

## Li-Huei Tsai: I well remember

Tsai studies how Cdk5 activity affects brain development, learning, and memory.

Cdk5 is a kinase expressed mainly in neurons, where it helps regulate the activity of a whole host of downstream targets, including ion channels and synaptic scaffold proteins. Thus, it's perhaps to be expected that Cdk5 dysregulation is associated with many neuropathologies (1).

Li-Huei Tsai cloned Cdk5 as a post-doc (2) and decided she wanted to study it further in her own lab. When asked during job interviews what she would do if Cdk5 wasn't involved in any interesting phenotypes, she replied that she would no doubt find something else interesting to study. She was soon hired on at Harvard. As it turns out, though, Cdk5 (and its regulation) is plenty interesting (3–5), as we learned when we called her at her current lab at the Massachusetts Institute of Technology.

### A VIVID MEMORY

*Was there something from your childhood that influenced your career?*

I was born and raised in Taiwan. My parents were in debt when they first got married, so I lived with my maternal grandmother while they worked in another city. I was very close to my grandma.

One of my strongest memories is that, while we were walking back from the market one morning, we took shelter from a thunderstorm at a bus stop. After the rain stopped, my grandmother was very confused, saying, "What are we doing here?" I said, "We were walking home from the market." And she said, "Home? Where's home?"

I was badly frightened. I had no idea what was going on. Later, I was told she was diagnosed with Alzheimer's disease around that time. That memory has had a huge influence on me.

*Did you want to be a scientist?*

No, that never occurred to me back then. I really enjoyed learning about science, but, growing up, I didn't have much oppor-

tunity to be exposed to research. When I went to college, I ended up in a veterinary school program, because I loved animals and I thought being a vet would be cool. Only during my last year in vet school did I notice that a few of our elder classmates actually went on to graduate school. That was the first time I realized there were other career possibilities.

After I graduated, I wanted to give myself an opportunity to get some new experiences. So rather than just rush into being a vet, I decided to apply for master's programs abroad. I applied to schools in the United States and finally got accepted in a master's program at the University of Wisconsin–Madison.

*You went on to do a PhD...*

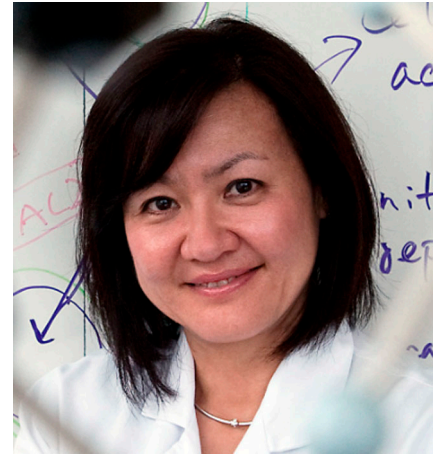
I applied for PhD programs when I was at Madison, and the microbiology department at UT Southwestern Medical Center in Dallas finally accepted me. I did my PhD with Bradford Ozanne, who had described autocrine growth mechanisms, where cancer cells secrete growth factors that support their own proliferation. I was involved in the biochemical purification of a growth factor. I learned a lot from him, and it was a very good experience.

After completing my PhD, I applied to Ed Harlow's oncology laboratory at Cold Spring Harbor Lab, and I got accepted. But Ed soon moved to Mass General in Boston. So that's how I ended up here in Boston.

### A MYSTERY SOLVED

*You started out cloning proteins related to the cyclin-dependent kinase Cdc2...*

Yes, and I quickly found one protein, Cdk5, that really challenged me. I just had the hardest time proving whether it was a real protein kinase or not. Based on its primary sequence, it was a bona fide protein kinase, but I could not detect its catalytic activity in cells.



Li-Huei Tsai

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I became a little bit fanatic about trying to figure this out, and I think one day I just decided to grind up every tissue and organ from a rodent and then do kinase assays to see whether I could find this catalytic activity anywhere. Finally, from that experiment, I realized this kinase is pretty much only active in the brain.

*This is what set you on the path for the rest of your career...*

Pretty much, because that was also when Ed connected me with Verne Caviness, who is a child neurologist at Mass General. Verne was like a second mentor to me. He taught me a lot of things about brain development and neuroscience, and I worked closely with people in his lab. That experience was what made me decide, "In my own lab, I'm going to study the brain."

*Does Cdk5 behave similarly to other cyclin-dependent kinases?*

Well, Cdk5 activity is not regulated over the course of the cell cycle like other cyclin-dependent kinases (Cdks) are. However, there are other aspects in which it's similar to them. One is that, like other Cdk proteins, this protein is very highly regulated. Once Cdk5 becomes active, it autophosphorylates its regulatory activator, p35, which triggers ubiquitin-

dependent degradation of p35 by the proteasome. So p35 has a very short half-life.

