HN

SEASON 2

Farah Saeed FS Farah Saeed

Understanding Cancer

Lecture 15

Types of signalling pathway: NFKB

DR HAFSA WASEELA ABBAS

www.hafsaabbas.com

RECAP:

What you hopefully should understand so far from Lecture 14

- The menstrual cycle is the **release of an egg from the ovaries every 28 days** and **changes in the thickness of the womb lining.**

- There are three phases of the menstrual cycle.

  Follicular phase: This occurs on Day 1 of the period until Day 14. Oestrogen and Follicle Stimulating hormone (FSH) levels rise.

  Ovulation: The release of the egg on Day 14 in most cases. The luteinizing hormone is responsible for its release.

  Luteal phase: Corpus luteum produces progesterone to store glycogen.

- **Oestrogen** has other functions beside**s period e.g. growth of the womb, pregnancy and Cell-to-cell communication in the breasts.**

- Dysregulation of the oestrogen signalling pathway: **overexpression of the receptors, overproduction of oestrogen, imbalance of hormones, coregulatory proteins and lifestyle factors.**

RECAP:

What you hopefully should understand so far from Lecture 15

Oestrogen binds to receptor → **Translocation with genome in the nucleus** → **Increase transcription**

Oestrogen binds to receptor → **Secondary messenger and kinase activity increase** → **Cellular response hormones increase growth of bones.**

What will we learn today?

- ***The structure of NFκB***
- ***Receptor activation: NFκB Normal signalling pathway***
- ***Signal transduction: Normal Notch signalling pathway***
- ***IκB proteins***
- ***IκK proteins***
- ***Cellular response: Normal NFκB signalling pathway***
- ***Dysregulated signalling pathway.***

GENTLE REMINDER
An ideal way of learning:

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday

Mini-lectures.

Approximate total time: 1 hour

Divide over 7 days at your **own pace**.

Challenge yourself with a quiz!

HN

www.hafsaabbas.com

RECAP: How to support your learning?

- **Key facts with diagrams by HN designs presented in a simplified way.**
- **Glossary to help understand what key words mean.**
- **Summary doodle revision posters by HN designs.**
- **Quizzes to test your knowledge and reflect.**
- **Reference list for further reading.**

Acknowledgements: Special thanks to my parents, family, friends and colleagues for their support and the respected teachers and health professions who taught me and installed the passion of cancer/oncology.

The structure of NFκB

The structure of NFκB

Nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) is a transcription factor made of five subunits:

- ❑ Rel (cRel)
- ❑ p65 (RelA, NFκB3)
- ❑ RelB
- ❑ p105/p50 (NFκB1)
- ❑ p100/p52 (NFκB2)

(Xia *et al*., 2018; Peng *et al*., 2020)

The structure of NFκB

There are three main types of domains found in NFκB.

Some member of some of the domains, other members do not.

Transactivation domain (TAD)	Rel-homologous domain (RHD)	Ankyrin repeats
• **Activate transcription**	• They initiate **DNA binding.** • They initiate **dimerization between the same members (homomeric) or different members (heterodimeric).** • The binding between subunits **helps reveal the nuclear localisation sequence** which is needed for the NFκB dimer to **translocate into the nucleus to start transcription.**	• They are present in **members that do not activate gene transcription.** • They provide an **inhibitory** effect.

(Xia *et al*., 2018; Peng *et al*., 2020)

The structure of NFκB

Type of NFKB	Transactivation domain (TAD)	Presence of ankyrin repeats	Rel-homologous domain (RHD)
Rel (cRel)	✓	✗	✓
p65 (RelA, NFκB3)	✓	✗	✓
RelB	✓	✗	✓
p105/p50 (NFκB1)	✗	✓	✓
p100/p52 (NFκB2)	✗	✓	✓

(Xia *et al*., 2018; Peng *et al*., 2020)

The structure of NFκB

Some members have:

- ❑ Leucine zipper-like motif (LZ)
- ❑ Death domain (DD)

Leucine zipper domain

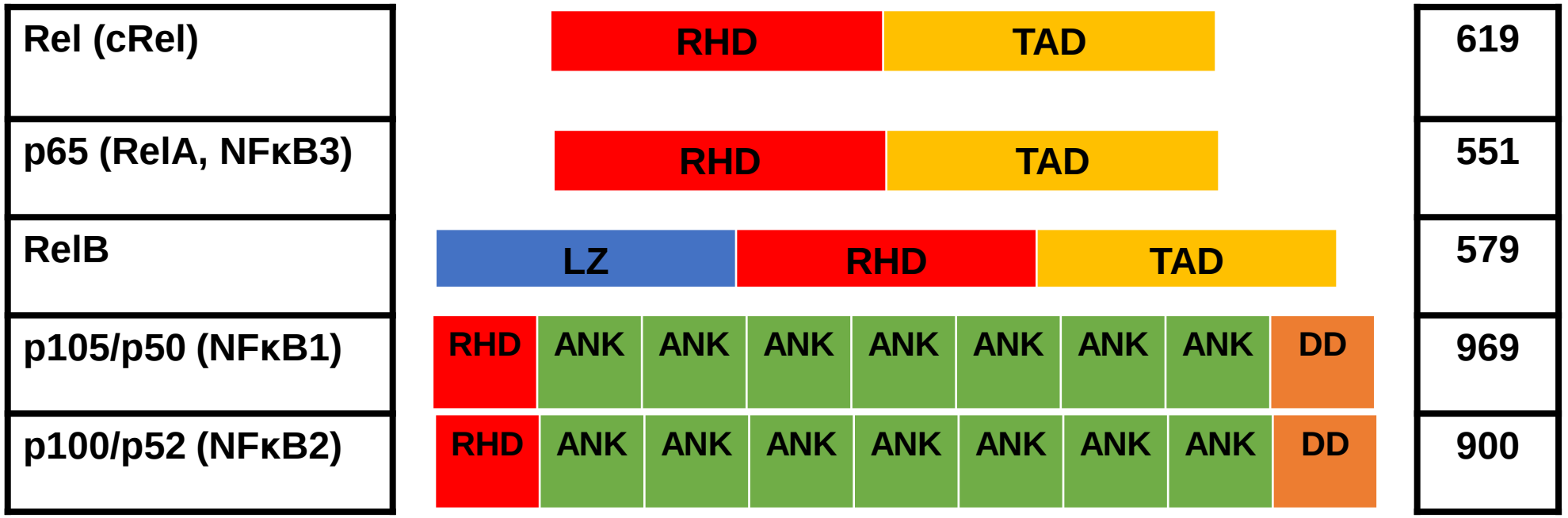
- **Basic domain:** It recognises specific DNA sequence.
- **Leucine residues:** This is found along alpha helix structure and mediates dimerization.
- **Dimers of LZD:** They recognise short, inverted and repeated sequences.

Death Domain (DD)

- They are adaptor proteins that induce protein-protein interactions.
- They can associate by themselves to form homodimers.
- They can associate with other members of DD superfamily including CARD (Caspase activation and recruitment domain), DED (Death Effector Domain), and PYRIN to form heterodimers.

(Park *et al*., 2007; Pollard *et al*., 2017)

The structure of NFκB



Receptor activation

Receptor activation

Some members can form dimers with specific members of NFκB but not with others.

The most common is RelB.

Other members of NFκB can form either homodimers or heterodimers.

The most common NFκB dimer is p65–p50.

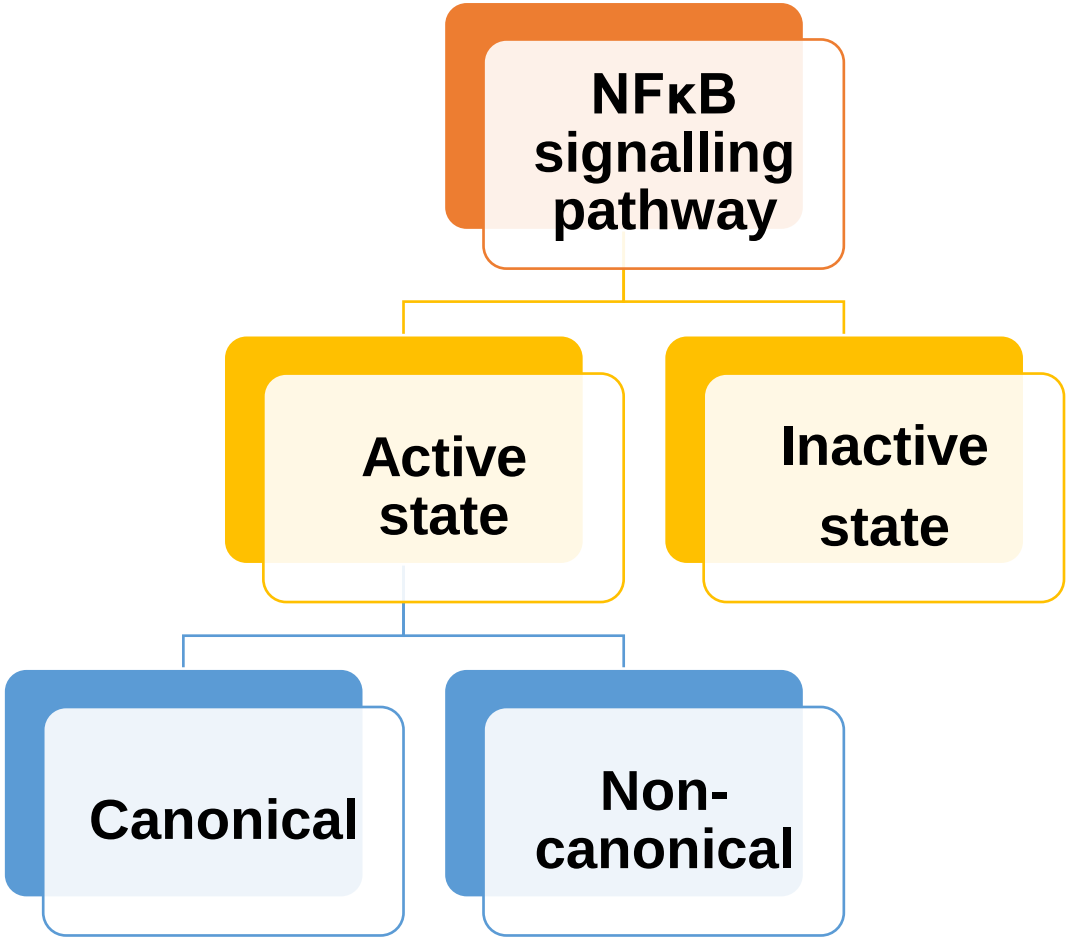
(Xia *et al*., 2018; Peng *et al*., 2020)

Signal transduction

Signal transduction

Following protein dimerization, two groups of proteins play a major role in transducing the signal inside the cell.

