RESEARCH NEWS

TREATMENTS

Figure 1. Ameliorating effect of CaCl₂ on NaCl-induced injury in rice. Treatment of rice seedlings to 200 mM NaCl reduced the seedling growth. Inclusion of varying concentrations of CaCl₂ from 1 to 20 mM had a remarkable effect in mitigating the adverse effect of NaCl (Pareek, A. and Grover, A., unpublished).

reduced expression of ENA1, which is responsible for Na⁺ efflux as stated above. In addition, it was found that the K⁺ transport system of cnb1 mutant cells was not converted to the high affinity state that facilitates better discrimination of K⁺ over Na⁺.

If CAN coordinates gene expression and activity of ion transporters to facilitate ion homoestasis, can overexpression of this protein improve salt tolerance in plants? CAN homolog in yeast is a heterodimer consisting of a catalytic subunit (CAN A or CNA) and a regulatory subunit (CAN B or CNB). The regulatory subunit binds up to four molecules of free Ca²⁺, stimulating the protein phosphatase activity of the CNA. CNB complex that is inactive in the absence of Ca²⁺. Recently, Pardo et al.10 co-expressed catalytic and regulatory subunits of yeast CAN in transgenic tobacco plants and reconstituted a constitutively-activated phosphatase in vivo. Importantly, several different transgenic lines that expressed activated CAN also exhibited substantial NaCl tolerance and this trait was linked to the genetic inheritance of the CAN transgenes. It was further seen in this study that the enhanced capacity of plants expressing CAN to survive NaCl shock was similar when evaluation was conducted on seedlings in tissue culture conditions or plants in hydroponic culture. From the above work, it was concluded that in plants, like in yeast, a Ca²⁺ and calmodulin-dependent CAN pathway regulates determinants of salt tolerance required for stress tolerance.

As mentioned previously,3 tolerance to abiotic stresses is mediated by multiple genes and for pyramiding useful genes it is important to search novel candidate genes. Calcinurin emerges as one such important gene which can be a candidate for pyramiding. More importantly, it has recently been shown that application of regulatory genes (such as transcriptional factor genes) which can influence the whole cascade of genetic alterations is a more rewarding approach for raising abiotic stress tolerant crops.11 Calcinurin is a focal component of the Ca²⁺-dependent signal transduction pathway and recent success with this component may lead to a new dimension of genes for stress tolerance. Further work on calcineurin must address the following points: (i) Is this pathway universal for explaining Na⁺/K⁺/Ca²⁺ interactive responses for diverse crops? (ii) Is there a limit to which Ca²⁺ mitigates Na⁺-induced injury and what metabolically determines this limit? (iii) To what extent do the accompanying anions influence the balance/ration of cations in the cell?

(iv) How do the laboratory conditions of altering Na⁺/K⁺/Ca²⁺ match with the field-conditions where there are multi ions species which are under constant dynamism? (v) Can the results of such experiments be extrapolated to the performance of crops at critical stages like flowering, etc.?


Anil Grover is in the Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi 110 021, India.

The debate on the dawn of multicellular life on earth

A. V. Sankaran

Few episodes in the long march of time on earth have been under such prolonged debate as the bouts of explosion of life during early Cambrian or their extinction during late Cretaceous. In spite of considerable interdisciplinary research to close or narrow down many of the controversial issues concerning them, a consensus has been eluding the scientists. Currently, the controversy is over the earliest appearance of multicellular organisms (metazoans) on earth. Palaeontologists had, for long time, settled down to the view that evolution of life proceeded very slowly during the Precambrian times (4500–570 m.y ago) even though life, as unicellular forms, had begun as early as...
forms, all of a sudden, during early Cambrian, appeared discordant to the basic tenets of Darwinian evolution step-by-step. To them, the metazoans must have been developing gradually all through the Precambrian and the observed gap in fossil record is related to the poor fossilizing potential of the organisms which were soft-bodied and microscopic, and possibly, they were entombed in unfavourable fossilizing conditions. Their proliferation, however, at the start of Cambrian period is ascribed to the advent of favourable external factors—climatic, atmospheric and ecologic over earth, aided by intrinsic genetic changes like gene doubling in a set of critical genes called Hox clusters\(^4\). These, they say, must have led to the development of improved skeletonized taxa having better fossilization potential. As a result, geological strata had greater fossil abundance creating an impression of an abrupt explosion of life.

