A novel cross-linking technique to study nuclear lamina–membrane interactions

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Protein cross-linking studies using various bifunctional reagents can provide information about the organization of complex proteinaceous structures. We have studied the association of the nuclear lamina with membrane proteins using bifunctional cross-linkers of the bis-(imidoester) class. Analysis of cross-linked products by two-dimensional diagonal gel electrophoresis demonstrates that the lamins are closely associated with three nuclear membrane proteins (54, 50 and 45 kDa) in intact nuclear envelopes. This interaction does not occur when the envelope organization is disrupted with detergent or urea.

THE inner surface of the nuclear envelope in a eukaryotic cell is closely associated with a filamentous network or lamina, composed of intermediate filament-like proteins called the lamins[1–4]. The lamins may be classified into two major types: the A-type, which get solubilized during mitosis[5,6], and represented by lamins A and C in most cells; and the relatively insoluble B-type lamin. Lamin B is strongly associated with the nuclear membrane[7] and remains bound to membrane vesicles during nuclear-envelope disassembly at mitosis and subsequent reformation of the envelope[5]. The biochemical basis of interaction of the lamina with the inner nuclear membrane is not clearly understood. Recent studies with purified lamin B and lamin-depleted nuclear membranes suggest that lamin B is anchored to the membrane via a 58-kDa receptor protein in avian and yeast cells[8–10].

In the present study, we have adopted a direct approach to look at the interactions of the lamina with nuclear membrane proteins in mammalian cells by chemical cross-linking studies with purified nuclear envelopes using bifunctional imidoesters and analysis of cross-linked products by two-dimensional diagonal gel electrophoresis.

Nuclear envelopes were isolated from purified mouse liver nuclei by Kaufmann's procedure[11], and characterized in detail by biochemical and morphological criteria as described earlier[12]. Nuclear envelopes were obtained as intact, double-membrane vesicles, similar in size to nuclei and devoid of intranuclear and cytoplasmic contaminants. Envelopes were fractionated with (i) 8 M urea, or (ii) 2% Triton X-100 and low or high concentrations of salt (20 mM or 300 mM KCl) or 4 M urea by published methods[8,13,14].

In a typical cross-linking reaction, intact or extracted nuclear envelopes (~100 μg protein) were incubated with 5 mM dimethyl suberimidate (DMS; Pierce Chemicals, USA) for 30 min at 30°C in 100 μl of 100 mM triethanolamine·HCl, pH 8.0 (refs. 15–17). The reaction was quenched with excess glycine and the samples separated by SDS–polyacrylamide gel electrophoresis (8% polyacrylamide gels)[18]. These conditions were found to be suitable after standardization with respect to concentration of cross-linking reagent (0.5–20 mM) and time (15 min–24 h). Under these conditions, up to 10% of cross-linked higher-molecular-mass species were detectable for a known tetrameric protein, alcohol dehydrogenase, the remainder being mostly products of addition of several molecules of cross-linker to monomers of the protein. Experiments were also carried out with dimethyl pimelimidate (DMP) and dimethyl adipimidate (DMA) (which form shorter bridges) under similar conditions. Samples of cross-linked proteins (in triplicate) were separated by SDS–polyacrylamide gel electrophoresis. One lane was stained with Coomassie blue and the second lane was treated with methylamine exactly as described[16]. The treated lane and the third, untreated lane were separated in the second dimension by SDS–polyacrylamide gel electrophoresis (8% polyacrylamide gels). The two-dimensional gels were stained by the more sensitive

silver-staining technique[19], which was applied subsequent to or as an alternative to staining with Coomassie blue.

Cross-linking of highly purified, intact nuclear envelopes with DMS gives rise to extensive cross-links, as indicated by the high-molecular-mass (~200 kDa) material seen in the first-dimension gel in Figure 1, *a*. Treatment of the first-dimension gel with methylamine followed by electrophoresis in the second dimension (Figure 1, *b*) indicate the presence of proteins that were originally cross-linked as spots off the diagonal, and give a major set of spots in the range 70–67, 62, 54, 50 and 45 kDa. A second set of spots is also seen at 70–67 and 62 kDa. The spots at 70–67 and 62 kDa migrate to positions identical to those of lamins A, B and C respectively in the first-dimension gel. Slight cross-linking between the 54 and 45 kDa proteins is also observed but it is not significant enough to suggest that these proteins are associated with each other. Although some spots are also observed just below the diagonal, their molecular masses do not indicate that they were cross-linked initially and so they may have arisen through methylamine cleavage of the non-cross-linked proteins in the first-dimension gel, which is known to occur[16]. Visible cross-linking with DMA or DMP was not observed with intact envelopes.

