Dual Philosophy in Death Receptor Signalling

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Abstract: Tumour necrosis factor (TNF) is the founding member of a cytokine family with important roles in both, physiology and pathological conditions. The two seemingly opposing cellular responses to stimulation by TNF itself are death and induction of pro-inflammatory signalling. TNF and other TNF superfamily (SF) members signal by crosslinking their cognate receptors. These form part of the TNF receptor SF (TNFRSF). Members of this family have between two and six characteristic cysteine-rich repeats in their extracellular domain. These repeats are crucial for receptor-ligand interaction. Members of the TNFRSF come in three flavours: as type I transmembrane proteins, attached to the plasma membrane by a glycosylphosphatidylinositol (GPI) anchor, or as secreted soluble proteins. The latter receptors act as decoys for their respective ligands. To date 30 members of the TNFRSF are known. Six of them form part of the subfamily of the death receptors. Death receptors are characterised by the presence of an intracellular death domain (DD). Amongst the death receptors there are again at least two subclasses, the ones which recruit the Fas-Associated Death Domain (FADD) and the ones that recruit the TNFR-Associated Death Domain (TRADD) protein. The primary function of FADD-recruiting receptors is to induce apoptosis whilst the primary function of the TRADD recruiters is to activate pro-inflammatory signalling (Fig. **1**). However, from a second platform both systems are also capable of triggering the respective other signalling outcome.

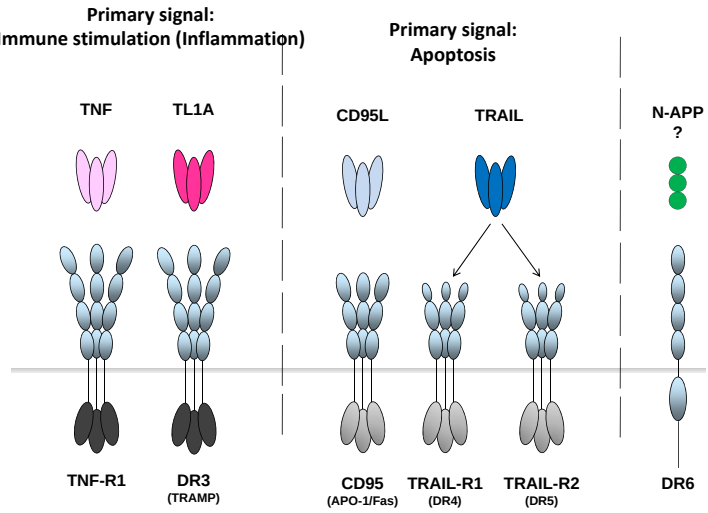

Fig. (1). The 6 human DD-containing receptors and their known ligands. The six human DD-containing receptors, TNF-R1 (p55/p60 TNF-R), CD95 (Fas, APO-1), death receptor 3 (DR3, TRAMP), TRAIL-R1 (DR4), TRAIL-R2 (DR5) and DR6 (TNFRSF21) are activated by their respective ligands: TNF, CD95L (FasL/APO-1L), TL1A, TRAIL (Apo2L), and a specific amino-terminal cleavage fragment of the β-amyloid precursor protein (APP), N-APP. They are transmembrane proteins which contain repeats of 2-4 cysteine-rich domains (CRDs) in the extracellular portion required for ligand binding and an intracellular death domain (ICD) capable of recruiting specific adaptors proteins. Whilst the primary signal output of the TRADD-recruiting ICDs of TNF-R1 and DR3 (shown in dark grey) is the activation of inflammatory signalling, the FADD-recruiting ICDs of CD95 and the TRAIL death receptors (shown in light grey) induce apoptosis as their primary signalling output.

Keywords: Death receptors, TRAIL, TNF, apoptosis, NF-κB, signal transduction.

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In this review we will exemplarily explain these two classes of death receptor signalling systems on the basis of the TRAIL and the TNF pathways, respectively.

1876-3901/11 2011 Bentham Open

THE TRAIL SYSTEM

In 1995, two groups, one at Immunex in Seattle and one at Genentech in San Francisco, noticed an expressed sequence tag (EST) in the public data base that was annotated as homologous to the ligand of the apoptosis-inducing receptor CD95 (Fas/APO-1), the CD95 ligand (CD95L) also know as FasL or APO-1L. The TNF-related apoptosis inducing ligand (TRAIL) or Apo2L, as the newly identified protein was then named by these two groups, respectively [1, 2], was found to kill a number of cancer cell lines whereas it appeared that normal cells could not be killed by TRAIL [3]. Walczak *et al.* and Ashkenazi *et al.* next determined that systemic treatment of tumour-bearing mice with recombinant TRAIL killed tumour cells *in vivo* without harming normal tissue [3, 4]. It is important to note that the form of TRAIL used in at least one of these studies was indeed capable of killing TRAIL-sensitive mouse tumour cells very efficiently but nevertheless did not exert any toxicity [3]. More than two decades after the discovery of TNF [5], this represents the first successful systemic application of a TNF-like cytokine that resulted in specific killing of tumour cells in the absence of toxicity. Based on these findings, Immunex and Genentech joined forces to co-develop Apo2L/TRAIL.