In spite of the strong belief that metazoan beginnings did not predate Cambrian, three molecular biologists, Gregory A. Wray, Jeffrey S. Levinton and Leo H. Shapiro (State University of New York, Stony Brook, New York\(^5\), hinted couple of years ago that unfolding of multicellular organisms and major phyla must have taken place between 2000 and 1000 m.y. ago, much ahead of the Cambrian period. The trio studied nucleotide and amino acid sequences, which diverge at a regular rate and used the regular cycles of change as a molecular clock to time the point of diversification of life forms. Since this rate is known to vary with time and taxa, they derived a mean rate of sequence of divergence, using seven genes and recorded such genetic changes in different animal groups including very simple forms like yeast and fungi. Their calculations revealed that ‘there was an extended period of divergence during mid-Proterozoic commencing about a billion years ago’ (Figure 1). This conclusion went against the prevailing notions that complex life forms did not exist prior to early Cambrian. Now, F. J. Ayala and others (US Geological Survey\(^6\) have questioned the validity of this mid-Proterozoic dawn of metazoans. While not denying the soundness of the principle behind the use of normally constant gene-change rate to time remote events, they caution that they can

3800 m.y. ago\(^1\), during very early Precambrian (Figure 1). Fossil records from geological strata prior to 565 m.y. ago, i.e. early Cambrian, indicate that life in those times was marked by the enigmatic Ediacaran fauna\(^2\) and microscopic unicellular forms. Rapid evolution to complex multicellular forms is believed to have taken place suddenly during the Cambrian times. Palaeontologists refer to the latter proliferation as ‘Cambrian explosion of life’ or the Cambrian ‘big-bang of animal evolution’. This point in earth’s history represented a significant stage in the evolution of life as the newly evolved organisms carried the basic genetic codes for the development of all major phyla of the invertebrates and vertebrates which appeared in the next few tens of millions of years.

Fossil records in geological formations form the basis to trace the march of evolution of life but often these guides become unavailable owing to hiatuses or gaps that intervene at various geological intervals giving room to speculative interpretations about the possible trends evolution took. For example, a major gap during the Precambrian period has been interpreted by many scientists, as a consequence of slow evolution and an evidence that the main diversification to advanced forms did not take place in the Precambrian but only during the Cambrian. At the same time, to some others, this view about the emergence of multicellular

---

**Figure 1.** Precambrian and Palaeozoic time scale showing multicellular fossil finds prior to the Cambrian explosion of life (big-bang).
yet evolve at disparate rates at different times or in different taxa and hence one can err if this heterogeneity across taxa and or through time is ignored. Wray and others, they say, had evidently neglected these departures in the evolutionary rates and were thus misled to an erroneous date. By adopting special statistical procedure, Ayala et al. systematically eliminated such erratic rates of divergence, and revised the time of radiation of life to 160 m.y. preceding Cambrian, i.e. around 700 m.y. ago (Figure 1).

The DNA studies have modeled the evolution to higher metazoans from simple forms thus: sponges→diploblastic (2 germ layered forms like jellyfish, sea anemone)→triploblastic acelomate forms→triploblastic coelomates having distinct three-layered body plan (arthropods, molluscs, annelids). However, fossils representing these stages predating Cambrian were not reported for a long time and the few claims of discoveries from strata older than 1500 m.y. were discredited as being non-biogenic. Only recently, existence of these metazoans is reported from Precambrian strata from a number of countries—Scotland, Canada, Mongolia, Namibia, Russia, China and India (Figure 1).

Martin D. Brasier and Owen Green (both from Oxford University, UK) reported sponges from southwestern Mongolia, considered to be first evidence for a filter-feeding metazoa a little earlier than the Cambrian big bang from late Precambrian rocks10. Along with D. McLay, Brasier announced another trace fossil evidence (chain of pellets) of a coelomate or pseudocoelomate metazoa in sedimentary rocks in western Scotland which is inferred to be older than the 597 m.y. old igneous rock (339/U/Pb dating) lying above11. Some burrow-like structures, apparently dwelling traces, made by possible diploblastic cnidarian from Mackenzie Mountains in Northwest Canada are dated to belong to 1100–800 m.y. period and similar burrow, made by a vermiform metazoa recorded from Namibia are believed to belong to 900–700 m.y. period12. The Russian occurrences from the late Precambrian described by Mikhail A. Fedonkin (Russian Academy of Sciences) and Benjamin W. Waggner (University of California, Berkley, USA)12 are fossils (Kimberella) which are similar to those reported from Australia during the 1980s. These are supposed to be sea-bottom dwelling triploblastic univalved forms resembling closely molluscs and ranged in size between 3 and 105 mm. Their fossilization had preserved both the hard and soft parts of the body. The Chinese fossils, reported during early 1998, are phosphatized sponges and algae, discovered by two independent groups—Li et al.13 and Shuhai Xiao et al.14 (both of Harvard University, Mass., USA) from the Doushantuo phosphate deposit (Central Guizhou, China). The specimens, ranging in size 150–750 μm, are of early Vendian age (~580 m.y.) and phosphatization had preserved their soft tissues, different types of cells with their cytoplasm and nuclei, and interestingly, several embryos in various stages of blastula development. The fossils of algae reported by Shuhai Xiao and others belonging to neo-Proterozoic time (570 ± 20 m.y.) are also phosphatized specimens. These too reveal all the minute details of the cells, anatomy and reproductive biology. Particularly impressive is the preservation of cell-division pattern confirming that these may be animals more complex than sponges or jellyfish. These fossil occurrences from diverse countries indicate that development of complex anatomy, typical of much younger age animals, had commenced very much before the Cambrian.

