cycle with terminal differentiation. A unipotent host cell population acts as stem cells, which proliferate and migrate from the center of each lobule to the periphery, cease division, and die through apoptosis. During this journey they carry along their symbionts, which divide in the center, stop dividing, and subsequently become digested in the periphery. Digestion thus serves both in host nourishment and the control of the symbiont population density.

What do we know about the symbionts? The *Endoriftia* 16S rRNA phylotype was detected in the environment, both on surfaces and in the water. Its metagenome shows the presence of genes for the oxidative TCA cycle indicating the ability to live as heterotrophs outside the host. Genes for chemotaxis suggest that *Endoriftia* actively seeks the prospective host. This points to a highly versatile bacterium capable of surviving in the biofilms of hydrothermal vents and adjacent deep sea as well as thriving under host control as endosymbionts.

How do vent and seep tubeworms differ? Some tubeworms, such as *Riftia*, inhabit hydrothermal vents, while other species live on whale bones or at seeps, deposits of oil and gas leaking through sediments to the sea floor. Vents are highly disturbed, short-lived ecosystems, while seeps may persist for ten thousands of years. Consistent with the temporal dynamics of these contrasting ecosystems, *Riftia* grows fast and is short-lived, whereas *Lamellibrachia luymesi* from the seeps of the Gulf of Mexico grows slowly and is — with estimated ages of up to 300 years — among the longest lived of any of the non-colonial invertebrates. Although carbon fixation rates of *Riftia* exceed those of *Lamellibrachia*, both symbionts are highly active and support similar, high cell proliferation rates. However, apoptosis rates are low in *Riftia*, but in *Lamellibrachia* match those of proliferation. Thus *Riftia* grows fast, whereas *Lamellibrachia* grows slowly and consistently renews its tissue, supporting longevity.

Who are the tubeworms' relatives? Vestimentiferan tubeworms belong to the small polychaete family Siboglinidae (Figure 1). Unlike other polychaetes, siboglinids share an obligate symbiotic life style in chemosynthesis-based ecosystems, such as vents, seeps, and whale bones. The thiotrophic symbiosis probably evolved once rather than several times independently. This is supported by the position of the trophosomes at the exact same location in the first segment of the different worms. The remarkable differences of trophosome origins, however, suggest that the last common ancestor harbored symbionts in several tissues. Consequently, in frenulates, the trophosome became restricted to the gut, in *Osedax* to the somatic mesoderm, and in vestimentiferans and its sister *Sclerolinum* to the visceral mesoderm. Horizontal transmission not only ensures continuation of symbiosis, it also allows for the uptake of appropriate symbionts, which can be selected in each generation anew. Most remarkably, *Osedax*, which colonizes whale bones, must have replaced its thiotrophic symbiont with heterotrophic Oceanospirillales nourished by the host.

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Polo-like kinases

Conrad von Schubert and Erich A. Nigg*

What is Polo and what are Plks? Plk stands for Polo-like kinase. In the 1980s, genetic screens in budding yeast and *Drosophila* identified several key regulators of mitosis, including the founding member of the Polo kinase family. Since then, a total of five mammalian paralogs of the *Drosophila Polo* gene have been discovered. These exhibit largely non-redundant functions and are differently expressed, localized, and regulated. The *Polo* homolog *Plk1* is common to all eukaryotes, apart from plants and certain protozoan parasites. *Plk4* is also present in most vertebrates and invertebrates and probably arose early on, in a first round of gene duplication. The evolutionarily 'younger' *Plk2* sub-family is only found in some bilaterian animals and comprises two genuine kinases, *Plk2* and *Plk3*, as well as the kinase-deficient *Plk5*.

