A biochemical model of necroptosis explains cell type-specific responses to cell-death cues

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***Short Abstract* — We present a detailed computational model of tumor necrosis factor (TNF)-induced necroptosis. The model is fit to experimental time course data for phosphorylated mixed lineage kinase domain-like protein (pMLKL) and a dynamical systems analysis identifies four distinct modes of necroptosis execution, distinguished by rate constant values and roles of the deubiquitinating enzymes A20 and CYLD. Sensitivity analyses based on initial protein concentrations and rate constants identify potential targets for modulating necroptosis sensitivity within each mode. We conclude by associating numerous experimental studies from the literature with different model-predicted execution modes and address unresolved controversies regarding cell-type- and context-specific necroptosis execution.**

***Keywords* — Necroptosis, computational modeling, sensitivity analysis, modes of execution.**

Necroptosis is an alternative form of programmed cell death in which the cell membrane is ruptured, leading to immune response activation [1]. Although many of the primary molecular species involved in necroptosis have been identified [2], including receptor interacting protein kinase-1 (RIP1), RIP3, and mixed lineage kinase domain-like protein (MLKL), efforts to target necroptosis dysregulation or leverage it therapeutically are hindered by the lack of a detailed, mechanistic understanding of the biochemical pathways driving necroptosis execution [3].

Numerous published experimental studies have shown that RIP1 deubiquitination in complex I is driven by A20, CYLD, or both, depending on cell type. These varying reports have led to unresolved controversies about the specific molecular mechanisms driving necroptotic cell death [3]. Here, we present a detailed biochemical model of TNF-induced necroptosis derived from decades' worth of published experimental studies. The model was calibrated using Bayesian parameter inference to experimental protein time course data for phosphorylated mixed lineage kinase domain-like protein (pMLKL), an established necroptosis reporter, from a well-established necroptosis-executing cell line. A subsequent dynamical systems analysis identifies four distinct modes of necroptosis signal execution, which can be distinguished based on rate constant values and the roles of the deubiquitinating enzymes A20 and CYLD in the regulation of RIP1 ubiquitination. In one case, A20 and CYLD both contribute to RIP1 deubiquitination, in another RIP1 deubiquitination is driven exclusively by CYLD, and in two modes either A20 or CYLD acts as the driver with the other enzyme, counterintuitively, inhibiting necroptosis.

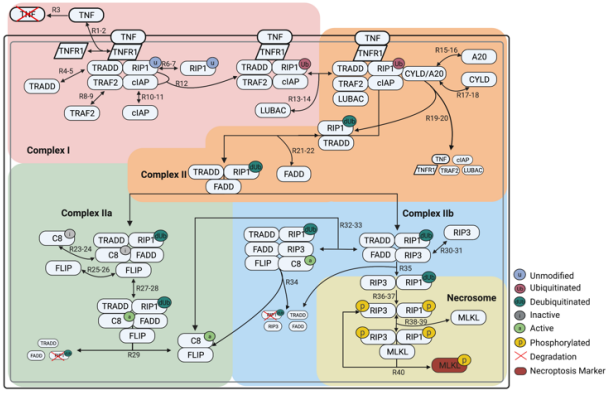

Fig. 1. Schematic of the necroptosis execution model. The diagram is color coded to highlight the processes involved in formation of complex I, complex II, and the necrosome. Arrows are labeled with 'R_N' or '$R_{N\text{-}M}$', where N and M correspond to reaction indices in the model. In many cases, '$R_{N\text{-}M}$' denotes a set of reversible reactions, with N the index of the forward direction and M the index of the reverse. Note that unmodified (u) and deubiquitinated (dUb) RIP1 are considered distinct states and are involved in different reactions. Created with BioRender.com.

We also performed sensitivity analyses of initial protein concentrations and rate constants and identified potential targets for modulating necroptosis sensitivity among the biochemical events involved in RIP1 ubiquitination regulation and the decision between complex II degradation and necrosome formation. We conclude by associating numerous contrasting and, in some cases, counterintuitive experimental results reported in the literature with one or more of the model-predicted modes of necroptosis execution. Overall, we demonstrate that a consensus pathway model of TNF-induced necroptosis can provide insights into unresolved controversies regarding the molecular mechanisms driving necroptosis execution for various cell types under different experimental conditions.

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