Does the MK2-dependent production of TNFα regulate mGluR-dependent synaptic plasticity?

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Abstract: The molecular mechanisms and signalling cascades that trigger the induction of group I metabotropic glutamate receptor (GI-mGluR)-dependent long-term depression (LTD) have been the subject of intensive investigation for nearly two decades. The generation of genetically modified animals has played a crucial role in elucidating the involvement of key molecules regulating the induction and maintenance of mGluR-LTD. In this review we will discuss the requirement of the newly discovered MAPKAPK-2 (MK2) and MAPKAPK-3 (MK3) signalling cascade in regulating GI-mGluR-LTD. Recently it has been shown that the absence of MK2 impaired the induction of GI-mGluR-dependent LTD, an effect that that is caused by reduced internalisation of AMPAR. As the MK2 cascade directly regulates tumour necrosis factor alpha (TNFα) production, this review will examine the evidence that the release of TNFα acts to regulate glutamate receptor expression and therefore may play a functional role in the impairment of GI-mGluR-dependent LTD and the cognitive deficits observed in MK2/3 double knockout animals. The strong links of increased TNFα production in both aging and neurodegenerative disease could implicate the action of MK2 in these processes.

Keywords: p38 MAPK, MK2, TNFα, AMPAR trafficking, GI-mGluR-LTD, hippocampus.

1. Introduction

Synaptic plasticity in neurons is an activity dependent change in synaptic efficacy, which is believed to be an experimental correlate of learning and memory [1]. The two primary types of synaptic plasticity are long-term potentiation (LTP) an increase in synaptic transmission strength and long-term depression (LTD) a decrease in synaptic transmission strength [1,2]. In the hippocampus there are two principal types of LTD, one form of LTD is induced by activation of ionotropic N-methyl-D-aspartate receptors (NMDAR), known as NMDAR-dependent LTD. The other form of LTD is induced by the activation of group I-metabotropic glutamate receptors (GI-mGluR-LTD). The known molecular mechanisms underlying these two types of LTD have been extensively reviewed elsewhere [2,3]. GI-mGluR-LTD can be induced either by the application of the group 1 selective agonist (R,S)-3,5-dihydroxyphenylglycine (DHPG) or by paired-pulse low frequency stimulation (PP-LFS) in the hippocampus [2,3]. The induction of GI-mGluR-LTD is dependent on the activation of p38 mitogen-activated protein kinase (MAPK) in the hippocampus [4,5]. The downstream effectors for the p38 action during synaptic plasticity were unknown until recent evidence discovered that p38 regulates GI-mGluR-LTD via the activation of the MAPK-activated protein kinases 2 and 3 (MAPKAPK-2 and MAPKAPK-3, also known as MK2 and MK3) [6]. Activated p38 binds to and phosphorylates MK2 to induce a conformational change that allows the binding and/or phosphorylation of the p38-MK2 complex to its substrates (the p38 and MK2 localisation and interaction mechanism has been described elsewhere [6-8]).

The involvement of the p38-MK2 signalling cascade in regulating inflammatory responses, in particular the production of the pro-inflammatory cytokine tumour necrosis factor alpha (TNFα) has been well described in mammalian cells and in the spinal cord [9-11]. However, not much information regarding the functional involvement of the p38-MK2 complex is known in the brain. The expression of p38 and MK2 proteins has been detected in neurons and microglia in the brain, principally in the cortex and the hippocampus [12-14]. It is known that TNFα is predominantly synthesised and released by microglia in the brain [15,16]. However, the implications for the p38-MK2-pathway activation in the production and release of TNFα in the brain has not yet been well characterised. TNFα production and release has in recent years been shown to have a wide range of functions in the brain such as apoptosis, cell migration and proliferation [16]. In addition to these traditional roles for TNFα there is a growing body of evidence for the function of TNFα in the regulation of

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glutamate receptor trafficking and synaptic transmission [16-19].