Also like cyclins, p35 transcription is sensitive to stimulation. But, whereas transcription of cyclins responds to mitogens, p35 transcription responds to neuronal activity. What's interesting is that there is another, very rapid way to up-regulate Cdk5 activity that's transcription independent. How does that happen? It turns out that p35 is also the substrate of a calcium-dependent protease known as calpain.

Calpain cleaves p35 at a very specific site, which truncates the p35 protein. We don't know exactly how, but this smaller form—we call it p25—can activate Cdk5 rapidly and more efficiently than p35 does.

#### A FINE BALANCE

##### *Can p25 become dysregulated?*

It's been shown over and over that neurotoxic conditions promote p25 production and that p25 overproduction can cause pathological conditions. For instance, there are many human amyloid models in mouse, and in many of those models people have demonstrated elevated p25 levels. Other people have shown that more p25 gets produced during aging.

We have some unpublished data indicating that aging animals are also under chronic stress. This causes yet more p25 to get produced, which in turn tends to lead to undesirable pathologies.

##### *Is p25 production during aging compensating for something?*

You know, I love the concept of compensation, but I think this is extremely diffi-

cult to prove. [Laughs] As I mentioned, we now have very strong evidence that p25 is physiologically produced during aging, which is really not surprising. p25 production is part of the normal synaptic plasticity process. Our feeling is that the primary role of p25 is to reset the system.

In the brain, neurons can be in either a resting state or an active/excited state, depending on extracellular input or stimulation. In their excited state, neurons can increase the expression of certain genes that facilitate the formation of new memories. After stimulation, they de-potentiate and then recalibrate to the baseline state so that they can be stimulated again. Our

new results suggest that p25 plays a very important role in this process. We have done a lot of proteomic screening to see how gene expression patterns respond to Cdk5 activity and found that, in the absence of Cdk5, hundreds of proteins show huge differences in abundance at the synapse. So p25 and Cdk5 strongly affect synaptic architecture.

##### *In what directions are you taking your work right now?*

Studying Cdk5 really gave me an opportunity to look at neurological disorders that have a huge impact on cognitive function. Cdk5 is important in brain development, and we are interested in whether we can identify specific cellular or molecular defects associated with developmental disorders such as autism or epilepsy. But Cdk5 has also been implicated in Alzheimer's disease, which affected my grandmother.

It's known that Cdk5 phosphorylates Tau, a microtubule-associated protein that is important in Alzheimer's. A few years ago we created a p25 gain-of-function model, which is an inducible transgenic mouse model that selectively expresses p25 in excitatory neurons. This mouse manifests a very severe neurodegeneration phenotype, and among its pathologies is hyperphosphorylation of Tau protein. This animal has very severe

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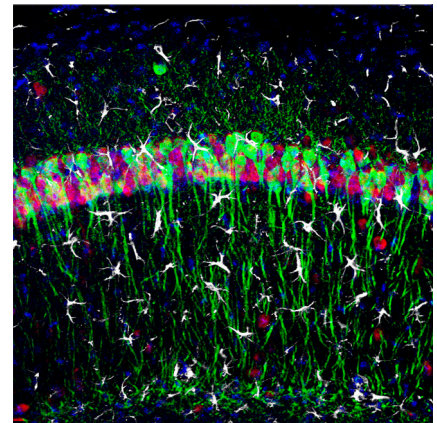


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Brain tissue is shown from transgenic mice with induced p25 expression (green), alongside markers for neurons (red) and glial cells (white). Nuclei are shown in blue.

neuronal loss, Tau pathology, and even elevated  $\beta$  amyloid. There's tremendous inflammation in the brain, loss of synaptic density, and very severe learning and memory deficits.

This has given us a great opportunity not only to better understand the disease mechanism but also to try out new therapeutic approaches. And it was through one of those adventures that we came across epigenetic gene regulation. Histone deacetylase is part of the transcriptional corepressor complex that inactivates gene expression. We found that we can promote gene expression by broadly inhibiting histone deacetylase and that this has an unbelievably beneficial effect on learning and memory, even after severe neurodegeneration and neuronal loss had occurred in the animal models. We recently worked out some mechanisms underlying these observations, and we would like to know whether we can exploit further information from this line of work and whether we can identify potential targets for small molecules to enhance cognitive functions.

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Tsai and co-workers are decoding some of the mysteries behind learning and memory.