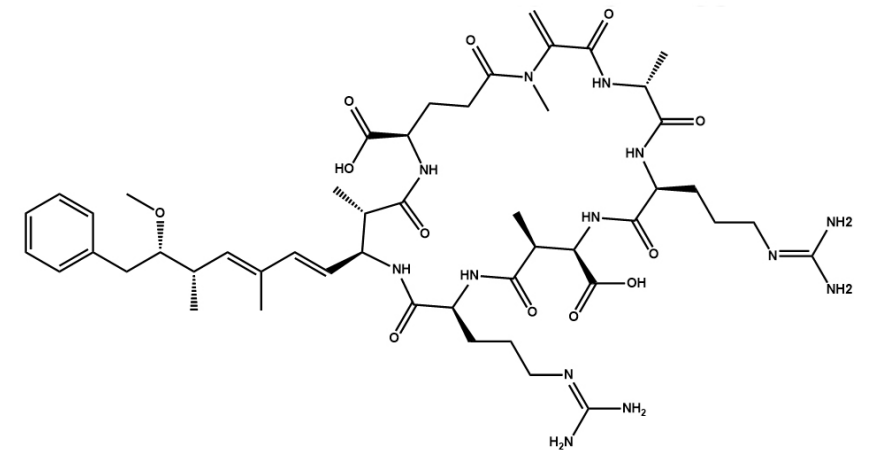
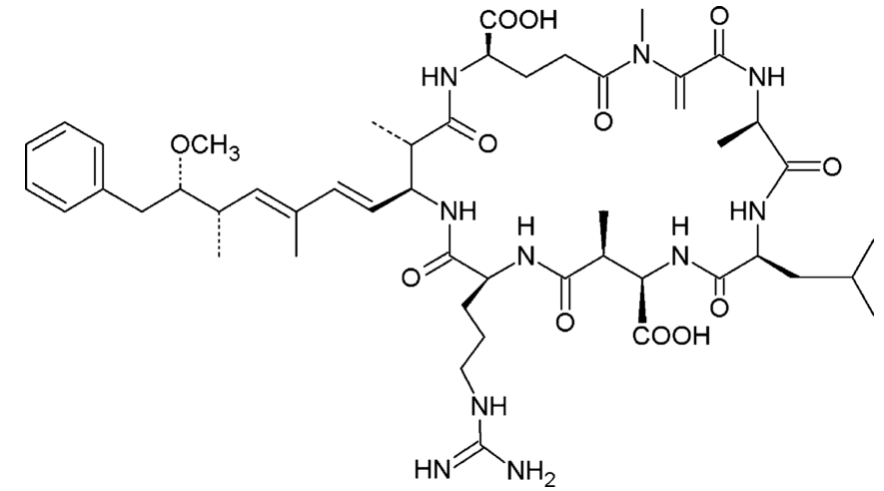


Global transcriptomic profiling of microcystin-LR or -RR treated hepatocytes (HepaRG)