The latest discovery in India9 has pushed the metazoa evolution still further, to >1000 m.y., some half-billion years before the Cambrian. The discovery was made by Adolf Seilacher (University of Tübingen, Germany and Yale University, New Haven, USA) along with Pradip Bose (Jadavpur University, Calcutta) and Friedrich Pfüger (University of Tübingen, Germany and Yale University, New Haven, USA) who found 5 mm diameter branching fossilized mini-tunnels in rocks underneath microbial mats which they said were burrows made by worm-like organisms. These were noted in Sone Valley in Central India, within Chhorhat Sandstone, which is a member of the Semri Group of the lower Vindhyan formations (Figures 2a and b). The latter expose an unmetamorphosed sequence of shales and algal limestones, besides this Chhorhat sandstone, which occupies the middle stage (Keinjua Stage) of this Semri Group. These rocks and the upper Vindhyan formations lying above this Group are considered by some to belong to middle to late Proterozoic times (~1400–570 m.y.) and as per the K/Ar radiometric dates quoted by Seilacher et al., the Chhorhat Sandstones are ~1100 ± 40 m.y. old15. Occurrence of fossils in the L. Vindhyan formations is doubted, so far, and the few earlier finds have been dismissed as inorganic impressions, though undoubted unicellular fossils and algae have been noted by some recently. According to the authors, these burrows are distinctly biogenic and differ from non-biogenic syngenesis cracks that sometime form during compaction of sediments as they appear in considerable numbers on top of sandstone beds and are too irregular and sharply delineated to be syneresis cracks or wrinkles, too large to be made by protists (unicellular organisms) or fungal rhizoids (filamentous outgrowths from surface cells). Neither could they be modern artifacts like plant roots or termite burrows, as their architecture do not conform to this class. These tunnels, they argue, were made by peristaltic movements of worm-like organisms scavenging the overlying biomat which served as a food source as well as an oxygen mask. The authors argue that such organisms must be quite advanced having a nervous system to control body movements and digestion of ingested food, all indicative of triploblastic anatomical organization. Martin Brasier (University of Oxford, UK)16, who has considerable knowledge of the global Precambrian–Cambrian successions, is not convinced by views of Seilacher et al. and feels that similar burrows made by unicellular organisms and simple Ediacaran fauna are known and hence need not require triploblastic body organization. Besides, their branching form is too sophisticated a work to be achieved by simple multicellular forms that lived as early as 1100 m.y. ago.

A recent investigation by R. J. Azmi (Wadia Institute of Himalayan Geology, Dehra Dun)17 disputes the age assumed by Seilacher et al. (1100 ± 40 m.y.) for Chhorhat sandstone on the basis of biostratigraphic and biochronologic correlation of Lower Vindhyan formations.
RESEARCH NEWS

(Figure 2). Azmi had undertaken micropalaeontological studies in the Rhotasgarh limestone lying above the Chorhat sandstone and had come across rich, well preserved, typically early Cambrian fauna (small shelly fossils and inarticulate gastropods). These fossils, he says, compare well with the well studied Blains-Krol-Tal (BKT) sequence elsewhere, in the Lesser Himalayas, a recognized standard Terminal Precambrian-Early Cambrian sequence in global correlation\(^6\) and like the BKT, the lower Vindhyan (Semri Group) also starts with a basal conglomerate bed. Azmi strongly feels that the entire Vindhyan basin formed between end Proterozoic and early Palaeozoic and not older as many believe. Brasier also doubts the reliability of the range of K/Ar ages 1200-590 m.y. quoted for the Lower Vindhyan, particularly as these datings were carried out about 40 years back, which, perhaps, may be the reason for the conflict with established biostratigraphic and biochronological classification\(^10/19\).

The identity of Azmi’s fossils has not been accepted by all. S. C. Morris and colleagues Soren Jensen and Nicholas J. Butterfield (Cambridge University, UK) who had the opportunity to examine samples under scanning electron microscope are sure they are not fossils but only inorganic artifacts or mineral growths. Azmi’s identification of these features as fossils was probably created by the texture of the rocks abetted by the oddly-shaped bits produced during acid treatment of the samples while extracting the fossils\(^11/12\). But Azmi is unconvinced and says that these critics have not examined the actual specimens reported in his paper.

