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EDITORIAL

Life versus 'biomass'—why application needs cell biology

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For centuries, biology had been driven by the desire to order and understand the multitude of life forms. However, the last century saw the discovery of unifying molecular principles for life, which caused a paradigm shift; now, molecules, rather than shape, were invoked to explain life. This molecular explanation of life not only shifted biology into the centre of scientific progress but also stimulated a vigorously developing new branch of technological application. The success of biotechnology demonstrated impressively that tools and concepts derived from chemistry could be employed to manipulate biology. Interestingly, over the entire first half of the last century, the breakthroughs of biochemistry were made on the base of quite simplistic concepts of the cell. Only from the 1950s, it became progressively clear that a cell is more than just a 'bag of enzymes'. However, bioengineering still tends to speak about the cells growing in biofermenters as 'biomass'. In his famous Science publication, Paul Srere (1967) compared enzyme concentrations in tissues, and he concluded that the local concentrations found in vivo are orders of magnitudes higher than those used in vitro. This was nothing else than the rehabilitation of space as relevant category also for molecular biology. In consequence, the spatial organisation of molecules and the compartmentalisation of metabolic activity become highly relevant, if one wants to understand and to manipulate biosynthetic pathways. The secondary metabolism of plants with its estimated more than a million specific molecules provides impressive examples for metabolic compartmentalisation. To exploit the biotechnological potential of this biochemical proficiency requires insight into the cell biological aspects. Several contributions in the current issue add new aspects that are relevant, if one wants to tailor the biotechnological use of plant secondary compounds.

A central element of the chemical toolbox for secondary metabolites are the cytochrome P_{450} enzymes. This highly diverse group of proteins has virtually exploded during the evolution of terrestrial plants, giving rise to several hundreds of members in angiosperms. The cytochrome P_{450} proteins are endowed with a high substrate-specificity and often determine the time-limiting step of a pathway, driving oxidative and mostly irreversible reactions such as hydroxylations, epoxidations, dealkylations, dehydrations, or carbon-bond cleavages. The evolutionary background for the wealth of these enzymes has to be seen in the rich and sophisticated interactions entertained by terrestrial plants with other organisms. The review by Rasool and Rozi (2016) in the current issue gives an overlook of the current knowledge on this pacemaker of secondary metabolism focussing on examples with relevance to pharmacological applications such as the terpenoid indole alkaloids. On the examples of traditionally used medical plants such as *Withania somnifera* (Srivastava et al. 2015), they illustrate how advances in genomics, if they are well integrated with cellular and regulatory viewpoints, can propel the biotechnological use of such secondary compounds to a new level.

Many of the pharmaceutically interesting compounds produced by plants are derived from lipids, and the respective pathways are often strongly compartmentalised between different organelles. This involves considerable trafficking of lipids, an aspect that is often overlooked. Lipid transport is anything else than trivial in an aqueous environment. The review by Lung and Chye (2016) in the current issue shifts important players of this transport into the focus: the Acyl-CoA-binding proteins not only act as binding partners for important intermediates of lipid metabolism but also fulfil

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important signalling functions. These proteins have been found meanwhile in most membranes including plasma membranes, ER, Golgi, peroxisomes and nuclear envelope. These diverse patterns of intracellular localisation are linked with a wide range of functions in developmental and stress responses. Acyl-CoA proteins are for instance relevant when membrane surfaces have to be extended, as during the formation of the surface coat in pollen (Murphy 2006). A very new, but metabolically highly relevant, aspect of these proteins is the export of fatty acids from the plastid to the ER, which might proceed without cytosolic intermediates, and thus links two of the central metabolic compartments of plant secondary metabolism.

While these two reviews give a general survey on important molecular players, two original publications describe different stages on the long path towards efficient metabolic engineering:

Triterpenoids from birch, including betulinic acid and oleanolic acid, are pharmaceutically interesting due to their therapeutical potential against tumours and HIV. Birch triterpenoids are traditionally extracted from plant tissue, such as bark or leaf material, but the yields are low and variable, because they depend on environmental conditions, limiting any biotechnological approach. In the attempt to get access to the key enzymes for the pathway, Zhang et al. (2016) in the current issue have cloned squalene synthase (the first committed enzyme of the steroid branch of the basal isoprenoid pathway) and squalene epoxidase (the first committed step of triterpenoid synthesis by epoxidation of squalene into 2,3-oxido squalene). They show that both enzymes are strongly expressed in the leaves of birch seedlings and can be induced by activation of ethylene and jasmonate signalling, whereas abscisic acid can stimulate only the squalene synthase. To get deeper insight into the regulatory patterns of these enzymes that were found to be located in the cytoplasm, the authors cloned out the respective promoters and found, by mapping the cis elements, signatures for the binding of MYB transcription factors. They further could confirm, using recombinant expression in yeast, the squalene synthase and epoxidase activities of the two enzymes. They have, thus, established an interesting toolbox for future metabolic engineering of this pathway.

Using the terpenoid indole alkaloids as target, Sun and Peebles (2016) in the current issue have proceeded further towards metabolic engineering, using hairy-root cultures of *Catharanthus roseus*. The *Vinca* alkaloids produced by this plant belong to the most potent anticancer compounds (reviewed in Dostal and Libusova 2014). Since these compounds are too complex to be synthetised chemically, they still have to be extracted in a cumbersome way from the plants. The cellular and regulatory aspects of this pathway have been analysed in great detail (reviewed in Verma et al. 2012), which opens avenues for sophisticated tailoring. Since the pathway is activated by jasmonic acid, dependent on the transcriptional activator octadecanoid response *Catharanthus* AP2-domain protein, authors have used the strategy to overexpress this regulator under a glucocorticoid inducible promoter. Surprisingly, this did not stimulate the accumulation of the tested alkaloids, although the transcripts of all the synthetic enzymes were upregulated, with one exception. This exception was strictosidine glucosidase, which was downregulated, thus creating a bottleneck that was concluded to be responsible for the negative output of this approach. To overcome this limitation, the authors engineered strictosidine glucosidase under control of the same glucocorticoid inducible promoter. This time, their approach was successful yielding some 50 % of increase in the accumulation of the terpenoid indole alkaloids of interest. This work shows nicely that knowledge on the regulation of metabolic pathways directly translates into a better efficiency of biotechnological exploitation.

Compliance with ethical standards

Conflict of interest The author declares that he has no conflict of interest.

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