Immediately following identification of TRAIL the race for cloning of the TRAIL receptor began. Both EST-based bioinformatic as well as biochemical and/or molecular biological approaches were taken to identify the TRAIL receptor. Cloning this protein was potentially very valuable as an antibody against this receptor may serve as a new drug to treat cancer. Suprisingly, these efforts resulted in the cloning of not only one but a whole handful of receptors that bind TRAIL. Two of them were capable of killing cells [TRAIL-R1 (DR4) [6, 7] and TRAIL-R2 (DR5) [8-10]]. Apart from these two apoptosis-inducing receptors, two other cell-surface expressed TRAIL receptors, TRAIL-R3 (DcR1) [8, 9, 11, 12] and TRAIL-R4 (DcR2)[12-14] were discovered. These two receptors, however, did not induce apoptosis and due to the absence of an intracellular death domain they were thought to exert a decoy function for TRAIL (hence the name "decoy receptor" [DcR]). It was hypothesised that these receptors would be expressed by normal cells, thereby protecting them from TRAIL-induced apoptosis and that this could be responsible for TRAIL's tumour-selective activity. However, an expression pattern of TRAIL-R3 and/or TRAIL-R4 in line with the decoy hypothesis could never be verified. Finally, it was found that TRAIL can also bind to a fifth receptor, Osteoprotegerin (OPG) [15]. OPG is a soluble TNFRSF member whose main function is to regulate the development and activation of osteoclasts in bone remodelling [16-18]. The OPG-TRAIL interaction is only of low affinity and OPG's high-affinity ligand in the TNFSF is RANKL [19]. The reported interaction of TRAIL with OPG is most likely not of high *in vivo* relevance as transgenic mice which express high levels of TRAIL do not develop any phenotype reminiscent of the OPG-deficient mice [20]. In summary, TRAIL has been shown to bind to five different receptors: the four membrane-bound TRAIL receptors TRAIL-R1 to TRAIL-R4 and the soluble receptor OPG.

TRAIL-R1 and TRAIL-R2 share 58% sequence homology and so far it has not been possible to identify clearly distinct functions of one receptor versus the other. They both trigger apoptosis *via* the same pathway. TRAIL-R3 lacks an intracellular domain and is inserted into the plasma membrane *via* a GPI anchor. TRAIL-R4 has a cytosolic domain but there is only a truncated DD of 15 instead of 80 amino acids which is not capable of inducing cell death [13]. However, TRAIL-R4 can activate NF-κB [13]. As mentioned above, TRAIL-R3 and TRAIL-R4 are often referred as "decoy receptors" as they were shown in some of the cloning papers to sequester TRAIL upon over-expression, thereby inhibiting TRAIL-induced apoptosis [9, 12]. Yet, to exert this death-inhibitory effect, TRAIL-R3 and TRAIL-R4 would have to present with a higher affinity for TRAIL or be expressed at substantially higher levels than TRAIL-R1 and/or TRAIL-R2. However, this is not the case [21]. Others have proposed a model in which TRAIL-R3 and TRAIL-R4 interact *via* a pre-ligand assembly domain to inhibit ligand binding [21]. A third notion suggests that the NF-κB-inducing activity of TRAIL-R4 may antagonise the death signal [13]. In summary, more than a decade after these receptors were cloned, we still know very little about the physiological function of these non-apoptosing receptors of TRAIL.

The apoptotic signalling pathways triggered by TRAIL is very similar to the one described for CD95. Apoptosis induction by TRAIL or CD95L is triggered by ligand-mediated cross-linking of the cognate receptor(s) resulting in recruitment of FADD which in turn recruits caspase-8, caspase-10 and the cellular FLICE-like inhibitory protein (cFLIP). Together, these proteins constitute the death-inducing signalling complex (DISC) [22]. FADD is recruited by a homotypic interaction of its DD with the cytoplasmic DD of CD95, TRAIL-R1 or TRAIL-R2 whilst recruitment of caspase-8, caspase-10 and cFLIP requires homotypic interactions between the DED of FADD and the N-terminal DED of the caspases of cFLIP. Activation of caspase-8, like activation of caspase-10 occurs *via* DISC-recruitment-induced homodimerisation of the caspases which induces an activating conformational change. Importantly, it is not the cleavage that activates the DISC-associated initiator caspases but the conformational change induced by DISC recruitment enabling homotypic interaction [23]. Recently it was also reported that ubiquitination of caspase 8 at the DISC was a crucial event for its activation [24]. Ubiquitination is an important mechanism of regulation of the TNFR signalling complex TNF but less described for the CD95 and the TRAIL system. The study of Jin *et al.* shows for the first time that death receptor ligation induces polyubiquitination of caspase-8 through a previously unknown interaction of the DISC with the E3 ligase cullin-3, and that the ubiquitin-binding protein p62 promote aggregation of polyubiquitinated caspase-8, leading to full activation and processing of the enzyme crucial for the induction of cell death.