- ❑ IκB – inhibitory proteins of dimers
- ❑ IKK



(Xia *et al*., 2018; Peng *et al*., 2020)

IκB proteins

Types of IκB

IκBα (alpha)

- **Inhibits the p50/RelA heterodimer.**

IκBβ (beta)

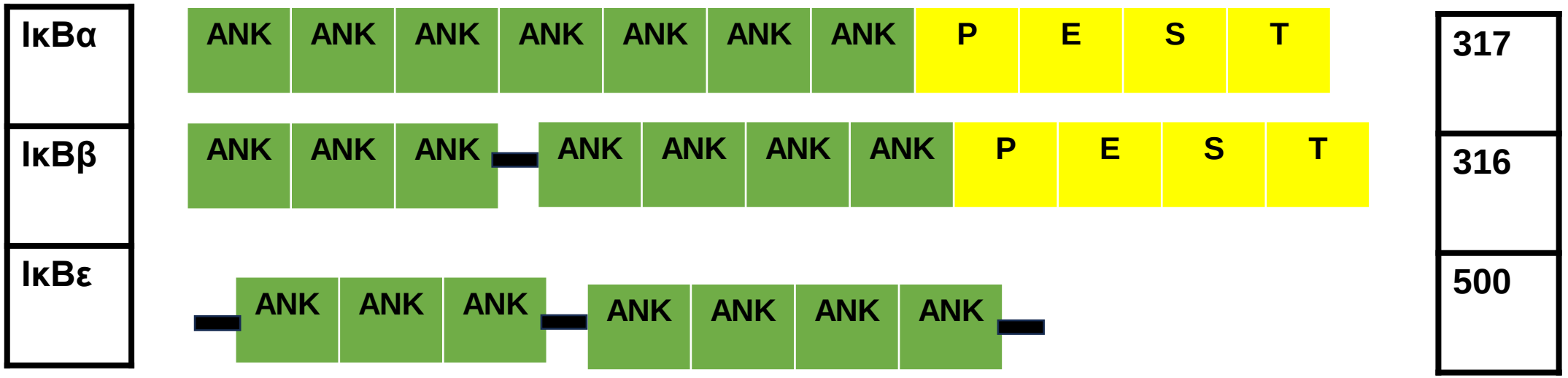
- **Inhibits the RelA/cRel heterodimer**

IκBε (epilson)

- **Inhibits the RelA and cRel dimers.**

(Xia *et al*., 2018; Peng *et al*., 2020)

Types of IκB



Anykrin repeats – They attach to the DNA-binding domains of NF-κB dimers

PEST – A domain that comprises of proline (P), glutamate (E), serine (S) and threonine (T).

(Xia *et al*., 2018; Peng *et al*., 2020)

Some nuclear IκB proteins can both inhibit and activate NF-κB transcription of target genes.

Bcl-2

(Xia *et al*., 2018; Peng *et al*., 2020)

Precursors of IκB

There have two precursors:

- p105/IκBγ
- p100/IκBδ.

(Xia *et al*., 2018; Peng *et al*., 2020)

Inactive state process

NFκB dimers binds to three inhibitory factors (IκBα, IκBβ, and IκBε) in the cytoplasm.

This blocks the nuclear localization sequence and prevents the NFκB from translocate into the nucleus.

(Xia *et al*., 2018; Peng *et al*., 2020)

IκK proteins

What are IκK proteins?

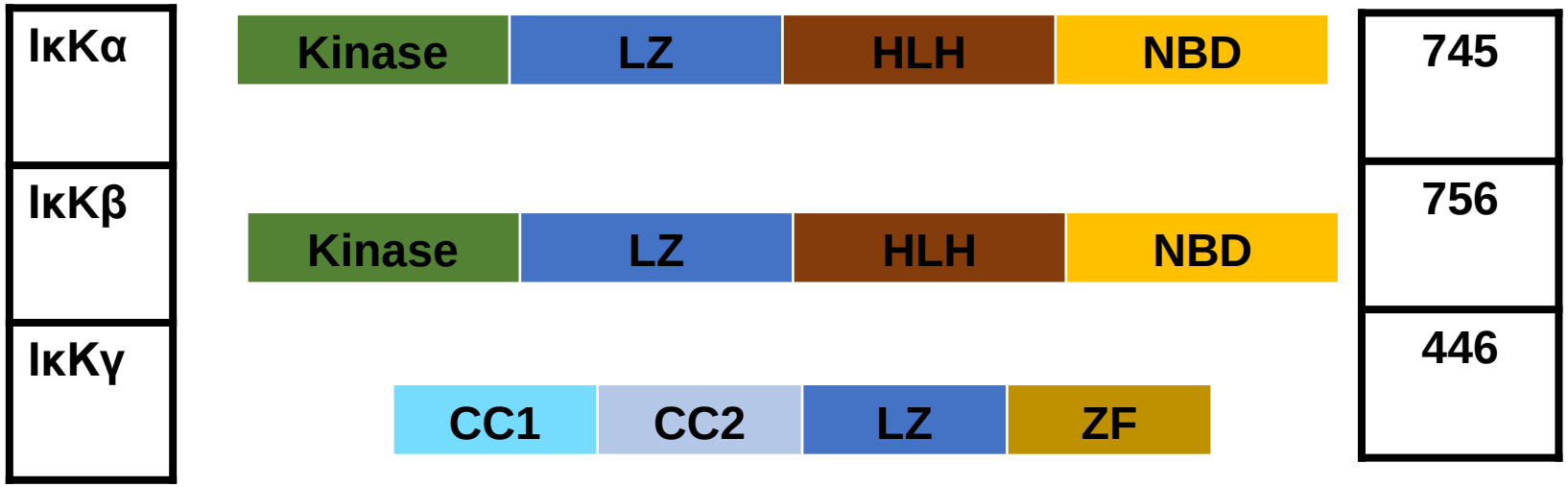
There are three types of subunits found in IκB kinase (IκK) proteins:

- ❑ **Catalytic subunit IKKα**
- ❑ **Catalytic subunit IKKβ**
- ❑ **Regulatory subunit IKKγ also known as NFκB essential modifier (NEMO).**

What are the features of IκK proteins?

IκK	Structure	Function
IKKα	It is the type of the specific upstream kinase. Other domains are present: • Leucine zipper-like motif (LZ) • helix-loop-helix domain (HLH): regulate transcription and function in determination of sex and development of the muscles and nervous system (Jones, 2004) • NEMO-binding domain (NBD): It inhibits the IKK complex.	• It phosphorylates the serine amino acid residues present in IκBβ. • This causes the degradation of IκBβ by proteosomes.
IKKβ	A type of kinase • LZ • HLH • NBD	• It phosphorylates the serine residues of IκBα and IκBβ. • It strongly attaches to IκBα than IκBβ
IKKγ	It has the following features: • N-terminal coiled-coil (CC) domain: Two alpha-helices wound together to provide structural rigidity. • ZF regulate gene transcription. It is characterised by the DNA binding motifs e.g. C2H2 and Gag knuckle. • LZ.	• Regulate the activity of IKK. • It interacts with IKKα and IKKβ.

What are IκK proteins?



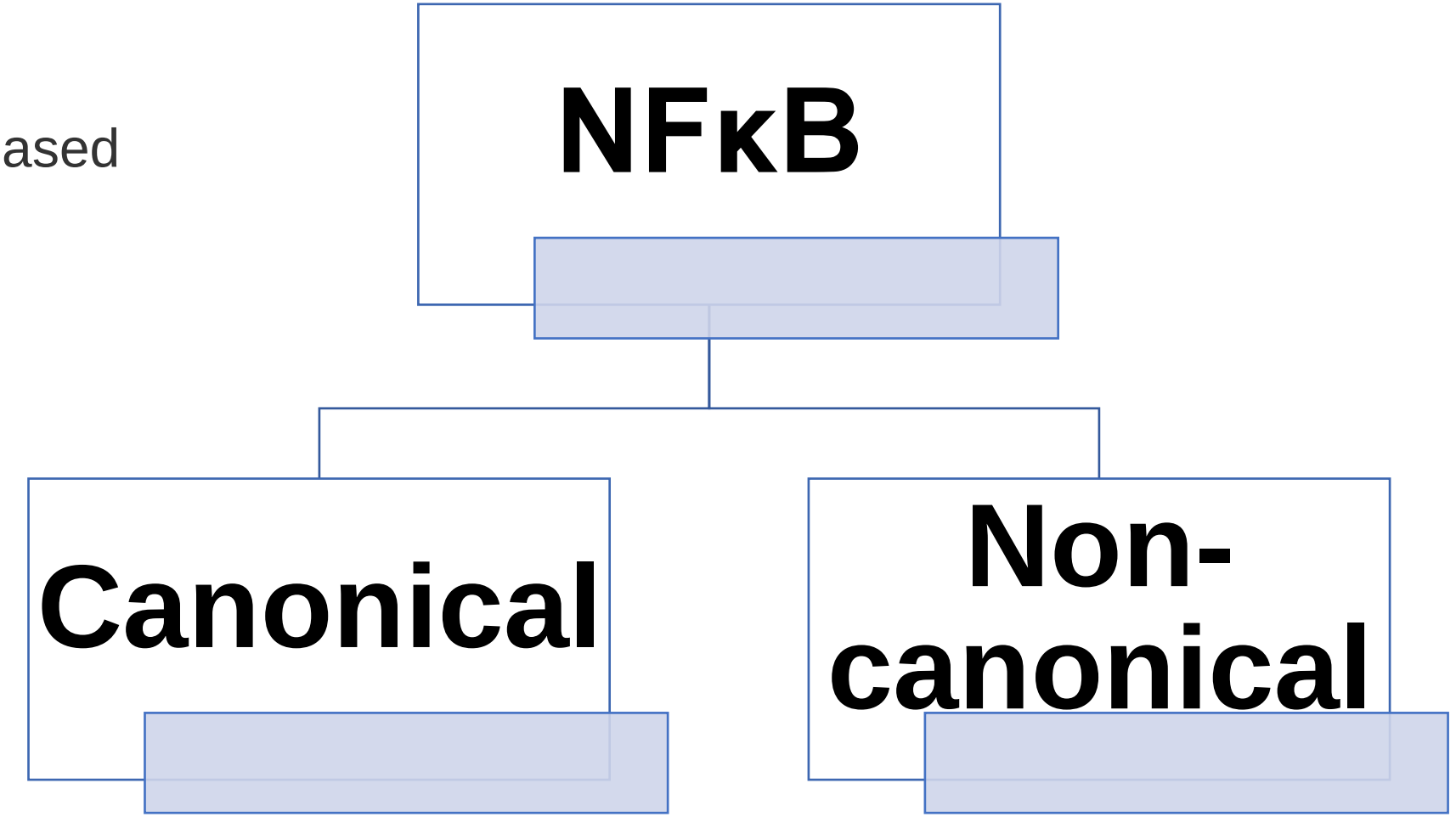
- **Leucine zipper-like motif (LZ)**
- **helix-loop-helix domain (HLH)**
- **NEMO-binding domain (NBD)**
- **coiled-coil domain (CC)**
- **zincfinger domain (Z).**

(Lu. *et al.*,2011)

The complexes NFκB–IκBα or NFκB–IκBε form links between the cytoplasm and nucleus.

