Expression and Purification of Full-Length bRaf

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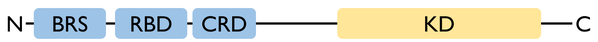
Raf

The serine-threonine kinase Raf plays a key role in the MAPK pathway. Upon activation by the small GTPase Ras, Raf phosphorylates and activates another kinase, MEK, leading to further signal transduction.

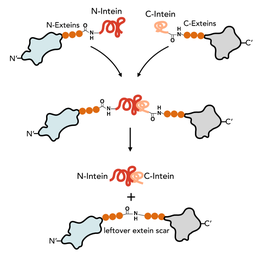
The interaction between Ras and the Ras binding domain (RBD) of Raf is well studied, but the exact mechanism of Raf activation remains unclear. It is difficult to study this mechanism without the full-length Raf protein, which is extremely difficult to purify in the quantities required. Establishing a protein purification protocol for Raf will allow us to investigate this important step in the MAPK pathway.

Along with the RBD, Raf also contains a cysteine rich domain (CRD) with two zinc finger domains, as well as the kinase domain (KD). The bRaf isoform also contains a bRaf specific domain (BRS).



Intein

My approach to purifying full-length Raf makes use of intein chemistry. Similar to introns in DNA, inteins in proteins can excise themselves and splice together the remaining pieces.



http://2014.igem.org/Team:Heidelberg/Project/Background

Abstract

The serine-threonine kinase Raf is a member of the MAPK pathway, an essential signaling pathway involved in cell growth, differentiation, and survival. Mutations in Raf are often linked to cancer, particularly mutations of Val-600 in the bRaf isoform. As such, it is important to understand how Raf is normally regulated and activated, as well as how mutations affect this regulation. However, it is difficult to study Raf in vitro because the full-length protein does not express well in bacteria and there is no established protocol for purifying it.

I have developed a protocol for human bRaf expression and purification using the E. coli expression system in combination with intein chemistry. First I screened a range of domain boundaries until I found the optimal N- and C-terminal domain constructs. I then used intein chemistry to combine the two to produce the pure full-length protein, which could then be used in biochemical assays and structural studies. This work has opened up new possibilities in studying Raf *in vitro*, hopefully leading to a better understanding of the protein and potentially developing more effective treatments for related diseases.

Conclusions

I have established a protocol for expression and purification of full-length human bRaf. This work makes it possible to study the full-length protein *in vitro*, hopefully leading to further insights into the mechanism of Raf activation.

Moving forward, the next step is to determine the structure of full-length bRaf using electron microscopy. Another future direction is studying binding partners by coexpressing them with bRaf.

Acknowledgements

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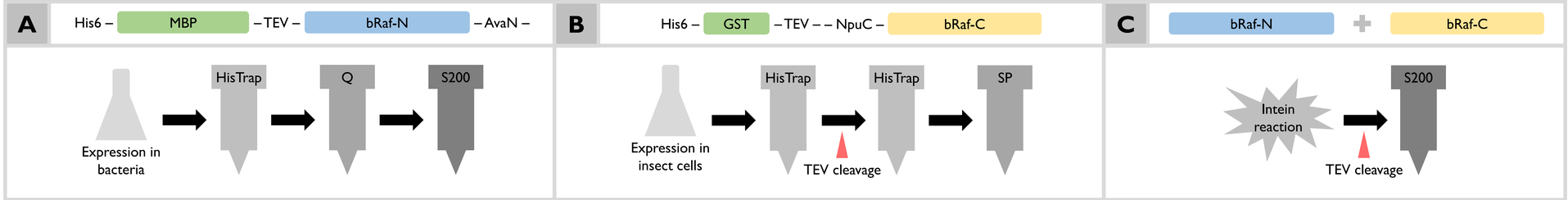
Methods

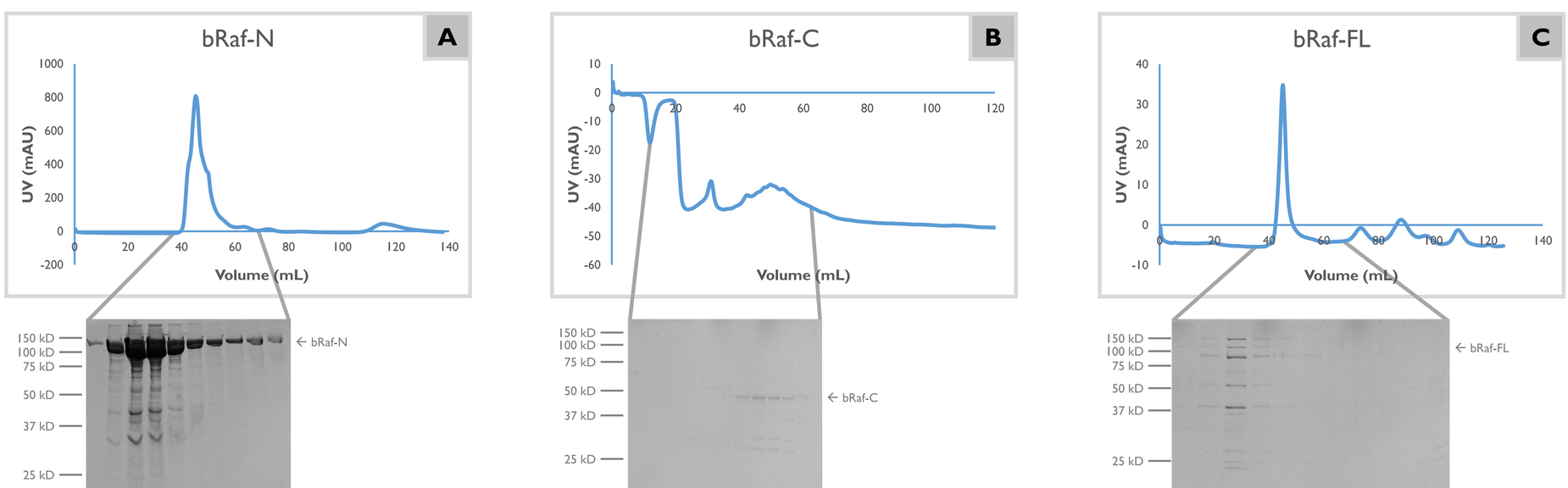


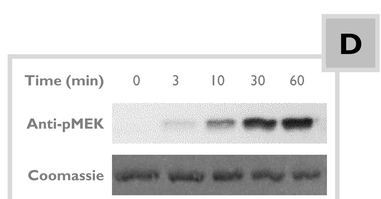
A. Purification scheme for bRaf-N. bRaf-N contains the BRS, RBD, and CRD. Attached to the C-terminal end is the intein fragment AvaN.

B. Purification scheme for bRaf-C. bRaf-C contains the KD. Attached to the N-terminal end is the intein fragment NpuC.

C. Purification scheme for bRaf-FL. Upon mixing bRaf-N and bRaf-C, AvaN and NpuC react and bRaf-FL is formed.

Results





A. Purification of bRaf-N: S200 column. Overall yield was 64.4 nmol per 4L culture.

B. Purification of bRaf-C: SP column. Overall yield was 4.7 nmol per 4L culture.

C. Purification of bRaf-FL: S200 column. Overall yield was 1.3 nmol. Approximately 4.1 nmol bRaf-N and 5.8 nmol bRaf-C were used for the intein reaction.

D. Kinase activity assay of bRaf-FL. bRaf-FL shows ATP- and time-dependent phosphorylation of kinase-dead MEK. Approximately 10 pmol bRaf and 100 pmol MEK were used.