The active caspase-8 or -10 are then able to cleave caspase-3 and BID. Caspase-3 is the most important effector caspase which will cleave a number of vital cellular proteins including structural components such as lamins and gelsolin but also other proteins like poly(ADP)-ribose polymerase

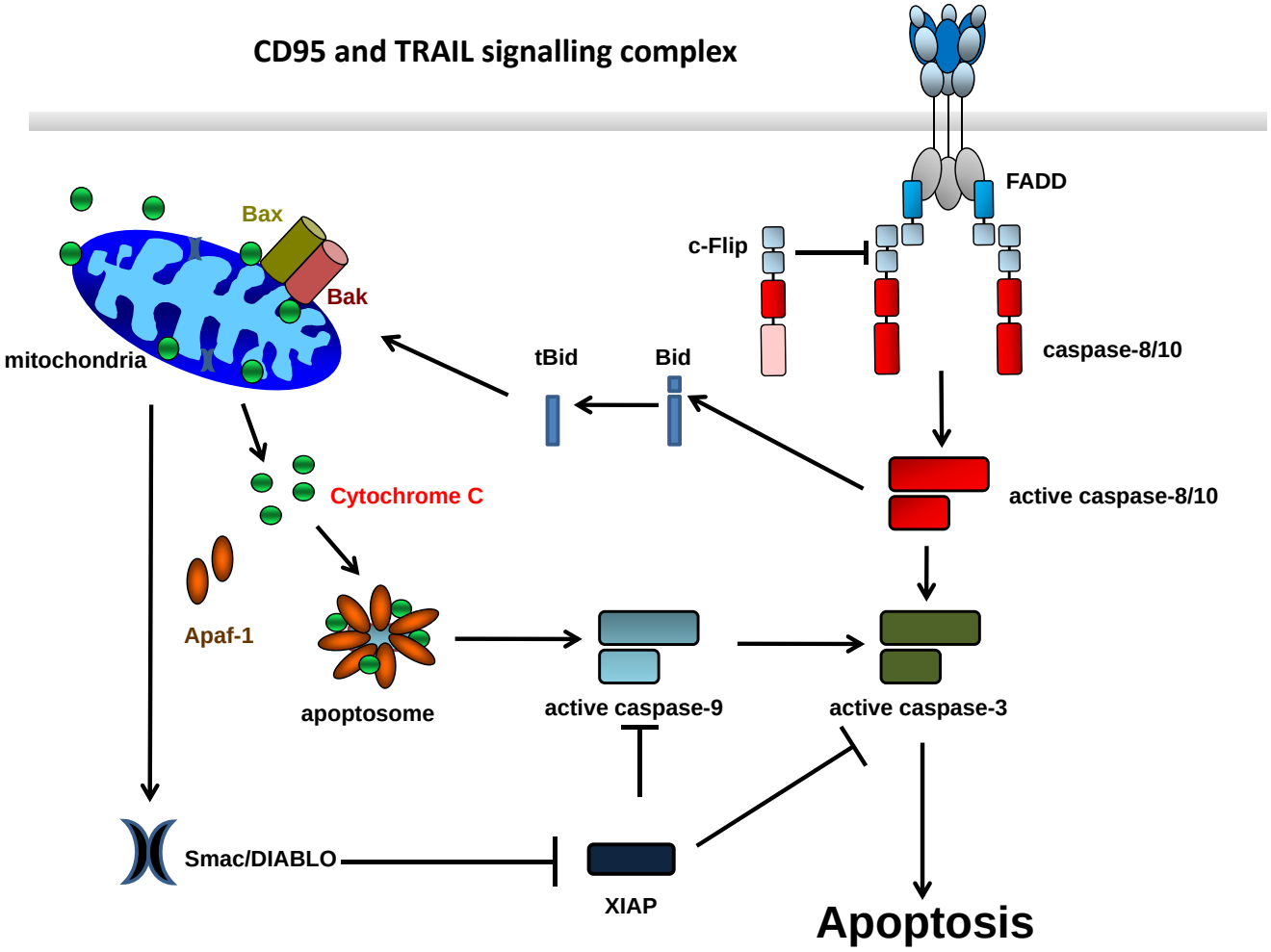

Fig. (2). Schematic representation of the TRAIL and CD95 signalling network. Binding of CD95 or TRAIL to their respective receptors leads to receptor trimerisation and formation of the death-inducing signalling complex (DISC). The adaptor protein FADD is recruited to the DISC, this results in recruitment of procaspase-8 and -10. cFLIP can compete with caspase-8 for the binding to FADD. DISC-activated caspase-8 and -10 trigger a caspase cascade *via* cleavage of consequent activation of caspase-3. In addition, Bid is cleaved to tBid. which induces MOMP resulting in release of cytochrome c (CytC) and Smac/DIABLO from the mitochondrial intermembrane space. CytC, together with Apaf-1 and caspase-9 forms the apoptosome, which serves as the activation platform for caspase-9. Smac/DIABLO counteracts the inhibitory function of XIAP, thereby allowing for full activation of caspases 3 and 9, ultimately leading to cell death.

