

# Discovery and characterization of the cadherin family of cell adhesion molecules

An interview with Masatoshi Takeichi

DOUGLAS SIPP\*

*Office for Science Communications and International Affairs, RIKEN Center for Developmental Biology, Kobe, Japan*

This article presents an interview with Masatoshi Takeichi, who is best known for his discovery of cadherins, which are of fundamental importance in the mechanisms of intercellular recognition and adhesion. He was the first to recognize that cell-cell adhesion involves two distinct mechanisms – calcium-dependent and calcium-independent – and to identify molecular bases for each. He named the molecule responsible for calcium-dependent adhesion ‘cadherin,’ and went on to identify a group of related molecules, now known to form the core of what are collectively referred to as the cadherin family. These molecules are differentially expressed by tissue type and developmental stage, and function by allowing cells with compatible cadherins to recognize and bind to each other. His recent work focuses on the role of cadherins in neural network formation and cadherin-mediated controls of morphogenetic cell behavior. In addition to his ongoing research into cell adhesion and tissue patterning, he serves as director of the RIKEN Kobe Institute and Center for Developmental Biology (Kobe, Japan), one of the world’s largest research institutes dedicated entirely to the study of the mechanisms of development and regeneration. He has received the Keio Medical Prize, the Ross Harrison Prize (International Society of Developmental Biologists) and been named a member of the Japan Academy in recognition of his scientific achievements.

In our interview in his laboratory on September 8, 2003, Dr. Takeichi’s continuing enthusiasm for his research, his gentle

sense of humor, and the acuteness of his perspectives into the shifting nature of scientific research, administration and education were evident. He spoke candidly and in detail about his education, his early postdoctoral work, the events leading up to the discovery of the first cadherin molecule, and how the field has changed and grown over the years. While acknowledging the contributions of others to the advancement of the field, he stressed several times the need for conceptual independence, and cited the freedom he was given as a student and young researcher as fundamentally important to his growth as a scientist.

## **What first prompted your interest in science? Can you remember any specific person or event that made you think “This is what I want to do”?**

Of course I have been influenced by a number of people in my career, but really what first got me into science was my interest in nature and living things. Even as a young boy, I had always liked insects – other kids called me “the bug expert” – and as I got older and entered junior high school, I started to keep tropical fish and got interested in bird watching, and even now I’ve started to raise orchids. You could say I’ve always just been interested in biology. If you look in the back of books on biology, you see the authors’ names and they’re all Ph Ds, so I guess that’s one thing that got me started thinking about a career in science.

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\*Address correspondence to: Douglas Sipp, Office for Science Communications and International Affairs, RIKEN Center for Developmental Biology, 2-2-3 Minatojima Minamimachi, Chuo-ku, Kobe 650-0047, Japan. Fax+81-78-306-3090. e-mail: sipp@cdb.riken.jp



**With Goro Eguchi (left) in Baltimore, 1975.** *He was my first mentor, who supervised my studies of lenses at the graduate school of Nagoya University. In this photo, he was visiting me while I was working at the Carnegie Institution Department of Embryology. Around this time, I was busy with the first characterization of the trypsin/calcium sensitivity of what was later identified as cadherin.*

The problem was, when I was seriously considering what field of science to major in as an undergrad at Nagoya University, the job prospects for biology majors were not good. So when I first entered school, I wasn't sure whether to enroll in a more applied field like engineering where the future was clearer, especially since my father ran his own small company and I knew he would have preferred for me to be able to continue the family business. Fortunately, at the time Nagoya University allowed undergrads to defer the decision to declare a major for the first two years. At first I was thinking about chemistry, which was closer to my father's business, but in the end I chose biology, even though I was worried about being able to make a living at it.

Of course there were teachers along the way who helped to encourage my interest in biology. In high school, I remember one teacher in particular, Mr Ogasawara, who ran an after-school bird-watching club. So when I was debating whether or not to major in biology, I went to him for advice, and actually he told me "It'll be tough for you to make a living if you major in biology, so I wouldn't recommend it." [laughs] But actually I had already pretty much made up my mind at that point, and I declared biology anyway.

#### **What did he recommend you do instead?**

He said that you can still have a hobby like bird-watching even if you're working for a company, so that would be a safer route. So I guess you could say that my decision to study biology was just an extension of an interest I'd had since I was a boy.

#### **So how was your experience at university?**

When I entered the Nagoya University biology program, it was a really great environment – they had spaces for ten students, but there were only four in the biology program. Of course, physics, math and chemistry were more popular and biology was kind of a dark horse – actually, I think there were only two students who entered the program the following year. One reason for that was the lack of careers – teaching high school was about the only clear option. But the fact that there were only four students was really a plus – especially as Nagoya had an excellent teaching faculty at the time; biology was one of the strongest departments at the school. The small size of the class allowed us to have a lot of freedom in our education – in essence we had our own lab to work in. Teachers would come by and give lessons in the lab, and we had our own equipment, so we could do whatever experiments we wanted at any time. It was like we lived in the lab. So those two years were kind of like a paradise for an undergrad.

