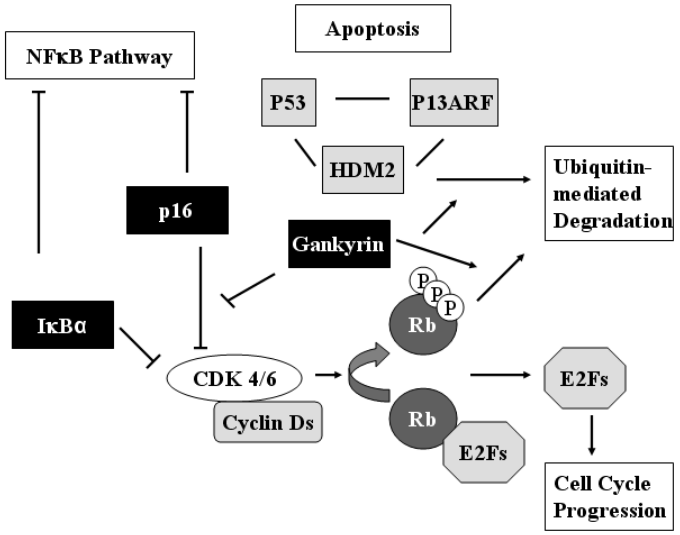
Tumor Suppressors and Cancer Proteins (1996-present). We have been working on the structure-function relationship of the INK4 tumor suppressor proteins, and a number of related proteins related to cancer.



The tumor suppressor p16, discovered in 1995, was found to be mutated more frequently than the best known tumor suppressor p53 in various cancer cells. Its main function is to serve as a negative regulator of the cell cycle by binding to and inhibiting cyclin-dependent kinase 4 (CDK4). Our lab was the first to solve the tertiary structure of free p16 and identified key binding residues (*Molecular Cell*, 1998). Several more papers have been published subsequently to address the structural and functional properties of p16 and its homologous proteins p15, p18, and p19. Nikola Pavletich's group has later solved the structure of p16-CDK6 complex by X-ray.

Gankyrin. Our studies with the INK4 family, which are ankyrin-repeat proteins, led us to study another newly discovered ankyrin-repeat oncogenic protein called gankyrin, which contains six ankyrin repeats. It has been reported to be involved in the phosphorylation and degradation of the retinoblsatoma gene product, Rb. Using *in vitro* systems, we have identified a peptide fragment of gankyrin responsible for binding of gankyrin to Rb. We further demonstrated a different mechanism for gankyrin to facilitate the phosphorylation of Rb – by binding with CDK4. This binding does not inhibit the Rb-phosphorylating kinase activity of CDK4, but it competes with p16 binding to CDK4 and counteracts the inhibitory function of p16. We then showed that the two mechanisms involve different structural regions of gankyrin: the Rb-binding motif is located at the fifth ankyrin repeat, whereas the CDK4-binding region is located in the first three or four ankyrin repeats. Subsequently we solved the structure of gankyrin by NMR.

Other Ankyrin-repeat Proteins: In another study, we showed that the Tax oncoprotein encoded by human T-cell leukemia virus 1 (HTLV-1) binds to and activates CDK4 *in vitro*, and that such binding counteracts against the inhibition of p16 and p18, and acts as the major path to regulate Tax-mediated activation of CDK4. We have identified the binding region lies in the first 40 residues. These results of *in vitro* studies demonstrate a potentially novel, p16-independent route to regulate CDK4 activity by the Tax oncoprotein in HTLV-1 infected cells. Likewise, we

investigated the biochemical and functional properties of the ankyrin-repeat fragment of IkBα and the oncogenic protein p34[SEI-1].

Future Directions: In collaboration with Dr. Junan Li of the Ohio State University, we are proceeding in the following directions: (a) comparison of structural and biophysical properties of various ankyrin repeat proteins involved in cell cycles; (b) structure-based protein engineering to change the CDK4-binding and modulating of gankyrin; (c) examine the biological effects of the mutants with interesting properties by cell culture studies; and (d) apply the structural and functional information obtained to drug discovery. Furthermore, in collaboration with Dr. Wen-Hwa Lee of UC Irvine, we are tacking the structure-function relationship of a number of other cancer-related proteins.