Adam Biales

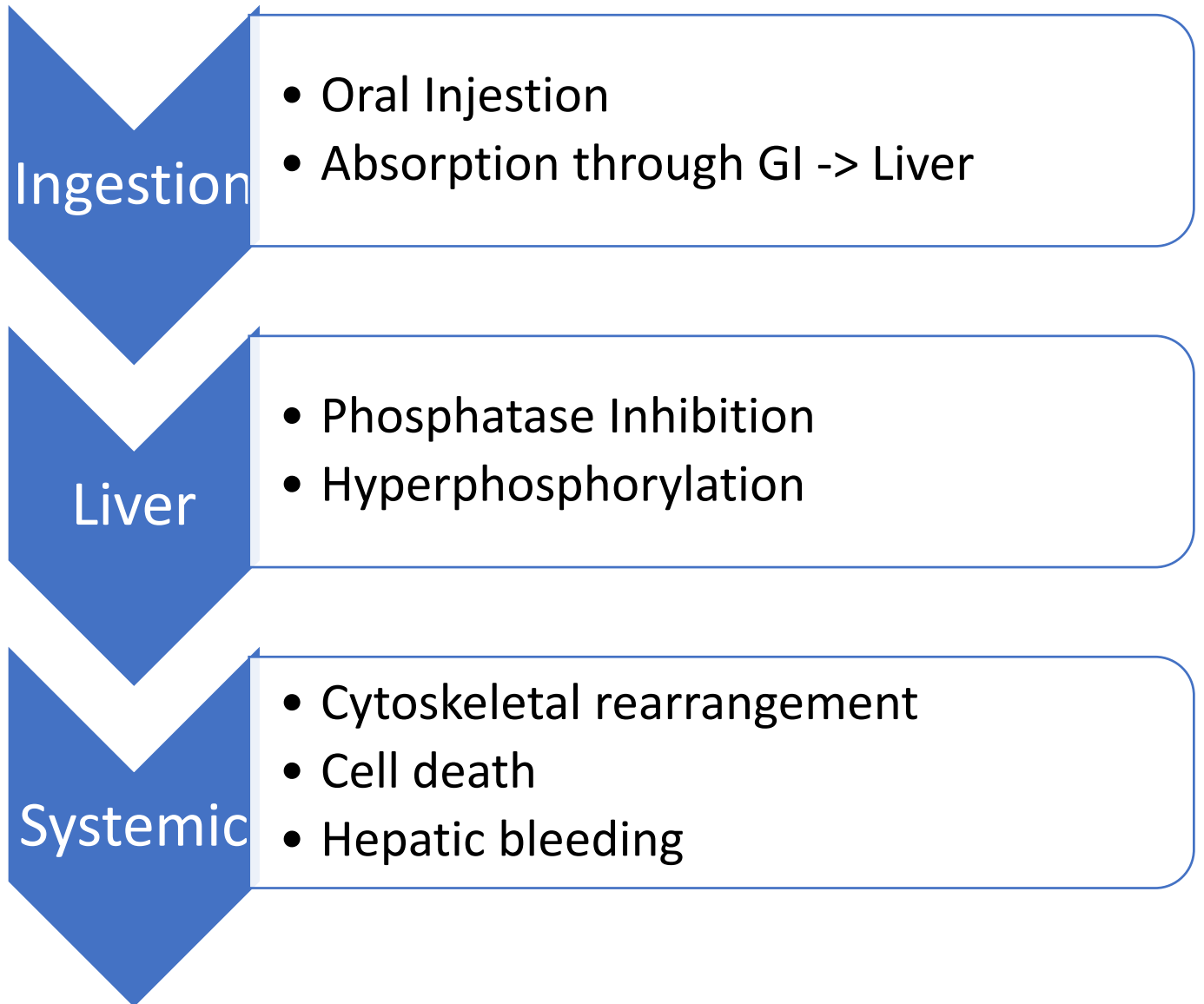
Microcystin congeners

- 100s of congeners
- Differentiated by structure
- Occur in mixtures
- MC-LR best characterized
- MC-RR commonly found w/ -LR