To confirm that the 70–67- and 62-kDa spots are the nuclear lamins, envelopes were extracted with 8 M urea to preferentially solubilize the lamins, leaving membranous material in the high-speed pellet[8]. Cross-linking of proteins in the 8-M-urea supernatant with DMS (or DMA or DMP) gave a faint set of spots in the range 70–67 and 62 kDa, corresponding to the lamins, and did not give spots at 54, 50 and 45 kDa (see Figure 2). This indicates that the interaction between the lamins and the 54-, 50- and 45-kDa proteins is lost upon urea extraction of envelopes, and suggests that the 54-, 50- and 45-kDa proteins may be integral membrane proteins. Their membrane location was confirmed as follows.

When envelopes were extracted with Triton/low-salt or Triton/high-salt buffers to solubilize membrane proteins, and the supernatants subjected to cross-linking with DMS, no significant cross-linking was discernible (data not shown). Extraction of envelopes with Triton/urea buffer (which also partially disrupts the lamina) followed by cross-linking of proteins in the supernatant, gave cross-linked spots at 70–67 and 62 kDa (Figure 3). However, no spots were seen at 54, 50 and 45 kDa, suggesting that the interaction of the lamins with integral membrane proteins seen in intact envelopes is lost when the envelope architecture is disrupted with detergent.

Our data demonstrate that (i) the nuclear lamins are closely associated with three membrane proteins (54, 50 and 45 kDa) in intact nuclear envelopes; (ii) this association does not occur when the envelope organization is disrupted, either by solubilizing the membrane with detergent or by extracting the lamina with 8 M urea. The requirement for a definite length of cross-linker bridge, viz. 11 Å for DMS, and the absence of visible cross-linking of intact nuclear envelopes with reagents forming shorter bridges (8.6 Å for DMA and 9.2 Å for DMP) are further evidence for a specific interaction between the lamins and their membrane-binding sites. Our observations on the cross-linking of the lamins to each other is consistent with the available data on the strong interactions between the different lamins. Recent reports have suggested that lamin B in avian and yeast cells is anchored to the membrane via a

