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EDITORIAL

Ars comparandi: "molecular convergence" versus "functional homology"

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Physicists measure—biologists compare. This statement is of course oversimplified, but describes a principal difference between the two sciences (ignoring the fact that also measuring is a kind of comparison, because objects or phenomena are compared to a unit of measurement). Life is manifold, and in order to describe this complexity, one has to filter out the particularities of a given life form, and to focus on its general features. To tell apart what is "particular" and what is "general" appears far from trivial, though. The art of comparing requires not only a thorough understanding, how a life form is structured, but also insight into development, physiology, interactions with the environment, and evolutionary history. Morphological similarity may lead astray, when these facets are ignored, because similar selective pressures may channel evolution towards similar shapes, a phenomenon termed as convergence. A meaningful biological comparison requires, however, that the similarity stems from a common origin, i.e. that the life forms to be compared show true homologies. In practice, it can be very tricky, to distinguish homology and convergence, especially in life forms where the evolutionary relationships are not clear. As a guideline, comparative morphology employs three classical criteria to validate homology (Remane 1971): continuity, specific quality and position.

The ample use of sequence information (of both nucleotide and protein sequences) has meanwhile made life much easier, because sequence similarity immediately allows to infer on phylogenetic relationship, a methodological twist that has not only diluted the term homology, but also has undermined the *ars comparandi*: to sequence the genome of an organism appears more convenient than to deal with all the anatomical and functional details of its body structure. In this context, it is often overlooked that also molecular phylogenies are based upon homology criteria: When we align two sequences, we use the criterion of position to define corresponding base pairs or amino acid residues, which is not so different from the procedure used to tell that a bird wing is homologous to a human arm. To tell that two proteins are corresponding and therefore informative for a comparison, we use presence, order and sequence motifs of characteristic domains, which is nothing else than applying the criterion of specific quality. And in order to validate a common origin of two sequences, we use the criterion of continuity by including into our phylogenetic tree numerous sequences from related organisms.

As long as we compare sequences from closely related life forms, this approach works neatly. Things become more challenging, when we have to work without the continuity criterion, for instance, because our life form is isolated, or because we want to conduct higher-level comparisons of more distant life forms. Here, the term "homology" can readily turn into something void of any meaning (Zuckerkandl 1987). More than ever, molecular data have to be integrated with a deeper understanding of the functional context, in which a life form develops and survives. Three contributions to the current issue show how such knowledge on functional and cellular details helps to understand molecular data.

The small heterocyclic molecule indole-acetic acid was originally predicted as transportable growth signal (auxin) in plants by the work of Darwin, Cholodny, Went and many others. Since its molecular identification by Thimann, auxin belongs to the most intensively studied plant signals, because it is transported directionally, providing a flow that orients and guides plant development from the first division of the zygote. The work by Borchers et al. (2018) adds a seemingly exotic new facet to our understanding of auxin as directional cue for plant development: These authors investigate a rotational movement of leaves in the model plant *Arabidopsis thaliana*. Although organ movements in sessile organisms may appear as nothing more than a playful detail of Nature, such twisting

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movements are clearly of adaptive value, because they adjust the position of the leaf blade with respect to light to ensure photosynthetic efficiency. By asymmetric stimulation with gravity or light, authors can elicit an organ movement. In contrast to tropistic bending, where the direction of the movement is determined by the direction of the stimulus, the direction of the response is here constrained by the geometry of the organ (a situation for which classical plant physiology has coined the term photo- or gravinasty, respectively). Interestingly, the authors can modulate the response by administering gradients of auxin, and they can also show that responses of a mutant, *tortifolia*, where the petiole is twisted into a right-hand helix, are qualitatively different from the wild type. This exotic phenotype is linked with the failure of cortical microtubules to reorient into a transverse array in response to auxin, a phenomenon that is generally found in higher plants (Takesue and Shibaoka 1999), and has been shown to be central for classical tropism (Nick et al. 1990). From these data, the TORTIFOLIA gene product emerges as linker between directional transport of a signal (auxin), a directional cellular process (orientation of microtubules) and a directional organ movement (nastic torsion of the petiole).