This review will examine the relationship between the reduction in glutamatergic synaptic transmission seen in MK2/3 double knockout (DKO) neurons that is promoted by reduced surface expression of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPAR) [6] and correlate these findings with the possible MK2-dependent production of TNFα in the brain. The potential consequences of this MK2 regulated production of TNFα in the brain and the alterations in synaptic transmission that are observed in MK2/3 DKO animals will also be discussed.

2. MK2/3 DKO mice have deficits in GI-mGluR-LTD and cognitive flexibility.

The recently published Eales *et al.,* paper examined the changes in synaptic transmission and in cognition in the MK2/3 DKO mice [6]. Cultured hippocampal neurons and CA1 pyramidal neurons obtained from MK2/3 DKO animals showed altered spine morphology with an increase in the length of the spine neck and a decrease in spine head diameter compared to wild-type cells. These changes in spine morphology are promoted by the disruption of the p38-MK2-cofilin cascade observed in MK2/3 DKO cells that regulate the depolymerisation of actin filaments [6,20]. The increased activation of cofilin caused a shift from filamentous actin to monomeric globular actin in MK2/3 DKO mice [21].

In addition to these changes in spine morphology, electrophysiology recordings in cultured hippocampal neurons from MK2/3 DKO mice showed a reduction in AMPAR-mediated miniature excitatory post synaptic current (mEPSC) amplitude under basal conditions (Figure 1 A-D). This decrease in amplitude of mEPSC suggests that there are less AMPAR expressed at the post-synaptic density [6]. AMPAR are ionotropic glutamate receptors that mediate fast excitatory synaptic transmission, they are tetramer structures constructed of the four subunits, GluA1-4 [22]. In mature cultured hippocampal neurons AMPAR are normally expressed as hetrotetramers composed of dimers of the GluA2 and GluA1 subunits [23]. Eales *et al.,* 2014 demonstrated that hippocampal cultures of MK2/3 DKO mice displayed a reduction in both AMPAR-mediated mEPSC amplitude and reduced expression of the GluA1 subunit at the cell surface. Interestingly, there was no reduction in the surface expression of the GluA2 subunit in MK2/3 DKO mouse cultures. In agreement with the observation in hippocampal cultures, a reduced expression of GluA1, but not GluA2, was observed in hippocampal lysate obtained from adult MK2/3 DKO mice [6]. However, the mechanism behind this alteration in AMPAR expression in MK2/3 DKO mice at the cell surface was not determined. Therefore further investigation is necessary to address whether the release of glutamate is compromised in these animals, as this could cause a reduction in AMPAR surface expression.

The decrease in surface expression of the GluA1 AMPAR subunit in MK2/3 DKO animals is significant as GluA2 lacking AMPAR are highly permeable to calcium [24, 25]. This enhanced conductance to calcium has given GluA1 expression interesting links to excitotoxicity and cell death [25,26]. Therefore decreasing GluA1 surface expression by the removal of MK2 protein expression could be neuroprotective in neurons by reducing excitatory activity in the brain and decreasing the amount of calcium induced apoptosis.

Cultured hippocampal neurons obtained from MK2/3 DKO mice showed a clear impairment in GI-mGluR-LTD induced by DHPG exposure (Figure 1 E). Impaired GI-mGluR-LTD induced by either DHPG or PP-LFS was also observed in hippocampal slices obtained from MK2/3 DKO mice [6]. Importantly, GI-mGluR-LTD is dependent on the endocytosis of glutamate receptors, most likely GluA1-containing AMPAR [3,6]. In agreement with impaired GI-mGluR-LTD observed in hippocampal neurons obtained from MK2/3 DKO mouse, a reduction in AMPAR subunit 1 internalization is also observed after DHPG exposure when compared to wild-type cells [6]. Explanations for this impaired mGluR LTD are that under basal conditions, MK2/3 DKO neurons already display a reduced amount of GluA1 subunits at the surface and therefore the subsequent endocytosis of AMPAR by the induction of GI-mGluR-LTD is not sufficient to reach the threshold to trigger sufficient endocytosis of AMPAR to induce GI-mGluR-LTD. Alternatively the knockdown of MK2 expression in MK2/3DKO has mimicked mGluR-LTD and therefore occludes the induction of mGluR-LTD. Investigating the relationship between MK2 and AMPAR regulation is an important step in understanding the synaptic deficits observed in MK2/3 DKO mice as well as the role of p38 and MK2 in GI-mGluR-LTD. In accordance with the impaired mGluR-LTD, the MK2/3 DKO mice displayed cognitive deficits specifically in hippocampal-dependent spatial reversal learning when their learning and memory was tested using a modified Barnes maze task [6].