But on graduation, I knew I couldn't just go on living in the same way forever, and I went out and interviewed with two or three companies, but I ended up not getting hired anywhere, so I went back to grad school [laughs]. I chose to study animal development, although Nagoya had a very strong grad program in molecular biology at the time – actually it was an entirely separate department from the biology department – and they had state of the art equipment. Nagoya also had famous professors like Reiji Okazaki [for whom the Okazaki fragment is named], and Sho Asakura, who studied the patterning of flagella, so it was an exciting place to be. But I decided to go into developmental biology, I think mainly because it was closest field to the direct observation of animals, which was the reason I was interested in biology to begin with. I've always enjoyed observing, and through my hobbies and practice, I'd developed some observation skills, so I thought development, rather than molecular biology, was the field where I could put them to the best use.

But I also purposely stayed out of subjects that require fieldwork like ecology – I wanted to work in something with a strong academic aspect. That's not to say that ethology and ecology aren't academic, I suppose in the end I just didn't find the idea of counting and tracking the number of birds in a flock to be appealing. Bird-watching is fun, but I wouldn't want to do it for a living.

Anyway, I went into development under Goro Eguchi, and started to work on lens differentiation.

#### **That was in chick?**

Well, Dr. Eguchi was working with lenses taken from newts, but I thought that kind of classical approach needed new technical advances in order for it to be successful, so I decided to try culturing lenses from chickens and see if that would reveal something about their development. Molecular biology was making progress as a field in those days, but was still mainly using bacteria and phages, and those techniques still didn't have applications in developmental biology. One of the interesting things about Nagoya at around that time was that Tsuneo Yamada (who had already left before I entered the school) and others had been trying to apply chemistry and molecular biology and other new approaches to classical developmental biology. Unfortunately, that work didn't produce any real breakthroughs, but I had already decided that the classical approach of dissecting the lens and examining it under a microscope wasn't leading anywhere, and I decided that developmental biology needed

to start using new experimental techniques. I thought cell culture was worth giving a try, to see if I could learn something about development by altering culture conditions.

If you study the lens, you can see it's got two different epithelial surfaces, one that faces the retina like this [cups fist in hand], and if you turn it around it tries to revert to its original state. So the theory was that there was some factor in the retina that presented positional information to the lens. So for my master's work, my idea was to try culturing lens cells and introducing retinal cells, or culturing lens and retinal cells and combining them under different conditions to what effects that would have.

#### **So your experience at Nagoya was positive overall?**

The good thing about the education there was the freedom allowed by the high teacher-student ratio. Of course there was structure, and they'd hold training courses that would run for a month or so, but the approach wasn't rote or mechanical, and we had relative freedom to set up and run our own experiments. So when we were studying bacteria, one of the students was looking into bioluminescent bacteria and he went to the store and bought some squid and kept it in our refrigerator. It didn't smell very nice, but it just shows how curiosity was encouraged and rewarded.

In my courses at Kyoto University, I can't give that kind of individual freedom to every student, which is a shame, but that's the reality of the larger class sizes now that biology is becoming more popular.

#### **Was Nagoya special in that ability to provide an individualized education?**

I think that to some extent it was that way all over – but perhaps Nagoya was even more so. I can't say from firsthand experience, but I think class sizes were generally smaller across the country. One good thing was the level of interaction between the grad students and undergrads at Nagoya – there were a number of grad students who were really active in talking with the undergrads. That may have been possible because they weren't as busy with research in those days – there were less of the urgency and demands on time that you see nowadays.

I remember there was some distilling equipment in the lab and we used it to distill wine in the lab [laughs]. We got caught and had to hand over all the brandy we'd made. A few people also tried keeping chicks that had hatched from eggs used in experiments.

#### **Like in other parts of the world, the late 1960s were a turbulent time in Japan. Did that have any effect on your studies?**

It's probably the same everywhere, but students were politically active. There were some protests against the use of Japanese bases by American nuclear subs. I joined in a few demonstrations myself, but I've never been a very political person. It was more just one of those things that students did back then.

There were also mass student demonstrations across Japan just around the time when I was starting to work at Kyoto. It wasn't the most ideal environment to do research in, but the head of the lab, Tokindo Okada, still held his seminars and overall the effects on our research were minimal.

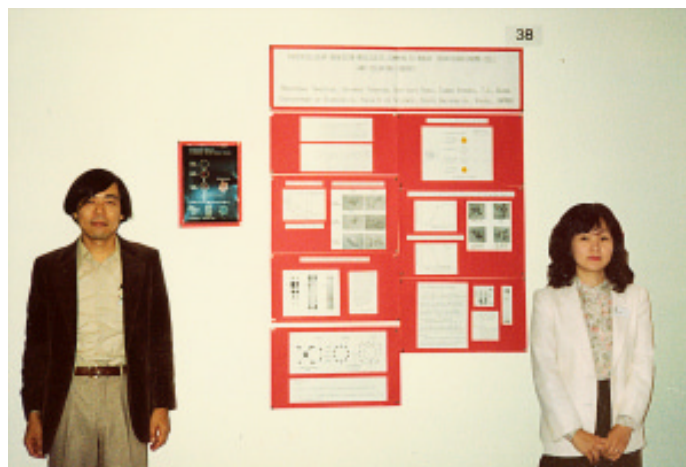
#### **How did your school days and early postdoc experiences shape your philosophy about education?**

