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14. ABSTRACT The tumor suppressor protein merlin interacts with ERM (ezrin, radixin, moesin) proteins and components of cell-cell adherens junctions (AJs) and plays major roles in stabilizing AJs, cell migration and invasion, cell proliferation, contact-mediated cell inhibition, and regulating ligand-mediated cell signaling. Although merlin shares sequence and structural similarity with ERM proteins, and its capacity to bind F-actin and the plasma membrane, it is unique in its function as tumor suppressor. We propose structural characterization of merlin by X-ray crystallography to determine the structure of merlin, its domains, and in complex with its interacting partners. We solved the crystal structure of the human merlin-1 head:tail complex. Surprisingly, unlike other ERM head-tail interactions, the merlin-1 tail provokes dynamic movement and unfurling of the F2 motif of the FERM domain directing dimerization. This dimer formation may control its homeotypic and heterotypic interactions necessary for tumor suppression. Further, crystallizations of merlin and various complexes are in progress.					
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Table of Contents

	<u>Page</u>
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	7
Reportable Outcomes.....	8
Conclusion.....	9
References.....	9
Appendices.....	9

INTRODUCTION:

Neurofibromatosis type 2 (*nf2*) gene produces two functionally characterized proteins merlin-1 and merlin-2 through alternative splicing. The previously reported crystal structures of ERM (ezrin, radixin, moesin) family proteins have been used for structural and conformation characterization of merlin-1 because of its large sequence and domain similarities to ERM proteins. Merlin interacts with other ERM proteins and has functional similarities with ERM proteins in binding actin and plasma membrane. However, it is unique in its ability to suppress cell proliferation and ligand-induced cell signaling in many cell types, and to function as tumor suppressor. The purpose of this postdoctoral trainee award has been to understand the tumor suppressor function of merlin by its structural characterization to address its homeotypic and heterotypic interactions which have been believed to have role in its tumor suppressor function.

BODY:

I seek to understand molecular mechanisms of the tumor suppression function of merlin by solving the structure of full-length merlin, the merlin head:tail complex, and in complex with its binding partners.

Aim 1. Crystal structure determination of merlin-1 head:tail complex and of full-length merlin-1

- a. Solving the structure of merlin-1 head:tail complex structure
- b. Improving the merlin full length crystals

Aim 2. Crystal structure determination of the merlin-1:ezrin and merlin-2:med28 complexes as heterotypic and other protein interactions are known to regulate the merlin's function.

- a. Solving crystal structure of ezrin head and merlin-1 tail complex
- b. Solving crystal structure of Med28 and merlin-2

For Aim #1a, we have indeed solved the merlin-1 head:tail complex crystal structure as proposed. Similarly to other FERM domain structures, our merlin head:tail complex structure shows three subdomains F1, F2, and F3, with fold similarities to known structures (1), namely ubiquitin, acyl-CoA binding protein, and PTB (phosphotyrosine binding)/PH (pleckstrin homology), EVH1 (Enabled/VASP Homology 1) signaling domains respectively. Surprisingly, we found that the F2 subdomain unfurls and α -helix α 3 rotates away from the remainder of the subdomain (Fig. 1). The α -helix α 3 region no longer interacts with the remainder of F2 but with the α 1 α -helix of F3 from a two-fold related molecule (Fig. 1 and 2). There is further movement in the β 6- β 7 loop of the F3 subdomain (Fig. 3). We thus generated several mutations in the unfurled regions of F2 and the β 6- β 7 loop. We are in the process of introducing these mutants into *Nf2*^{-/-} background to correlate the crystallographic results to biology. We have obtained SC4 cells that are spontaneously *in vitro* transformed *Nf2*^{-/-} adult mouse Schwann cells from Dr. Marco Giovannini (House and Ear institute, CA).