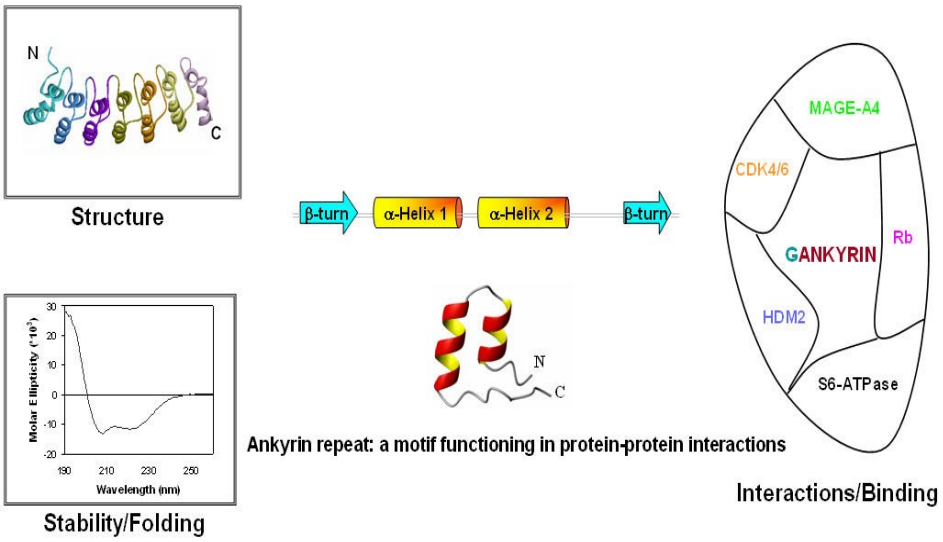


Figure. Illustration of our current approaches to elucidate the relationship between structure, folding, and biological functions of ankyrin-repeat proteins in cancer-related signaling pathways.

Publications:

1. "Tumor Suppressor $p16^{INK4A}$: 1. Structural Characterization of Wild-type and Mutant Proteins by NMR and Circular Dichroism". A. Tevelev, I.-J. L. Byeon, T. Selby, K. Ericson, H.-J. Kim, V. Kraynov, and M.-D. Tsai, *Biochemistry 35*, 9475-9487 (1996).

2. "Tumor Suppressor $p16^{INK4A}$: Determination of Solution Structure and Analyses of Its Interaction with Cyclin-Dependent Kinase 4". In-Ja L. Byeon, Junan Li, Karen Ericson, Thomas L. Selby, Anton Tevelev, Hee-Jung Kim, Paul O'Maille, and Ming-Daw Tsai, *Molecular Cell 1*, 421-431 (1998).

3. "Tumor Suppressor INK4: 3. Determination of the Solution Structure of $p18^{INK4C}$ and Demonstration of the Functional Significance of Loops in $p18^{INK4C}$ and $p16^{INK4A}$." Junan Li, In-Ja L. Byeon, Karen Ericson, Ming Jye Poi, Paul O'Maille, Thomas Selby, and Ming-Daw Tsai, *Biochemistry 38*, 2930-2940 (1999).

4. "Tumor Suppressor INK4: Comparisons of Conformational Properties between $p16^{INK4A}$ and $p18^{INK4C}$." Chunhua Yuan, Junan Li, Thomas L. Selby, In-Ja L. Byeon, and Ming-Daw Tsai, J. Mol. Biol. 294, 201-211 (1999).

5. "Tumor Suppressor INK4: Quantitative Structure-Function Analyses of $p18^{INK4C}$ as an Inhibitor of Cyclin-Dependent Kinase 4." Junan Li, Ming Jye Poi, Dongyan Qin, Thomas L. Selby, In-Ja Byeon, and Ming-Daw Tsai, *Biochemistry 39*, 649-657 (2000).

6. "Structure-Function Relationship of the INK4 Family of Tumor Suppressors". Junan Li, In-Jia L. Byeon, Ming Jye Poi, Karen Ericson, Thomas Selby, Paul O'Maille, Dongyan Qin, and Ming-Daw Tsai, in *DNA Alterations in Cancer: Genetic and Epigenetic Changes*, Melanie Ehrlich, Ed., BioTechniques Books, Eaton Publishing, pp. 71-84 (2000).

7. "Tumor Suppressor INK4: Refinement of $p16^{INK4A}$ Structure and Determination of $p15^{INK4B}$ Structure by Comparative Modeling and NMR Data". Chunhua Yuan, Thomas L. Selby, Junan Li, In-Ja Byeon, and Ming-Daw Tsai, *Protein Science 9*, 1120-1128 (2000).

8. "Somatic *INK4a-ARF* Locus Mutations: A Significant Mechanism of Gene Inactivation in Squamous Cell Carcinomas of the Head and Neck." Ming J. Poi, Thomas Yen, Junan Li, Huijuan Song, Jas C. Lang, Dave E. Schuller, Dennis K. Pearl, Bruce C. Casto, Ming-Daw Tsai, and Christopher M. Weghorst, *Molecular Carcinogenesis 30*, 26-36 (2001).

9. "Novel Insights into the INK4-CDK4/6-Rb Pathway: Counter Action of Gankyrin Against INK4 Proteins Regulates the CDK4-Mediated Phosphorylation of Rb". Junan Li and Ming-Daw Tsai, *Biochemistry 41*, 3977-3983 (2002).