I think being able to set up and conduct my own master thesis research work was important. I was also lucky to be able to study

first under Prof. Eguchi and then Prof. Okada at Kyoto. In retrospect, moving to Kyoto was especially fortunate because Okada had returned from the Carnegie Institute after studying what were at the time cutting-edge techniques like clonal cell culture, and he had probably the only lab in Japan equipped to do that kind of work in developmental biology. Certainly Nagoya was poorly equipped to do the kind of culture of lens cells that I wanted to work on. But Okada had brought these new culture techniques back to Japan and set up his lab in Kyoto so you could culture lens cells *in vitro* and observe their responses to changes in culture condition.

I think it was good for me to study in an environment that gave me the freedom to make my own decisions about my research projects. That meant that I was able to choose lens differentiation for my thesis work, and also later to focus on cell adhesion when that started to interest me. It takes a special kind of professor to give his students that kind of freedom – I think that my own approach to education was shaped by that. I think it's important for teachers and mentors to have that same tolerance and breadth of knowledge so that they can guide their students toward finding their own way, rather than steering them down one specific path. I think at least part of Okada's heart is in the humanities. He was interested in philosophy in high school, but like me he also loved to collect insects.

Back then developmental biologists had already realized that they needed to start taking more new approaches other than just repeating classical embryology explanation experiments and looking at morphological changes. There was talk of a new "chemical embryology" and people were starting to focus on molecules. But the mechanisms were still a complete mystery, and many of the necessary techniques had yet to be developed. So even mentors couldn't really point their students down a path with any certainty – the field was still too young. So I was also lucky to be a grad student during those days. The situation is completely different now, where there's, if anything, too much information and students need to be directed more closely to make sure that they're learning what's really important. Still, I try to encourage students to explore by themselves and guide them by comments, and there have been a number of times when a student has



**My graduate student, Chikako Yoshida (on the right; currently named Yoshida-Noro) and I, presenting a poster describing the first identification of future E-cadherin at the 1981 meeting of the ISDB in Basel.**



**At a party with other members of the Okada laboratory in 1984.** The name “cadherin” was first proposed that year in a paper in *Developmental Biology*.

ignored what I said, done some experiment without telling me and come up with an interesting result. So I think being permissive is important in teaching basic science. I think that’s part of the general culture in biology departments.

**Your earliest published work was in cell-substrate adhesion in the chicken lens. How did those studies help to guide your later research?**

My idea was to use cell culturing to look for signaling between retinal and lens cells, and the effect of the retina on lens differentiation. I tried culturing them in different ways, for example, transferring retinal cells into lens cell cultures, and looking for changes. I watched them every day and... absolutely nothing happened [laughs]. So you could say I was running into a wall with that line of research.

But doing all that work with cell cultures wasn’t a total waste of time. I can’t remember exactly what it was that caught my attention, but during the course of that work I started seeing that cells in some Petri dishes sometimes stick to the dish while others cultured under different conditions would not. I thought that was interesting. So was the fact that in some cases, for unknown reasons, trypsin took much longer than normal to detach cells from the plate. So it wasn’t directly related to the theme of lens differentiation, but that was when I started thinking about investigating the relationship between cultured cells and substrates.

One of the main attractions for me was that I could design and conduct an experiment and see the results the same day. It gave me the chance to do empirically analytical science where I’d try a putting a plastic coating on the plate and see whether it affected the ability of cells to adhere to it, or add divalent cations to cells and see whether they could stick together. What I liked about it was the opportunity to do analytical science. Studying differentiation, you just had to watch and wait. But this was the kind of

science I enjoyed. And when I wrote it up in a lab report, Dr Okada agreed that it was interesting. Actually, he had been interested in the same kinds of phenomena from before. When I was still in Nagoya I didn’t know about it, but he already had another master’s student in his lab working on cell assembly, I think he was looking at the effects of adding serum to cells in culture.

Even Okada himself had published about the ability of renal cells to re-aggregate and resume function after dissociation. Moscona had studied that as well, but what Okada brought to the field was immunological techniques, antibodies, to mark specific cell types. Before that, it had been possible to disaggregate cells and then see if they reaggregated, but not to track the activity of individual cells. Okada made an antibody specific to epithelial cells in the kidney, and used it to stain cells that he’d dissociated and follow their activity. That work showed that when dissociated cells reorganized, they were following some pre-existing blueprint or plan, which was an important finding in the field at the time. (Although I have to admit I wasn’t aware of it.)

So Okada was important for introducing immunological techniques to the study of development, and he was also interested in cell adhesion. His experience and network overseas was also important – he worked at Carnegie at the same time as Malcolm Steinberg, who proposed the differential adhesiveness hypothesis in 1962 (Steinberg 1963b), and he had contact with Moscona in the US as well, and in England, Adam Curtis, who was also very active in cell adhesion at the time. I think that that exposure made him interested in the field, and maybe that one of the reasons why he was supportive, or at least didn’t say no, when I told him I wanted to quit my work on lens differentiation so I could study the phenomena. I think he was also attracted by fact that the research was analytic, not simply descriptive.