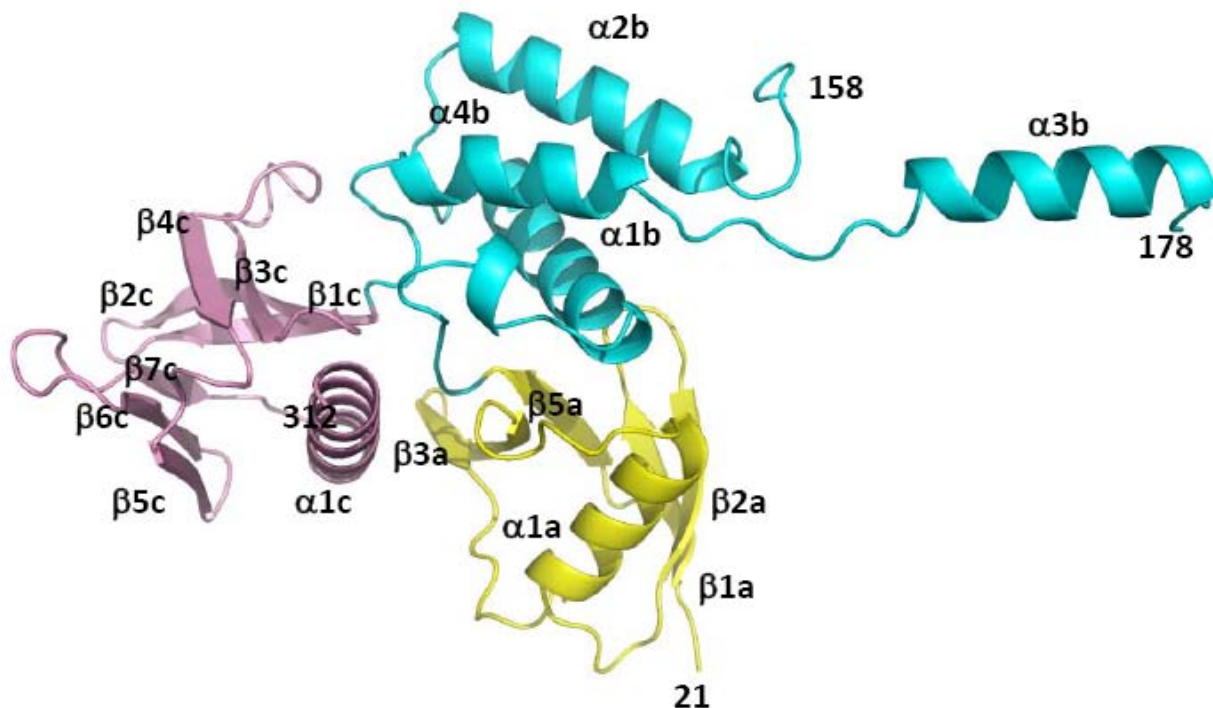


Fig 1| Crystal structure of the human merlin FERM domain. The F1 subdomain is shown in yellow, F2 in cyan, and F3 in pink. Some termini and secondary structure elements (“a” belonging to the F1, “b” to F2, and “c” to the F3 subdomains) are labeled.

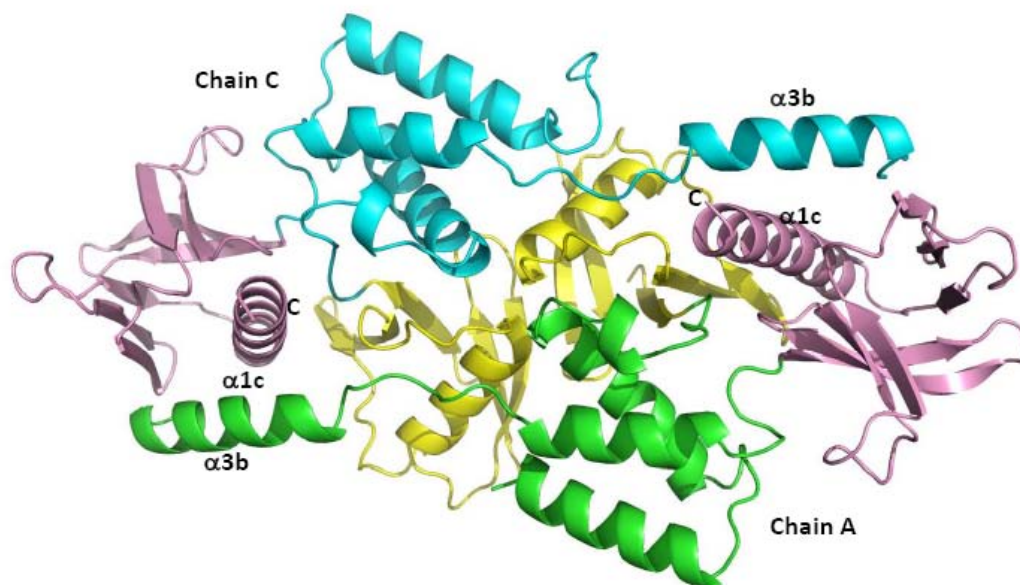


Fig 2| Dimeric structure of the human merlin FERM domain. The F1 subdomain is shown in yellow, F2 in cyan/green for chain C and A, and F3 in pink. C-termini and secondary structure elements [$\alpha3b$ of F2 (b) and $\alpha1C$ of F3(c) subdomains] are labeled.