Is Plk1 the leader of the pack? Plk1 is a wizard of both mitosis and meiosis (M phase of the cell cycle). It is expressed in proliferating cells and regulates many aspects of M-phase progression — notably mitotic entry, spindle architecture and positioning, sister-chromatid separation, and cytokinesis. Hence, inactivation of Plk1 in cultured cells leads to cell-cycle arrest in early mitosis, followed by apoptosis. In addition, Plk1 is also involved in key processes, such as release of amphibian eggs from cell-cycle arrest upon fertilization, recovery of mammalian cells from DNA damage, and RNA polymerase III-dependent transcription. At this point in time, it seems fair to state that Plk1 is top dog of the family. But Plk4, another key regulator of cell division (see below), is increasingly challenging Plk1's leadership position. In contrast, the roles of the younger Plk2 family members still remain sketchy.

How does Plk1 manage all these different functions? The answer lies in the structure. Plk1, like all other family members, has a topology with two conserved domains: an amino-terminal serine/threonine kinase

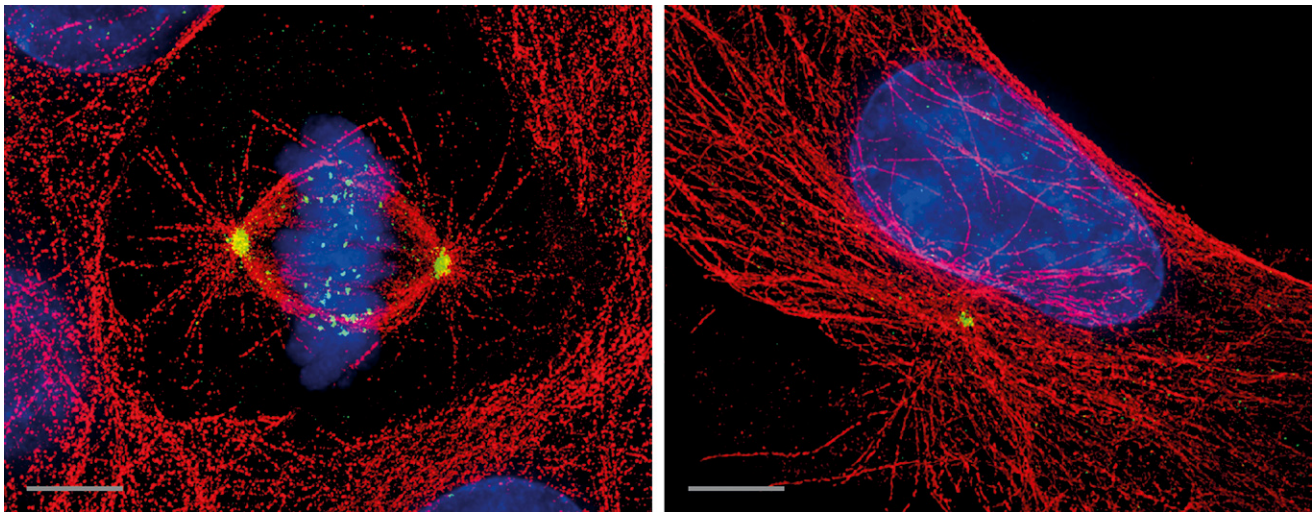

Figure 1. Immunofluorescence analysis of human hTERT-RPE1 cells showing Plk1 (green, left panel) at kinetochores as well as spindle poles and Plk4 (green, right panel) at centrosomes. Microtubules are shown in red, DNA in blue. Scale bars represent 5 μm. (Image: C. von Schubert; A.I. Ferrand, IMCF Biozentrum.)

