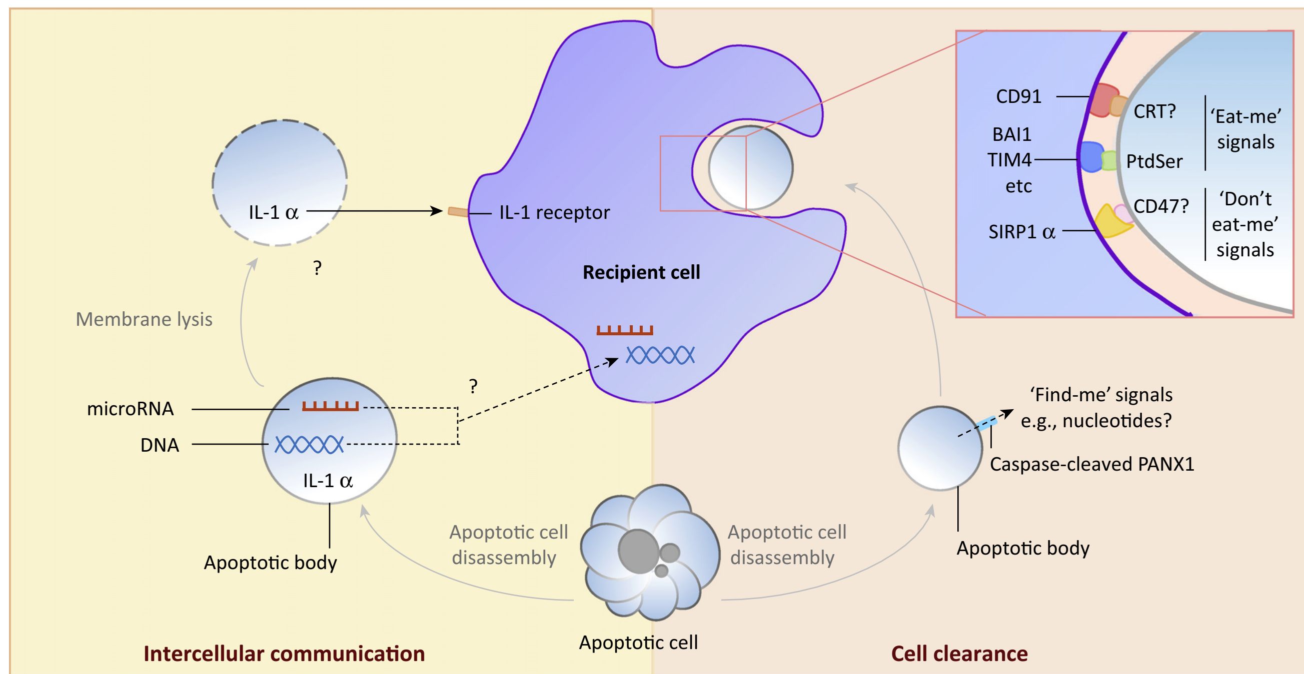

Trends in Cell Biology

Figure 3. Interactions between Apoptotic Bodies (ApoBDs) and Recipient Cells. ApoBDs can mediate the transport of biomolecules including DNA, microRNAs, and cytokines to recipient cells to aid in intercellular communication. Although the mechanism underpinning the transfer of biomolecules from ApoBDs to recipient cells is undefined, membrane lysis may facilitate the release of cytokine IL-1α. ApoBDs can function as small 'bite-sized' fragments to promote cell clearance. Similar to apoptotic cells, ApoBDs expose the 'eat-me' signal PtdSer to promote recognition and uptake by phagocytes. Whether ApoBDs can release 'find-me' signals (e.g., nucleotides) or expose other 'eat-me' signals (e.g., CRT) and 'don't eat-me' signals (e.g., CD47) is currently undefined. It should be noted that, if apoptotic cells are unable to disassembly into ApoBDs, the presence of bridging molecules such as MFG-E8 can facilitate their uptake by macrophages.

cells of aortic root plaques and recruiting endothelial progenitor cells to mediate vascular repair [13]. It is interesting to note that DNA and RNA were packaged into separate ApoBDs generated from apoptotic HL-60 promyelocytic cells [73]. Although the mechanism and functional significance of this phenomenon is unclear, this observation highlights the ability of ApoBDs to carry different cellular contents (e.g., either DNA or RNA) and may represent different functional properties in ApoBDs.