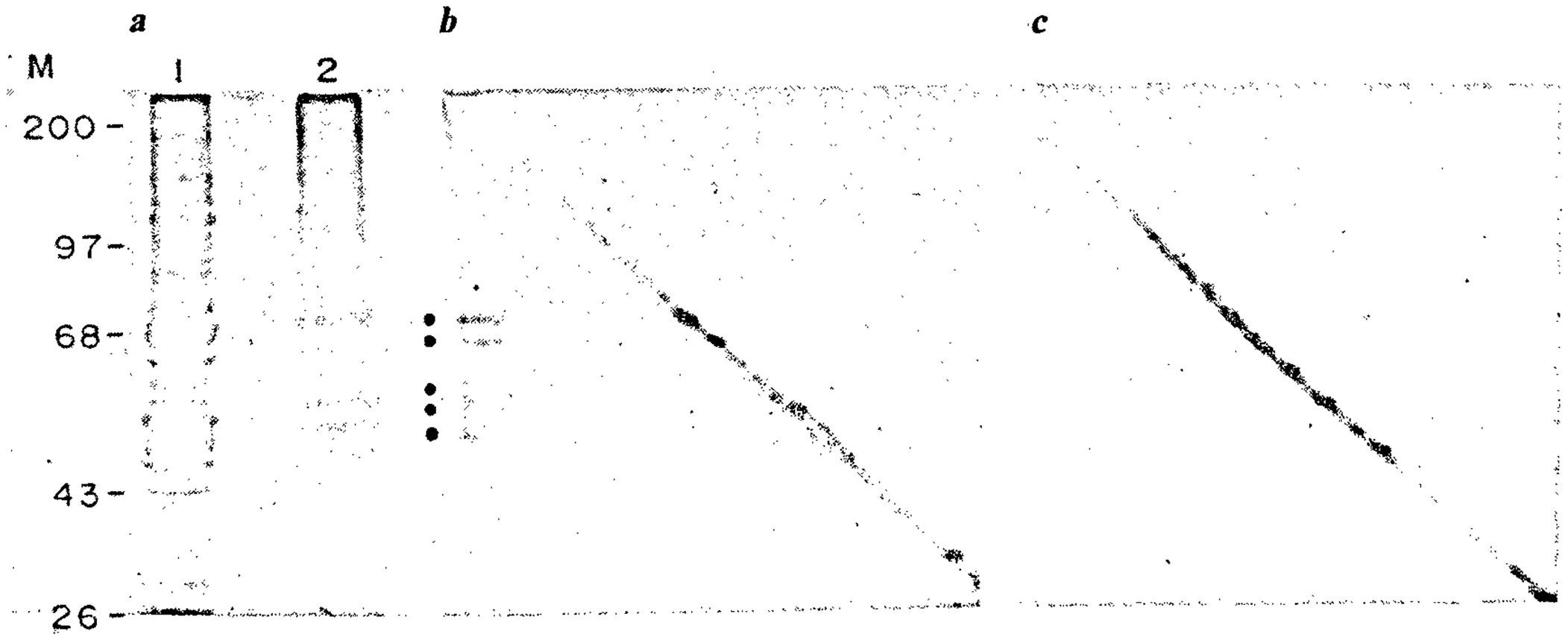

Figure 1. Cross-linking of intact nuclear envelopes with DMS. *a*, First-dimension gel: lane 1, intact envelope proteins; lane 2, cross-linked envelope proteins. *b*, Second-dimension gel, after methylamine treatment. Bold dots indicate cross-linked polypeptides. *c*, Untreated second-dimension gel; molecular mass markers (M) are myosin (200 kDa), phosphorylase *b*, (97 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa), α-chymotrypsinogen (26 kDa).

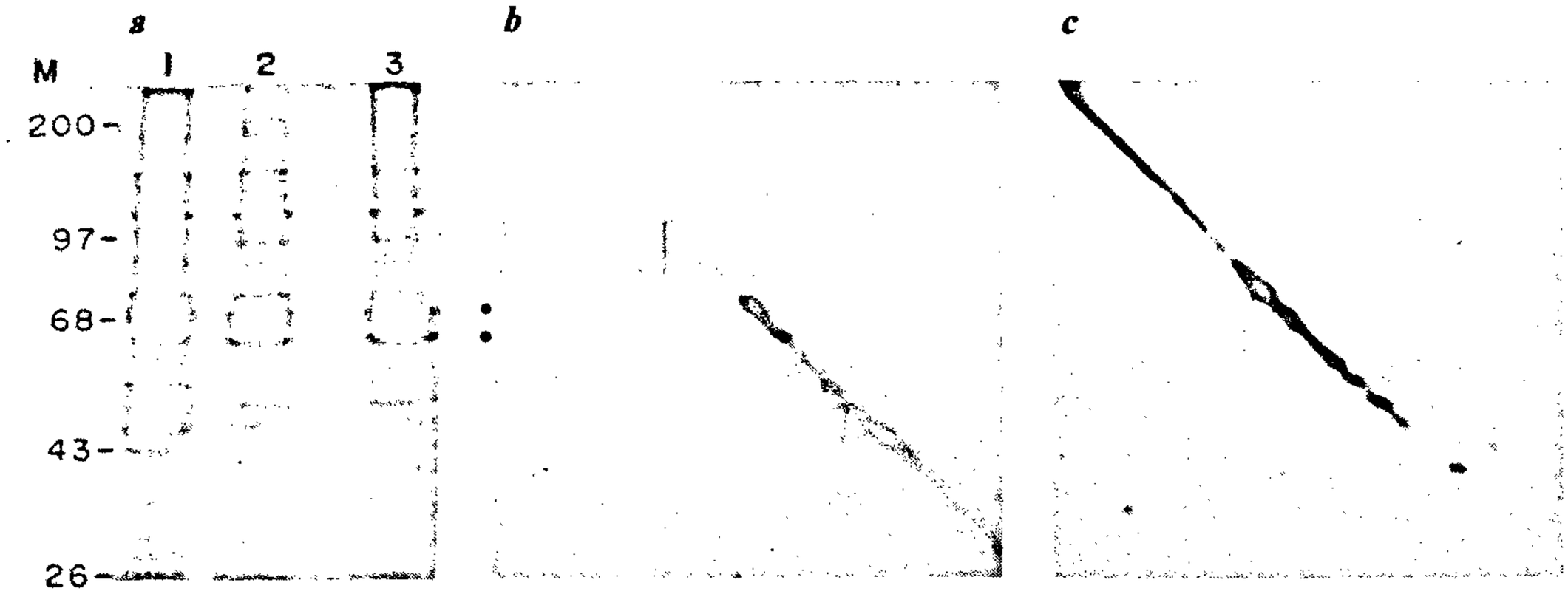

Figure 2. Cross-linking of proteins solubilized with 8 M urea. ***a***, First-dimension gel: lane 1, intact envelope proteins; lane 2, supernatant after 8 M urea extraction of envelopes; lane 3, cross-linked proteins from urea supernatant. ***b***, Second-dimension gel, after methylamine treatment. **c**, Untreated second-dimension gel; molecular mass markers are as in legend to Figure 1.