Prototypical Toxicity Pathway

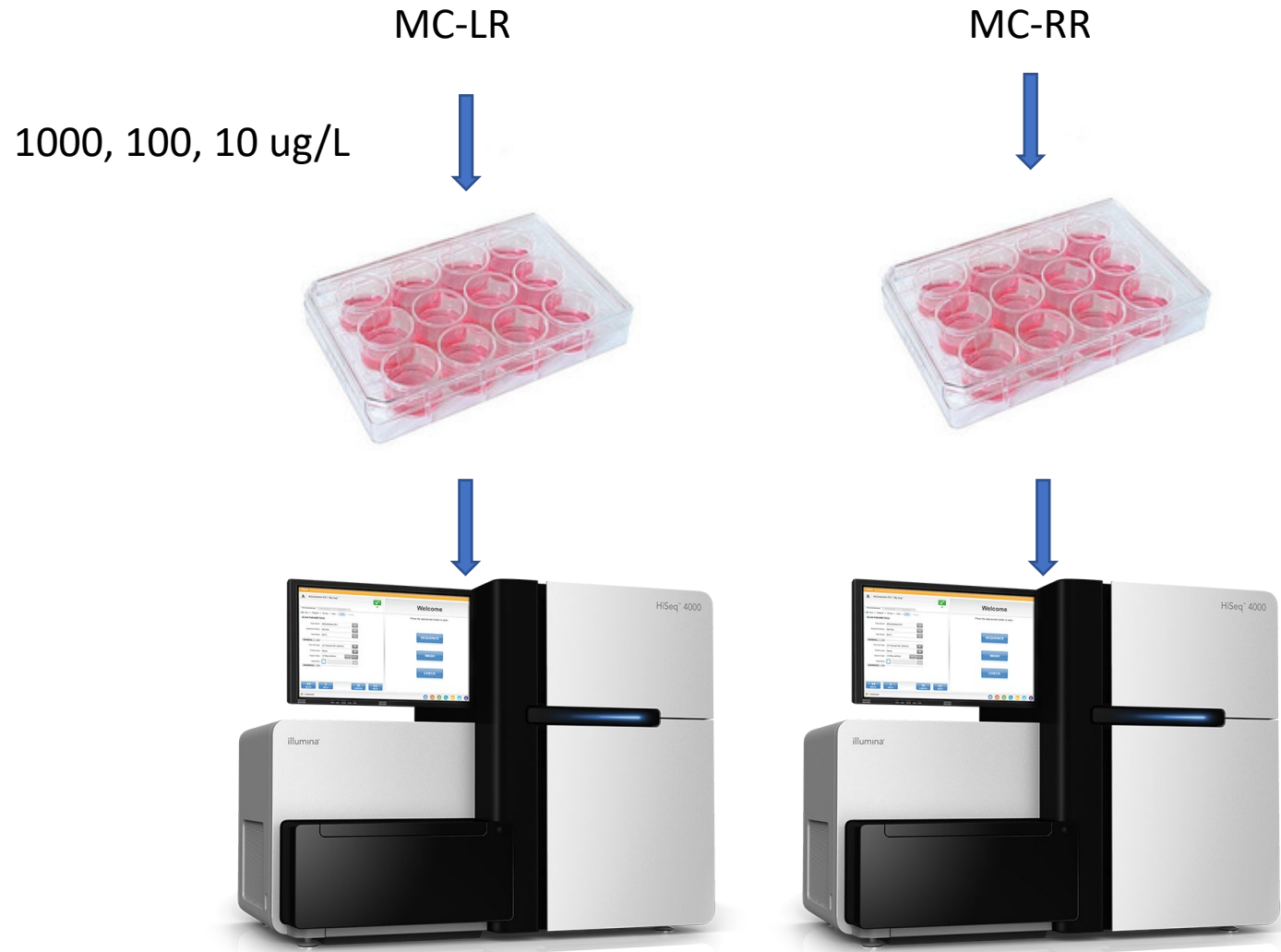
- MC congeners poorly characterized
 - No mechanistic data
 - Potential for unidentified mechanisms
- MC-LR > -RR toxicity
 - MC-LR \approx -RR in PP inhibition
 - Toxicokinetics
 - Molecular targets?



Molecular characterization of MC targets

Gene transcription

- Underlies cellular processes
- Likely to be impacted via phosphatase inhibition
- Generation of hypothesis



Design and Overview

				Batch N			
Group	Total	Up-regulated	Down-regulated	1	2	3	N total
LR10	339	230	109	7	6	6	19
LR100	171	116	55	10	6	6	22
LR1000	2098	1740	358	6	6	6	18
RR10	12	11	1	7	6	0	13
RR100	1255	1130	125	10	6	6	22
RR1000	1279	1138	141	6	6	6	18
Solvent				18	12	6	36

Cytotoxicity

Treatment	XTT
LR10	0.0592
LR100	7.24e-05
LR1000	9.48e-12
RR10	0.3178
RR100	0.7634
RR1000	0.4227

Consistency of DEGs and enrichment

LR1000		LR100	
FOS	15.5	FOS	2.64
ATF3	8.22	ATF3	1.98
FOSB	6.31	FOSB	1.85
IER2	4.08	IER2	1.57
RND1	3.94	GADD34	1.56
GDNF	3.69	CREB5	1.45
NFKBIZ	3.08	KLF6	1.37
GADD34	3.00	NOXA	1.34
JUN	2.68	DUSP1	1.31
SLC25A25	2.45	DUSP8	1.30

