part of my lab in that direction with work on human embryonic stem cells which, as we have come to learn, show aberrant epigenetic regulation. There is a lot to learn from this much needed human model.

What do you enjoy most about science? The challenge, the discovery, and the people. In this profession, one meets some of the brightest (and most eccentric) minds on Earth. Although I dislike airports and flying, I do enjoy the chance to see different cultures, experience science as it is done in other countries, and taste great food (I always enjoy myself once I am there). Through science, one has the chance to make a lasting positive contribution to our society and planet.

Are there any disappointments in science? For me, one of the biggest attractions to science is that it is inherently value-free. There is one truth that cannot be changed by culture, history, and politics. But I no longer think that science is devoid of politics. Science itself is objective, but the practice of it is about people, personalities, and perceptions of what is important. We see this in the way grants are awarded, papers published, and policies established by the government. I see great ideas that are not given a chance at being tested because of diminishing resources; or great ideas not finding an appropriate place in the literature because of a general unwillingness to take risks on very imaginative thinking. Perhaps there is no way around this, especially with the current crisis in grant funding. Both junior and seasoned investigators spend a lot of time writing grants to keep their laboratories alive when their time would be more productively spent on experiments.

Any career advice for someone just starting their own lab? Stay at the bench for as long as possible, because it is more rewarding to do experiments than to exercise your administrative skills in the office. Nowadays, I am more coach, cheerleader, and psychologist than experimentalist — sadly. The people you hire are your greatest assets — choose and treat them well. Hire people with complementary skills and always try to keep a balance of talent and personalities in the lab. Over the years, I have come to value talent, work ethic, honesty, and collaborative spirit equally.

Are there gender differences in science? As someone who makes a living out of studying male and female differences, you might think that I am hardwired to think so! But I am not a big proponent of the idea that men and women inherently do science differently. Whatever difference there is, I am certain that there are many more differences among individuals and cultures than between the sexes. This I see in practice everyday. From what I can see in my own lab, there is not a significant 'delta' in work hours, talent, and commitment.

What emerging problems interest you most? There are so many problems I would work on now. If I were a student thinking about science, I might focus on environmental issues, energy, neurobiology, or cognitive science. I also think that the areas of genome–environment interactions and environmental toxicology (branches of epigenetics) will blossom in the coming years.

What would you like to see solved in the next 10 years? An end to global warming and pollution. I would also like to see better land stewardship and conservation. Do you think I'm unrealistic?

What advice would you give a student thinking about a career in academic science? You must love it — everything about it, from writing and speaking to thinking and doing. As with anything else in life, it is easier to love something when you are good at it. So find a problem that suits your talent and personal goals. Expect no immediate rewards (money, fame, prizes). If you are passionate about science, you will survive grant writing, paper rejections, and the long road to tenure. A life in science is one of the best there is.

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Quick guide

Tight junctions

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What is a tight junction? Tight junctions (TJs) are intercellular contacts that seal the space between the individual cells of an epithelial sheet so that they can collectively separate tissue compartments. The barrier is required to accomplish vectorial transport of material from one side of the compartment to the other and to limit paracellular entry of undesirables like toxins, antigens and microbes. The tight junction forms a continuous intercellular contact at the apical-most end of the lateral side of epithelial cells (Figure 1), above other specialized cell contacts like adherens and gap junctions and desmosomes. Its name derives from early transmission electron microscopy images showing membrane 'kiss points' between adjacent cells where the outer membrane leaflets appeared to fuse. It is also called the occluding junction (OJ) and the zonula occludens (ZO). TJs do not just act as barriers but are also sites for vesicle targeting, cytoskeletal dynamics, signals controlling proliferation and transcription, and for defining cellular polarity between the functionally distinct apical and lateral membrane surfaces.

What are TJs made of? Compared with other junctions, TJs include a surprisingly large number of different proteins — at least 40. The actual barrier is formed by continuous adhesive strands of transmembrane proteins that interact to seal the paracellular space (Figure 1). These transmembrane proteins are called claudins and form the hallmark network of interconnected strands revealed by freeze–fracture EM. Many different claudins are often expressed in a single cell and their expression profiles create the variations in barrier properties observed among different tissues. Other adhesion proteins collect in the strands but their function remains unclear; these include tricellulin (which tends to concentrate where three cells come together), its homolog occludin and an immunoglobulin