While the use of auxin as orienting cue is common for a multicellular terrestrial plant, such as *Arabidopsis thaliana*, the work by Zivanović et al. (2018) in the current issue asks the question, whether auxin plays a role in the phototropic bending of *Phycomyces blakesleeanus*. This fungal model had been intensively studied over decades by Max Delbrück and others for its sophisticated and complex responses to directional stimuli. To assume that these should be under control of auxin, as it has been worked out for higher plants, might appear as a clear case, where a convergence has been mistaken for a homology. The presence of auxin in *Phycomyces blakesleeanus* had been demonstrated in the past, but given the large evolutionary distance between fungi and plants, this auxin might have a completely different function, or it might even be a byproduct of a different process with no specific function whatsoever. Authors probe for a potential signalling function in a series of careful experiments, where they modulate growth and bending with different concentrations of the natural auxin, indole-acetic acid, but also by synthetic auxins that differ in their uptake and transport properties, as well as by a specific inhibitor of auxin-efflux. They obtain patterns that are specific and complex, and also differ between wild type and the phototropically insensitive *madC* mutant. The specific quality of auxin signalling in tropism would support a homology with plant tropism, which is certainly not expected for such a distant organism. In fact, authors are able to show that the *Phycomyces* genome harbours candidates for different components of plant auxin transport, including the auxin-influx carrier protein 1, the auxin-efflux facilitator PILS, and the putative auxin-efflux carrier ATP-binding cassette transporter B. In contrast, *Phycomyces* seems to lack the PIN proteins that are central components of directional auxin transport in plants. Is this now a case of molecular convergence? Not necessarily—the PIN proteins are also absent in the algal ancestors of land plants, while the PILS facilitators and the ATP-binding cassette transporters are already present (Reeck et al. 2013). Indole-acetic acid can permeate membranes at slightly acidic pH as it is common in cell walls, it is trapped in the neutral cytoplasm by deprotonation and then has to be pumped out by efflux carriers, whose asymmetric localisation is the cellular base for directional transport. Why should a fungus use the same molecule as a plant to set up directionality? The answer may be hidden in a closer look on the evolutionary context. *Phycomyces*, while commonly classified as "fungus", originally had been described as a member of the algae, and, in fact, is evolutionarily as distant from true fungi as it is from plants or animals. Thus, the functional context of using auxin for directionality, although put into action by partially different proteins, might represent a case of "functional homology", i.e. overlap by common origin.

The third contribution by Cavalier-Smith et al. (2018) in the current issue sheds light into the evolutionary mysteries of unicellular eukaryotes by using a comprehensive molecular and cellular approach to understand a very diverse group within the Chromista (eukaryotes that are neither plants, nor animals, nor fungi). In their previous work, they had already used cellular functions (mainly protein targeting into the periplastid) to understand the overall structure of this still enigmatic eukaryotic kingdom (Cavalier-Smith 2018). In the current work, they zoom into the Rhizaria, one of the four major infra-kingdoms. By critical integration of the available genetic information with careful analysis of the specific features in the movement system (such as details of flagella), as well as ecological aspects (such as benthic versus planktonic lifestyles), they elaborate a complex phylogeny, placing the Chromista as sister to the plants. This pioneering work shows impressively how genetic changes can be integrated into a larger functional context to explain evolution.

The unicellular eukaryotes with their extremely diverse representatives, conspicuous differences of even related species and often still mostly mysterious relationships illustrate that the art of comparing extends beyond computer-assisted sequence analysis, and also has to consider functional relationships. This allows detecting homologies that would go unnoticed, if only the sequence levels were considered. What appears as "molecular convergence", because it can be difficult to identify true sequence homology, might turn into a hallmark of common origin, i.e. a homology trait, when functional details are taken into account. All three contributions show different facets of this "art to look at organisms" (Ledford 2018). This art, while becoming progressively endangered, helps to connect otherwise disconnected observations into something that generates sense. To allow this classical expertise to get lost would be a serious mistake.

Compliance with ethical standards

Conflict of interest The author declares that there is no conflict of interest.

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