Modeling FLASH Participation in CD95-mediated Apoptosis using Pi-Calculus

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ABSTRACT

Mathematical models are often very useful in different fields such as Biology and Chemistry to provide a deeper and more insightful understanding of systems. One of the least explored and researched biological processes, is Apoptosis, which safely carries out cell suicide in multicellular organisms. We used Pi Calculus, a model of computation for concurrent systems, to represent this process and based it on a structured information model created by Martin Bentele. We only focused on the Apoptosis' extrinsic pathway, specifically the DISC system, and included FLASH(FLice ASsociated Huge Protein) - a component necessary in CD95-induced apoptosis - as proposed by different papers by Imai et al and Milovic-Holm et al. Imai et al states that FLASH is essential for the activation of caspase-8 and that the DED domain of FADD can interact with the DRD of FLASH, proving that FLASH is indeed a component of DISC. On the other hand, Milovic-Holm et al's model shows that CD95-induced activation of caspase-8 signals FLASH to be translocated to the cytoplasm from the nucleus, and then interacts with endogenous caspase-8 at the mitochondria. In this paper, we modeled the DISC system of Apoptosis and its interaction with FLASH using Pi-Calculus.

Keywords

CD95-induced Apoptosis, FLASH, Pi Calculus

1. INTRODUCTION

Apoptosis is a very complex signaling transduction that plays a crucial role in maintaining biological processes that occur in multicellular organisms. Defects in apoptosis can result in cancer, autoimmune diseases and spreading of viral infections [3]. Apoptosis is induced in a good number of ways with CD95-induced being one of the best studied. CD95 is a member of the death receptor family, TNF-R. When the CD95 receptor binds with its complementary CD95 ligand, apoptosis is induced. However, the eventual death of the cell is dependent on its overall state. The Jurkat cancer cell, for instance, is capable of inhibiting apoptosis [3].

CD95 induces two different apoptotic pathways: intrinsic and extrinsic. In the intrinsic pathway, the activated receptor was not able to generate a signal strong enough for the execution of cell death [1]. The signal must then be amplified through the mitochondria where pro-apoptotic proteins such as Bcl-2, BAX and BID release signals to promote the activation of the cytochrome c. Cytochrome c will form a complex system in the cytoplasm. This complex system will activate Caspase-9, and together will activate the effector-protein, Caspase-3, which is responsible for the degradation of the cell. In the extrinsic pathway, the activated receptor starts the caspase cascades, through the activation of Caspase-8. This is done by recruiting the death-inducing signaling complex (DISC), which is responsible for Caspase-8 activation.

Bentele's CD95-induced apoptosis is roughly divided into three compartments: DISC System, Caspases and the Mitochondria. The DISC System determines whether an extrinsic pathway is successful or not. This is because the activation of Caspase-8, which activates the caspase cascades, is determined in the DISC system. Understanding apoptotic pathways of Type I cells (cells undergoing the extrinsic pathway) can then be confined to the study of the DISC system making the model a lot simpler.

2. PI-CALCULUS

Pi-calculus, a member of the process calculi family, is a calculus of communicating systems (CCS) that allows the representation of concurrent computations whose configuration may change during the computation. It is developed by Engberg and Nielsen who decided to extend this calculus with mobility while maintaining its algebraic attributes. Pi-Calculus was developed by Robin Milner, Joachim Parrow, and David Walker as a continuation to CCS. The role of names is central to pi-calculus since it is used to identify variables, constants, and communication links – the basic entities used in pi-calculus. Computations, on the other hand, can be represented as communications between these units. In contrast to λ-calculus, which represents computations through functions, pi-calculus uses the process as an abstraction of an independent thread of control [12]. A channel or link is an abstraction of the communication link between processes. Processes interact by sending information through these channels. Furthermore, mobility has an important role in pi-calculus since links are also allowed to be exchanged through links themselves. The following constructs, presented with their notations and the operations defined are available in the calculus. Let P and Q represent processes.