10. "Expression and Characterization of Syrian Golden Hamster p16, a Homologue of Human Tumor Suppressor $p16^{INK4A}$." Junan Li, Dongyan Qin, Thomas J. Knobloch, Ming-Daw Tsai, Christopher M. Weghorst, W. Scott Melvin, and Peter Muscarella, *Biochem. Biophys. Res. Commun. 304*, 241-247 (2003).

11. "Direct Binding of the N-terminus of HTLV-1 Tax Oncoprotein to Cyclin-dependent Kinase 4 Is a Dominant Path to Stimulate the Kinase Activity". Junan Li, Hongyuan Li, and Ming-Daw Tsai, *Biochemistry 42*, 6921-6928 (2003).

12. "An NF-κB-Specific Inhibitor, IκBα, Binds to and Inhibits Cyclin-Dependent Kinase 4". Junan Li, Sang Hoon Joo, and Ming-Daw Tsai, *Biochemistry 42*, 13476-13483 (2003).

13. "Frequent *$p16^{INK4A}$/CDKN2A* Alterations in Chemically Induced Syrian Golden Hamster Pancreatic Tumors". Junan Li, Christopher M. Weghorst, Masahiro Tsutsumi, Ming J. Poi, Thomas J. Knobloch, Bruce C. Casto, W. Scott Melvin, Ming-Daw Tsai, and Peter Muscarella, *Carcinogenesis 25*, 263-268 (2004).

14. "The Nuclear Protein $p34^{SEI-1}$ Regulates the Kinase Activity of Cyclin-dependent Kinase 4 in a Concentration-dependent Manner." Junan Li, W. Scott Melvin, Ming-Daw Tsai, and Peter Muscarella, *Biochemistry 43*, 4394-4399 (2004).

15. "Solution Structure of Human Oncogenic Protein Gankyrin Containing Seven Ankyrin Repeats and Analysis of Its Structure-function Relationship". Chunhua Yuan, Junan Li, Anjali Mahajan, Ming Jye Poi, In-Ja L. Byeon, and Ming-Daw Tsai, *Biochemistry 43*, 12152-12161 (2004).

16. "Dissection of CDK4-binding and Transactivation Activities of $p34^{SEI-1}$ and Comparison between Functions of $p34^{SEI-1}$ and $p16^{INK4}$". Junan Li, Peter Muscarella, Sang Hoon Joo, Thomas, J. Knobloch, W. Scott Melvin, Christopher, M. Weghorst, and Ming-Daw Tsai, *Biochemistry 44*, 13246-13256 (2005).

17. "Ankyrin repeat: a unique motif mediating protein-protein interactions". Junan Li, Anjali Mahajan, and Ming-Daw Tsai, *Biochemistry 45*, 15168-15178 (2006).

18. "Human p16γ, a novel transcriptional variant of $p16^{INK4A}$, coexpresses with $p16^{INK4A}$ in cancer cells and inhibits cell-cycle progression". You-Chin Lin, Mitchell B. Diccianni, Youngjin Kim, Hsin-Hung Lin, Chien-Hsin Lee, Ruey-Jen Lin, Sang Hoon Joo, Junan Li, An-Suei Yang, Huan-Hsien Kuo, Ming-Daw Tsai, Alice L. Yu, *Oncogene 26*, 7017-7027 (2007).

19. "Dissection of Protein-Protein Interaction and CDK4 Inhibition in the Oncogenic versus Tumor Suppressing Functions of Gankyrin and P16". Anjali Mahajan, Yi Guo, Christopher M. Weghorst, Chunhua Yuan, Ming-Daw Tsai, Junan Li, *J. Mol. Biol. 373*, 990-1005 (2007).

20. "Comparisons of the Conformational Stability of Cyclin-dependent Kinase (CDK) 4-Interacting Ankyrin Repeat (AR) Proteins". Yi Guo, Anjali Mahajan, Chunhua Yuan, Sang Hoon Joo, Christopher M. Weghorst, Ming-Daw Tsai, and Junan Li, *Biochemistry 48,* 4050-4062 (2009).

21. "Contributions of conserved TPLH tetrapeptides to the conformational stability of ankyrin repeat proteins." Yi Guo, Chunhua Yuan, Feng Tian, Kun Huang, Christopher M. Weghorst, Ming-Daw Tsai, Junan Li, *J. Mol. Biol. 399,* 168-181 (2010).

22. "Regulatory Mechanisms of Tumor Suppressor P16INK4A and Their Relevance to Cancer". Junan Li, Ming-Jye Poi, and Ming-Daw Tsai, *Biochemistry 50,* 5566-5582 (2011). (Review)

23. "Evidence that P12, a specific variant of $P16^{INK4A}$, plays a suppressive role in human pancreatic carcinogenesis". Poi MJ, Knobloch TJ, Yuan C, Tsai MD, Weghorst CM, Li J., *Biochem. Biophys. Res. Commun*. *436*, 217-222 (2013).