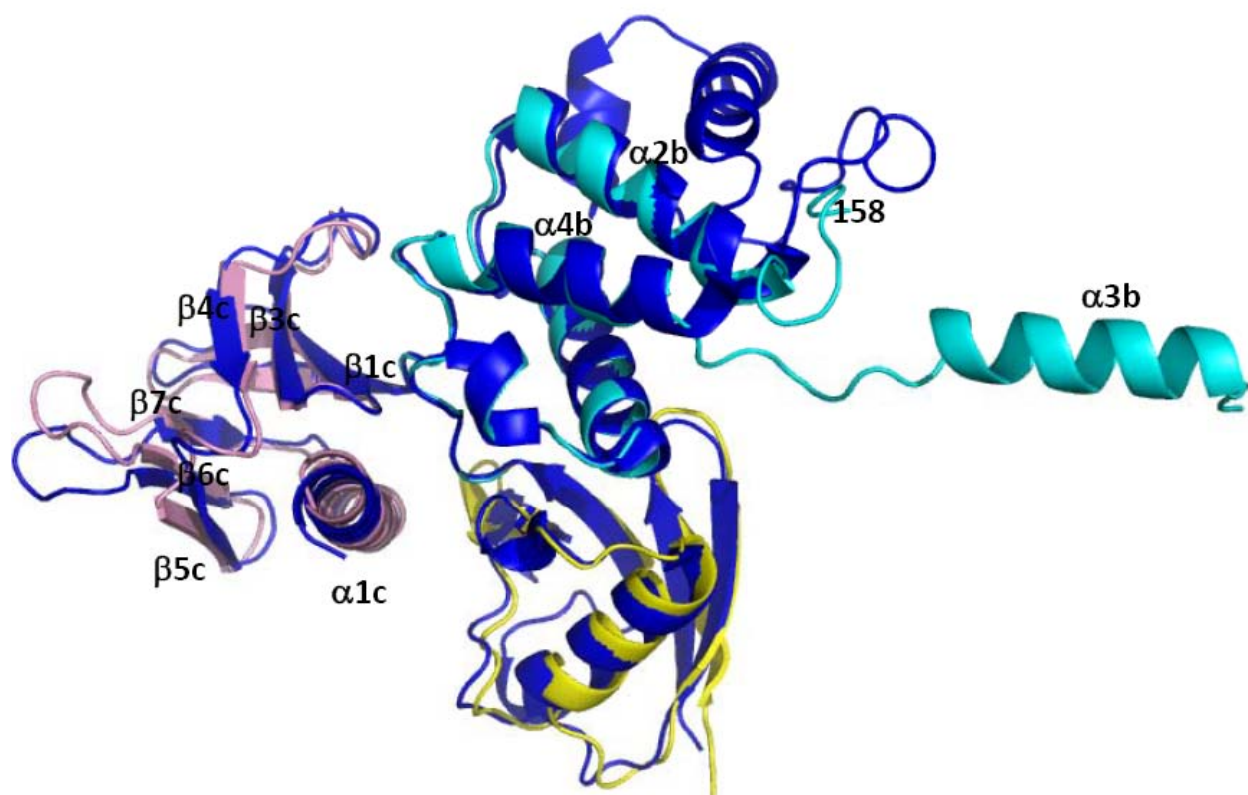


Fig 3| Superposition of the merlin FERM domain structure onto the moesin FERM domain structure (PDB entry 1ef1). The F1 subdomain is shown in yellow, F2 in cyan and F3 in pink for the merlin FERM domain, and moesin structure is shown in blue. $\alpha 3b$ and $\beta 6$ - $\beta 7$ loops are labeled along with some secondary structure elements in F2 and F3 subdomains.

For Aim #1b, to improve our crystals of full-length merlin, we requested and received vectors from Dr. Lars Pedersen (NIEHS-NIH, Research Triangle Park, North Carolina), which have been successfully used to yield crystals for several proteins that are difficult to crystallize (2-6). Further, these vectors are a one-step advancement from the vectors we have used for merlin-1. In our earlier constructs, we engineered entropy mutations on to merlin surface. Surface entropy mutations are expected to enhance the crystallizability of protein by generating new crystal contacts. The vectors from Pedersen Laboratory have entropy mutations that were introduced on surface of MBP (4). There are five different vectors carrying different sets of mutations present on the MBP surface that are listed in table 1.

Vector	SER mutations
pMALX(A)	D82A/K83A
pMALX (B)	E172A/N173A
pMALX(C)	D82A/K83A/K239A
pMALX(D)	E172A/N173A/K239A
pMALX(E)	D82A/K83A/E172A/N173A/K239A

Table 1. Entropy mutations present on MBP surface in the vector

Full-length merlin-1 wildtype cDNA was cloned into the five vectors, which have different sets of entropy mutations and the selection of clones and optimization of expression are underway. Once we have the clones in our hand, we will express, purify, and crystallize these proteins as we did to obtain our original crystals presented in our grant application.