Operation	Notation	Meaning
Prefix	π.P	Sequence
Action	a(x).P, ā<x>.P	Communication
Summation	a.P + b.Q $\sum_{i=1..n} P_i$	Distributed Choice
Recursion	P={.....}.P	Repetition
Replication	!P	Repetition
Composition	P | Q	Concurrency
Restriction	(νx)P	Encapsulation
Nothing	0	Do nothing

Table 1. Pi-Calculus Constructs [8]

These operations have the following meaning:

· τ.P reads as τ and then do P. ;

· a(x).P, this binds the name y in P and read as "input some name called x on the link called a and then use it in P";

· ā<x>.P reads as "output the name y on the port named x, and then do P";

· P+Q means that either P or Q may occur, which gives the pi-calculus the ability to model distributed choice. This denotes a process that behaves like P or Q;

· Σi=1..n Pi means that any of Pi may occur and is a shorthand form of the "+" operator being equivalent to P1 + P2 + … Pn;

· P|Q means that the processes P and Q are concurrently active (this is the construction which really gives the power to model concurrency to the pi-calculus);

· (νx)P, which binds the name x in P, means that the usage of x is "restricted" to the process P. It ensures that x is a fresh channel in P;

· !P means that there are infinitely many processes P running in parallel;

· 0 is the null process that does nothing.

Aside from representing processes, concurrency within processes, communication between processes through links, restriction of new channels, and replication of processes, pi-calculus can also express non-determinism. On the other hand, stochastic pi-calculus allows rates to be assigned to communication actions. This is useful in simulating systems such as chemical reactions wherein probability plays an important role.

3. PI-CALCULUS AND BIOCHEMICAL SYSTEMS

According to a paper by Regev and Shapiro [9], computer science plays an important role in providing a better understanding of biochemical systems through abstractions. The paper also discussed the properties of a good abstraction – relevant, computable, understandable, and extensible.

Biochemical systems can be divided into four groups that require different approaches.[9]

a. Chemical Kinetic Models - Compared to the others, this approach is already well developed, and well understood. These models are based on the 'cell-as-collection-of-molecular-species'. Although it is considered as extremely powerful, it lacks in relevance and understandability when it comes to biological systems. Biological models are composed of molecular objects, unlike chemical models, which consider the cell as one monolithic entity.

b. Generalized Models of Regulation - Boolean network models or generegulatory circuits are simulated using a 'cell-as-logical-expression' abstraction. These are normally used in studying properties of larger networks, and other systems that possess only qualitative properties. Some limitations are evident when it comes to relevance, computability, and extensibility.

c. Functional Object-Oriented Databases - Pathway databases are viewed as a 'molecule-as-object' abstraction. Molecular interactions and pathways are stored in databases that are equipped with querying tools. One disadvantage of these models is that they provide little if any dynamic capabilities, thus, leading to very limited querying tools.

d. Abstract Process Languages Applied to Biomolecular Systems – The focus of this paper will be on this last approach, which is based on the 'molecule-as-computation' abstraction. λ-calculus and Petri nets are included in this classification. Petri nets have been widely used for representation, simulation, and analysis of metabolic pathways. Pi-calculus belongs to this approach, since it is an abstract process[9].

4. BENTELE ET. AL'S MODEL

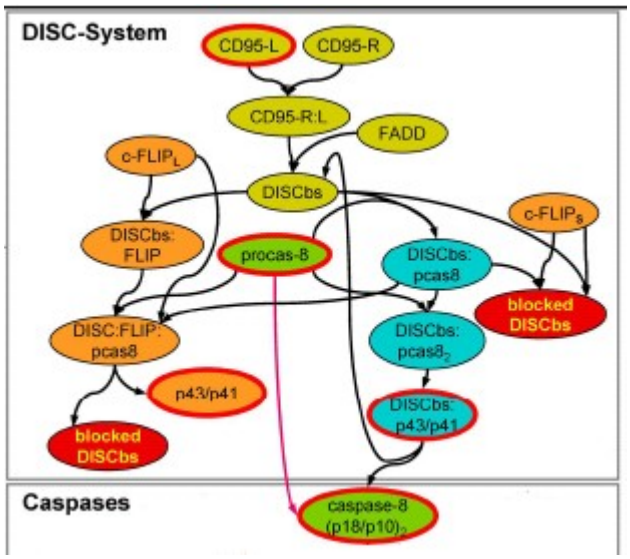

Figure 1. Bentele et al's model

Bentele et al's model is roughly divided into three compartments: DISC system, Caspases and Mitochondria. Since we focused on the extrinsic pathway induced by the CD95 receptor, we removed other compartments in the model. What remained are the DISC system and the active Caspase-8 in the Caspases.