LR1000:LR100 (59% overlap)

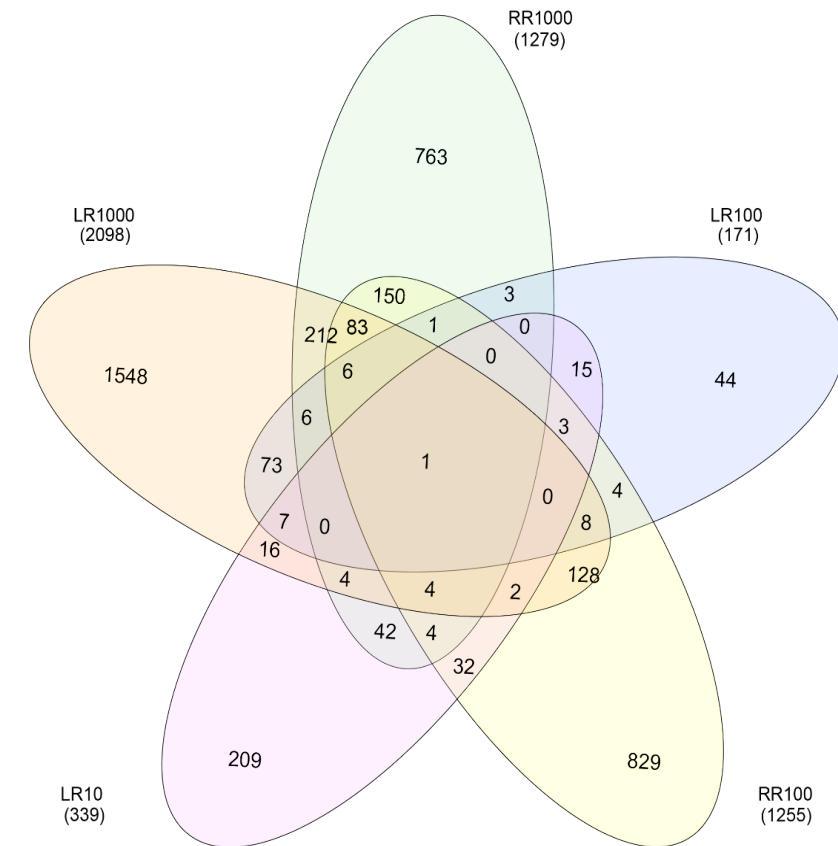
- bZIP TFs
- FOS family of proteins
- TNF-a signaling pathway

LR1000:RR1000 (25% overlap)