For Aim #2a, since the crystals we generated by using the ezrin head domain with an uncleavable His-tag and the merlin tail domain could not be improved in size and diffraction, we decided to remove the His-tag from the *N*-terminal ezrin head domain. We thus constructed the ezrin FERM domain fused to an *N*-terminal cleavable His₈-tag. We have now cloned the ezrin head domain into a modified pET-28 vector, which has a precision protease site between the His₈-tag and the ezrin FERM domain. After co-purification using a chelating Nickel affinity chromatography column, the protein complex was digested using precision protease cleavage overnight at 4°C to cleave the His₈-tag. The protein without the tag was purified using a supredex-75 sizing chromatography column to obtain the pure complex for crystallization trials. The protein was screened at 10 mg/ml at 4°C in 672 conditions.

For Aim #2b, although we were able to successfully purify the complex of med28 with the merlin-2 tail domain, the crystallization trials at both 4°C and room temperature in 672 conditions did not result in any crystallization hits. A probable reason could be that med28 is part of a large multi-protein mediator complex and stability the complex between med28 and merlin-2 has not been studied. Therefore, a larger biophysical characterization (limited proteolysis) of the complex might help to understand the stability of complex in solution over a time.

KEY RESEARCH ACCOMPLISHMENTS: Bulleted list of key research accomplishments emanating from this research.

1. As proposed, we have successfully completed the structure determination of merlin-1 head:tail to 2.7Å resolution with a manuscript currently in revision.
2. We have cloned wildtype full-length merlin-1 and mutant proteins into eukaryotic expression systems to validate our unfurling model.
3. We have cloned wildtype merlin-1 into new MBP vectors carrying entropy mutations and will screen these proteins for crystallization.
4. We have generated new constructs for the ezrin head domain and produced the ezrin:merlin complex which we screened for crystallization.
5. Initial results suggest that we need to biophysically characterize merlin-2:med28 complex.

REPORTABLE OUTCOMES:

1. **Abstract** submitted for poster presentation to American Crystallographic Association Meeting (ACA 2011) in New Orleans, LA, May 28- June 2.

Unfurling of the F2 FERM domain of merlin tumor suppressor

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Merlin is a tumor suppressor encoded by the *Neurofibromatosis-2* (*NF2*) gene and inactivation of *NF2* through mutations leads to development of nervous system tumors. Merlin is an ERM (ezrin, radixin, moesin) family cytoskeletal protein that interacts with other ERMs and with components of cell-cell adherens junctions (AJs), stabilizing these complexes. Loss of merlin destabilizes AJs, promoting cell migration and invasion, which in *Nf2*^{+/-} mice leads to highly metastatic tumors. Merlin shares sequence and structural similarity with ERM proteins, and its capacity to bind F-actin and plasma membrane. But it is unique in its ability to suppress cell proliferation and ligand-induced cell signaling in many cell types, and to function as tumor suppressor. Paradoxically, the ‘closed’ conformation of merlin-1, where its *N*-terminal four-point-one/ezrin/radixin/moesin (FERM) domain binds to its *C*-terminal tail, directs its tumor suppressor functions. In this work, we report the crystal structure of the human merlin-1 head domain when crystallized in the presence of its tail domain. Remarkably, unlike other ERM head-tail interactions, this structure suggests that binding of the tail promotes dynamic movement and unfurling of the F2 motif of the FERM domain. Our ‘open’ structure of merlin-1 suggests that this unfurling of F2 domain directs dimerization of merlin-1 and may control its interaction with other proteins required for tumor suppression. We conclude that the ‘closed’ tumor suppressor conformation of merlin-1 is in fact an ‘open’ dimer whose functions are disabled by *NF2* mutations that disrupt this architecture.

2. **Manuscript** entitled “Unfurling of the F2 FERM domain of merlin tumor suppressor” is in review.

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CONCLUSION: We were successful in solving the merlin-1 head:tail complex crystal structure. We have also adapted new strategies to tackle the crystallization of full-length merlin-1 and the ezrin:merlin complex. In the second year, we are hoping to solve the crystal structure of full-length merlin-1 and of the ezrin:merlin complex. The proposed med28:merlin-2 complex structure determination (aim #2b) has thus far been very difficult. Since med28 is a part of megadalton mediator complex, and thus perhaps unstable just bound to merlin-2. For this we will subject the full-length med28:merlin-2 complex to proteolysis to identify the stable domains to pursue further to crystallization trials.

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APPENDICES:

None

SUPPORTING DATA:

None