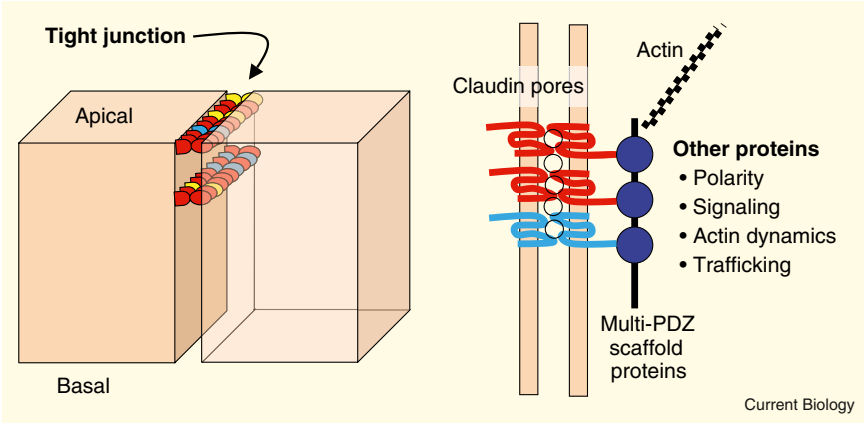

Figure 1. The tight junction barrier is formed by continuous rows of claudin proteins which ring the apical end of the cell. Different claudin gene products, depicted in different colors, assemble in the barrier and create paracellular pores through their extracellular contacts (shown on the left). Claudin, and other types of strand proteins, are attached to multi-PDZ proteins and the actin cytoskeleton (right). Many other functional classes of proteins are found at TJs.

superfamily member called JAM-A. The transmembrane proteins are attached to a network of multi-PDZ proteins (including ZO-1, ZO-2, ZO-3, MAGI-1, PatJ, PALS1 and MUPP1), which is thought to cluster the barrier-forming proteins and provide redundant attachments to the actin cytoskeleton. Actin dynamics appear to be the key to junction assembly as well as to the physiological and pathological control of the barrier.

How are TJs formed? This remains an active area of research. Assembly occurs downstream of signals initiated by other cell–cell adhesion complexes. Current evidence suggests a cascade initiated by intercellular adhesion between nectins, which, through several cytoplasmic proteins, recruit cadherin, which in turn, through other cytoplasmic linkers, determines the location of claudins. Proteins controlling actin dynamics (like Cdc42, RhoA, Rac, Rich1, GEF-H1, TUBA, cortactin and N-WASP) are also required, as are several kinase signalling pathways. The evolutionarily conserved polarity proteins (Par3, Par6 and aPKC) are also concentrated at the TJ and are required for TJ assembly and establishment of apical-basolateral cell polarity, in part through controlling actin dynamics. The TJ is intimately connected to and dependent on the cadherin-based adherens junction, and in endothelial cells the two often merge.

What gets through the TJ? Although an oversimplification, it is useful to think of passage through the TJ barrier comprising two components — physiological pores and pathological breaks. All epithelial TJs have a system of small 8 Å diameter pores that varies among cell types in ionic charge selectivity and in porosity, i.e. the apparent number of pores. The mechanism controlling overall porosity is unclear, but it is known that ionic charge preference is controlled by claudins through electrostatic effects of fixed charges on their extracellular domains. The claudins form the pores or at least line them. Of those studied, each claudin has a characteristic influence on the permeability for small cations and anions. Current research attempts to draw parallels between the nascent field of TJ pores and the well-established field of transmembrane ion channels. However, the TJ pore is structurally entirely different and is formed by repeating contacts of many proteins across the extracellular space as opposed to a few subunits perforating the bilayer. At this point we know painfully little about the structure of the TJ pore.

The passage of material larger than 8 Å through the TJ shows no size or charge selectivity. Flux through this pathway is normally quite limited, leading to speculation that this pathway represents a pathological break between cells. Such breaks can arise in response to proinflammatory factors like interferon-γ and tumor necrosis factor-α (among others), which induce RhoA- and myosin-dependent endocytosis of TJ membranes and cytoskeletal contraction. There would be great benefit to understanding the control of TJ integrity since its breakdown enhances tissue damage by allowing entry of proinflammatory bacterial cell wall products, antigens and microbes.

When did TJs arise in evolution? Although a paracellular barrier of some description is a hallmark of all metazoans, the TJ appears to be specific for vertebrates. Invertebrates do have a related barrier called the septate junction, but, while in vertebrates, the TJ is more apical than the cadherin-based adherens junction, in invertebrates, the order is reversed with the septate junction being basal to the adherens junction. Whereas the TJ contacts appear in EM images to occlude the intracellular space, the intercellular septate contact is widely spaced and spanned by a ladder-like series of septa. Some of the cytoplasmic proteins are homologous, for example ZO-1 in vertebrates is the homolog of *polychetoid* in flies. Knockdown of one of the three claudin-like proteins found in the nematode *Caenorhabditis elegans* causes leakiness of the gut epithelium. Analogous barrier defects follow deletions of the *Drosophila* claudins, Sinuous and Megatrachea, which are located at septate junctions. Interestingly, the phenotypes of *sinuous* and *megatrachea* mutants also reveal a clear role for these claudins in developmental signaling and epithelial morphogenesis; evidence for this role for vertebrate claudins is pending. Finally, there is an intriguing structural similarity between the proteins found at the TJ and at synapses, suggesting a possible evolutionary connection between these functionally distinct cell contacts. For example, a PDZ domain interaction mediates binding at TJs of claudin to ZO-1 and in synapses of the AMPA receptor regulatory subunit (claudin homolog) to PSD-95 (ZO-1 homolog). Both TJs and synapses are also hotspots for vesicle targeting.