One of the most important findings from Eales *et al.,* is the observation that re-introducing MK2-WT, but not MK3-WT, in MK2/3 DKO hippocampal neurons reversed the deficit in dendritic spine morphology, restored basal synaptic transmission and GI-mGluR-LTD to wild-type levels (Figure 1) [6]. These findings suggest that absence of MK2 is the causative factor for the alternations observed in MK2/3 DKO mice. However the question still remains: what is the mechanism linking the activation of the MK2 cascade to reduced surface expression of GluA1 and synaptic transmission. Here we propose that the deficits in synaptic transmission seen in MK2/3 DKO animals are due to reduced levels of TNFα production in the brain (Figure 2).

3. Is TNFα production and release the missing link between p38-MK2 pathway activation and the impairment of mGluR-LTD seen in the absence of MK2?

The absence of MK2 is known to reduce the amount of p38 protein expression and to regulate the production of TNFα in mammalian tissue [9-11]. In the spinal cord it has been shown that reduced levels of produced and released TNFα after injury are a direct consequence of MK2 regulating TNFα production at a post-transcriptional level [10]. The mechanism by which the MK2 cascade regulates TNFα mRNA stability and translation after lipopolysaccharide (LPS) stimulation has been described elsewhere [9,11]. However, the mechanistic relationship between the MK2 cascade activation and TNFα production after increased activity has not yet been fully established in the brain. The molecular mechanism regulating the release of TNFα at the synapse and the activation of the signalling cascades at postsynaptic neurons is not yet completely understood. In the central nervous system, there are two known receptors for TNFα, TNFα receptor 1 (TNFR1) and TNFR2 which are expressed in hippocampal neurons [28]. The activation of these two diverse receptors triggers different signalling cascades to cause greatly different effects for the action of TNFα. The activation of TNFR1 triggers apoptotic cascades and TNFR2 activation elicits cell survival cascades [28,29].

In the brain, TNFα concentration has been shown to have a role in the regulation of glutamate receptors, synaptic transmission, synaptic plasticity and excitotoxicity [15,17-19,30-33]. The role of TNFα in the regulation of these functions is important because the correct trafficking and regulation of glutamate receptors is vital for normal development and function in the central nervous system and the brain. For example, exposure of neurons to a high concentration of 60 nM of TNFα for 15 minutes has been shown to cause a rapid increase in the amount of AMPAR GluA1 subunit expression at the cell surface in neuronal cultures, this was shown to be dependent on the binding of TNFα to the TNFR1 [19]. Conversely, exposure of hippocampal cultures to a recombinant soluble form of TNFR1, which binds to endogenous TNFα and reduces the amount of TNFα at synapses, resulted in decreased GluA1 subunits expression at the cell surface and reduced basal synaptic strength in neurons [17,19]. This could imply that the reduction in surface expression of GluA1 that is observed in MK2/3 DKO mice [6] could be the result of a reduction in the amount of TNFα at the synapse released by microglia and/or neuron or by the effect of the activation of the p38-MK2 cascade in neurons. The p38-MK2 cascade has been shown to have a mechanistic role in mGluR-LTD by promoting the activation of cofilin to cause actin remodeling as well as having a role in the removal of AMPAR in neurons [6] but the downstream cascades that causes AMPAR internalization are not clear. The MK2-dependent production of TNFα mediating endocytosis of GluA1 could provide a mechanism for the reduction of AMPAR after the induction mGluR-LTD.