First, the CD95 receptor (CD95-R) binds with the CD95 ligand (CD95-L) to form the complex CD95-R:L. This complex will then bind with the adapter molecule, FADD, forming the DISC binding site (DISCbs). DISCbs may or may not activate Caspase-8 depending on the molecule it binds with. It is instantly blocked when it binds with the inhibitor short-strand c FLIP (c-$FLIP_S$). If, on the other hand, it binds with the long-strand c FLIP (c-$FLIP_L$), it still needs to bind with Procaspase-8 before it can produce a blocked DISCbs and p43/p41. Although a DISCbs bind to a p43/p41 is not instantly blocked, technically, it can be considered blocked, since there is no way p43/p41 can activate Caspase-8.

For DISCbs to be able to activate Caspase-8, it needs to bind with two Procaspase-8 molecules. In each of these two binding process, DISCbs can still bind with the inhibitor c-FLIPs. The complex that resulted from the binding of DISCbs and two Procaspase-8 molecules will eventually activate Caspase-8 and leave DISCbs intact and ready for binding once more with other molecules.

5. IMAI ET AL'S EXPERIMENTAL RESULTS

It is claimed that FLASH is necessary for the activation of Caspase-8 in Fas-mediated apoptosis[13]. The complex responsible for the activation of Caspase-8 is the DISC, which is composed of Fas, FADD and Procaspase-8. Procaspase-8 becomes proteolytically autoactivated by oligomerization, stimulating other caspases by cleaving them.

It was suspected that some other molecule(s) apart from the three component of the DISC could activate Caspase-8. It has also been shown that the adenovirus E1B19K, which is unable to bind with Fas and FADD, can indirectly inhibit the activation of Caspase-8. This implies an unknown protein that links E1B19K to the DISC - the Flice associated huge protein or FLASH. Once Fas signaling is stimulated, FADD and then the FLASH-Procaspase-8 complex are recruited.

Over-expression of Procaspase-8 or of the DED domains of Caspase-8 can induce apoptosis. It was proposed that FLASH may serve as a chaperone to control the conformation and local concentration of Procaspase-8 before stimulation of Fas; also, FLASH may promote the autocatalytic activity of Procaspase-8 with the help of FADD by binding to Procaspase-8 through DRD-DED interaction and oligomerizing Procaspase-8 through its CED-4-like domain after activation of Fas.

FLASH binds E1B19K, a member of the Bcl-2 family, preventing it from inhibiting Fas-mediated apoptosis. As E1B19K prevents apoptosis induced by FADD as well as by Fas, but not by Caspase-8, it probably works before Caspase-8 is activated and mitochondrial damage is induced by Bid. E1B19K could block formation of the DISC complex by binding to FLASH, and overexpressed FLASH might stop E1B19K binding to the complex FLASH with Caspase-8.

5.1 Integrated Model

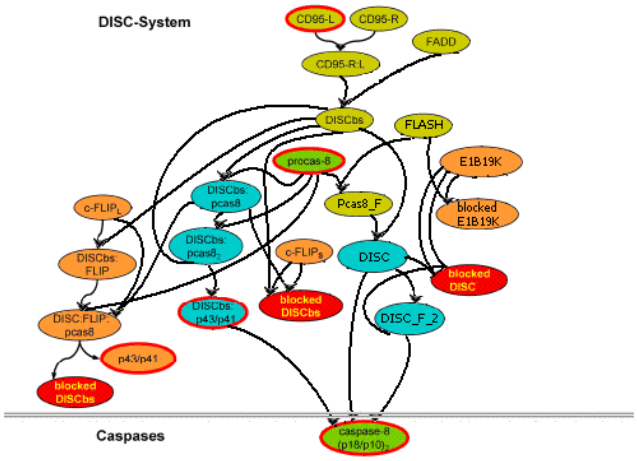

Figure 2. Bentele et al's model integrated with Imai et al's

We retained everything in Bentele's model of the DISC system. CD95L binds with CD95R to form the complex CD95LR which binds with FADD to form DISCbs. The DISCbs may or may not activate Caspase-8 depending on what molecules it binds with.

To integrate Imai's result, we added a new complex that is capable of activating Caspase-8. This complex, which is composed of the CD95LR complex, FADD, FLASH and Procaspase-8, can be form in two different sequences. FLASH may bind with Procaspase-8 and then bind with the DISCbs formed from the binding of the CD95LR complex and FADD. Another is, FLASH binds first with FADD and then binds with the CD95LR complex and finally with Procaspase-8. To keep the model simple, we assumed a particular sequence in the formation of DISC. Since Imai mentioned the first sequence more frequently, it is the binding sequence we will adopt.