As for lens differentiation, that research was taken up by other people in Okada’s lab. Okada eventually got interested in the culturing of lens cells himself, which led to the cloning of the genes of the lens proteins delta and alpha -crystallin *et al.*, 1982, Okazaki *et al.*, 1985). That work has continued even after Okada retired from research, and was taken up by Kunio Yasuda at NAIST and Hisato Kondo at Osaka University. It’s interesting to me that the project I took with me to Kyoto had a future in other hands.

**When you first started to pursue your interest in cell adhesion, it was already a very active subject of research. Could you talk a little about some of the work that was being done at the time?**

When I was getting started, the phenomena were known, but really very little was known about the mechanisms. Moscona focused really hard on identifying molecules involved in adhesion, and ultimately came up with the name “cognin.” Moscona of course had discovered that animal cells dissociated using trypsin could reassemble Moscona 1952). But he also noticed that some kind of slimy substance would form on the cell cultures and he

reported that that substance could cause cells to clump together. Malcolm Steinberg proposed that the substance was actually DNA from dead cells, and for a while there was quite a heated debate between the two.

So when I was getting started we didn't have any molecules. The first mechanism I discovered at that level was involved in cell-substrate adhesions. I found that if you culture on a clean plastic plate, the cells adhere immediately, but if you coat the plate with a protein, the adhesion is more gradual. In that form of adhesion, cations are important – magnesium, and particularly manganese – although calcium's activity is not so strong (Okada 1972, Takeichi and Okada 1974).

So I had published some work that showed that different cations play roles in different forms of adhesion, and I became interested in what calcium was doing. It had been known for a long time that divalent cations were important in adhesion because if you removed calcium from the medium by adding EDTA when you dissociated many types of animal cells, they failed to reaggregate. But the roles of the individual cations in cell adhesion still weren't clear. I'd made a little progress with the magnesium finding, but still had no molecules at that point and I was wondering where to take my research. And that was where the field was in general – there were these fundamental questions to be answered, and it didn't really matter whether you were competing with big names because everyone was really working from the same starting point.

The first adhesion molecule to be discovered was by Günther Gerisch who was using antibodies to study aggregation in slime molds and identified contact sites A and B (Gerisch 1978). This involved injecting cells into animals to prompt them to create antibodies against molecules present on the cells' surfaces. This results in the production of at least some antibodies against, for example, cell adhesion molecules which you can test for by exposing cells to serum taken from the injected animals and seeing if it has an inhibitory effect on adhesion, the idea being that an antibody with an adhesion-blocking effect is present in the serum. Gerisch was the first to succeed at identifying a cell adhesion molecule using this indirect process. At the Rockefeller Institute, Gerald Edelman, who had won a Nobel Prize for his research on the chemical structure of antibodies, was working along with Jean-Paul Thiery, Urs Rutishauser and Robert Brackenbury using similar methods to try to identify cell adhesion molecules in animal cells and found N-CAM *et al.* 1977, Thiery *et al.*, 1977).

So that was the state of things – I had my findings about magnesium and calcium, and was thinking about what to do next when Prof. Okada asked me if I'd be interested in doing a fellowship overseas. He made some inquiries with Jim Ebert who was the director of the Carnegie Institution Department of Embryology, which resulted in an opportunity for me to work at Dick Pagano's lab. They were studying the interactions between liposomes and the cell surface, which I thought sounded interesting because it offered a simpler experimental system for studying membrane interactions.

**The Carnegie Institution is where you first found a direct link between calcium and cell-cell adhesion. What was it that led you to make that connection?**

I was working with a lung cell line from Chinese hamster called V79, using trypsin to dissociate the cells and incubating them in

suspension. When I had done similar experiments in Kyoto, the cells always reaggregated, but at the Carnegie they did not. I thought that was strange, so I checked the ingredients of the trypsin solution and saw that it contained EDTA, which sequesters divalent cations. I thought there might be something interesting in this, started experimenting with cations and found that if I added calcium to a trypsin solution, it failed to cause dissociation. These same cells could be dissociated by washing them in a calcium-free solution, and they would reaggregate when calcium was re-introduced. And as I had seen with trypsin plus EDTA, cells that were dissociated in the absence of calcium never reaggregated. Magnesium on the other hand had no effect.

The results were striking and clear, and I really wanted to focus on the mechanisms behind it. This was right about the time when Edelman published his first paper in PNAS on N-CAM, presenting his model of cell adhesion, which was not calcium-dependent. Needless to say, I was a little anxious to be working on the same problem as such a renowned scientist. Saul Roseman at Johns Hopkins, which shares a campus with the Carnegie, also had developed a cell adhesion model involving interactions between glycosyltransferases and carbohydrates which also enjoyed some currency at the time *al.* 1971). But I was convinced that I was on the right track with calcium.