The developmental importance of TNFα release in the regulation of glutamate receptors in synaptic scaling, plasticity and excitotoxicity has been documented. Synaptic scaling maintains the delicate balance between excitatory and inhibitory activity, which is required for the regulation of homeostatic responses in the brain to maintain optimum neuronal activity. Exposure of neurons to TNFα disrupts synaptic scaling by increasing excitatory AMPAR expression and decreasing inhibitory γ-aminobutyric acid ($GABA_A$) receptor expression [19]. There have also been associations of TNFα with synaptic plasticity, although LTP induction is normal in TNFα knockout animals [34]. Importantly it was observed that GI-mGluR-LTD is dependent on TNFα as GI-mGluR-LTD is impaired in TNFR1 null mice [30]. Therefore the reduction in GluA1 expression and impaired GI-mGluR-LTD observed in the hippocampus when there is a reduction in TNFα, correlates with the reduction of GluA1 expression and impaired GI-mGluR LTD seen in MK2/3 DKO animals. These findings give a strong possible link for the synaptic transmission alterations in MK2/3 DKO mice being regulated by the potential MK2 mediated reduction in TNFα.

TNFα has been shown to be both neuroprotective and to potentiate excitotoxicity depending on its concentration at the synapse. Over stimulating glial cells in hippocampal culture increases TNFα release which induced apoptosis in neurons. The induction of apoptosis was TNFα mediated as it could be prevented by TNFα antibody application, which binds to TNFα and prevents its interaction with TNFα receptors [35]. TNFα exposure exacerbates AMPA toxicity, this can be demonstrated as a subtoxic dose of AMPA induced cell death when combined with TNFα, suggesting that TNFα can potentiate AMPA toxicity [29, 36]. TNFα can also increase neuronal susceptibility to neurotoxic insults by causing an increase in GluA1 receptor expression [37]. High concentrations of TNFα (10 ng/ml), enhanced the induction of neurotoxicity, whereas pre-incubation with TNFα (1 ng/ml) for 24 hours has a neuroprotective effect on CA1 hippocampal neurons when they are exposed to toxic levels of AMPA [29]. Interestingly, despite this neuroprotective role, there is a lack of information associating an endogenous reduction in TNFα release with its effect at glutamatergic synapse. Therefore the use of MK2 knockout (MK2 KO) mice could be an excellent model to study whether the reduction of TNFα release at excitatory synapses could have a neuroprotective effect. Evidence supporting this neuroprotective hypothesis can be demonstrated in MK2 KO animals, which have a significant reduction in TNFα production. MK2 KO mice have decreased cell death and increased recovery activity after spinal cord injury [10]. MK2 KO mice were also protected against a high bacterial lipopolysaccharide insult that was lethal in wild-type littermates [11]. In both cases the neuroprotective effect for the absence of MK2 was shown to be dependent on the reduction of cytokine production, particularly TNFα.

The concentration of TNFα in the brain is high during development, low during adulthood and increases in aging

and disease states. Microglia are the primary producers of TNFα in the brain [13,14] and because aging has been associated with increased microglia activity, this is thought to cause an over production of TNFα in the normal aging brain [38,39]. In addition to this, the proliferation of microglia in response to stress stimuli is much higher in aging brains when compared to young brains, meaning higher quantities of microglia and TNFα [40]. This increase in TNFα during aging could be having numerous harmful neurotoxic effects and contribute to the decline in cognitive ability during aging. However, considerable research is needed to establish the role of TNFα in normal aged brain models. It would be interesting to investigate if an endogenous reduction in TNFα mediated by the absence of MK2 could be neuroprotective in aging and prevent cognitive decline.