Active state

NFKB needs to be released from inhibitors.



Extracellular ligands

Interleukin-1

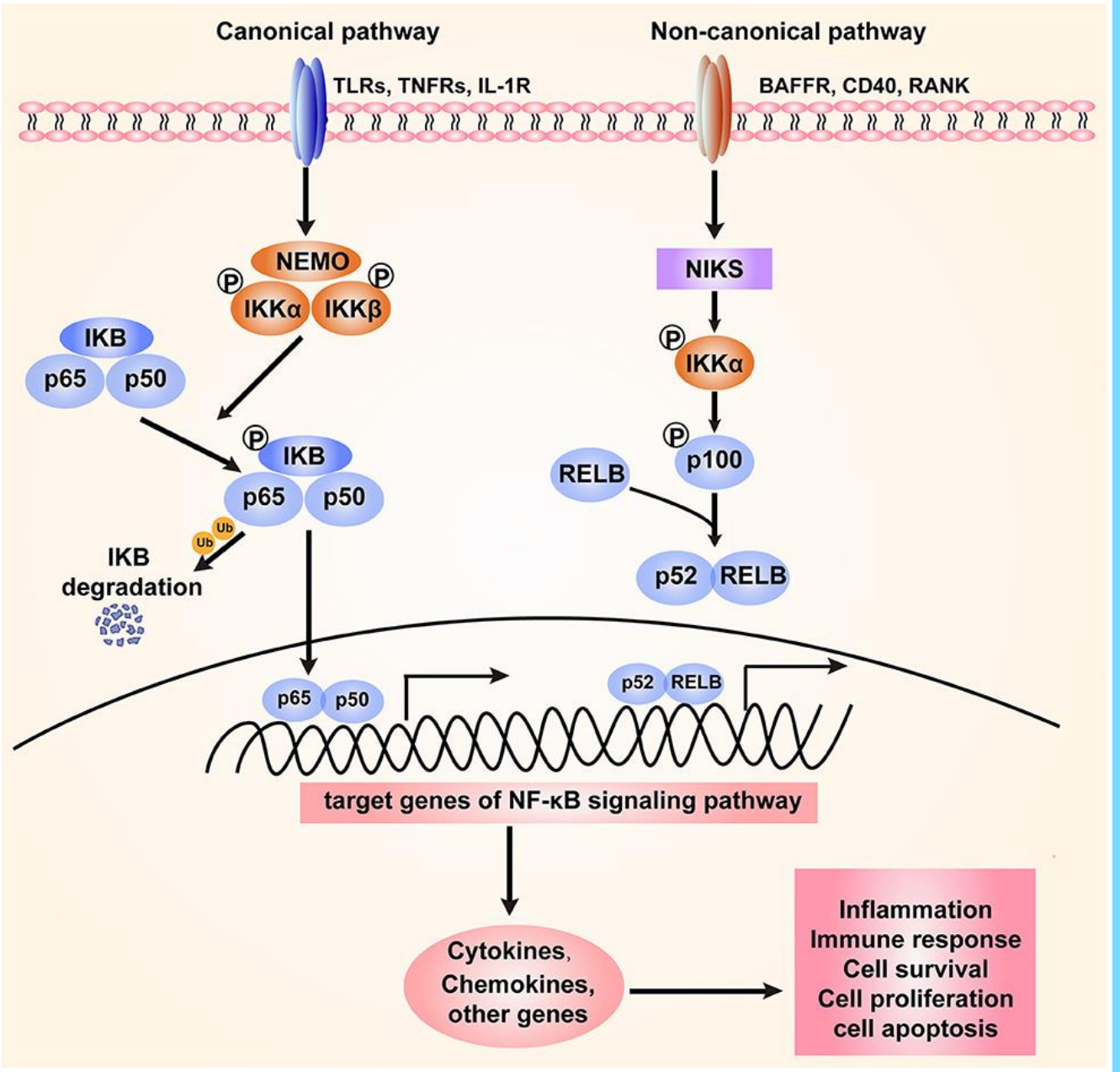
Lipopolysaccharide

Tumour Necrosis Factor –alpha (TNFα)

Double-stranded RNA in viruses

Radiation

Signal transduction



Permission from Creative Commons

(Peng *et al*., 2020)

Signal transduction Canonical pathway

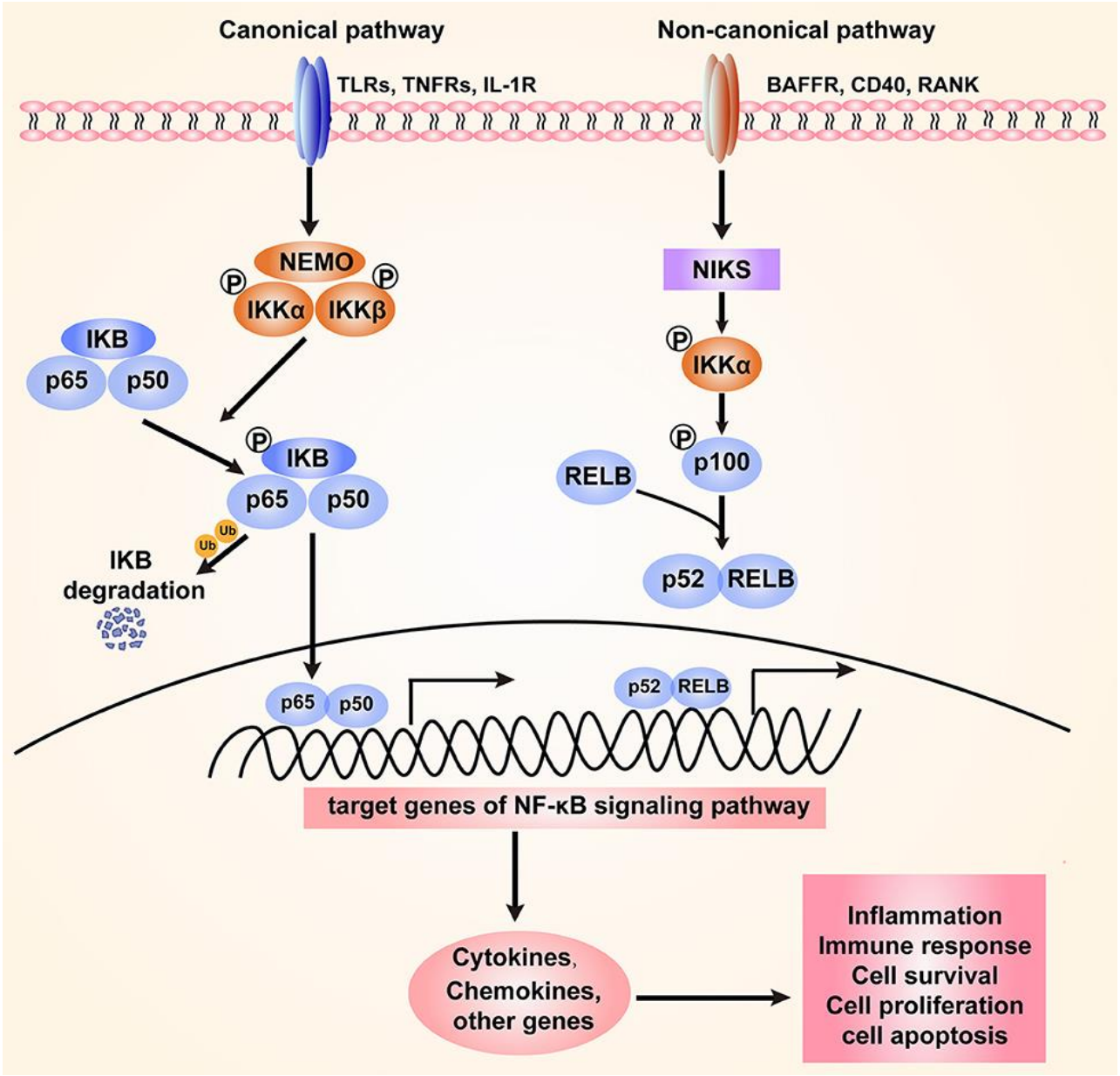
Step 1

NFκB consists of an inactive dimer p50-p65 complex and is situated in the cytoplasm.

To be activated, the p65 or p50 heterodimers need to be formed.

The formation of p65 (RelA)–p50 or p65–cRel heterodimers causes activation.