I was just a visiting researcher, but one of the nice things about working at the Carnegie when I was there was the freedom that was given to the individual researchers. Jim Ebert's policy as a director was to let people study what they wanted. And the labs were quite compact – usually just a few postdocs and technicians per lab – it was an environment that encouraged people to pursue their own scientific interests. I remember being told I could do whatever I wanted. Dick Pagano also never said a word to me about the fact that I was studying these mechanisms that really didn't have anything to do with his official research theme [laughs]. When I look back now, I'm a little embarrassed, astonished really, about what I got away with as just a postdoc, but I was young at the time and never gave it much thought.

I authored my first paper on the calcium effect in 1977, after returning to Japan, and published in the *Journal of Cell Biology*. To tell the truth, after I had seen the initial results of those experiments with trypsin, EDTA and calcium I couldn't wait to get back to Japan so that I could work on the problem full time. The environment at the Carnegie Institution was very free, but I was still working in someone else's lab, so I wanted to get back to where I could do my own research in Kyoto. Fortunately, the timing was good, as I was just coming to the end of my two-year term.

**So trypsin had been used for dozens of years and no one had much of idea of how it caused cells to dissociate? Had you yourself thought about the mechanism prior to that?**

I guess that most people were just accustomed to the trypsin effect and found it useful as an experimental tool. The sense was just that "trypsin dissolves the bonds between cells," but I don't know if anyone was actively looking for the mechanism behind that. Of course, Moscona had looked for the molecule and came up with that "slimy" coating on cell cultures I mentioned earlier, but I think that was about as far as it went. I myself hadn't thought too deeply about it. It wasn't until I noticed the different response to trypsin dissociation at the Carnegie Institution that I really began to think in depth about the molecular aspects of the trypsin effect.



With Ken Yamada (left) and Jean-Paul Thiery (right). We organized an international research team under the support of the Human Frontiers Science Program to study the crosstalk between cell-cell and cell-substrate adhesion, and met in Paris to hold a workshop in 1993.

returning to Japan that was what I used to try to identify the cell adhesion molecule. At around the same time, Gerald Edelman was in the process of identifying an adhesion molecule he named L-CAM (al. 1983), and Francois Jacob at the Pasteur Institute was working on an antibody that affected compaction morphology in the early mouse embryo (al. 1981). Caroline Damsky and Clayton Buck also isolated an antibody that interfered with cell adhesion of epithelial cells (al. 1983), and Walter Birchmeier at Tübingen (al. 1983) and Barry Gumbiner at EMBL (Simons 1986) also found similar antibodies, as did Benny Geiger at Weizmann Institute in Israel (Geiger 1984). So I knew there was a lot of related research into these kinds of antibodies.

**Was there much collaborative work in the field or was most of the research being done in isolation?**

That's an interesting point. In my case, our lab was of course geographically remote and we didn't attend international meetings so often, so you could say we were working in isolation. I didn't know what was going on in

**Other than your research results, what did you feel was the most important thing you gained from your time overseas? And what was in your mind when you returned to Kyoto in the fall of 1976?**

Well, I spent most of my two years on research of course, but I did have some chances to get out and see the country. I took one twenty-day trip where I drove all the way from Baltimore to Banff in Canada, passing through places like the Great Lakes, Saskatchewan and Jasper. So I had a nice time when I was in the States, but I never really felt like I wanted to spend the rest of my life there. And of course, my research was calling me back to Japan, so when my two years ended I went back to Kyoto.

One time when I was still at the Carnegie Institution, Prof Okada was coming to America for a conference or something and arranged a meeting for us with Malcolm Steinberg at Princeton. But on the day of the meeting, he was late for some reason, and I ended up meeting with Steinberg alone, which could have been uncomfortable, but he was very gracious and it turned out to be a very positive experience for me, meeting with a well-respected scientist whom I'd only known by reputation until then. Maybe that was the most important result of my time in America, the ability to meet in person with other scientists in the field. Having personal contact makes a big difference.

At Carnegie too, there were some very important molecular developmental biologists, such as Igor Dawid and Don Brown, who took over as director after Jim Ebert left the Carnegie. I felt very fortunate to be able to attend his talks while I was at the Carnegie.

**What was the next step after recognizing that the adhesion molecule was calcium-dependent?**

The only real experimental option at the time was to use the immunological method that Gerisch had demonstrated, so after

other labs, but in some senses that worked in my favor because I could pursue my own research without being too concerned with what other people were doing. Of course, I knew in general terms that there were other labs working on the same kinds of questions, but certainly not in any detail, and I think that that lack of contact actually let me go at my own pace. If you know too much about what others are working on, it might make you steer away from areas which you might otherwise pursue, and in the end may have a negative effect on a researcher's freedom and ability to do original work. Of course, I did hear from time to time what was going on in other labs, and I can't say I was completely unaware or unconcerned.