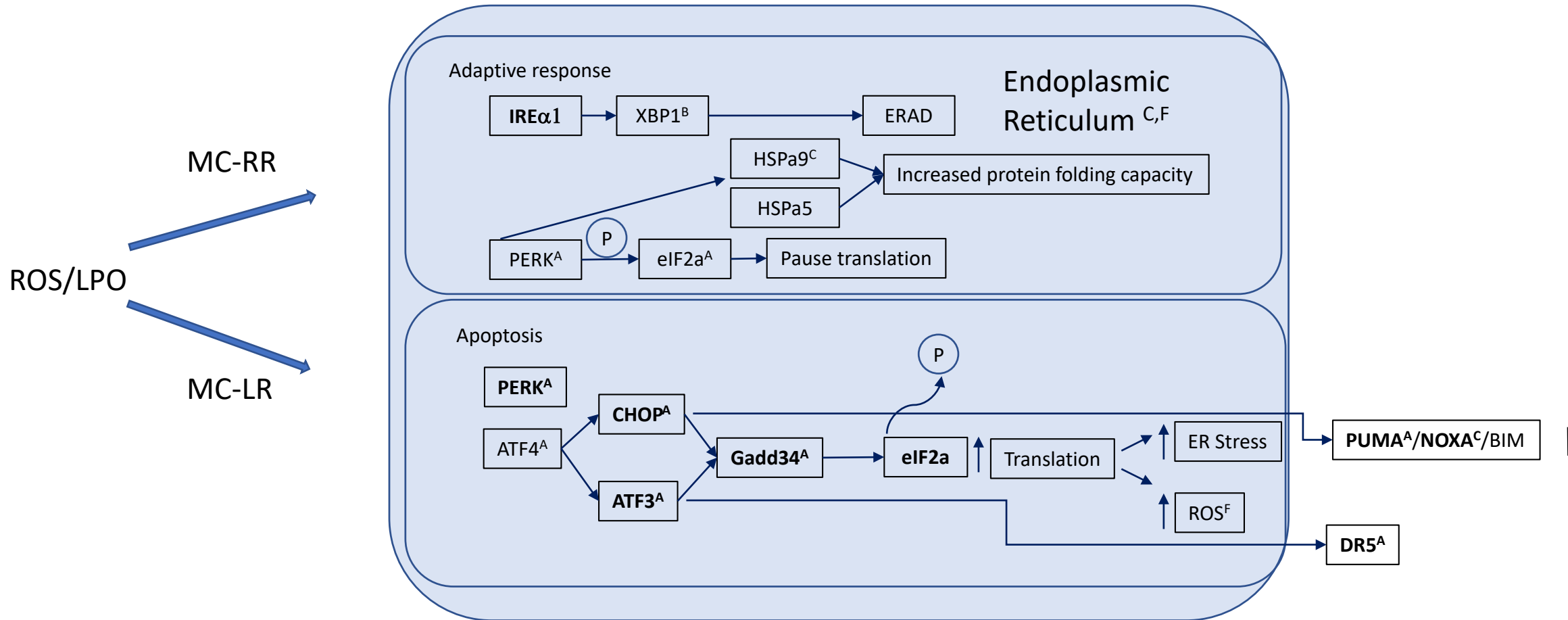
- Aldehyde Dehydrogenase family
- 7/8 members in common

RR1000:RR100 (20% overlap)

- Complement
- Extracellular exosome
- acetylation



Endoplasmic Reticulum Stress Response

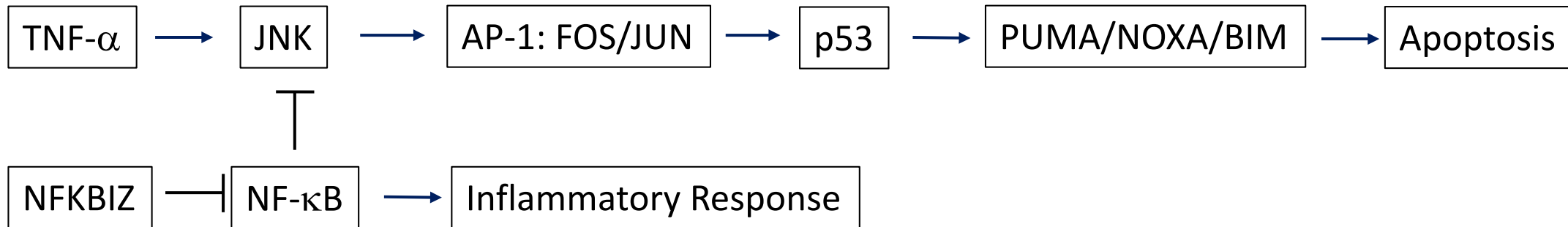


Other common identified targets/processes

- Lipotoxicity
 - ALDH family up-regulated in MC-LR & - RR
 - ROS
- Extracellular Exosomes
 - Highly enriched in MC-RR
 - Less so in MC-LR
 - Off-loading misfolded proteins?
 - Extracellular signaling - sensitization

MC-LR specific

- AP-1 constituents
 - Among the most consistently and highly expressed
 - FOS, JUN, ATF
 - TNF- α /JNK signaling



- Protein Phosphatases – PP1 and 2

MC-RR Specific Response

- Enrichment of complement genes
 - Immunity and defence
 - Liver damage —| Apoptosis
- Diversity of protein phosphatases
 - Little overlap with MC-LR

Conclusions

- Consistent response
 - w/in study and across
- Oxidative Stress is a key driver of MC toxicity
- ER-stress is important
- MC-LR and –RR differ
 - Toxicity
 - PP targets
- Toxicity confounded with congeners

Acknowledgements

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