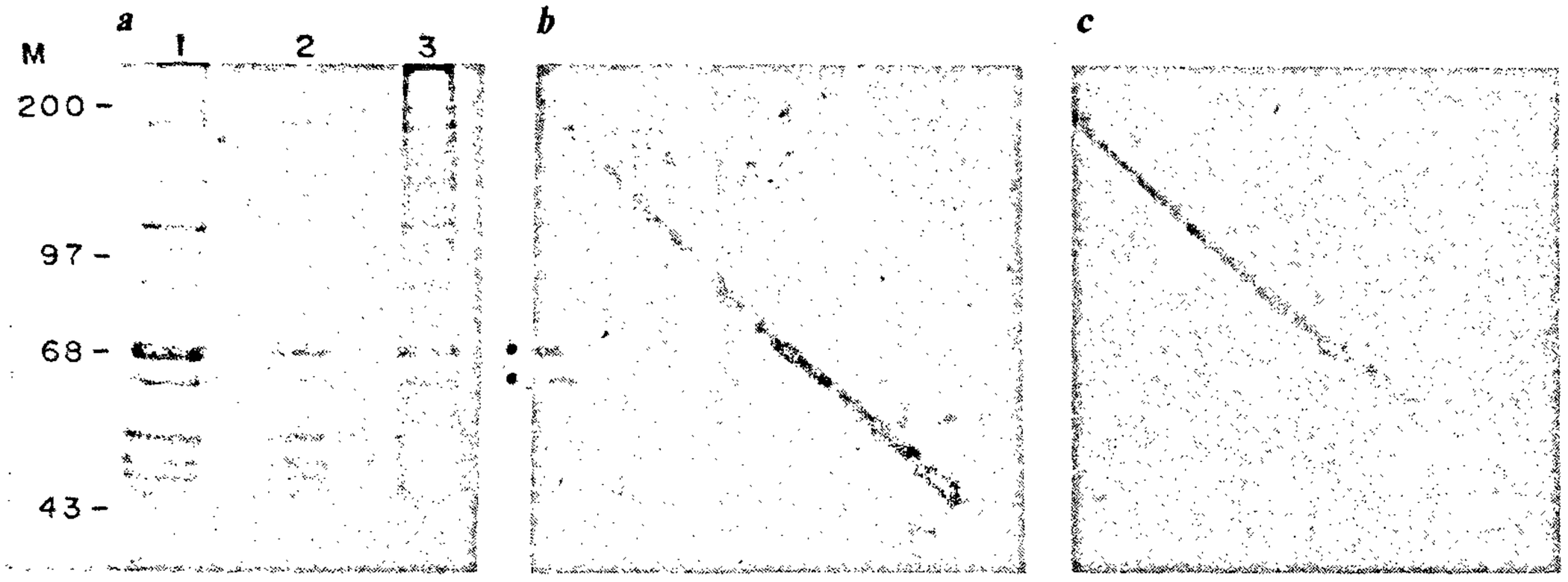

Figure 3. Cross-linking of proteins solubilized with Triton/urea buffer. ***a***, First-dimension gel: lane 1, intact envelope proteins; lane 2, supernatant after Triton/urea extraction of envelopes (contains half the amount of protein in lane 3); lane 3, cross-linked proteins from Triton/urea supernatant. ***b***, Second-dimension gel, after methylamine treatment. **c**, Untreated second-dimension gel; molecular mass markers are as in legend to Figure 1.

58-kDa membrane receptor protein[8,9]. Our preliminary evidence on binding assays with labelled lamin B and nuclear envelopes from mouse liver nuclei suggests that the molecular mass of the lamin B receptor in the mouse system is 54 kDa (S. Pandey and V. K. Parnaik, unpublished results), and this may be similar to the 54-kDa membrane-binding protein that we have observed in this study. Further studies on these proteins and their exact sites of attachment to the different lamins should be feasible once purified proteins are available.

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A timely collection

Plants for Reclamation of Wastelands. Publications and Information Directorate, CSIR, New Delhi. Price: Rs 325, $110, £65. 1990. 684 pp.