In addition to nucleic acids, the transfer of proteins via ApoBDs to professional phagocytes such as macrophages and DCs could play a role in regulating immunity. Proteomic analysis of thymocyte-derived ApoBDs demonstrated enrichment of autoantigens and proinflammatory molecules in ApoBDs, including various nuclear proteins and heat shock protein 90 [40]. Similarly, ApoBDs generated from apoptotic lymphoblasts contained nuclear proteins including histones 1, 2A, 2B, 3, and 4, as well as the autoantigen La/SSB [74]. Multiple groups have reported the distribution of protein autoantigens into apoptotic membrane blebs and ApoBDs of various cell types (e.g., epidermal cells, T cells, cardiomyocytes) *in vitro* and *in vivo* [75,76]. Notably, primary human monocyte-derived macrophages were able to engulf ApoBDs containing autoantigens, indicating that autoantigens could be transferred to professional phagocytes via ApoBDs [74]. However, whether the trafficking of autoantigens via ApoBDs can affect the maintenance of immune tolerance is not well defined. In addition to autoantigens, endothelial cell-derived ApoBDs were found to contain the precursor and processed form of proinflammatory cytokine IL-1α [77]. ApoBDs containing IL-1α were able to stimulate the production of chemokine IL-8 by healthy endothelial cells *in vitro* and to induce the infiltration of neutrophils into the peritoneal of mice [77]. Although the mechanism underpinning the release of IL-1α from

ApoBDs is not defined [77], early membrane lysis could release intracellular contents within ApoBDs, as seen in a subset of ApoBDs [23].

The disassembly of apoptotic cells has also been proposed to aid in the transfer of infectious agents. For example, phagocytic uptake of apoptotic fragments derived from HIV-infected T cells by neighbouring epithelial cells mediated the transfer of HIV proteins and genome into epithelial cells, resulting in the transcription and expression of HIV proteins [78]. Furthermore, efficient removal of ApoBDs generated from prion-infected apoptotic neurons may be required to prevent the onset of prion disease [79]. Thus, ApoBDs can function as a 'Trojan horse' for infectious agents [80,81].

Concluding Remarks and Future Directions

It is becoming increasingly clear that apoptotic cell disassembly is a complex process, involving highly coordinated morphological steps. Depending on the mechanism used by a particular cell type to undergo apoptotic cell disassembly, a different quantity and quality of ApoBDs will be generated. These observations raise several unanswered questions as to why different cell types need to disassemble differently and the functional significance of such diversity (see Outstanding Questions). Determining the function of ApoBDs in different physiological and pathological contexts may shed light on the importance of apoptotic cell disassembly for different cell types. Lastly, it is worth noting that several compounds, including clinically approved drugs, have been identified that can either block or enhance ApoBD formation [9]. Thus, it is feasible to modulate apoptotic cell disassembly pharmacologically, and this may represent a novel therapeutic approach to treat diseases associated with the formation of ApoBDs.

Outstanding Questions

Why do different cell types disassemble differently during apoptosis?

What are the key cytoskeletal components that regulate apoptopodia and beaded apoptopodia formation?

How does PANX1 regulate the formation of apoptopodia and beaded apoptopodia?

What is the mechanism that controls the final fragmentation stage of apoptotic cell disassembly?

Does the formation of apoptotic membrane protrusions occur *in vivo*?

What is the purpose of generating apoptotic bodies (ApoBDs) of specific sizes and contents?

Are ApoBDs preferentially engulfed by a specific type of phagocytes?

Can ApoBDs travel vast distances within an organism to regulate intercellular communications?

Would defects in regulators of apoptotic cell disassembly lead to disease phenotypes?

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