(PARP), and the inhibitor of caspase-activated DNAse (ICAD).

Unexpectedly, some similarities between the CD95 and TRAIL death-receptor signalling pathways and the Hedgehog signalling can be highlighted. Indeed, as discussed in depth in this edition by Mark Ditzel, even if Sonic-Hedgehog signal is generally considered as a survival signal, it also seems to play a pro-apoptotic role in some developmental death processes. Interestingly the Hedgehog receptor Patched has recently been described as a dependence receptor able to transmit a pro-apoptotic signal when its cognate ligand Hedgehog is absent. Similarly to CD95, TRAIL-R1 or TRAIL-R2, Patched receptor contains an intracellular domain which is essential for its pro-apoptotic function since it leads to the formation of a pro-apoptotic complex defined as “dependosome”, which leads to caspase activation.

Proteolysis of effector caspase substrates is then responsible for the biochemical and morphological hallmarks of apoptosis. The anti-apoptotic factor cFLIP can prevent TRAIL- or CD95-induced apoptosis at the level of DISC-associated activation of caspases 8 and 10. cFLIP is structurally very similar to these caspases but lacks enzymatic activity as a protease due to absence of a cysteine residue in the position that otherwise would be its active centre.

BID is a pro-apoptotic BH3-only family member. Caspase-8 and -10 can cleave BID resulting in the generation of truncated BID (tBID). Truncation of BID activates its pro-apoptotic activity by enabling its translocation from the cytosol to the outer mitochondrial membrane. At this site tBID induces mitochondrial outer membrane permeabilisation (MOMP) if the molecular make-up of the cell regarding other members of the Bcl-2 protein family

allows it to do so (excellently discussed by Grant Dewson in this edition). In the context of CD95 and TRAIL-induced apoptosis it is important to note that cleavage of BID to tBID provides the link between the death receptor and the mitochondrial pathways of apoptosis induction. Although BID cleavage occurs in many cells that undergo CD95- and TRAIL-induced apoptosis it is only required for apoptosis induction by TRAIL and CD95L when the cells belong to a type of cells referred to as type II cells. Type I cells on the other hand are cells that do not require BID cleavage for CD95L- or TRAIL-induced cell death. The differentiation into type I and type II cells was first thought to be due to differences in DISC formation. However, from a number of biochemical studies published over the last decade it became clear that the actual culprit for the difference between type I and type II cells was further downstream in the pathway, namely the absence (in type I cells) or presence (in type II cells) of a protein called X-linked inhibitor of apoptosis protein (XIAP). Finally genetically proof for this was recently provided in an elegant study by Jost *et al.* [25]. Apart from inducing the release of cytochrome C from the mitochondrial intermembrane space, MOMP also induces the release of a second mitochondrial activator of caspases (SMAC), also known as direct inhibitor of apoptosis-binding protein with low pI (DIABLO) [26]. Cytochrome C release leads to apoptosome formation and activation of caspase-9. Release of SMAC/DIABLO, on the other hand, results in neutralisation of XIAP [27]. Once XIAP is inhibited, the effector caspases 3, 7 and 9 can fully mature and apoptosis can ensue (Fig. **2**). Therefore, cells which express high levels of XIAP cannot directly activate caspase-3 following DISC-induced caspase-8/10 activation which is why in these cells BID cleavage and subsequent pro-apoptotic events at the mitochondria are required for apoptosis to occur. Thus, XIAP expression or lack thereof classifies cells as type I and type II cells for CD95-induced apoptosis. It is possible that the intensity of DISC formation, first thought to be causative for type I/type II classification, either contributes to this or represents a marker that correlates with presence and/or absence of XIAP expression. The reasons for this correlation remain to be uncovered and it may be rewarding to elucidate them.

The high divergence between cells in terms of TRAIL-sensitivity is still not completely understood. Spencer *et al.* [28] proposed an interesting hypothesis stating that differences in the levels or states of proteins regulating receptor-mediated apoptosis would be the primary causes of cell-to-cell variability and probability of death and that these differences are not genetically determined.

As elegantly discussed in Gentle and Nachbur's review in this edition, "Scorched earth or viral birth", many viruses have evolved strategies to interfere with death-receptor mediated pathways which represent a potent and rapid mechanism to drive infected cells to kill themselves. Viruses can interfere with the main steps of the signalling pathways we have just described. They can lead to the sequestration of death ligands, to the downregulation of the death receptors themselves, they are also able to encode serpins that directly inhibit caspase-8 or viral FLIP which can inhibit the proteolytic processing of pro-caspase-8 to its active form.