I think that Japanese researchers in particular ought to try to take better advantage of the positive aspects of their relative isolation. It certainly allows you to avoid or screen out some of the noise you'd encounter working in a less isolated environment. I think it gives us a kind of freedom. Of course, there's the danger of spending a lot of time working on something only to find that another lab has beaten you to the punch, and there are advantages to being in close communication and physical proximity with other labs in the same field. I suppose the important thing for scientists is to make the best of whatever situation they find themselves in.

**You also determined that there had to be more than one mechanism for cell-cell adhesion, and that at least one of the mechanisms must work independent of calcium. What led you there?**

The discovery that multiple mechanisms were at work was an important step in understanding cell adhesion. That allowed us to take a more scientific approach to the dissection of the whole system and look at its individual components, not just the concept of cell adhesion in general, but at the specific molecular programs. In our lab, one grad student named Hideko Urushihara was

working on calcium independent adhesion, and that work produced a paper in *Cell* (Takeichi 1980). I focused on calcium-dependent adhesion, and my research actually fell behind because we weren't able to get an antibody to block its function.

#### **Was that because the technology wasn't yet available?**

It's not that the technology wasn't there. The process was as I described before, injecting V79 hamster cells into rabbits and checking the serum for antibodies. But we had tried this with rabbits at Carnegie and later at Kyoto, but were never able to find an antibody. So we couldn't use the immunological approach. We knew that trypsin plus EDTA resulted in the loss of adhesion, while cell adhesion was maintained in trypsin plus calcium, so we compared surface molecules. We found a marked difference in a molecule called p125, and I thought it must be important, but without an antibody to block its function, we had no way of proving it.

In the end, we were only able to develop an antibody by switching from V79 cells to a different cell line named F9, which somewhat resembles an ES cell line in its properties. After we had a useable antibody, research went ahead quickly and we published a paper in *Cell* (relatively soon thereafter Takeichi 1982). I had the idea to switch cell lines after reading a paper by Rolf Kemler and colleagues from the Pasteur Institute that described an antiserum against F9 cells that altered cell morphology in early mouse embryos (al. 1977). I already suspected that they were working on the same molecule, so I tried using that line instead and was able to observe the same effects I'd seen in V79 cells, and so I was finally able to do the inhibition of function experiments.

I never went back to find an antibody that worked against V79, and that remains kind of a mystery. For whatever reason, F9 cells express E-cadherin with a high antigenicity, so that mouse cells injected into rabbits produce an immunological reaction. We later discovered that there were a number of molecules in the cadherin family, and for example, mouse N-cadherins produce no blocking antibodies in rabbit. So the antigenicity varies from molecule to molecule, and it must be that V79 cells have a different antigen profile F9. This story relates back to that question of isolation – I guess this example shows the importance of keeping an eye on the literature.

#### **How did you settle on the name “cadherin?”**

I had a few ideas for names, but I wasn't confident about what would sound good in English. There was a researcher named David de Pomerai on a one-month visit to the Okada lab at the time, so I asked him his opinion and he said cadherin worked well. Actually, it was Chikako Yoshida who first proposed 'cadherin,' combining elements from 'calcium' and 'adhere'.

#### **It must be gratifying that the name has been applied to the entire family of molecules, as there were other groups at the same time who were also identifying molecules that later came to be grouped in the classic cadherins.**

Other groups gave different names to the molecules they found, but we were the first to recognize that there were different molecules with similar properties, which were later found to form a gene family, and I think that made the difference to the scientific community at the time.

#### **One of your first projects back at Kyoto was an immunoassay for cell surface components relevant to aggregation. Your lab's first successes were actually with calcium-independent glycoproteins. What was your approach there?**

Our lab used the same approach to calcium-independent molecules, which would now be classed in the Ig superfamily, as we did for the cadherins. Immunoglobulin molecules are divalent, which means they tend to increase rather than block cell adhesion. So we used a Fab fragment strategy in which only one of the two Fab arms of the antibody is used, which gave monovalent rather than divalent binding and allowed us to identify both calcium-dependent and independent adhesion molecules (Takeichi 1982).

#### **By the early 1990s, the cadherin-catenin complex was coming much more clearly into view. What were some of the labs that were at work in cloning the catenins?**

The first step forward in identifying beta-catenin was the cloning of the gene for human plakoglobin in 1989 by Franke and Cowin (al. 1989). Then Mark Peifer in Eric Wieschaus's lab identified the gene deficient in a mutant named *armadillo*, which had been genetically linked to *wingless*, and found that it was homologous to plakoglobin (Wieschaus 1990). Next, Barry Gumbiner cloned the beta-catenin gene in *Xenopus*, and showed its homology with *armadillo* and plakoglobin, demonstrating its high level of evolutionary conservation (al. 1991). Akira Nagafuchi and Rolf Kemler first purified the alpha-catenin protein and isolated its cDNA, and showed its similarity to vinculin (al. 1991, Herrenknecht *et al.*, 1991). So you can see there was a lot of interest and work being done during the late 80s and early 90s in respect to what turned out to be the cytoplasmic cadherin-catenin complex.