Given the impending threat of global warming, rising sea level, advancing desertification and burgeoning population, this publication on greening of 175 million hectares of India's wastelands is most timely. The book is divided into three parts. The first, covering 31 pages, deals with generalities like geography, climates, soils, definitions and estimates of wastelands. It is the second part, from page 32 to page 524, that forms the bulk of the book. After an introduction to green cover are discussed the roles of plants in controlling erosion, in nitrogen fixation, and as source of energy. Then follows a list of nearly 1000 species (in alphabetical order) that have contributed to wasteland reclamation in one form or another. Sufficient information is compiled for each species under several heads like vernacular names, morphology, habitat, distribution, silvicultural characters, cultivation/regeneration and utilization; the latter is further subdivided into reclamation value of the plant, and timber, fuel, fodder and other types of utility like medicinal or food value. One may be in for some surprises because included in the list is teak, the king of timber species, which one would not as a rule associate with wastelands. Other examples are *Bischofia javanica* and the bamboo *Bambusa arundinacea* of moist habitats. However, their use in the context of reclamation is properly explained. Genera like *Acacia* and *Eucalyptus*, which have made a mark in India's plantation programmes, receive a good deal of attention; besides the common ones included in the list, the lesser known species are also treated in tables with relevant facts. Part III is a short chapter of seven pages dealing with derelict mined areas. Whereas the bibliography is not exhaustive, there are a number of useful appendices enumerating species according to their adaptations to different types of wastelands and according to their economic value, such as fuel, fodder, green manure, etc. Indices have been provided for Latin, common English, vernacular, regional and trade names, which fact, combined with the size of the book, makes the publication worthy of its price.

The following printing error may be the sole addition to the errata list: page 48, line 22, read 'heave' for 'have'. References to the sources of some of the tables are not complete. Data in tables 2 and 4 reveal a gradual increase in forest cover parallel to a decline in barren and unculturable land from 1950–51 to 1982–83; these are contrary to the known trends. As proof of this, tables 5 and 11 contradict the figures of forest percentages given in tables 2 and 4. On page 13 there is a reference to Kudremukh mining area 'where lush green forests existed not long ago'. As a matter of fact, this iron-rich area was never under forest. The wooded tract to suffer damage in the wake of the mining project is the Bhagwati valley.

On page 17, the classification of coastal wetlands (mangroves) and grasslands–rangelands under 'degraded land with special problems' may not be universally acceptable. The valuable contribution of the mangroves in protecting the coastline and that of grasslands in preventing erosion need not be questioned. Saline-wetland vegetation has been receiving worldwide attention for the part it will have to play in gene-transfer biotechnology especially with the menace of greenhouse effect and submergence of islands and continental shoreline looming large on the horizon. The salt marshes are also significant sinks of carbon dioxide.

On page 34, the temperature-reducing property of mulching also needs to be stated, particularly of coconut coir dust. On pages 44–45, discussion on AVM is conspicuous by its absence. *Copaifera langsdorfii* should find a mention among petro-crops. On page 71, the fact that *Acacia holosericea* has given excellent results in Pondicherry region has not been recorded. On page 92, the lower limit of 25 mm for *Albizia amara* is erroneous. Rainfall range of *Dichrostachys cinerea* (page 215) may also be revised from 25–400 mm to 250–1300 mm. On page 252, relationship between *Eucalyptus* and soil moisture has been discussed but much of it remains to be verfied by serious experimental evidence.

Legends of photographs are too brief and do not mention locality. The plate facing page 5, of two *Acacia* spp., seems to show only one species. *Acacia holosericea* with its characteristic silvery foliage (phyllodes) cannot be made out. There is a mix-up in *Euphorbia* spp. also: no resemblance is noted between *Euphorbia royleana* on the plate facing page 189 and the one on page 279; the former (also given on the back cover) looks more like a species of *Cercus* (Cactaceae) than of Euphorbiaceae. Also *E. nivulia* on page 271 and the one given in the plate between pages 188 and 189 differ considerably, the latter being the authentic one. *E. neriifolia* on page 270 would be *E. caducifolia*.

It is hoped that a second edition, which seems likely given the usefulness of the publication to all those engaged in the conquest of wastelands, will take care of these minor errors.

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Precambrian assemblage

Archaean Greenstone Belts of South India. B. P. Radhakrishna and M. Ramakrishnan, eds. (GSI Memoir 19) Geological Society of India, Bangalore. 1990. 490 pp.

This volume is among a series of outstanding memoirs of the Geological Society of India brought out through the dedicated efforts of the editors on the occasion of the birth centenary of B. Rama Rao, who made fundamental contributions in the Precambrian geology of Karnataka craton in South India. The memoir, subtitled Bellur Rama Rao Volume, begins with a tribute to him by the senior editor. Then follows a five-page introduction to the 32 selected papers by early pioneers