(Peng *et al*., 2020)



Signal transduction Canonical pathway

Step 2

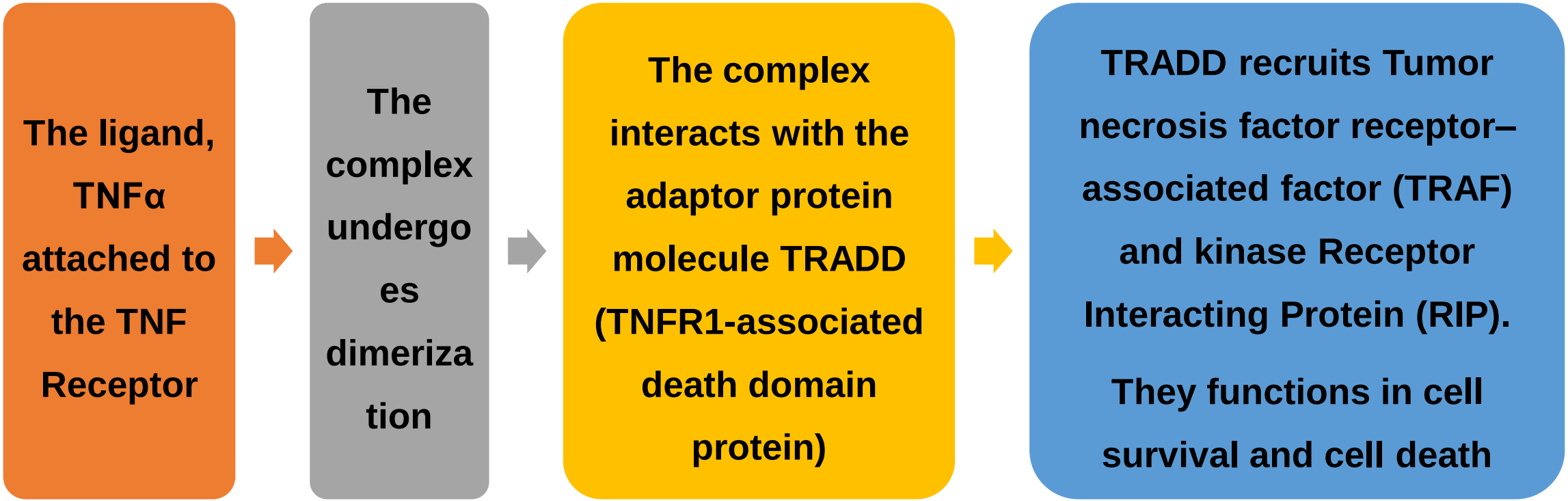
Cytokine proteins that promote inflammation, for example:

- **TNFα**
- **Interleukin 1 – phosphorylates MMK4 which processes p100 to the p52-active form and translocates p52 to the nucleus.**
- **Toll-like receptors**

It phosphorylates and activates IKKβ complex.

(Peng *et al*., 2020)

Signal transduction Canonical pathway



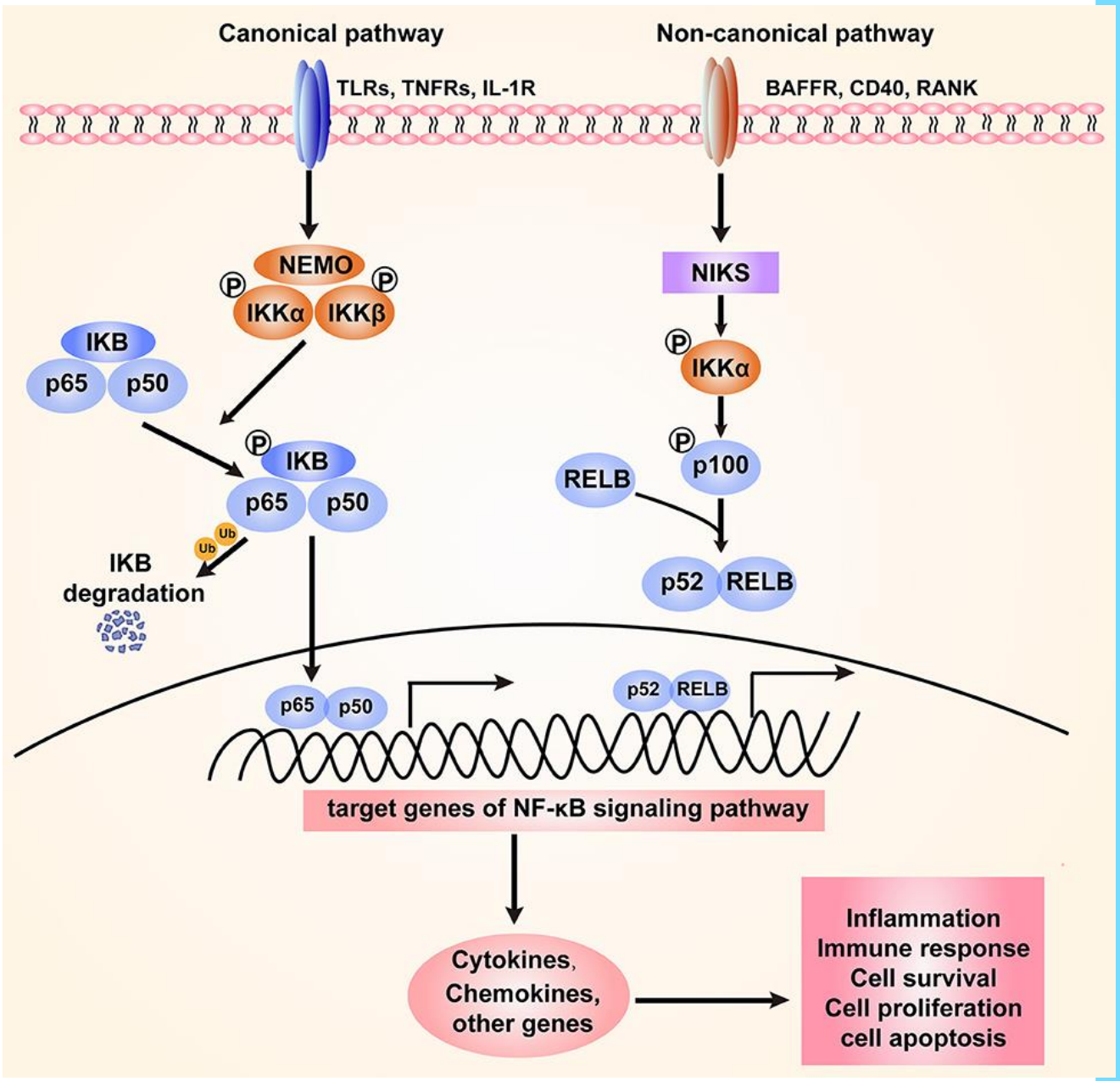
(Park 2018; Peng,*2012)*'; Pobezinskaya and Liu, 2012)

Signal transduction Canonical pathway

Step 3

The IκB protein dissociated from the p50-p65-IκB trimer.

IκBα phosphorylates two serine amino acid residues at Ser32 and Ser36.

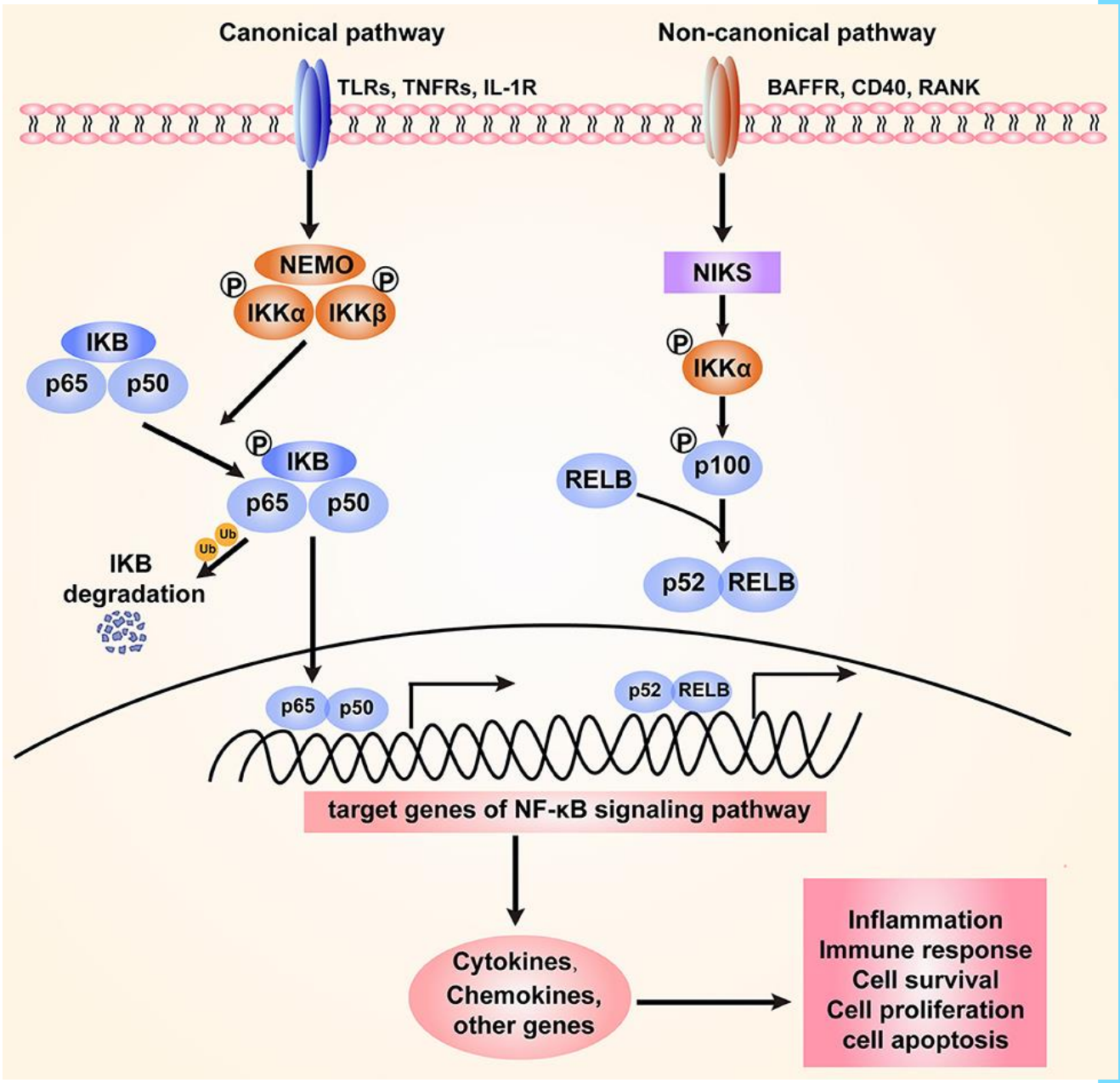


(Peng *et al*., 2020)

Signal transduction Canonical pathway

Step 4

The complex undergoes ubiquitination and is degraded by proteasomes activating the NFκB pathway.