Are TJs associated with diseases? When the TJ barrier fails, material can cross the epithelia in an unregulated fashion. Transepithelial absorption and secretion stop and antigens and microbes can breach the barrier. Almost anything that alters cell metabolism (toxic, metabolic, neoplastic or immune-based insults)

will break the barrier, making the TJ a frequent contributor to a vast range of pathologies of blood vessels and epithelial organs. Since most of these insults have pleotropic cellular effects, it is difficult to envision therapies that could target specific TJ components or functions. In contrast, some assaults on the barrier are quite specific. For example, house dust mites excrete a fecal protease that cleaves occludin and claudin, loosens the barrier and in theory allows allergens to cross the airway and skin epithelia. Similarly, some types of allergenic pollen produce a protease which cleaves occludin, enhancing allergen entry. Specific claudins are receptors for a cytotoxic diarrhea-inducing enterotoxin produced by *Clostridium perfringens* and other claudins are co-receptors required for hepatitis C virus to enter cells.

The growing list of diseases caused by mutations in the genes encoding TJ proteins has provided significant insight into how the TJ works. For example, claudins 16 and 19 form cation-selective pores and are located in a segment of the kidney tubule where Mg^{2+} ions return to the body by passing through the TJ. Mutations in either protein are associated with a failure to reabsorb Mg^{2+}, leading to low serum Mg^{2+} levels and resultant weakness and seizures. Loss of ion selectivity may also explain why claudin-14 mutations lead to deafness. Mutations in PMP-22, a claudin which seals myelin, lead to peripheral neuropathies through an unknown mechanism. Hopefully our increasing knowledge of TJ structure will lead to specific therapies to preserve or restore integrity of the barrier.

Can we manipulate TJs to enhance drug delivery? In principle it might be desirable to open TJs in order to enhance drug delivery across epithelia like the gut and airway surfaces or across blood vessels like the blood–brain barrier. Manipulation of the barrier may be required for the delivery of emerging therapeutic agents based on peptides, proteins and DNA. Despite wide interest and research, however, no agents have yet reached clinical application. Early efforts employed non-specific attacks on general signaling pathways that led to cells being pulled apart or alternatively used detergents to disrupt epithelial integrity. More recent approaches specifically target the intercellular interactions of claudins or occludin. Given the role of TJs in limiting drug delivery, there is a lot of creative energy applied to this problem; however, we will have to wait to see whether it's a good idea to break the barrier even transiently.

Is there a link between TJs and cancer? As a rule, cells use every point of cell–cell and cell–substrate contact to transfer information. Although we do not yet know what information the cell receives at TJs, the importance of TJs for differentiation and proliferation is suggested by the frequent alteration in claudin profiles seen in specific cancers. During 2007 alone there were >60 publications on PubMed using different claudins to classify and even to prognosticate outcome in various human carcinomas. Beyond these correlations, there are a few papers that report the manipulations of claudin levels in human cancer cell lines and show a correlation with metastatic potential when injected into mice. A link to Wnt signaling has also been implicated. The massive overexpression of specific claudins in specific cancers has led to several proof-of-principle studies targeting those claudins for chemotherapy. Specifically, in mouse models, the previously mentioned *C. perfringens* toxin will effectively eliminate human ovarian and pancreatic cancers, which overexpress the toxin receptors claudins 3 and 4. However, much remains to be learnt before TJ signaling can be targeted for cancer therapy.

Where can I find out more?

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Primer

Wnt–β-catenin signaling

Ken M. Cadigan

Wnts are a family of extracellular cell–cell signaling molecules which act in a wide range of developmental processes in metazoans; the name derives from the *Drosophila* gene *Wingless*, and the related mammalian oncogene *Int-1*. No-one knows for sure when the first Wnt evolved, but the existence of fourteen *Wnt* genes in the sea anemone *Nematostella vectensis* — by comparison, there are seven Wnts in *Drosophila*, five in *Caenorhabditis elegans* and nineteen in mice and humans — indicates that this gene family had already evolved and diversified more than 600 million years ago. Wnts are proteins defined by a conserved primary sequence that includes twenty-one specifically spaced cysteines. The expression pattern of the sea anemone *Wnts* suggests that they form a 'Wnt-code' that specifies its basic body plan and recent functional studies indicate that Wnts are required for primary and axial patterning in several cnidarian species. For the metazoan species that have been exploited by developmental biologists for their ease of genetic analysis, such as flies, worms and mice, there is abundant evidence that many developmental decisions are controlled by Wnts, from gastrulation and early pattern formation to organogenesis. Although Wnts are known to influence cell behavior through several different signaling pathways, many act by regulating the stability and subcellular localization of β-catenin. This intensively studied signal cascade is known as the Wnt–β-catenin pathway, the focus of this primer.

While the Wnt–β-catenin pathway is often thought of as a major signaling pathway in animal development, it also plays important roles in stem cell maintenance in regenerating tissues such as intestinal epithelia and hair follicles. Wnt–β-catenin signaling