In summary, the CD95 and TRAIL systems are signalling pathwayswhich have been thoroughly studied and characterized, yet many of the regulatory mechanisms remain to be unravelled.

THE TNF SYSTEM

TNF is a homotrimer with a characteristic conformation known as the TNF fold. Binding of TNF to its two receptors, TNF-R1 and TNF-R2, primarily induces the activation of NF-kB and mitogen-activated protein (MAP) kinases, resulting in gene induction which often drives an inflammatory response (Fig. **3**), whereas induction of cell death can be regarded as the fail-safe, alternative result of TNF stimulation [29, 30]. TNF-R1 is expressed almost ubiquitously on all cells; TNF-R2, on the other hand is only expressed on cells of lymphoid origin [30]. TNF-R1 contains a DD and initiates the majority of TNF-induced biological activities including induction of cell death. Despite being devoid of a DD, TNF-R2 is also capable of inducing cell death but it remained elusive for quite some time how this receptor achieved this detrimental outcome [31-33]. Recently it could, however, be demonstrated that TNF-R2-induced apoptosis works indirectly by modulating the input and output of TNF-R1 signalling. The input modulation is due to TNF-R2-induced activation of TNF transcription mediated by non-canonical NF-kB activation. The output modulation is achieved by TNF-RS-induced depletion of TRAF2 and cIAPs which results in diminished gene-inducing and enhanced cell death-triggering capacity of TNF-R1. Thereby, expression of TNF-R2 can significantly modulate TNF-R1 signal transduction and other non-DD-containing members of the TNFRSF including CD40, CD30 and the TWEAK receptor FN14 may work in a similar manner.

As mentioned above, the main signalling receptor for TNF is TNF-R1. Binding of TNF to TNF-R1 induces receptor oligomerisation and recruitment of cytoplasmic signalling proteins leading to the formation of the TNF-R1 signalling complex (TNF-RSC) [30, 34]. TRADD is recruited to the DD of TNF-R1 *via* a homotypic DD interaction [35, 36]. TRADD in turn recruits the TNFR-associated factor-2 (TRAF2). Apart from TRADD also the receptor-interacting protein 1 (RIP1) is recruited to TNF-R1 *via* its own DD which can occur even in the absence of TRADD [37, 38]. Recruitment of TRAF2 (or TRAF5) by TRADD enables recruitment of cIAP1 and/or cIAP2 to the TNF-RSC [39]. Both, recruitment of TRAF2/5 and cIAP1/2 are required for poly-ubiquitination of RIP1 as well as activation of gene induction by NF-κB and MAP kinases and recruitment of the linear ubiquitin chain assembly complex (LUBAC) [39-44]. Ubiquitin chains can be formed *via* linkages of the ubiquitin subunits on different ε-amino groups of the 7 different lysines present in ubiquitin [45] or *via* the α-amino group at the amino-terminus of ubiquitin, the latter creating linear ubiquitin chains. Until recently it had been thought that poly-ubiquitin chains involved in TNF signalling are either linked *via* the ε-amino groups of ubiquitin's lysine 63 (K63) or K48. However, recent data obtained by us and others revealed that linear ubiquitin chains also play an important role in this process. TRADD/TRAF2/cIAP-mediated recruitment of LUBAC into the TNF-R1 signalling complex enables linear ubiquitylation