In a recent publication, Rajesh Vishwakarma (Banaras Hindu University, Varanasi) has argued\(^23\) that the brachiopod shell fossils in the Semri Group (Vindhyan Supergroup) described by Azmi\(^19\) should be assigned an age of at least 1070 m.y. on the basis of recent radiometric datings. He has referred to the 1080 ± 40 m.y. age (determined in 1977)\(^24\) for the glauconitic sandstone (Semri Group) and also the 1067 ± 31 m.y. (a 1993 Rb/Sr dating)\(^25\) old diamondiferous kimberlite intrusion into the older members of the same Semri Group, which therefore makes this Group and the Chorhat sandstone as older to the age of this kimberlite intrusion (Figure 2 c).

The debate on the beginnings of multicellular organisms is today far from settled, though evidences that are accumulating tend to push this event to times prior to the Cambrian big bang. This brings to the fore, the oft repeated but somehow dismissed question – did life really stop progressing after it commenced 3800 m.y. back? Did the genes known to mutate and evolve at regular rates stop doing so for full 3000 million years till Cambrian? The long held view linking Precambrian fossil gap to soft-bodied and microscopic quality of the organisms can no longer be valid inasmuch as fossils preserving excellently, not only minute details of cells of very small organisms, but also soft internal organs of such large animals as dinosaurs are increasingly being reported. Perhaps, intensive search for fossils within early Precambrian, notwithstanding the paucity of such terrain remaining unaffected by destructive geological processes, may be

---

Figure 2. a, Map showing the 1100 m.y. old trace fossil find in Sone Valley, Central India and Vindhyan succession; b, Tunnels in Chorhat sandstone (Lower Vindhyan), Sone Valley, made by worm-like metazoan (taken from ref. 16); c, discrepancy between biostratigraphic and geochronologic correlation of the lower Vindhyan.
able to narrow, if not close, the 3 billion year gap between life-beginnings and their diversification. At the same time, in the light of present revision of some of the Doushantuo fossils as embryos, earlier dismissed as green algae, it may be worthwhile to reexamine many of the previous findings rejected as non-biogenic.

The widely discussed Central Indian discovery by Seilacher et al. has raised two important issues – firstly, the age of the Chorhat sandstone wherein the burrows were found and secondly whether these are indeed biogenic and if so whether they are the work of triploblastic metazoa or simple unicellular forms. The answers to these questions are necessary to push back the dawn of multicellular life some 500 million years predating Cambrian and for a proper assessment of the existing views about Vindhyan correlation.

Finally, fixing age of a sedimentary horizon based on biostratigraphy and biochronology has pitfalls if the possibilities of fossils (or clasts containing fossils) that may be reworked from older to younger sediments are not properly recognized, in the same way as the likelihood of older grains that might have mixed up during Chorhat sedimentation giving these rocks a much older age, as some skeptics have pointed out. In this connection, the age of the kimberlite igneous intrusion (1057 ± 31 m.y.) into the lower Vindhyans are more reliable pointers that the Semri Group rocks may be indeed >1000 m.y. old, though the presence of this intrusive in the overlying younger Kaimur beds (dated to be around ~890–725 m.y.) poses a geological anomaly, which presently is viewed as a unique selective erosional consequence. Unless supported by unambiguous geochronological data, attempts to correlate sedimentary beds on basis of similarity of fossils or rock sequences may be hazardous. Future dating attempts may have to be carried out on samples undoubtedly contemporaneous with the sedimentation and by methods, which are not debatable.


A. V. Sankaran lives at 10, P & T Colony, J Cross, R. T. Nagar, Bangalore 560 032, India.

SCIENTIFIC CORRESPONDENCE

Can free fatty acids in the tiger pheromone act as an individual finger print?

Theoretically, the scent marking of animals must encode information on individuality. The animals can and do distinguish such individuality but the molecular basis thereof is ill-understood. Only in the study by Gorman on mongoose, was a clear cut molecular basis detected in their pheromonal secretion, namely, unique ratios of carboxylic acids characterizing each individual mongoose.

The data presented here suggest a possible molecular basis in the proportions of free fatty acids (FFA) in the marking fluid (MF), the most important source of pheromones in tigers, which is a lipid-rich, smelly fluid ejected upwards and backwards through the urinary channel.

Eleven FFA in tiger MF, from acetic to octanoic acid, have been quantitatively estimated with the help of an internal standard (crotonic acid) under isothermal conditions. The values for acetic and octanoic acid are shown in Table 1. GCMS on a 60 m long DB WAX capillary column (temperature 50°C–200°C programmed at 50°C/min)