FLASH that is in the DISC promotes the autocatalytic activity of Procaspase-8. This results in the activation of Caspase-8. Thus, in this new model, there are two pathways leading to the activation of Caspase-8: formation of the complex DISCbs_Pcas8_2 and formation of the DISC.

The addition of FLASH entails the addition of an inhibitor molecule that binds with it, the adenovirus protein E1B19K. E1B19K binds with FLASH in two different ways. When E1B19K binds with an isolated FLASH, the anti-apoptotic effect of E1B19K is inhibited. This is because E1B19K can only bind with FLASH once. Another is when E1B19K binds with FLASH that is in the DISC. FLASH bound to E1B19K will not be able to aid Procaspase-8 in proteolytically cleave Caspase-8.

In Bentele's model, after the complex DISCbs_Pcas8_2 has activated Caspase-8, DISCbs remains intact and can be used again as a component of a new DISCbs. In Imai's paper however, it is

not stated that the DISCbs, or the complex CD95LR with FADD, can bind again with other molecules. Since the paper has not mentioned anything about the reuse of the complex CD95LR-FADD, we assumed that the DISCbs will be used up in the process of Caspase-8 activation.

5.2 Specification Matching (Bentele et al. + Imai et al. Model)

The code in this section is based on the convention defined by SpiM (Stochastic Pi Machine).

The system starts with the binding of CD95L with CD95R forming the CD95LR complex:

CD95L ::= !bind_CD95_LR

CD95R ::= ?bind_CD95_LR; ***CD95LR***

This complex may then bind with FADD to form DISCbs:

CD95LR ::= !bind_CD95LR_FADD

FADD ::= ?bind_CD95LR_FADD; ***DISCbs***

DISCbs may bind with inhibitor molecules such as cFLIPs and cFLIPl:

DISCbs ::= do !bind_DISCbs_cFLIPs
or !bind_DISCbs_sFLIPs

cFLIPs :: = ?bind_DISCbs_cFLIPs; ***Blocked_DISCbs***

cFLIPl :: = ?bind_DISCbs_cFLIPl; ***DISCbs_FLIP***

Blocked_DISCbs ::= τ; ***Blocked_DISCbs***

DISCbs binding with cFLIPs is instantly blocked (blocked_DISCbs). This complex is no longer capable of binding with other molecules present in the system and thus in no way can activate Caspase-8.

Unlike cFLIPs, cFLIPl binding with Caspase-8 will not be instantly blocked. It still needs to bind with Procaspase-8 to produce a complex that will eventually block DISCbs and produce another substance, the p43/p41:

DISCbs_FLIP ::= !bind_DISCbs_FLIP_Pcas8

Procas8 ::= ?bind_DISCbs_FLIP_Pcas8;
DISCbs_FLIP_Pcas8

DISCbs_FLIP_Pcas8 ::= τ; (***Blocked_DISCbs*** | ***P43_P41***)

P43_P41 ::= ( )

It is possible that p43_p41 still plays a role in the apoptotic pathway, but at the moment, we do not have papers that support such claim.

Aside from the inhibitor molecules cFLIPs and cFLIPl, DISCbs may also bind with the pro-apoptotic molecule Procaspase-8:

DISCbs ::= !bind_DISCbs_Pcas8

Procas8 ::= ?bind_DISCbs_Pcas8; ***DISCbs_Pcas8***

DISCbs_Pcas8 may promote Caspase-8 activation by over-expressing Procaspase-8. This can be done by binding once more to Procaspase-8 to produce DISCbs_Pcas8_2. Over-expressing Procaspase-8 makes it possible to proteolytically cleave itself that will lead to the eventual activation of Caspase-8. Like in DISCbs_Pcas8, cFLIPs and cFLIPl are still capable of binding and blocking the DISCbs with over-expressed Procaspase-8:

DISCbs_Pcas8 ::= do !bind_DISCbs_Pcas8_2
or !bind_DISCbs_cFLIPs
or !bind_DISCbs_cFLIPl

cFLIPs ::= ?bind_DISCbs_cFLIPs; **Blocked_DISCbs**

cFLIPl ::= ?bind_DISCbs_cFLIPl; ***DISC_FLIP_Pcas8***

Procas8 ::= ?bind_DISCbs_Pcas8_2; ***DISCbs_Pcas8_2***

Blocked_DISCbs ::= ( )

The DISCbs used to activate Caspase-8 stays intact and can again bind with pro-apoptotic bodies to activate another Caspase-8 molecule.