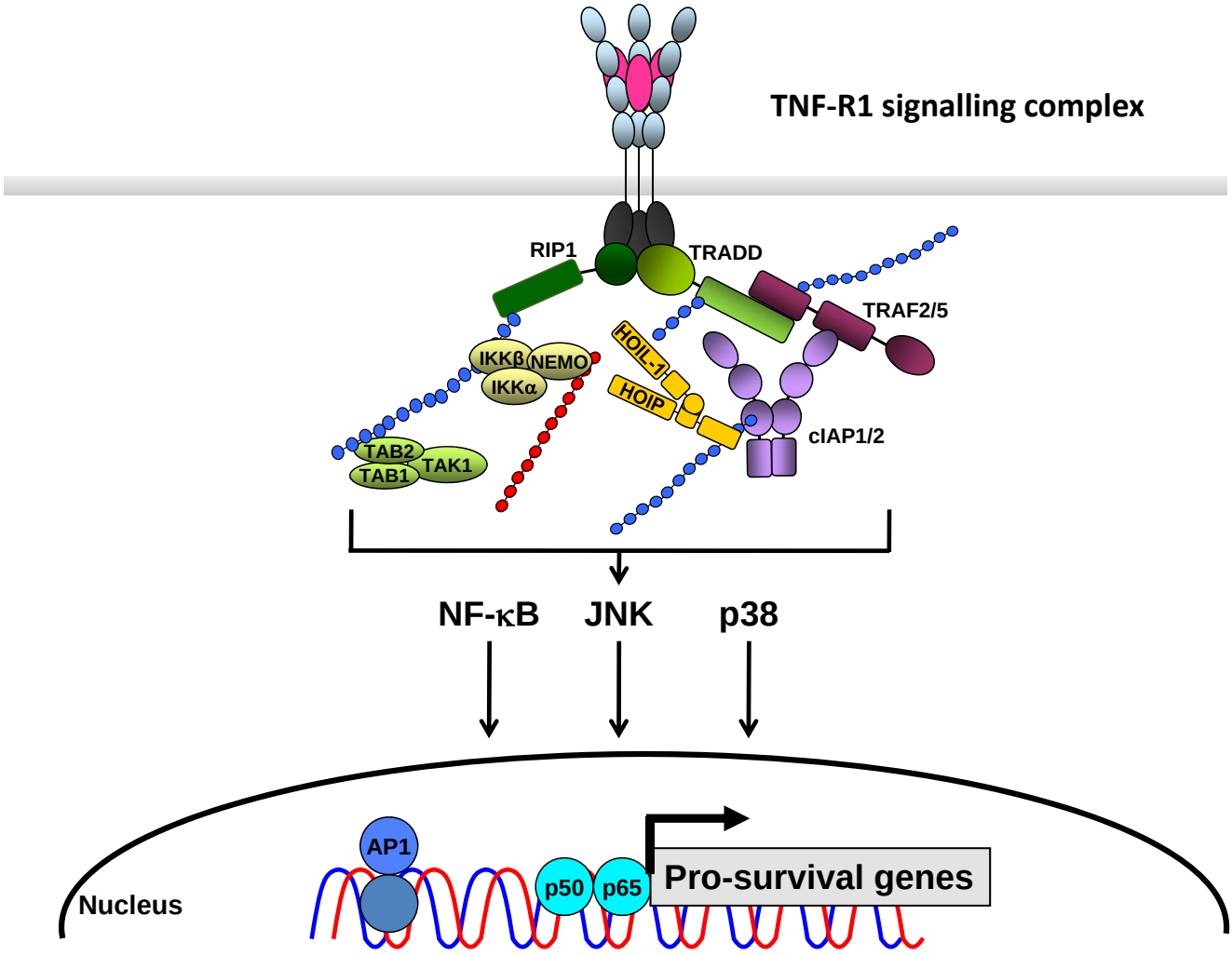

Fig. (3). Schematic representation of the TNF-R1 signalling complex. Binding of TNF to TNF-R1 leads to receptor trimerisation and formation of the TNF receptor signalling complex (TNF-RSC). Then, the signalling molecules TNF-R1 associated death domain (TRADD) and receptor interacting kinase 1 (RIP1) are recruited *via* their DDs to the receptor complex. TRADD then serves as an assembly platform for binding of TRAF2 which in turn recruits cIAP1 and cIAP2. These cIAPs then form ubiquitin chains which enable the recruitment of the linear ubiquitin chain assembly complex (LUBAC), the TAK-TAB complex and the IKK complex. RIP1 and TRAF2 are critical for the activation of the transcription factor nuclear factor κB (NF-κB) and the cJun-N-terminal kinase (JNK). Blue chains and red chains represent K63-linked and linear ubiquitin chains, respectively.

of NEMO and most likely other targets within this complex. We found that LUBAC activity is required for efficient TNF-induced NF-κB activation. This is a consequence of NEMO, not only being linearly ubiquitylated itself [46], but also binding more strongly to linear than to K63-linked ubiquitin chains [44, 47]. Together, K63-linked and linear ubiquitylation of different components of the TNF-RSC result in stable TNF-RSC formation and thereby enable the events which ultimately lead to activation of NF-κB and MAP kinases. Although we are only beginning to shed light on the role of LUBAC, its recent discovery as an integral component of the TNF-RSC and linear ubiquitylation as a central player in the organisation of this protein complex will undoubtedly substantially affect our current view of how these processes are regulated. It will be exciting to unravel these mechanisms at the molecular level and to understand how they control the function of TNF as well as other cytokines and immuno-stimulatory ligands.

FORMATION OF SEQUENTIAL SIGNALLING COMPLEXES AS A PRINCIPAL IN DEATH RECEPTOR SIGNAL TRANSDUCTION

The TRAIL- and TNF-induced receptor-associated signalling complexes described above form at the plasma membrane. However, they are not the only signalling complexes which form in the cell when TNFSF ligands activate DD-containing TNFRSF receptors. Following formation of the receptor-associated protein complex, referred to as complex I, biochemical changes within the complex which are not yet understood induce loss of affinity of the adaptor proteins FADD and TRADD for their respective receptors. Together with at least some of the factors they recruited to the respective receptors they then