#### **Once you had identified the E-cadherin molecule and cloned the cDNA, what was your next target?**

This was when the cloning of genes was just getting started. In Okada's lab, delta crystallin was the first gene cloned. Actually people didn't have much of an idea of what to do once you had cloned a gene – the mentality was anyway, just clone it. Promoter analysis was also a popular experiment. If you went to the Molecular Biology Society meeting, it seemed like everyone was talking about their work on some gene promoter.

Shigeo Hayashi, who was still a grad student at the time, made a good suggestion, which was to use cadherin cDNA to see if it could induce cell adhesion, which I hadn't given much thought to previously. That made sense, but that kind of experiment still hadn't become common practice at the time. It's similar to the way the human genome was sequenced first without there necessarily being a plan for how to use all that data once it became available. The applications come later.

Experiments with cadherin cDNAs, such as some done by Akinao Nose, clearly showed the specificity of adhesion was linked to different cadherin molecules (al. 1988). He made cells expressing P- and E-cadherins and showed that they didn't adhere to each other which gave solid molecular biological evidence of their functional specificity. At the same time, Akira Nagafuchi was working on the cadherin cytoplasmic domain – he showed that a deletion of this domain results in a loss of cadherin function (Takeichi 1988). Catenins still hadn't been cloned yet, so the specific reasons why the cytoplasmic domain is important weren't clear. So cDNA played a big part in developing a better



With Akio Ogasawara (right), my high school biology teacher, at an alumni party in 1996. He was also an ornithologist, and he inspired in me a sense of the importance of natural observations.

understanding of the molecular aspects of cadherins. It allowed us to move from immunologically based research, which only lets you block function, to the active manipulation of the molecules we were studying. We're really mainly working with the same kinds of technologies – immunochemistry and genetics - today. I was lucky to be doing my work when all of these powerful new techniques were being introduced, as they made it possible to make new discoveries.

**You must have been thinking about the developmental and medical implications of cell adhesion research all along, even before identifying the first cadherin. What do you see as the greatest contribution of your work in cell adhesion to the general fields of development and embryogenesis?**

I suppose the easiest way to answer that is that adhesion molecules are essential for the development of multicellular organisms, and that we were able to shed some early light on the complexity and multiplicity of adhesion mechanisms. There are a number of adhesion molecules, such as calcium-independent Ig superfamily molecules, but the cadherins have turned out to be the most important players. Without cadherins, cells fail to form stable junctions with each other.

In invertebrates, homologs of the classic cadherins exist but there appears to have been a diversification of function from species to species. There's DE-cadherin in *Drosophila* (Oda *et al.*, 1994), and its loss results in a total failure of cell adhesion in the developing embryo. On the other hand, it hasn't been shown that the loss of classic cadherin homologs causes cell disaggregation in *C. elegans*. Both species have about 20 genes that include cadherin repeats, but they don't necessarily have universally conserved function. In general though, cadherin-motif bearing proteins have been found in every organism studied to date, so the motif appears to be universally conserved in animals, even in very primitive multicellular organisms like sponges. I've thought I'd like to study that as well, but haven't yet found the time or the person willing to take that on.

**Cadherins have been pinpointed as important to cancer metastasis as well. When did you begin to have an interest in the cadherin-cancer connection?**

I've always thought that studying the relationship between cadherins and disease would be interesting. My background isn't medical, so I don't have a detailed knowledge of pathology, but Japanese research funding has historically emphasized cancer research and there have been a number of large grants available for work, including basic research, relating to cancer. Many of those projects involved the formation of multidisciplinary research groups, and so I've had chances to collaborate and share ideas with cancer researchers.

In some very basic senses, metastasis is a problem of cell adhesion.

That is, in order to metastasize, a cancer cell needs to disengage from its neighbors and move through the body. One concept that has been investigated is the role that cadherins, their dysregulation, plays in this process, the idea being that something must be happening to cadherins to disrupt normal cell adhesion in cancer. And a number of findings have been made on that front.

**Have they led to any applications?**

I think there's good potential for finding medical applications for cadherin research. In some cancers, you find mutations in or total loss of cadherins, resulting in the inability of the cells to form stable adhesions. But actually, in most cases, cancer cells metastasize while remaining adhered to each other. For example, colon cancer is highly metastatic, but it maintains cell-cell adhesion during the process, so in that case metastasis is not simply the result of a loss of adhesion. On the other hand, in scirrhous carcinomas of the stomach, the cancerous cells dissociate and invade the stomach lining, and at that point the prognosis for the patient is not good. That sort of invasion appears to be allowed by abnormal cadherin function. But for colon cancer, the picture isn't so clear, because the cells continue to adhere to each other. But when we studied the adhesion it turned out to be unstable under certain conditions. It looks like those cancer cells experience a failure or change in the regulation of adhesion, so it may be possible to find a pharmaceutical solution to the problem if you can identify the factor that's destabilizing that regulation.