(Peng *et al*., 2020)

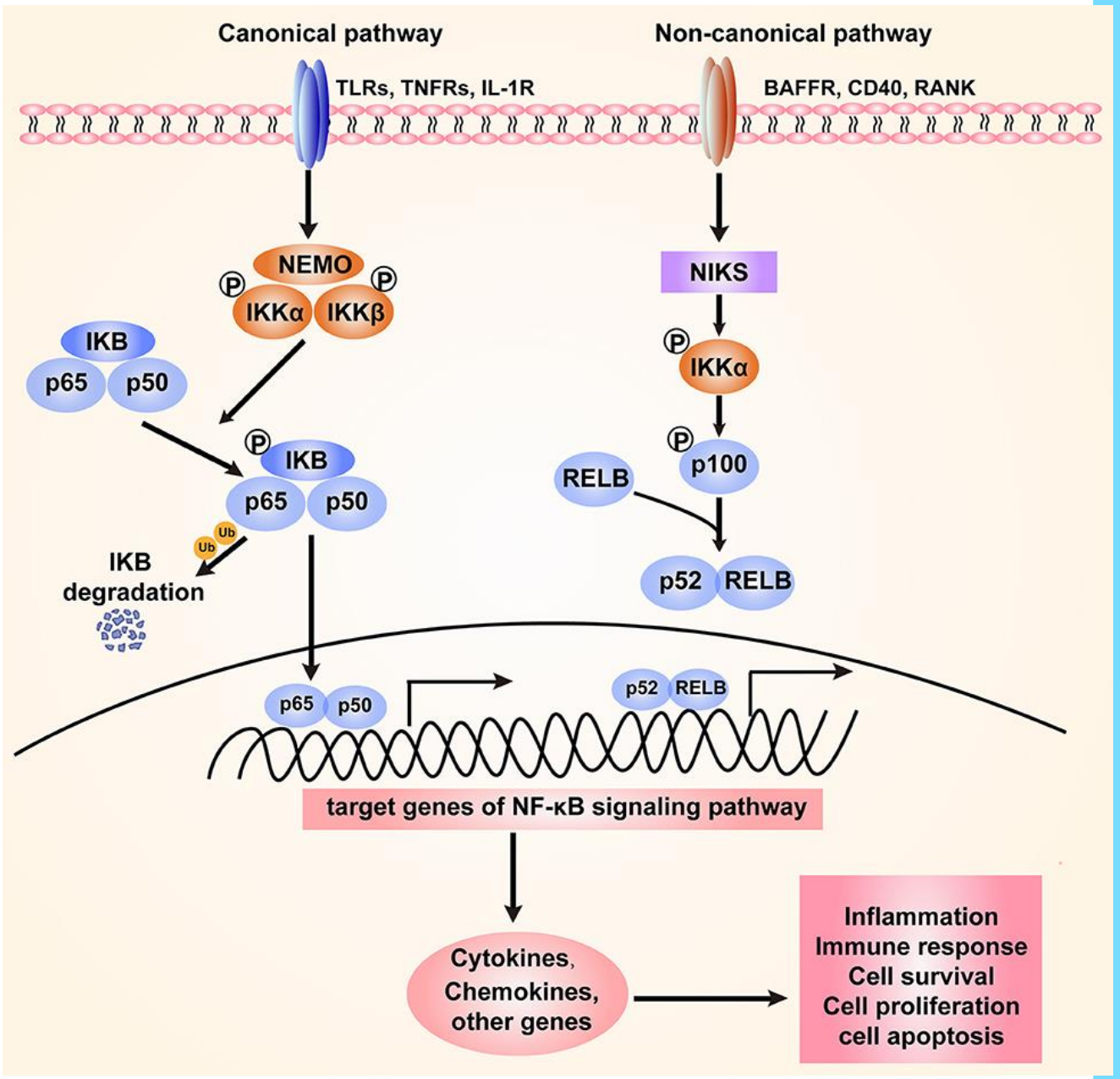
Gentle reminder - Polyubiquitination

- **Ubiquitination is the addition of the ubiquitin (Ub) protein.**
- **It has seven lysine (K) residues (K6, K11, K27, K29, K33, K48, and K63) and one methionine (M1). This can help link with other ubiquitins to form a polyubiquitin.**
- **The Ub is activated by the Ub-activating enzyme, E1.**
- **E2 transfers Ub from E1 to E3.**
- **K48-associated polyubiquitination helps release NFκB from IκBs. This is catalysed by the Skp1–Cullin–F-box (SCF)–βTrCP complex at K21 and K22.**

Signal transduction Canonical pathway

Step 5

Polyubiquitination will signal for degradation of IκBα by the proteosome

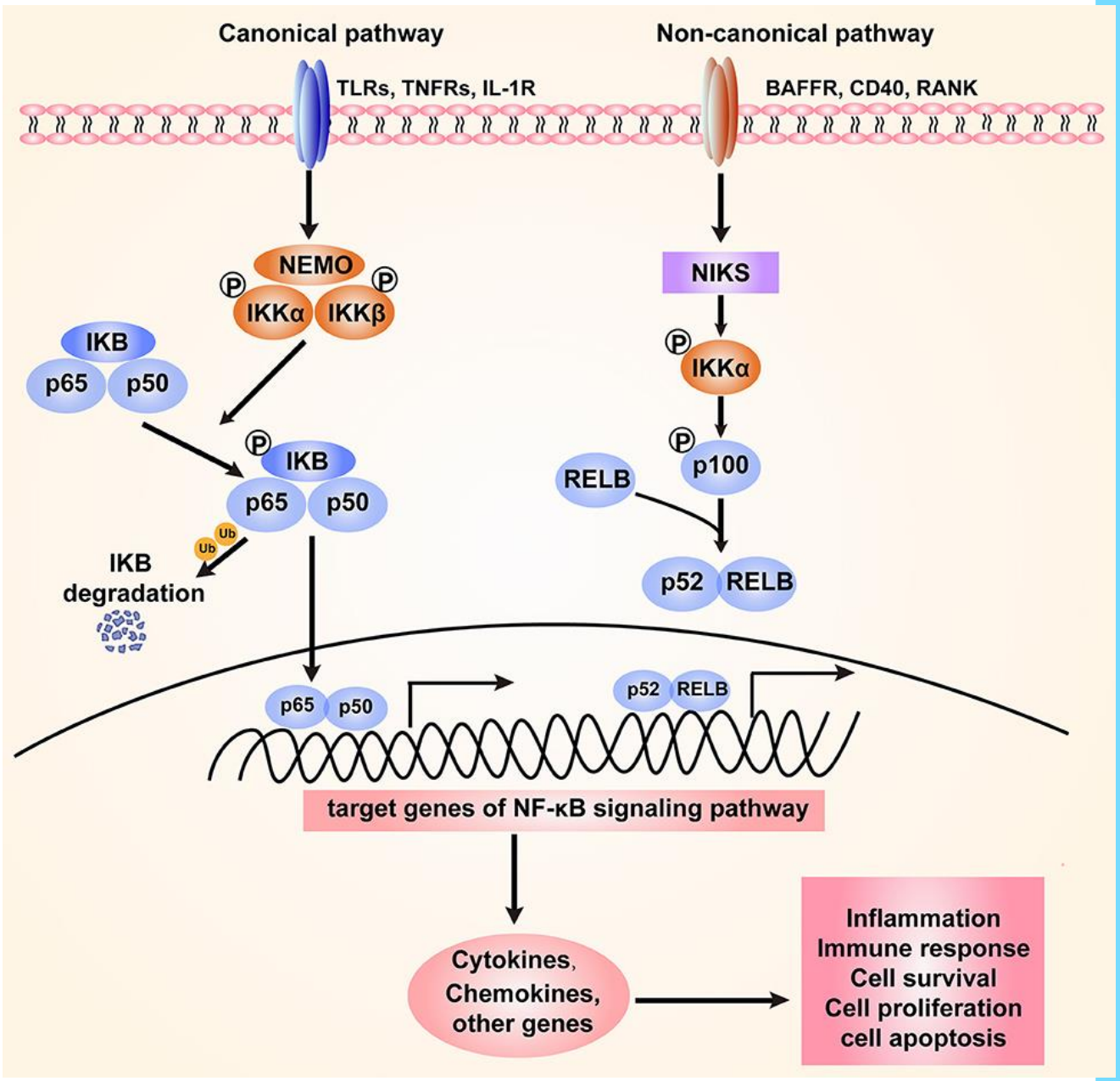


(Peng *et al*., 2020)

Signal transduction Canonical pathway

Step 6

p100 and p105 are phosphorylated and cleaved into p52 and p50.