form a secondary, cytoplasmic signalling complex, complex II. This complex can recruit further proteins to the liberated DDs of FADD or TRADD, respectively. Intriguingly, in both cases complex II is capable of inducing the very signals not induced by the respective complex I: i.e. complex II derived from TRADD-binding receptors induces signals which can lead to apoptosis and complex II of FADD-binding receptors induce gene activation resulting in pro-inflammatory signalling. However, when the primary signals of the respective complex I prevails, the outcome of secondary signalling from complex II is often neutralised by the very effects triggered by the primary complex (Fig. **4**). TRAIL and TNF can then both induce NF-κB activation and, consequently, the activation of pro-survival genes by different signalling complexes. As thoroughly described in Ekert and Jabbour's review in this edition, cytokines like IL-3 and GM-CSF also exert a role in cell survival. This interesting review highlights that the intracellular domains of the IL-3 and GM-CSF receptors can act as docking sites for adaptor molecules involves in survival signalling such as activation of the PI3K/AKT, RAS/RAF/ERK and JAK/STAT pathways.

The new concept of the formation of sequential signalling complexes in TRAIL and TNF signalling was first introduced for TNF-R1 in a landmark paper by Micheau & Tschopp in 2003 [48]. They found that signalling by this receptor involves the formation of two sequentially occuring complexes leading to activation of transcriptional programmes and induction of apoptosis, respectively. The first complex forms at the plasma membrane when TNF crosslinks TNF-R1. Formation of this protein complex, referred to as complex I, involves different biochemical steps which mainly comprise of phosphorylation and ubiquitylation events with the result of inducing transcriptional activity. The cytoplasmic complex II is derived from complex I and induces apoptosis, provided complex I-induced signalling does not impede this (Fig. **4**). It is likely, however not yet shown, that the same or at least a similar process occurs downstream of DR3 stimulation.