DISCbs_Pcas8_2 ::= τ; ***DISCbs_P43_P41***

DISCbs_p43_p41 ::= τ; (***Caspase8*** | ***DISCbs***)

Caspase8 ::= ( )

The addition of FLASH provides another way of activating Caspase-8. Procaspase-8 recruited to the DISCbs needs not be over-expressed. This is because FLASH aids Procaspase-8 to proteolytically cleave itself to Caspase-8.

DISC formation starts with the binding of FLASH with Procaspase-8:

FLASH ::= !bind_Pcas8_F

Procas8 ::= ?bind_Pcas8_F; ***Pcas_F***

Pcas8_F then binds with the DISCbs to form the DISC:

Pcas8_F ::= !bind_DISCbs_Pcas8_F

DISCbs ::= ?bind_DISCbs_Pcas8_F; ***DISC***

As said earlier E1B19K binding with FLASH can be viewed in two ways. First, E1B19K binds with FLASH that is in the DISC. E1B19K inhibits the DISC from activating Caspase-8:

E1B19K ::= !bind_E1B19K_DISC

DISC ::= do ?bind_E1B19K_DISC; ***Blocked_DISC***

or τ; ***Caspase8***

Blocked_DISC ::= ( )

Another is, E1B19K binding with an isolated FLASH. In this sense, FLASH inhibits the anti-apoptotic effect of E1B19K:

E1B19K ::= !bind_E1B19K_FLASH

FLASH ::= ?bind_E1B19K_DISC; ***Blocked_E1B19K***

Blocked_E1B19K ::= ( )

Once DISC was able to cleave Caspase-8, we can say that the CD95-signaling pathway was successful.

6. MILOVIC-HOLM ET AL'S EXPERIMENTAL RESULTS

Recent studies by Milovic-Holm [14] presented the role of FLASH in CD95-mediated apoptosis in a different light compared to Imai [13]. Milovic-Holm et al's paper indicates that FLASH is located in the nuclear bodies and cell nucleus. CD95-induced caspase-8 activation leads to the translocation of FLASH to the cytoplasm through the facilitation of Crm1-dependent nuclear export pathway. While FLASH was recognized to be capable of enhancing CD95-mediated apoptosis, Milovic-Holm et al proposed that FLASH interacts with endogenous caspase-8 at the mitochondria [14].

Unlike Imai [13], Milovic-Holm failed to detect an interaction between the CD95 DISC and FLASH. Imai et al used a different DISC precipitation protocol, which may have led to this discrepancy.

The anti-apoptotic adenovirus E1B19K was shown to trap endogenous procaspase-8 and FLASH at the mitochondria. Experiments confirm that CD95-induced caspase-8 activation was blocked in E1B19K-expressed cells, while the E1B19K-FLASH complex remained stable after CD95 activation [14]. This suggests that E1B19K interferes with caspase-8 activation at the mitochondria.

FLASH is illustrated to form a molecular complex with caspase-8, activating the mitochondrial apoptosis pathway by regulating caspase-8 activity. E1B19K can then interfere by forming a ternary complex with caspase-8 and FLASH at the mitochondria. This signaling pathway is significant, as apoptosis induction by other death receptors also needs caspase-8 activation.

Milovic-Holm et al further investigated how FLASH can affect CD-95-induced apoptosis by depleting FLASH through RNA interference. Results showed that depletion of FLASH led to inhibition of CD95-induced apoptosis as exhibited by the observation that the formation of the active p18 subunit was reduced [14].

6.1 Integrated Model

Like Imai et al's integrated model in section 5, we retained Bentele's model of the DISC System and added some molecules used in Milovic-Holm et al's experiment.

To integrate the Milovic-Holm et al's results, we included two blackboxes: the nucleus and the mitochondria. The endogenous FLASH protein is required to trigger cytoplasmic translocation through the activation of Caspase-8 at the DISC. After that, FLASH is targeted to the mitochondria and colocalizes with Caspase-8. We also added the inhibitor protein, E1B19K inside the mitochondria. It has been previously stated that the large fraction of this inhibitor localizes to mitochondria during adenovirus infection and through a confocal microscopy, a stably expressed E1B19K protein is predominantly present at the mitochondria [14]. The complex formation of FLASH and Caspase-8 is trapped by E1B19K protein at the mitochondria providing an explanation of its inhibitory effect. To be consistent, we still retained the Bid and tBid molecules of Bentele in the mitochondria. The cleavage of Bid is triggered by the activation of Caspase-8.