We actually found that if you treat one line of dispersed proliferating colon cancer cells with the tyrosine kinase staurosporine, you can get them to re-aggregate (al. 1999). That seems to me to be a good direction to look into for a metastasis-inhibiting drug. But of course there are some significant obstacles, such as staurosporine's toxicity and the lack of an understanding of the molecular mechanism by which it induces aggregation. But if you solve the question of how it regulates cadherin-catenin interaction to achieve stable cell-cell adhesion, it seems you ought to be able to design a molecule capable of producing the



same effect, and thereby block metastasis. The biggest problem in inhibiting metastasis, though, is that once the cancer starts metastasizing, it's already too late – you have to prevent the process before it starts. That's a big question in cancer surgery as well – it might be that by manipulating the tumor you're actually accelerating metastasis. Surgeons are aware of that, of course. Maybe one application for an anti-metastatic agent would be to stabilize cell adhesion, even partially, for the perioperative period to reduce the chances of that happening. So I'm hopeful that a drug company will look into the development of a non-toxic, cadherin-specific cell adhesion stabilizing agent, but so far there haven't been any takers.

**Your recent work has focused, among other things, on the role of cadherins in synaptogenesis. Could you talk a little about what you've been finding?**

I think of this as the last big challenge of my career. Really it's not just synaptogenesis, but the formation of neural networks. Of course for a biologist investigating how a functioning brain is constructed is intrinsically fascinating. It's enormously complex of course, but the complexity of the problem is a big part of what makes it so attractive. And although the end result is so elaborate and complex, I think that the basic principles are actually quite simple, and if you can work out those principles or mechanisms, then I think it's possible to answer the question of neural network formation. That's the same approach I had with cell adhesion – don't try to attack the problem as a whole, but break it down into its fundamental units and answer them one by one. I think the same will work for brain research – find the basic rules and figure out how they work and that will give you insights into the big picture.

In neural networks, the basic point of contact is the synapse. Of course, there are others, but the synapse is the most important junction. So if you can show the means by which neurons identify and recognize each other, then you've made a good start towards working out the problem of neural network formation. We already know that cadherins are involved in the process, and I believe that the molecular mechanism is fundamentally similar to that seen in simpler epithelial cells and fibroblasts. I think it's simply a question of modifications to the system.

One of my main goals right now is to study the relationship between the expression of different cadherins in different neuronal subgroups. We know that this kind of differential expression takes place, but it still isn't very well understood. I'd be happy if we could work that system out.

This kind of work might provide a molecular basis for a model like the one Roger Sperry proposed for neural network formation, based on interneuronal affinity gradients, which has already been demonstrated for ephrins and their receptors in general axon guidance. I think we might be able to show even greater specificity and precision in neuronal recognition using cadherins. That's the dream.

**You've also moved into a new phase in your career in science now, with increased administrative duties. How does work as the director of a large center compare with heading a lab at a national university?**

I'm quite happy to have made the move here to the CDB. I understood that taking on a director's responsibilities would

add to my workload and might tend to compete with research for my time, so for a while before I came I debated whether it wouldn't be better to stay at a university and just focus on research. But RIKEN offers advantages that aren't available in most academic research positions, and I can say that I really haven't encountered problems in continuing my research. I have more administrative duties of course, but they aren't overwhelming. When I accepted this position, it was with the understanding that I would be able to continue to do research, and the administrative support here has enabled me to do that. I think that the heads of research programs at national universities are actually much busier in that regard than I am. It's also been quite nice to be able to meet so many new people through the CDB. So overall, yes, it's been a very positive experience.

**I know you have strong feelings about the importance of educating new generations of scientists. Have you noticed any major changes in the education of researchers since the time you were a grad student? Is there anything you miss about the old days?**

That's an important point. Science is obviously changing – compared to when I was a grad student, the amount of information we have has grown tremendously and continues to grow faster all the time. Priorities have changed as well. All that has changed the way science is taught and learned. When I was a student, I was able to follow my own path and I got away with things that students today probably couldn't. It was easier to go your own way back then. The realities of research are different now, and I think figuring out how to give a good education tailored to the needs of the day is one of the big questions facing science educators now.

I think that there are some basic principles that remain unchanged though. Tokindo Okada used to warn students "Don't start a research project based on some paper you've just read." What he meant was that a finding that's been published in an important journal is already old news. That can particularly be a problem in Japan, where a lot of scientists are working somewhat removed from the networks in Europe and America. The important thing I think young researchers need to do is to look into what questions remain to be answered and begin establishing their own approach, and maybe that will bear fruit in ten or twenty years. Too many young scientists see an interesting paper in *Cell* or *Nature*, and think "I'll follow up on that!" but that will never lead to anything truly original. There's an unfortunate trend toward scientists getting their initial research ideas from reading something in a paper or having something assigned to them as a project by their lab head, rather than from an observation of a natural process or phenomenon. What I hope for my students is that they come up with their own ideas, based on what they've seen with their own eyes, look into what is known and unknown about a question, and then work out the means of solving the unknowns.

Europe and America have led the world in developmental biology, but I think that's the result of having a core of excellent scientists able to select and create an emphasis on and widespread interest in a limited number of research subjects which are then taken up by many others. This kind of research produces a lot of papers in major journals, but if you go back and look at who did what, and who asked the important questions, it's really that small core of scientists.

**Thanks very much for your time today. It was very enjoyable for me to have the chance to hear about your early education and experiences.**

Thank you.

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