The production of TNFα has been linked to many diseases such as ischemia, parkinsons, and multiple sclerosis [41-43]. However most relevant to this review is the over production of TNFα in Alzheimer's Disease, Fragile X syndrome and epilepsy [44-46] as these diseases have also been linked with altered glutamate receptor expression and excitotoxicity [26,47]. Alzheimer's is a neurodegenerative disease that causes global cognitive impairments and is characterised by amyloid plaques, neurofibrillary tangles and neuronal loss [48]. Microglia have been shown to be over activated in Alzheimer's disease which causes a rise in the release and synthesis of TNFα [49]. As TNFα can interact with amyloid precursor protein (APP) this rise in TNFα exposure has been shown to cause an increase in the production of Aβ [50,51] which is the main component of the hallmark amyloid plaques seen in Alzheimer's disease brains [48]. Importantly the reduction of TNFα production is neuroprotective in Alzhiemer's disease models. LTP in the hippocampus is blocked by exposure to oligomerised Aβ, this block of LTP can be rescued with the reduction of TNFα before exposure to Aβ or the removal of TNFR1 [34]. Additionally cognitive impairments are improved with the reduction of TNFα in Alzheimer's disease model mice [51]. This neuroprotective reduction in TNFα is also supported by preliminary human clinical trials for drugs reducing the levels of TNFα in Alzheimer's patients that resulted an improvement in cognitive deficits and a reduction in amyloid plaques and neurofibrillary tangles was observed [50,52]. Upstream of TNFα the production of MK2 is also upregulated in Alzheimer's disease transgenic animals, however less associations have been made between MK2 and Alzheimer's disease. Microglia obtained from MK2 knockout mice show significantly decreased TNFα production and interestingly there is a significant increase in cell viability on exposure to a neurotoxic amount of oligomerised Aβ when MK2 is knocked down. This neuroprotective effect of MK2 is thought to be mediated by a reduction in TNFα production levels [14]. The facilitation of mGluR-LTD by Aβ in Alzheimer's disease models has been established [53] and it would be interesting to see if Aβ exposure was able to facilitate mGluR-LTD in MK2/3 DKO mice when TNFα levels are reduced.

The reduction of TNFα concentration is also neuroprotective in epilepsy with mice lacking TNFR1 showing reduced susceptibility to epileptic seizures due to altered expression of glutamate receptors [54]. Additionally the potential involvement of MK2 and TNFα in Fragile X syndrome is an exciting possibility. Fragile X-related protein 1 (FXRP1) has been shown to regulate the production of TNFα at the post-transcriptional level and when FXRP1 is knocked down in neuronal cultures there is an enhancement in the production of TNFα [46]. In the disease model for Fragile X (FMRP1 knockout mice) there is an enhancement of mGluR-LTD [55], it is possible therefore that there are increased levels of TNFα in Fragile X disease that could be linked to the enhancement of mGluR-LTD seen in this disease model [55]. It would be interesting to further investigate both the role of TNFα in these disease states mentioned as well to see if there is an effect with the endogenous reduction of TNFα in these disease states. The links of TNFα to disease states and normal aging are particularly interesting as MK2 is potentially a good target for drugs, as its inhibitors have less side effects than drugs targeting TNFα and p38 [56].

CONCLUSION

In conclusion the synaptic and cognitive deficits that are seen in MK2/3 KO mice could be due to a reduction in the production and release of TNFα at the synapse. This reduction in TNFα at the synapse could provide a mechanism for the decrease in cell surface expression of AMPAR seen in MK2/3 DKO hippocampal neurons. This possible reduction in the concentration of endogenous TNFα production in the brain caused by the absence of MK2 could therefore be neuroprotective in aging and neurodegeneration where a fine balance in the concentration of TNFα production and release is needed.

Conflict of interest: The authors declare no competing interests

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Legend Figure 1:

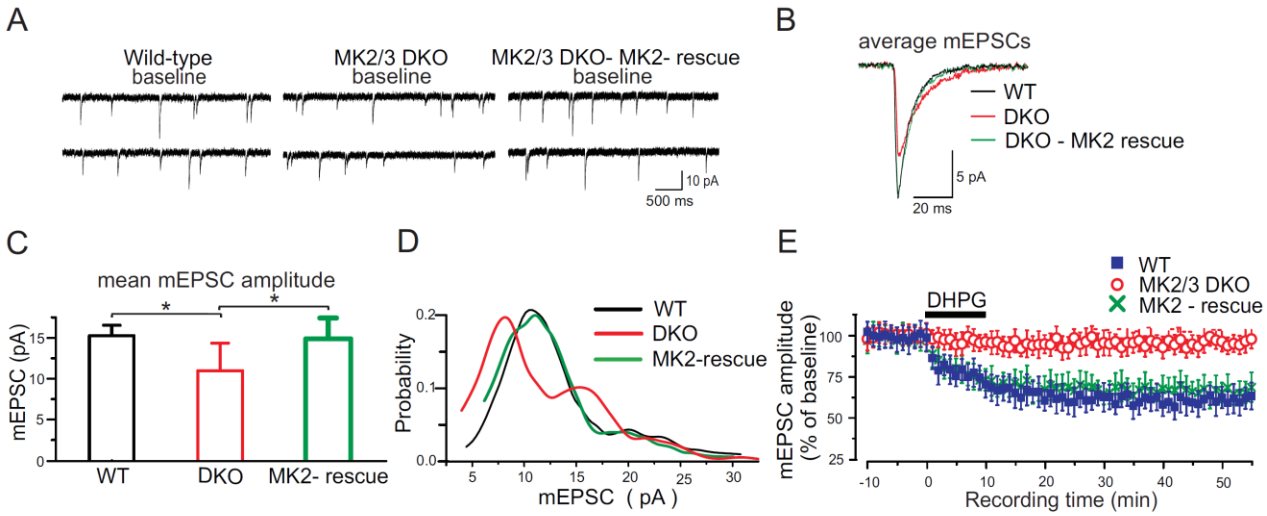
MK2 regulates synaptic transmission in hippocampal cultured neurons.

Electrophysiology experiments recorded from hippocampal cultured neurons reproduced from Eales et al., [6]. (A) AMPAR-dependent mEPSC traces from WT, MK2/3 DKO and MK2/3 DKO neurons expressing EGFP-tagged-MK2-WT (rescue) at baseline. (B) AMPAR-dependent mEPSC events recorded from WT, MK2/3 DKO and MK2/3 DKO neurons expressing MK2-WT. (C) Mean mEPSC amplitude showing that the decrease in amplitude observed in MK2/3 DKO is reversed by re-insertion of MK2-WT. (D) Release probability graph, the double peaks in mEPSC amplitude distribution in DKO cells suggest that multiquantal release is not seen in WT or in DKO expressing MK2-WT. Shown data are from WT (n=8 cells), DKO (n=10 cells) and DKO overexpressing MK2-WT (n=6 cells) from three to four independent preparations. (E) Graphs showing average time course of changes in mEPSC amplitude after DHPG (100 µM) induced mGluR LTD. Average mEPSC amplitude was 60.6±7.4% of baseline at 45±5 min (blue trace; n=8) in WT and 95.7±7.2% (red trace; n=10) in MK2/3 DKO. Note that LTD was rescued in MK2/3 DKO expressing MK2-WT 66.5±7.7% (green trace; n=6). Error bars represent ± s.e.m. and *P<0.04.

Legend Figure 2:

Putative working model linking the MK2-dependent production of TNFα to AMPAR trafficking in the central nervous system.

A schematic representation of the proposed mechanism by which the MK2-dependent reduction in TNF α production results in impaired GI-mGluR-LTD**. (A)** At non-stimulated dendritic spines of wild-type neurons there is a basal activity of p38, MK2 which results in basal levels of AMPAR at the surface and basal synthesis and release of TNFα primarily by microglia at synapses. By contrast, in spines of non-stimulated MK2 knockout (KO) neurons, where the p38-MK2 signalling cascade is blocked, there is a reduced number of AMPAR receptors at the cell surface and a potential reduction in the production and release of TNFα at the synapse. **(B)** Activation of GI-mGluRs in wild-type spines induces an increase in p38 and MK2 activity and endocytosis of AMPAR from the cell surface, which induces mGluR-LTD. However in MK2 KO neurons, the block of the p38-MK2 cascade results in no change in AMPAR at the surface and impaired mGluR-LTD. We hypothesize that this observation could be due to the inhibition of the MK2 downstream cascade in neurons and/or in microglia that promotes the reduction in TNFα synthesis and release primarily by microglia at the synapse.