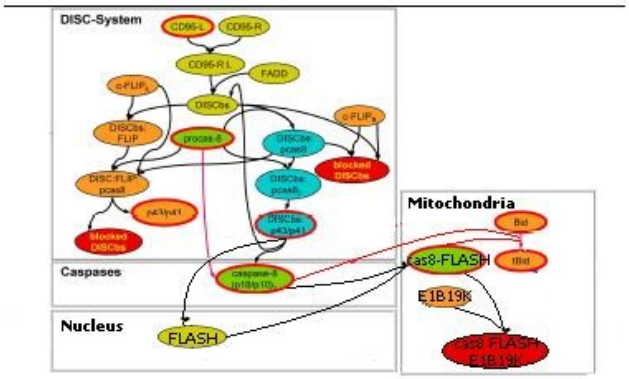

Figure 3. Bentele et al's model integrated with Milovic-Holm et al's

6.2 Specification Matching (Bentele et al. + Milovic-Holm et al. Model)

The code in this section is based on the convention defined by SpiM (Stochastic Pi Machine).

In this integration, we are no longer confined to the DISC system. FLASH is said to be translocated from the nucleus to the mitochondria once Caspase-8 is activated.

Since we only inserted Milovic-Holm's model to Bentele's model, it is no longer necessary to show that our Pi-calculus implementation of the DISC system matches the our model.

The system will start with the complex DISC_p43_p41 activating Caspase-8. During this activation, a signal is sent to the nucleus for the translocation of FLASH to the mitochondria:

DISC_p43_p41 := (!activate_Cas8 | !signal_nucleus)

Nucleus ::= ?signal_nucleus; ***FLASH***

The translocated FLASH may then bind with Caspase-8:

FLASH ::= !bind_FLASH_Cas8

Caspase8 ::= ?bind_FLASH_Cas8; ***Cas8_FLASH***

This complex can bind with E1B19K inhibiting it from cleaving Bid:

E1B19K ::= !bind_E1B19K_Cas8_FLASH

Cas8_FLASH ::= do ?bind_E1B19K_Cas8_FLASH;
*Blocked*_Cas8_FLASH
or !cleave_Bid

Blocked_Cas8_FLASH ::= ( )

Bid ::= ?cleave_Bid; **tBid**

tBid ::= ( )

7. CONCLUSION

Modeling of the whole process of Apoptosis according to Bentele et al's paper has been simulated in a previous study done by Jan Michael Yap [15]. In this paper however, we attempted to integrate FLASH into the existing model by Bentele based on two separate studies by Imai and Milovic-Holm, which showed the vital role of FLASH in both type I and type II cells. Through specification matching, we have modeled the DISC system of Apoptosis and its interaction with FLASH using Pi-Calculus.

Results of Imai's experiments indicate that FLASH is required in the activation of caspase-8, and that it is involved in CD95- and TNF-induced apoptosis, both mediated by activated caspase-8. Imai et al's paper identified FLASH through the process of cloning its complementary DNA. It contains the DED-like DRD domain, an adaptor sequence that mediates interactions between proteins of the apoptosis machinery such as FADD and caspase-8.

On the other hand, Milovic-Holm found FLASH interacting with Sp100 (speckled 100-kDa protein), a constitutive component of PML nuclear bodies. Their observation revealed that this protein harbors nuclear localization signals. This implies that FLASH and its nucleo-cytoplasmic translocation are important for CD95-mediated apoptosis.

The findings of these two models illustrate the mechanism of action of FLASH in both type I and type II cells. Although different protocols and means of identifying the location of FLASH were used in the two experiments, both illustrated that FLASH is significant in CD95-mediated apoptosis.

In addition, FLASH does not only transmit apoptotic signal during death receptor-induced apoptosis; but recent studies by Sug Hyung Lee showed that this pro-apoptotic protein contributes to biological roles such as cell-cycle progression and cell division.

It is suggested that another study be done to simulate these modified models with Stochastic Pi-Calculus. This can only be done if the required parameters such as the concentration and reaction rates are known.

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