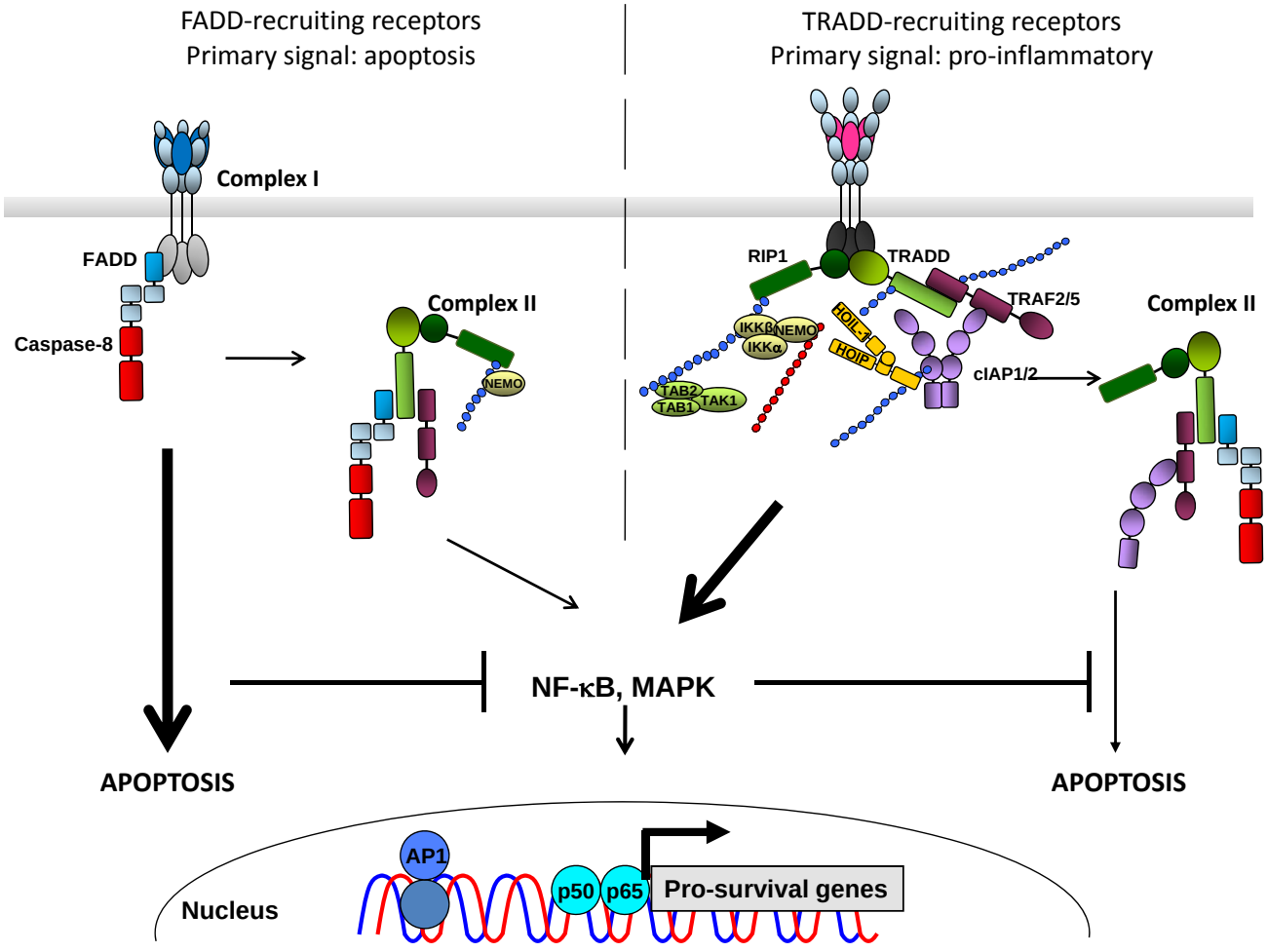

Fig. (4). Composition and signal triggered by complex I and complex II of FADD and TRADD-recruiting receptors. For the FADD-recruiting receptors (CD95 and TRAIL) as well as the TRADD-recruiting receptors (TNF and DR3), the complex defined as complex I is the protein complex which forms at the plasma membrane and exerts the primary function of the respective receptor, i.e apoptosis for CD95 and TRAIL-R1/R2 and gene induction *via* NF-κB and MAPK activation by TNFR-R1 and DR3. By an undetermined mechanism, the different complexes I dissociate from the DD of the respective receptor and recruit additional proteins from the cytosol and trigger the secondary function of each receptor. In the case of pro-apoptotic receptors this is gene induction *via* activation of NF-kB and MAPKinases pathways; in the case of the primarily immunostimulatory, pro-inflammatory receptors it is induction of apoptosis.

More specifically, release of TRADD from TNF-R1 together with the majority of the signalling proteins that it either directly or indirectly recruited to this complex leads to the formation of complex II. Complex II then recruits FADD, presumably to the DD of TRADD which is freed since it left the DD of the receptor behind. Then the initiator caspases 8 and 10 are recruited to FADD and, initiated by this intracellular secondary DISC, the cell can now undergo apoptosis. The outcome of complex II signalling depends on the result of complex I signalling; in most circumstances gene induction triggered by complex I which forms earlier than complex II leads to an increase in the expression of cFLIP. This is thought to be causative for interfering with effective pro-apoptotic complex II formation by inhibiting the activation of caspase-8 and -10 at this cytoplasmic DISC (Fig. **4**). However, the biochemical trigger for TRADD release from the receptor, and hence for complex II formation, remains elusive and it is possible that the TNF signalling output is also controlled at this level.

For the FADD-recruiting receptors it has in turn been shown that upon release of FADD from the TRAIL DISC, i.e. the complex I in this system, complex II recruits TRAF2, RIP, NEMO and possibly a number of other proteins – including TRADD and cIAP1/2 – required to induce the activation of NF-κB as well as the JNK and p38 MAP kinase pathways [49]. Obviously, this pathway would only be induced in a productive manner in cells in which proper execution of the apoptotic cell death programme were blocked. Thus, this pathway is not the primary reaction of the cell to the stimulus provided by TRAIL (or CD95L) but rather the secondary, alternative outcome of activation of the direct apoptosis inducers.

The spatial and temporal separation of different biochemical tasks into discrete signalling complexes which act in different cellular compartments, i.e. in this case at the plasma membrane and in the cytoplasm, respectively, and which are activated sequentially in a hierarchical manner is striking and makes biological sense. In case the first signal prevails you do not need the second one and in fact it should probably be minimised. However, if the primary signal is not achieved then the second, deferred signal comes to the fore, opening new avenues to achieve a very different, seemingly opposing outcome.

One may wonder why biology does not try to achieve the same outcome, albeit *via* a different route, in the second attempt. It might be, however, that this is exactly what happens, yet in an unexpected manner. When a certain signalling outcome cannot be achieved, this most likely means there is a problem in its execution. In such situations biology often follows a new path. If a cell that should die does not do so this signals potential danger. This danger is sensed by the activation of the opposite of cell death; the activation of pro-inflammatory signalling attracts other cells of the innate immune system, and possibly also adaptive immune cells at a later time. This process is likely to be capable of handling the situation caused by the refused cell death. With respect to the TRADD binders, if proper immunostimulatory signalling, as supervised by induction of cFLIP, cannot be achieved, then cell death induction prevails. Cell death can either be immunogenic or non-immunogenic. It is not clear which type of cell death is induced by TNF when the gene-inductive path does not prevail but it is known that TNF can induce apoptotic and necrotic – even necroptotic – cell death. Our prediction would be that the cell death would at least in part be immunogenic and that thereby the same biological outcome, i.e. the creation of an immunostimulatory, pro-inflammatory environment, could be achieved, yet in a manner very different from the originally intended gene induction. Hence, it appears that cellular suicide and inflammation may be more tightly linked to each other than it seems at first glance.

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Received: May 2, 2010 Revised: August 21, 2010 Accepted: October 22, 2010