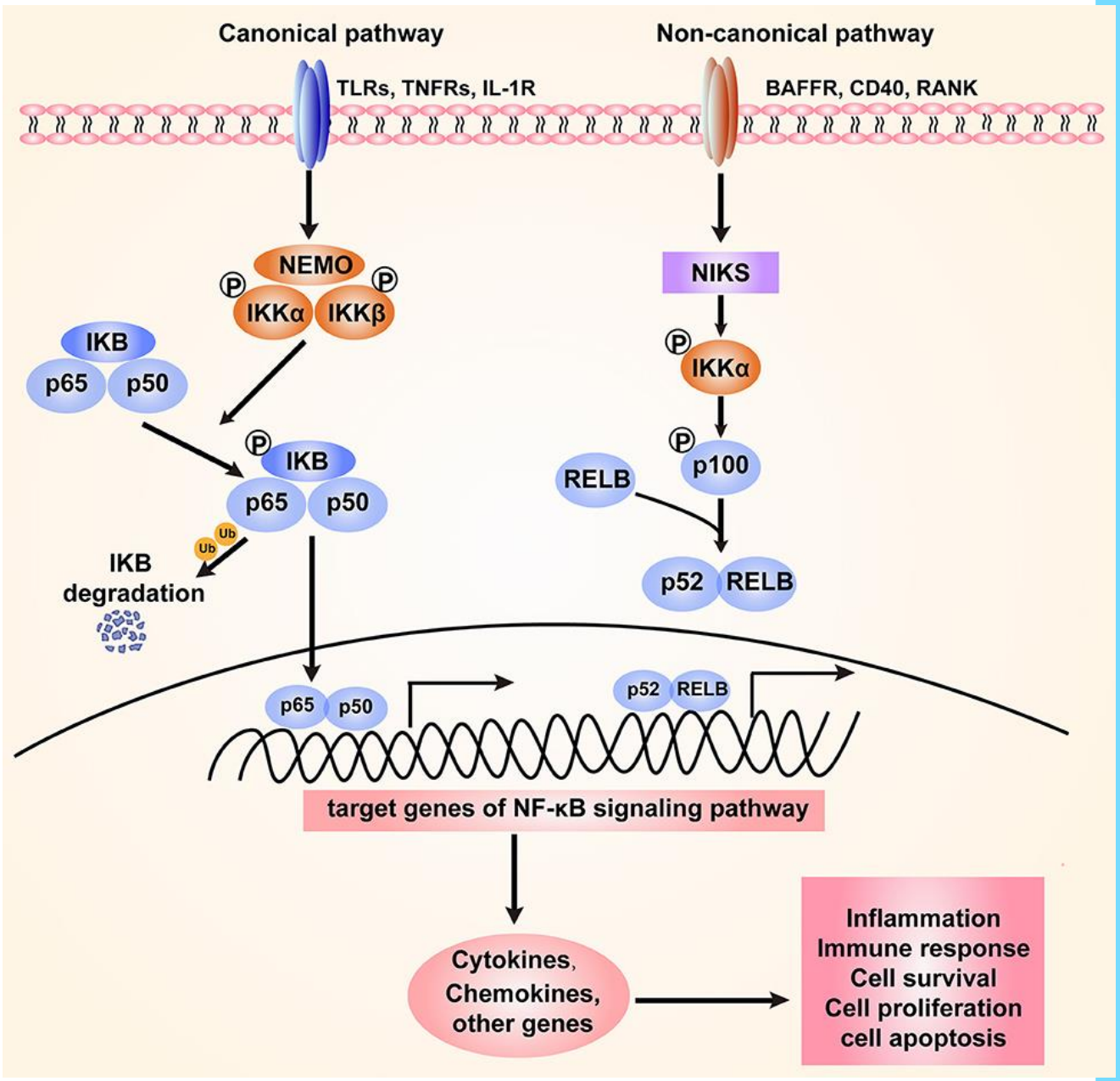


(Peng *et al*., 2020)

Signal transduction Canonical pathway

Step 7

Degradation of IκBα inhibitor releases the NFκB dimer p50-RelA/p65.

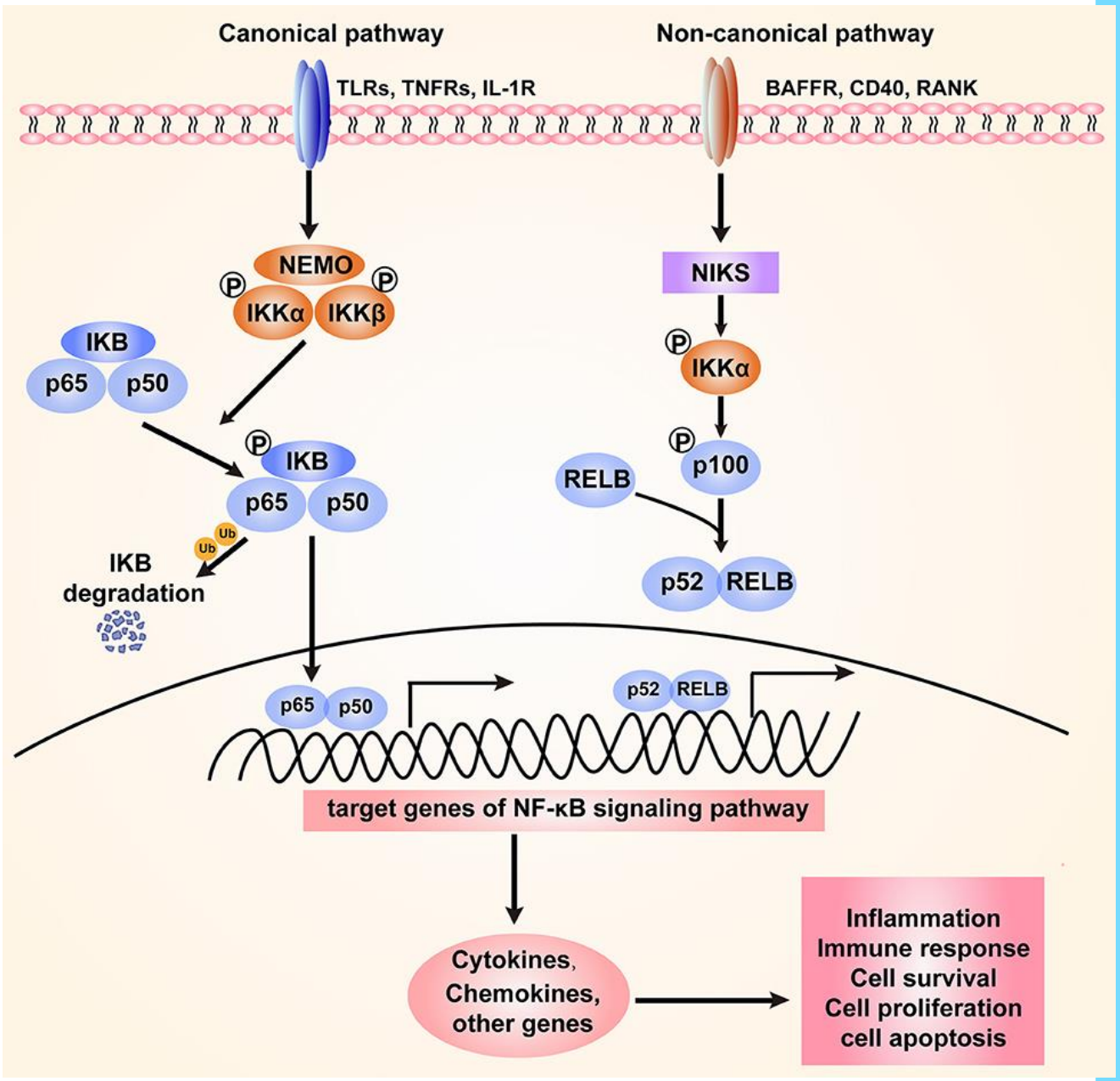


(Peng *et al*., 2020)

Signal transduction Canonical pathway

Step 8

The NFκB dimer p50-RelA/p65 translocate into the nucleus and activate gene transcription



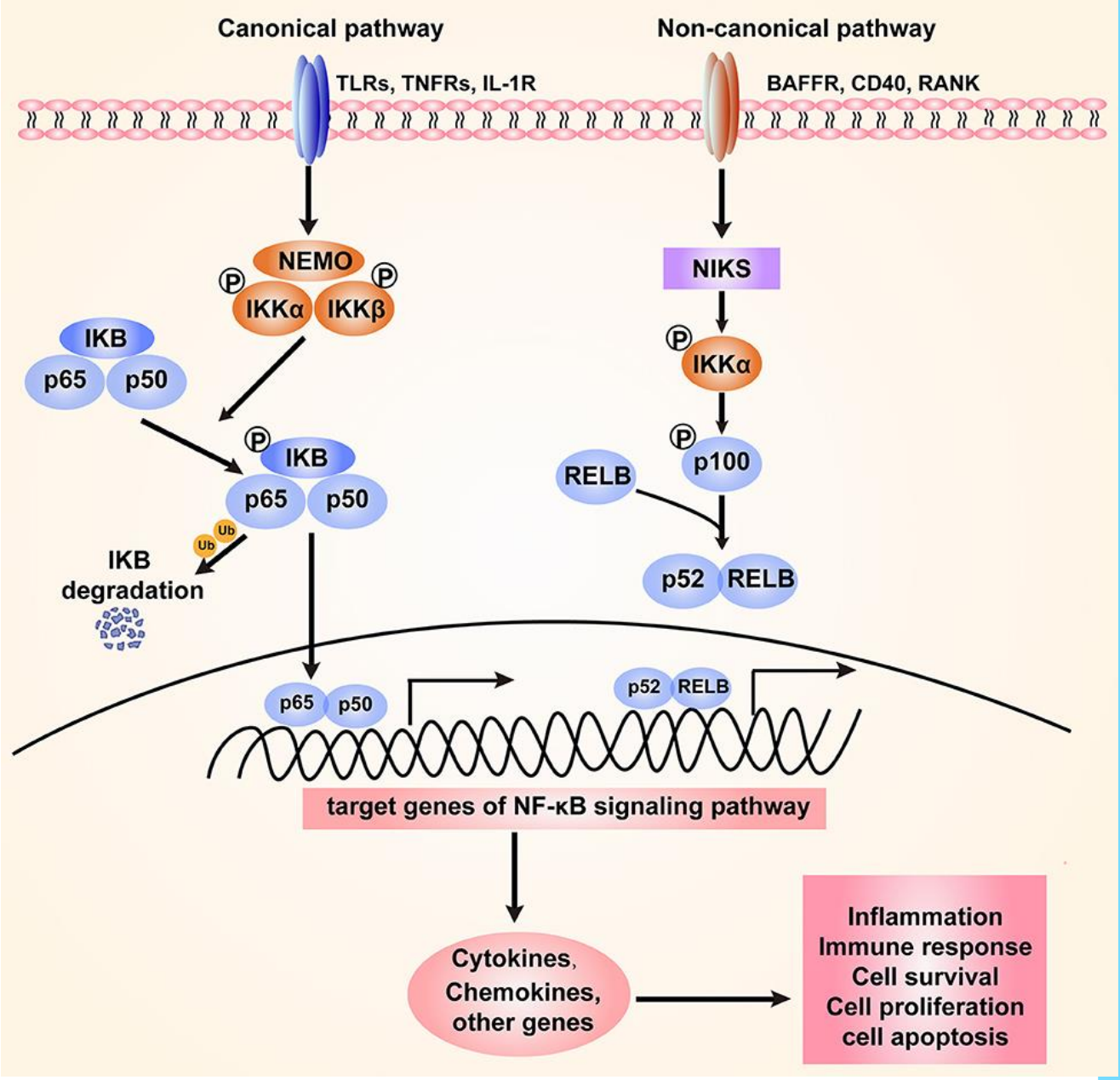
(Peng *et al*., 2020)

Signal transduction Non-canonical pathway

This pathway is stimulated by the TNF-receptor (TNFR) family. For example:

- LTβR
- BAFFR
- RANK

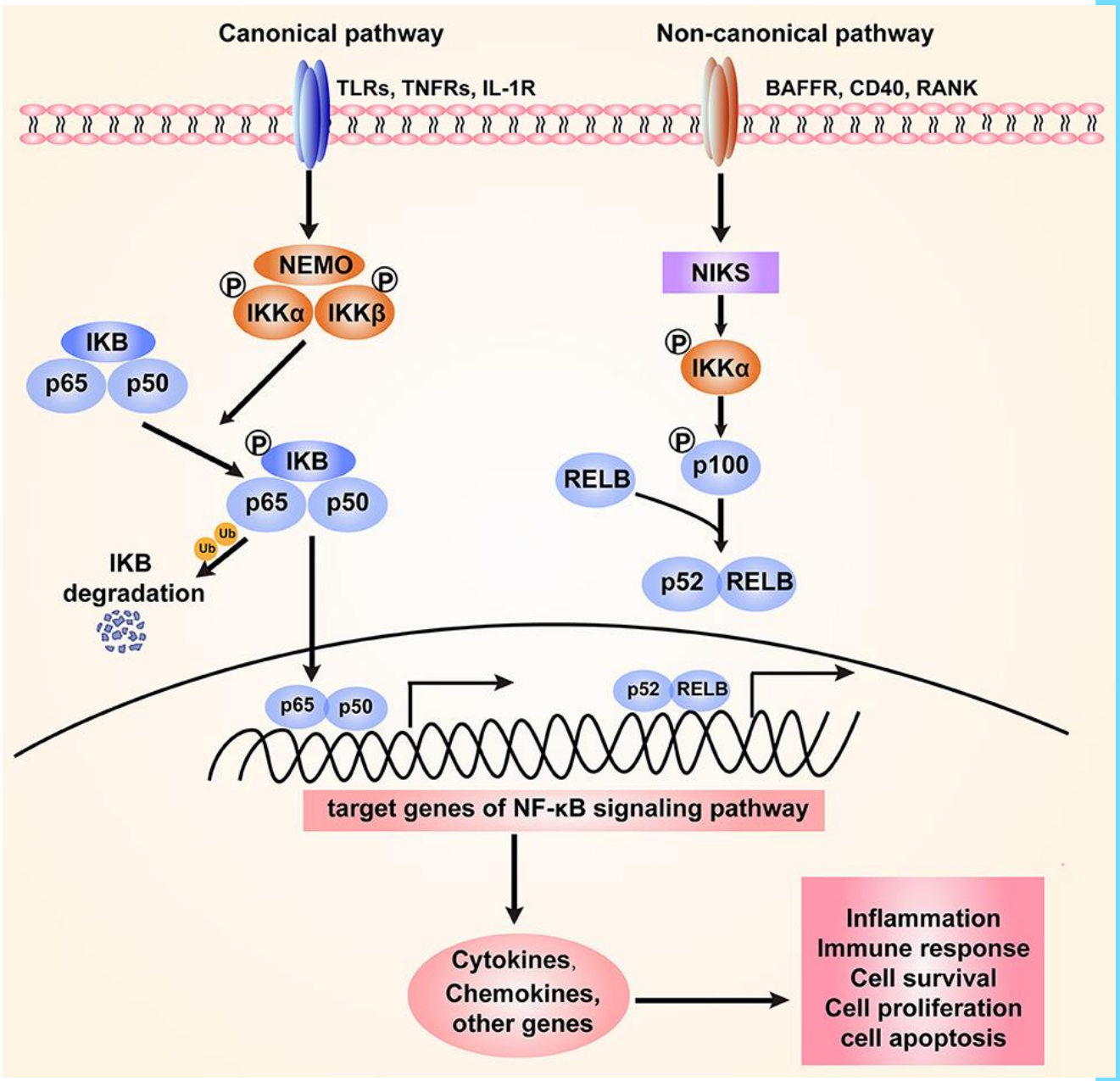
(Peng *et al*., 2020)



Signal transduction Canonical pathway

Step 1

The ligand binds to the receptor and becomes activated.

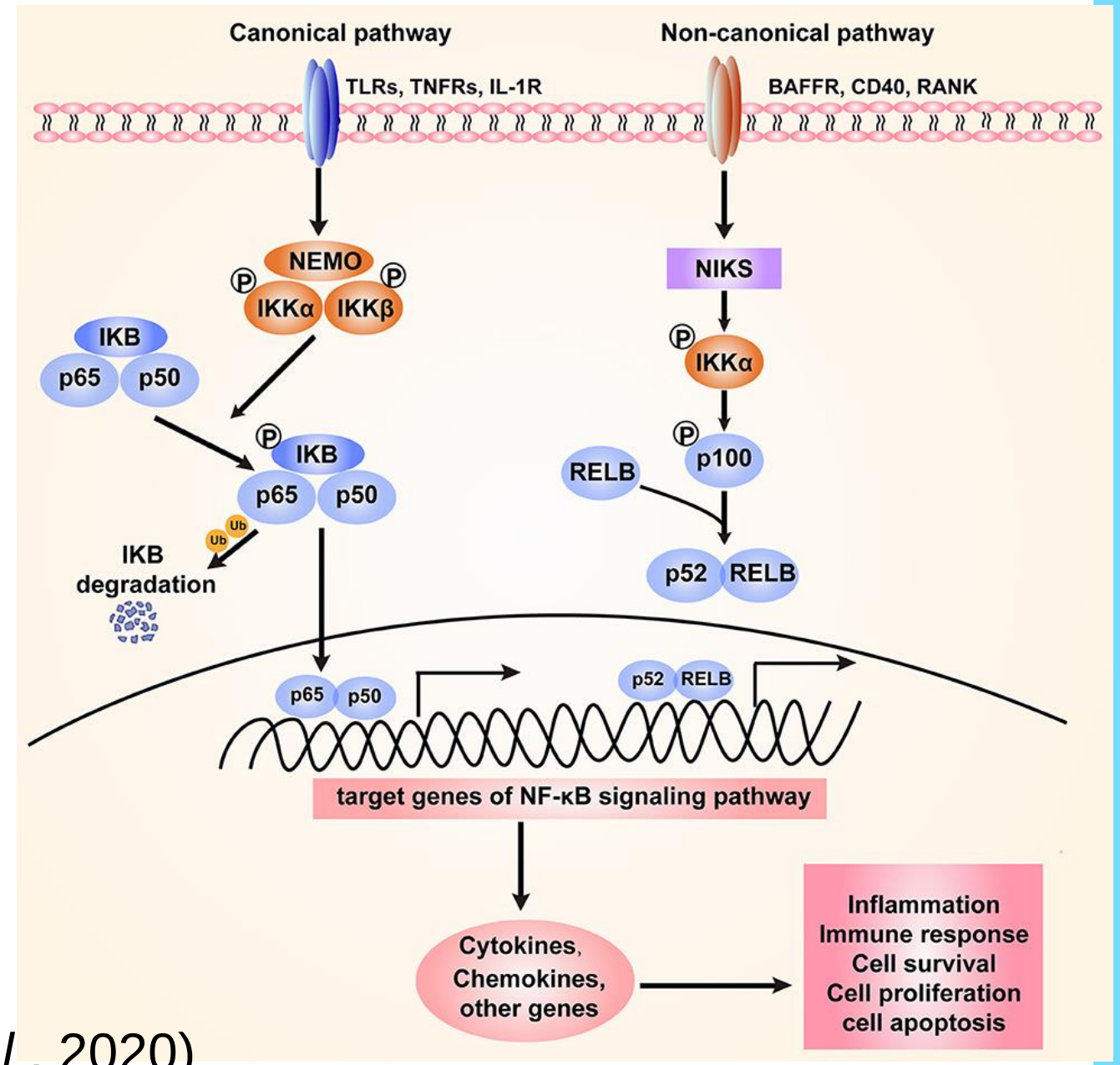


(Peng *et al*., 2020)

Signal transduction Canonical pathway

Step 2

TRAF proteins mediate the activity of NFκB-inducing kinase and activate an IKKα homodimer.



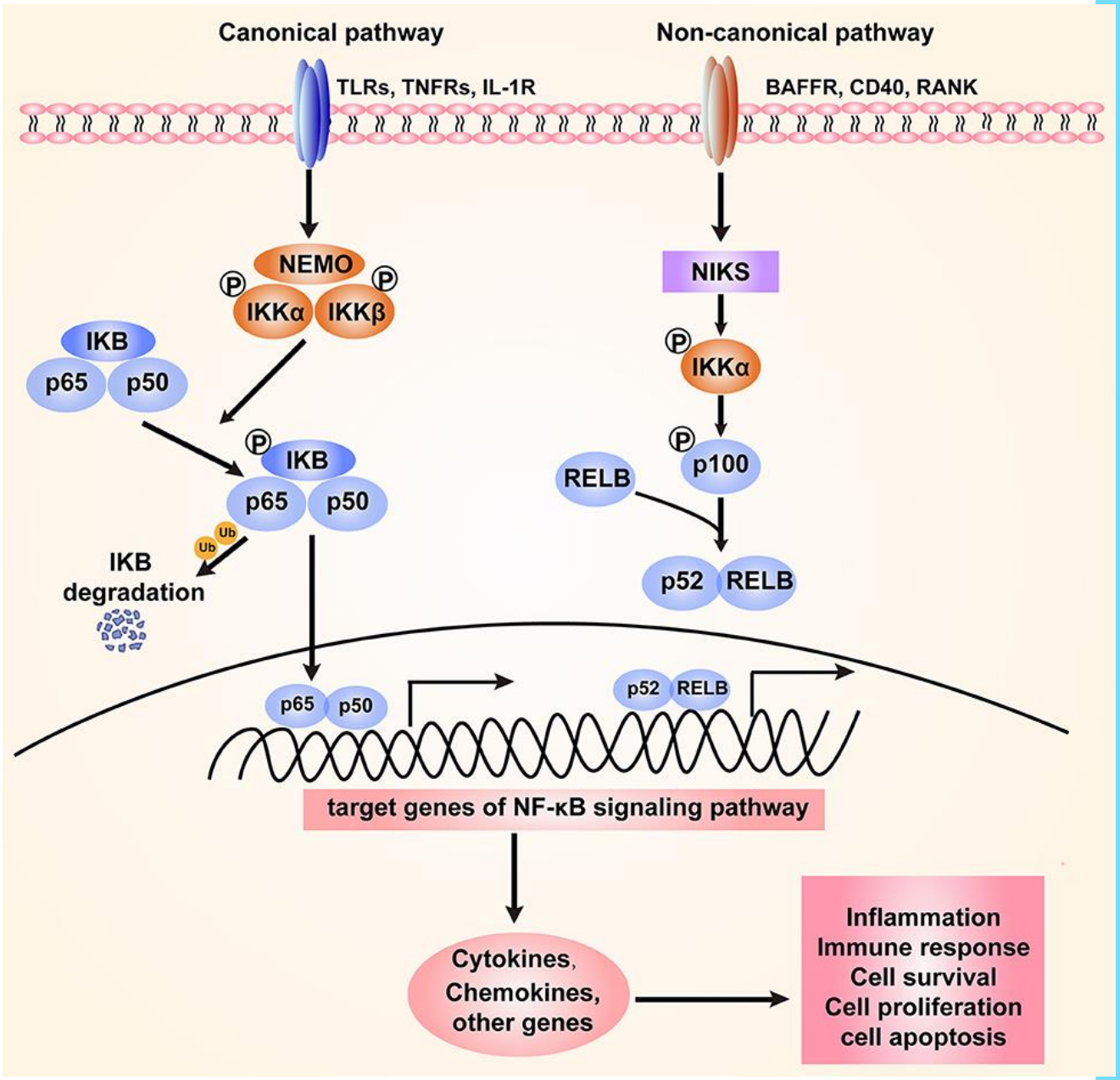
(Park, H.H. (2018); Peng *et al*., 2020)

Signal transduction Canonical pathway

Step 3

Heterodimerization of p100 and RelB.

