The control of bio-nanomachine using calmodulin-based iono-chromic nanodevice

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1. Background and Aim of Study

The small G Protein Ras is a guanine nucleotide-binding protein, functioning as a molecular switch, that cycles between inactive GDP-bound and active GTP-bound states to regulate a diverse array of cellular processes, including cell growth, apoptosis, and differentiation.

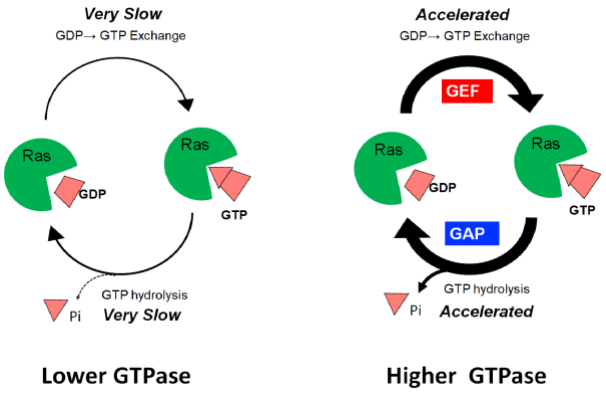

Fig. 1 Ras GTPase cycle

A class of regulatory molecules that lead to Ras activation are guanine nucleotide exchange factors (GEFs). Ras GEFs bind to Ras and facilitate GDP release, followed by GTP incorporation and Ras activation. Another regulatory molecule is the GTPase-activating protein (GAP), which can accelerate the hydrolysis of GTP. Ras has a functional domain HVR has an important physiological role in Ras, which can change Ras from monomer to multimer when incorporating with NBB into cysteine residues in HVR domain. Calmodulin is well-known as a Ca^{2+}-binding protein and is one of the essential regulatory proteins of cell signal transduction on pathways. CaM regulates the activities of numerous enzymes involved in cell function; Ca^{2+}/CaM binds to the well-conserved binding region M13 in CaM-dependent enzymes, resulting in the stimulation of the enzyme activities.

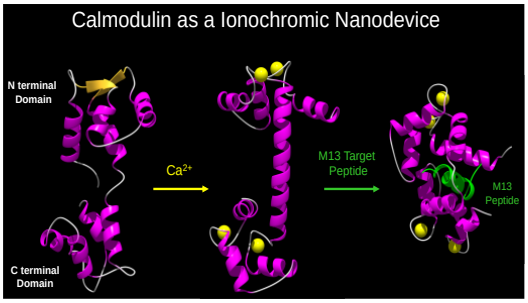

Fig.2 Mechanism of Calmodulin

Aim of this study To control Ras function by the mehod based on ionochromic phenomenon, we employed calmodulin as a ionochromic molecular device. H-Ras fused with calmodulin target M13 peptide at the HVR domain region in C-terminal were prepared. GTPase of H-Ras-M13 was regulated by Ca^{2+}-dependent calmodulin.

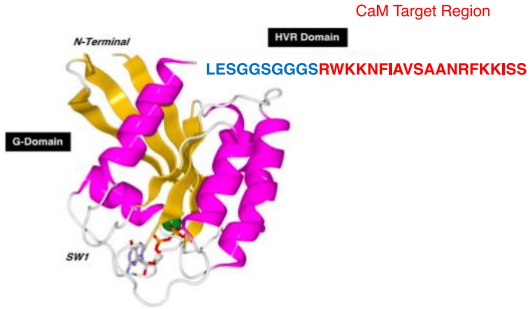

Fig. 4 Structure of H-Ras-M13

2. Materials and Methods

Expression and purification of H-Ras-M13 H-Ras-M13 DNA was incorporated into the pET21a vector. H-Ras-M13 expression plasmids were transformed into Escherichia coli Rosetta2 (pLysE). H-Ras-M13 was purified with a Co^{2+}-NTA column.