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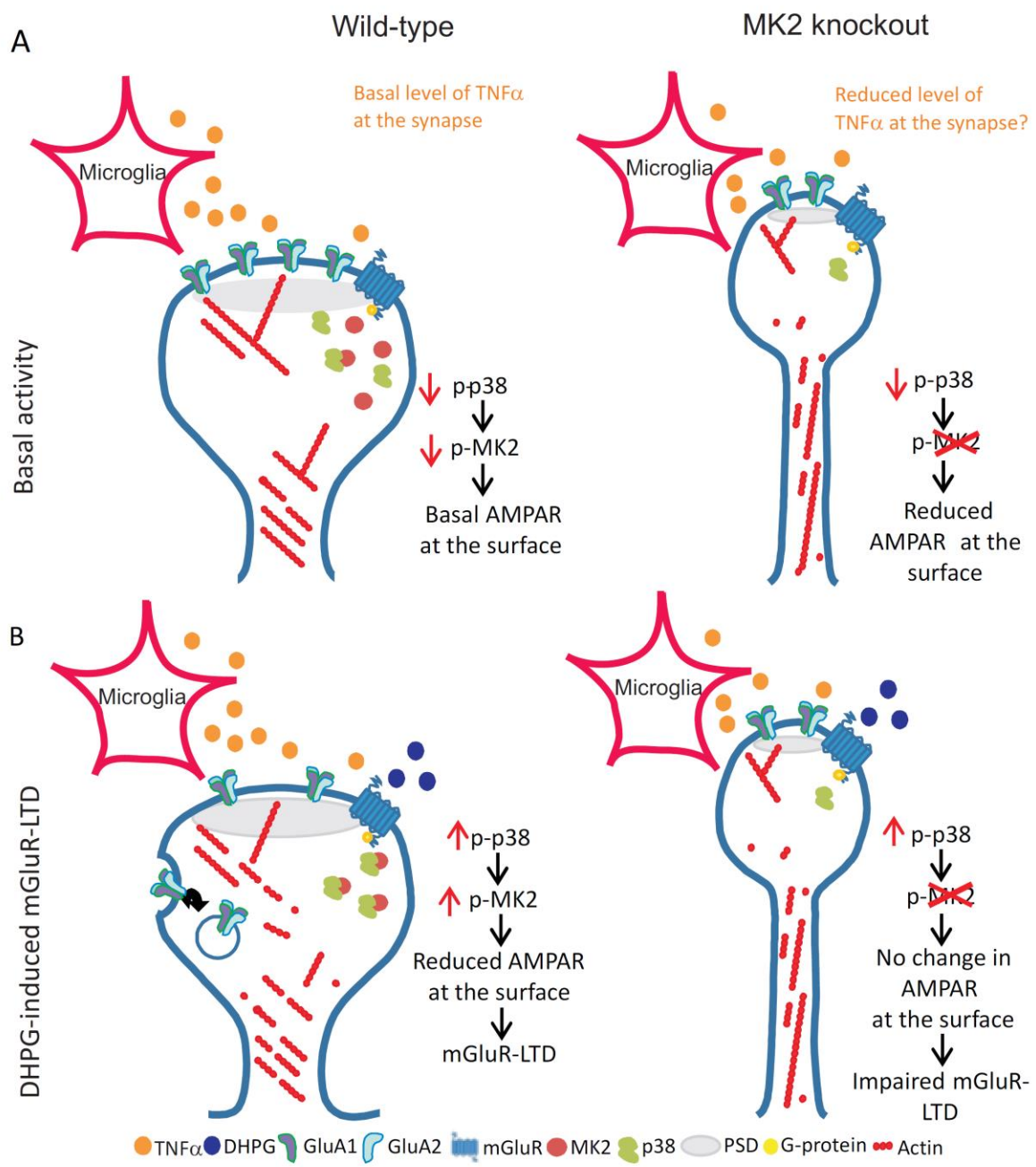

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