domain and a carboxy-terminal substrate-binding domain, known as the polo-box domain (PBD). Plk1 is a very busy kinase and several recent phospho-proteomics studies indicate that it targets a large number of physiological substrates. To perform its various tasks, Plk1 is targeted to distinct subcellular sites, such as centrosomes during G2 phase and kinetochores, spindle poles and the spindle midzone/midbody during M phase (Figure 1). Plk1 localization is governed by docking of the PBD to specific motifs (Ser–Ser/Thr–Pro) that have been primed by phosphorylation. This beautiful mechanism confers both temporal and spatial control over Plk1 activity. For example, Plk1 docking to early mitotic interaction partners is often primed by cyclin-dependent kinase 1 (Cdk1). Concomitantly, this same kinase prevents Plk1 from binding to late mitotic interaction partners through inhibitory phosphorylation adjacent to PBD-binding motifs. When Cdk1 activity is reduced at the onset of mitotic exit, this suppression is relieved and Plk1 primes its own recruitment to proteins that are important for the initiation of cytokinesis. The spectrum of Plk1 regulation also includes more conventional mechanisms, notably activation-loop phosphorylation by Aurora kinases and proteasomal degradation at the hands of the major mitotic ubiquitin ligase known as anaphase-promoting complex/cyclosome (APC/C).

I've heard that Plk4 is a master regulator of centriole and basal body formation — is this true? Yes, Plk4 indeed plays a key role in the biogenesis of centrioles and basal bodies. Centrioles are important for the assembly of centrosomes — the major microtubule-organizing centers of animal cells (Figure 1) — and as basal bodies they trigger the formation of cilia and flagella. Depletion of Plk4 results in loss of centrioles, whereas its overexpression triggers excessive centriole formation. Murine *Plk4*$^{-/-}$ embryos die early in development, confirming that Plk4-deficient cells are defective in cell-cycle progression. In most cells, Plk4 levels are extremely low and this reflects a self-destruction mechanism based on trans-autophosphorylation within Plk4 dimers, followed by ubiquitylation and proteasomal degradation. How Plk4 regulates centriole duplication remains to be understood, but clearly the PBD is important for Plk4 localization to centrosomes. Interestingly, Plk1 also plays an important role in centrosome biology in that it contributes to restrict centriole duplication to once per cell cycle.

Are Plk2 and Plk3 merely afterthoughts of vertebrate evolution? That's a bit harsh! Perhaps it will simply take more time to better understand what these kinases are actually doing — but, obviously, they are not required in many species. Both *Plk2* and *Plk3* were originally identified as early-response genes that are upregulated following serum stimulation of quiescent murine fibroblasts. Subsequently, both genes were also assigned tumor-suppressor roles: *Plk2* expression was reported to be downregulated in several types of cancer, whereas *Plk3*$^{-/-}$ mice are prone to tumor development. Remarkably, however, *Plk2* as well as *Plk5* are also expressed in non-proliferative tissue of the central nervous system and Plk2 was implicated in synaptic plasticity. Hence, although Plk2 family members may not be essential for life, they are likely to play important roles. This rings a cautionary bell with regard to the development of Plk1 inhibitors as anti-cancer drugs (see below).

What about Plk5, the new kid on the block? Plk5 probably functions as a decoy kinase. In humans, for example, the *Plk5* sequence contains a stop codon within the kinase domain, and a protein fragment is only resurrected thanks to a start codon downstream, resulting in expression of a catalytically inactive, truncated protein. Although expressed, *Plk5* was initially given the cold shoulder because it shows the characteristics of a pseudo-gene. However, recent studies have now shown that *Plk5* is expressed in non-proliferative tissues, mostly the brain. In addition, it was found to be upregulated in fibroblasts upon serum starvation or DNA damage, whereas

overexpression triggered a G_0/G_1-like arrest. Thus, it has been proposed that Plk5 function is related to stress responses.

Are Plks attractive drug targets for cancer treatment? Yes and no — the future will tell. So far, the focus has been on targeting Plk1: human Plk1 is highly expressed in proliferating tissues, often upregulated in tumors, and elevated expression in tumors is associated with poor prognosis. Furthermore, overexpression of Plk1 leads to transformation of cultured cells, likely via the stimulation of a mitotic transcription program involving the transcription factor FOXM1. In addition, it is in principle possible to interfere with Plk1 function not only via the usual route of ATP-competitive inhibitors (which of course raises concerns about specificity), but also by interfering with PBD binding to docking proteins. Several early cell-culture studies had suggested that tumor cells may be more sensitive to Plk1 inhibition than normal cells, but whether a sufficient therapeutic window can be found in a clinically relevant context remains to be determined. Several Plk1 inhibitors are presently in clinical trials and it will be interesting to see how these agents fare for the benefit of patients.