Ion-control of H-Ras-M13 GTPase assay The effect of Calmodulin bound to the M13 of H-Ras-M13 on the GTPase activity in the presence of GAP and GEF has been examined. The transition between cis and trans isomers of Calmodulin was achieved by present of Ca^{2+} and absent of Ca^{2+}

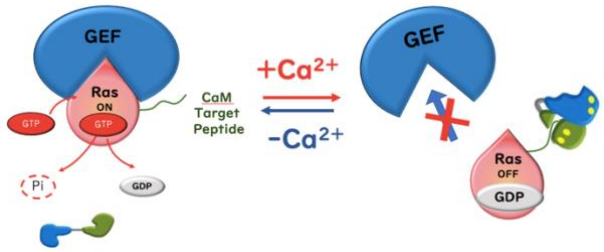

Fig. 4 Proposed possible mechanism for inhibition of H-Ras-M13 with Calmodulin.

3. Results and Discussion

GTPase assay of H-Ras-M13

To conduct the GTPase activity of H-Ras-M13 with Calmodulin. Due to the structure of calmodulin change in the presence and absence of Ca^{2+}. We use egtazic acid (EGTA) to create the absence of Ca^{2+} buffer solution. GTPase assay was conducted at four different conditions which were 5μM H-Ras-M13, 5μM GAP, 5μM GEF, 10 μM CaM, 2.5μM $CaCl_2$, 30 mM Tris-HCl (pH7.5), 120 mM NaCl, 2mM $MgCl_2$, 1mM GTP, and 5μM H-Ras-M13, 5μM GAP, 5μM GEF, 10 μM CaM, 2.5μM EGTA, 30 mM Tris-HCl (pH7.5), 120 mM NaCl, 2mM $MgCl_2$, 1mM GTP, and 5μM H-Ras-M13, 5μM GAP, 5μM GEF, 2.5μM $CaCl_2$, 30 mM Tris-HCl (pH7.5), 120 mM NaCl, 2mM $MgCl_2$, 1mM GTP, and 5μM H-Ras-M13, 5μM GAP, 5μM GEF, 2.5μM EGTA, 30 mM Tris-HCl (pH7.5), 120 mM NaCl, 2mM $MgCl_2$, 1mM GTP. The GTPase activity of H-Ras-M13 was measured at 25 °C.

Compare with in the absence of Ca^{2+}, in the presence of Ca^{2+} the GTPase of H-Ras-M13 was 43% decrease.

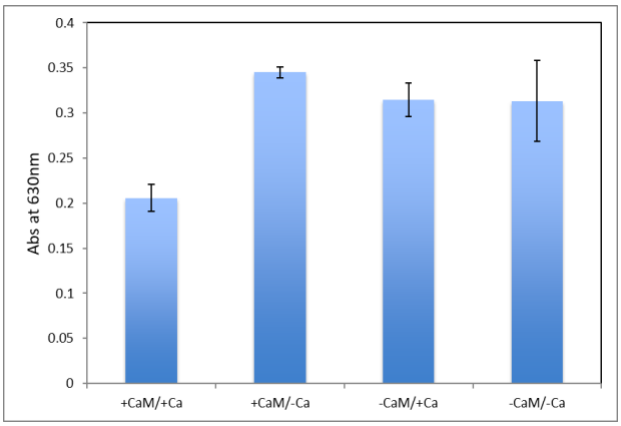

Fig. 5 GTPase of H-Ras-M13 with Calmodulin

4. Future Plans

To observe a more intense inhibition of H-Ras by Calmodulin, we will design a fusion protein of H-Ras, like a sandwich M13 will be both N and C-terminal of H-Ras. Moreover, our laboratory was already observed a stronger effect when the GS-Linker in photocontrol of H-Ras is shorter, so making GS-linker between H-Ras and M13 is shorter also is an excellent way to increase inhibition.

Conclusion

We have successfully demonstrated that incorporated M13 peptide into the HVR domain range in H-Ras, the control of GTPase activity be presence and absence of Ca^{2+}.

References

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