3.2 = RESPONSE TO METALS IN THE LIVERWORT *LUNULARIA CRUCIATA* AND IN THE CHAROPHYTE *NITELLA MUCRONATA*

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Our "perception" of the possible role of phytochelatins (PCs), that is metal(loid)-binding thiol peptides, is often erroneously biased by a "here and now" perspective, without considering their evolutionary debut in the distant past. We might in fact ask why the phytochelatin synthase enzyme (PCS), a gamma-glutamylcysteine dipeptidyl transpeptidase (EC 2.3.2.15) (1), is so widespread and constitutively present throughout the plant kingdom and beyond (2, 3). It is in fact not clear, for instance, how the sporadic need to sequester excess cadmium (Cd) or arsenic could have provided the selective pressure for maintaining a so ample PCS expression through a number of organisms (3). Another possibility is the involvement of PCs and/or PCS in essential functions, studies of which are only just getting underway (3). In this context, *Lunularia cruciata* (L.) Dumort. occupies a very basal position in the phylogenetic tree of liverworts (*Hepatophyta*, sin. *Marchantiophyta*), which in turn have been recognized as the basalmost clade of land plants (4). It would therefore seem appropriate to take *L. cruciata* as the starting point for deducing character evolution in the metal(loid) response of early land plants which possibly spread in the terrestrial environment from the Ordovician/Silurian periods onwards (4). Likewise, until now no information has been available regarding the PCS presence in charophytes (*Charophyta*) [i.e.: *Nitella mucronata* (A. Braun) F. Miquel], thought to be *sisters* of land plants (4). Accordingly, the hypotheses verified in this work are that: 1) the ability to synthesize PCs and the occurrence of an active PCS in *L. cruciata* gametophytes were early traits which responded to the need to regulate trace element homeostasis and to minimize the risk of exposure to toxic concentrations of certain metals; indeed, it might be of interest to note that the strongest evolutionary pressure for land colonization by plants came from potential access to much greater amounts of nutritive ions from surface rocks; 2) a PCS enzyme was also present in *N. mucronata*. Accordingly, we have demonstrated here that: 1) *L. cruciata* compartmentalizes Cd in the vacuoles of the phototosynthetic parenchyma, by means of a PC-mediated detoxification strategy, and possesses a constitutive PCS activated by Cd and homeostatic concentrations of iron(II) and zinc; 2) *N. mucronata* shows a western-immunoreactive PCS signal with a molecular mass of approx. 40 kDa; moreover, in 2D-western blot, this putative enzyme shows different immunoreactive signals distributed along an acidic pH range, presumably due to post-translational modifications (i.e. phosphorylations), the role of which is currently being investigated. Overall, the knowledge advancement in the field would make it possible to construct sound-based evolutionary hypotheses.

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