This activates p52

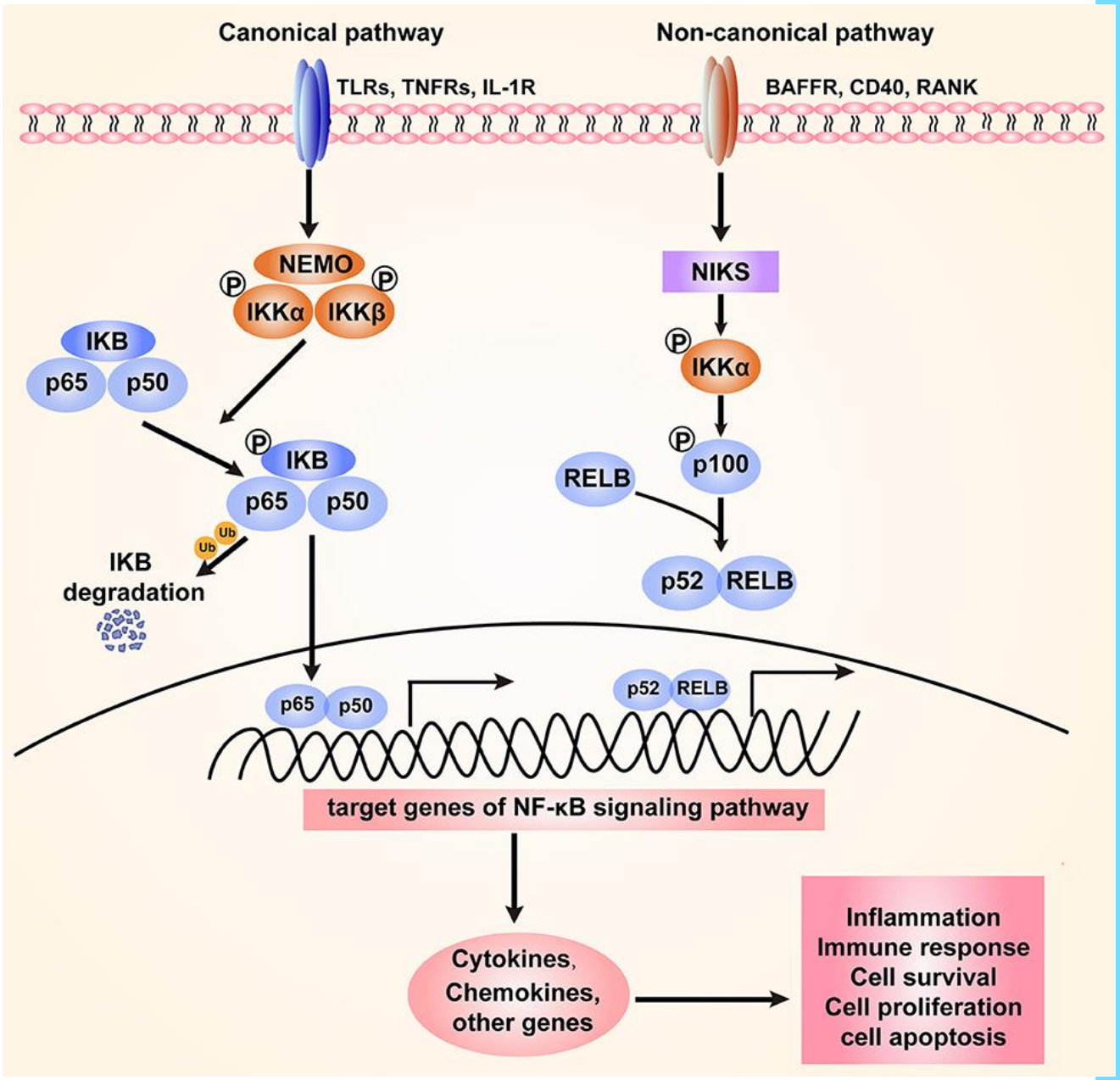


(Peng *et al*., 2020)

Signal transduction Canonical pathway

Step 4

This complex regulates gene transcription via κB DNA-binding sites.



(Peng *et al*., 2020)

Cellular response

Cellular response

Inflammation

Cell differentiation

Stress

Proliferation

apoptosis

Dysregulated signalling pathway

Dysregulated signalling pathway

- *Glioma*: interleukins e.g. the genes *IL1*, *IL6*, *IL8*, and *CCL2* are expressed and induce cancer progression.

- *Ovarian cancer*: TNF activates chemokines that induce inflammation when bound to their receptor CXCR2.

- NFκB can inhibit the production of inflammasomes by preventing the activation of caspase 1. This lowers inflammation and apoptosis and is facilitated by antiapoptotic proteins e.g. PAI2 and Bcl-xL.

Dysregulated signalling pathway

- *Breast cancer*: Mutations of *IKKA* increases tumour progression via the hormone progesterone.
- Other cancers e.g. *colon cancer, and lymphatic cancer*, leads to abnormal cell proliferation, metastasis, and treatment resistance.
- *Nasopharyngeal carcinoma*: it regulates energy metabolism

Dysregulated signalling pathway

- *Hepatocellular carcinoma*: Overexpression of *IKKβ* in NFκB signalling pathway can suppress the tumour progression.

- However, IKKβ can enhance hepatocellular cancer progression via JNK.

- LCN2 is a regulatory gene for NFκB–Snail pathway and can inhibit the phosphorylation of p65 to prevent activation of the NFκB pathway. This inhibits colorectal cancer cell epithelial–mesenchymal transition and metastasis.

By the end of this lecture, you should understand

Principles of NF-κB signalling

- ❑ NF-κB proteins: five members of NF-κB family: p65 (RelA), RelB, c-Rel, p50/105 (NF-κB1) and p50/105 (NF-κB2). They all share Rel homology domain responsible for DNA binding and multidimerization.
- C-terminal transcriptional activation domain (TADs) are present in RelA, p65 and RelB. They are not present in p50/100 or p52/105.
- ❑ IκB proteins
- ❑ IKK complex.

By the end of this lecture, you should understand

Canonical pathway

- ❑ This pathway is induced by TLRs, TNFRs, and IL-1R is bound to their specific ligand.
- ❑ This leads to phosphorylation and degradation of inhibitory protein IκB.
- ❑ NF-κB is released from the IκB-containing complex, then translocating into nucleus.

Non-Canonical pathway

- ❑ This pathway is dependent on the activation of NF-κB2 (p100)/ RelB complex by BAFFR, CD40, and RANK.
- ❑ It induces phosphorylation of NIK, which phosphorylates IKKα.
- ❑ The p52-RelB heterodimer is activated and translocate to the nucleus for transcription

Reference list for further reading

Ermine, K., Yu, J. and Zhang, L. (2021). Role of Receptor Interacting Protein (RIP) kinases in cancer. *Genes & Diseases*.

Gaurnier-Hausser, A., Patel, R., Baldwin, A.S., May, M.J. and Mason, N.J. (2011). NEMO-Binding Domain Peptide Inhibits Constitutive NF-κB Activity and Reduces Tumor Burden in a Canine Model of Relapsed, Refractory Diffuse Large B-Cell Lymphoma. 17(14), pp.4661–4671. doi:https://doi.org/10.1158/1078-0432.ccr-10-3310.

Li, X., Han, M., Zhang, H. *et al.* Structures and biological functions of zinc finger proteins and their roles in hepatocellular carcinoma. *Biomarker Research* **10**, 2 (2022).

Jones, S. (2004). An overview of the basic helix-loop-helix proteins. *Genome Biology*, [online] 5(6), p.226. doi:https://doi.org/10.1186/gb-2004-5-6-226.

Park, H.H. (2018). Structure of TRAF Family: Current Understanding of Receptor Recognition. *Frontiers in Immunology*,9, p.1999.

Park, H.H., Lo, Y.-C., Lin, S.-C., Wang, L., Yang, J.K. and Wu, H. (2007). The Death Domain Superfamily in Intracellular Signaling of Apoptosis and Inflammation. *Annual Review of Immunology*, 25(1), pp.561–586.

Peng, C., Ouyang, Y., Lu, N. and Li, N. (2020). The NF-κB Signaling Pathway, the Microbiota, and Gastrointestinal Tumorigenesis: Recent Advances. *Frontiers in Immunology*, [online] 11.

Thomas Dean Pollard, Earnshaw, W.C., Lippincott-Schwartz, J. and Johnson, G.T. (2017). *Cell biology*. Elsevier.

Xia, L., Tan, S., Zhou, Y., Lin, J., Wang, H., Oyang, L., Tian, Y., Liu, L., Su, M., Wang, H., Cao, D. and Liao, Q. (2018). Role of the NFκB-signaling pathway in cancer. *OncoTargets and therapy*, 11, pp.2063–2073.

Quizzes and Glossary will be released soon.

Thank you

SEASON 2

Understanding Cancer

Lecture 16

Types of signalling pathway: HER2

DR HAFSA WASEELA ABBAS

www.hafsaabbas.com