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Primer

Reptiles

Richard Shine

Most small children can tell you that ‘reptiles’ are the snakes, lizards, crocodiles, and turtles (perhaps with the dinosaurs thrown in) — suggesting that it’s easy to tell the difference between reptiles and other animals. Unfortunately, evolutionary biologists struggle with the same task, because phylogenetic analysis tells us loud and clear that these different types of what we loosely call ‘reptiles’ are not particularly closely related to each other (Figure 1). On the evolutionary tree, some of them (dinosaurs, crocodiles) are much more closely related to birds than to the other animals that we call reptiles. Other reptiles are the descendants of very ancient lineages; for example, turtles separated from the other reptiles, including the now-dominant Squamata (lizards and snakes), at least 200 million years ago. And another 200-million-year-old lineage has left just a single survivor, a lizard-like creature (the tuatara), on a few islands in New Zealand.

So, why do we still talk about ‘reptiles’, when an analysis based on shared derived traits (cladistics) says that the Reptilia are not a ‘natural’ (monophyletic) evolutionary group for which a single common ancestor can be defined that excludes all non-reptiles such as birds? The reason is that a comparison based on external morphology (phenetics) would yield the opposite conclusion: for example, crocodiles and tuataras really do look a lot like lizards. For example, they share a distinctive body shape, and are covered in scales. It is this outer resemblance which led to the concept of the Reptilia, and which has kept it alive and kicking even though the creatures known as reptiles are only distantly related to each other. So, the problem with defining the Reptilia actually throws up an interesting biological puzzle: given their divergent ancestries, why do these animals all look so much alike? The answer involves a fundamental feature of reptiles: the way in which they control their body temperature.

Taking the heat

By and large (with more than 8,000 species, there are exceptions to almost every rule), reptiles are ectotherms. That is, they rely upon ambient thermal heterogeneity to regulate their internal temperatures — for example, by basking in sunlight to become warm, and moving to shade to cool down. This tactic is in striking contrast to endotherms, such as birds and mammals, which rely upon metabolic heat production to maintain a high and relatively constant internal temperature. Endotherms are like racing cars — they keep their engines revving at high speed most or all of the time and so can perform at high speed. For example, they not only can move quickly, but they can also maintain that speed because their hearts and lungs can deliver extra oxygen to the muscles that are doing the hard work. And because they generate their own heat, endotherms can function effectively even in cold conditions.

At first sight, this looks like a clear case of an evolutionary advance: the primitive cold-blooded low-performing reptiles have been replaced by sophisticated high-performing mammals and birds. But that interpretation is wrong: first, ectotherms have not been replaced by endotherms, and when you include fish there are a lot more species of ectothermic vertebrates than endothermic vertebrates. Indeed, some authorities believe that crocodilians evolved from endothermic ancestors — something we wouldn’t expect to happen if endothermy was ‘better’. Second, ectotherms are not ‘cold-blooded’ — a desert lizard may run around with a higher body temperature than the rodent who lives in the adjacent burrow. The fundamental difference between endotherms and ectotherms is in the source of the heat used to regulate body temperature: endotherms make their own, whereas ectotherms exploit environmental heat. Because ectotherms do not need to create their own heat, their metabolic rates are about one-tenth of those of a similar-sized endotherm, massively reducing energy needs. They can’t fuel sustained muscular activity by aerobic means, but they have a fallback, as anaerobic metabolism usually can keep them going long enough to find the food item or shelter that they require. If endotherms are racing cars, ectotherms are pushbikes, less capable