Associations, mergers and acquisitions in the biological world

Ramesh Maheshwari

According to a bold and imaginative theory, cells of all plants, animals and fungi are a combination of two or more totally different microbial genomes. This theory has now been extended to understand evolutionary processes in eukaryotes: that is, speciation in eukaryotes has occurred (and continues) not only by accumulation of random mutations, but also by repeated physical merging of microbial cells in some specific order, and acquisition of different microbial genomes. The genomic sequence of organisms have provided evidence of alliances, mergers and acquisition of genomes between different individual species to form new species. This theory challenges biologists to recreate a eukaryotic cell in the laboratory from forced mergers of earliest extant microbes.

Keywords: Bacteria, eukaryotic cell, horizontal gene transfer, protists, symbiosis.

THE geneticist Theodosius Dobzhansky (1900–1975), acknowledged as 'the greatest evolutionary geneticist of our times' had remarked: 'Nothing in biology makes sense except in the light of evolution' (http://www.2think.org/dobzhansky.shtml). An upshot of this is that the vast DNA sequence data being obtained with increasing speed are being summarized in the form of phylogenetic tree diagrams linking various organisms based on resemblances in their order of descent from a common ancestor. Therefore the assertions that 'The family trees of life showing diverging branches' are wrong or that 'Bacteria lack species' are surprising. Lynn Margulis, the first author of the book (Figure 1) from which these statements are taken, is a distinguished university professor, a member of the US National Academy of Science, the Russian Academy of Natural Science, and a recipient of the National Medal of Science from the US President Bill Clinton in 2000. E. O. Wilson called Margulis 'One of the most successful synthetic thinkers in modern biology'. This article is a critique of a few of her ideas and thoughts.

Cells within cells

Margulis developed the 'endosymbiotic theory of eukaryotic cell' based on microscopic studies of single-celled, microscopic, nucleated eukaryotes (protists), their developmental life histories, and their morphological, cell biological and ecological relationships[1]. According to this theory at least three organelles – the mitochondria, chloroplast and cilia – originated through mergers of different kinds of bacteria by endocytosis (a capacity apparently evolved to take in solid food). Mitochondria evolved from purple bacteria, chloroplasts from cyanobacteria, and cilia originated from an earliest eukaryote that formed a centrosome – an organelle that organizes the cytoskeleton for intracellular movements of chromosome, vacuole transport, fertilization, phagocytosis, etc. Mitochondria and chloroplasts – the acquired microbial cells – have their own DNA and multiply in the cytoplasm. The symbiotic mitochondria help in generating energy; and when the partners also include chloroplasts, these not only help in food utilization but also food production by photosynthesis. Over evolutionary time, the organelles transferred most of their genes[2–4], to the nucleus and reimported the nucleus-encoded proteins made in the cytoplasm for service in the mitochondrion or

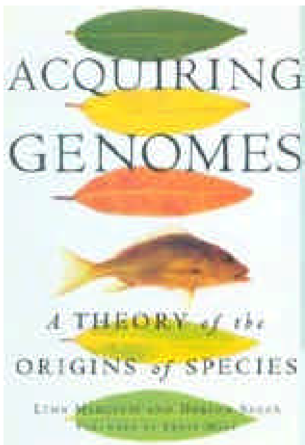

Figure 1. Jacket figure of a book that presents a radical view that species can transmute into a novel form by acquiring genomes in evolutionary time and revert to their original form by loss of acquired genes.

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the chloroplast. As a result, the bacterial endosymbionts – once-upon-a-time free living – have lost their ability to multiply outside of their host. In other words, the free-living bacteria now residing as endosymbionts cannot be cultured on artificial media *in vitro*. This is an example of mutual obligate dependency.

Serial endosymbiotic theory

The radical idea of symbiosis in cell evolution[1] has now been accepted and extended to speciation occurring through a specific sequence of mergers[5–9]. Margulis and Sagan[10] called this, the 'serial endosymbiotic theory (SET)'. Remarkably, the theory was developed based on microscopic observations of live and fixed material, independent of molecular data. Since the formulation of the endosymbiotic theory, sequences of mitochondria and chloroplast DNA from several organisms have been found to be strikingly similar to those in bacteria but distinct from nuclear DNA, confirming that all the higher forms of life are composites, i.e. they have descended through associations, alliances and mergers. Margulis has recently elaborated this theory in a book co-authored with her son, Dorion Sagan. The jacket figure of this book – depicting a plant leaf being transformed into a fish that reverts to the original form – pictorially conveys the message that new species originate by acquisition of a whole set of genes and may revert to their former form by loss of the acquired genome. According to Margulis[11], 'These ideas were rejected as obsolete nonsense by the scientific worldview', and 'My earliest complete statement of "serial endosymbiosis theory" was published after fifteen or so assorted rejections … the idea that new species arise from symbiotic mergers among members of old ones is still not even discussed in polite scientific society'.

Creative power of symbiosis

Does association between organisms result in mutual benefit or is the association lopsided? Is it chance or is it necessity that results in symbiosis? A few examples are selected that substantiate the creative power of associations in evolutionary change.

Animal symbiosis with alga (sea slug)

Recently, the prestigious plant journal *Plant Physiology* had a cover photograph of utter disbelief: A green leaf with a slug-like head with the footer 'Plant or animal' (Figure 2). When larvae of *Elysia chlorotica* suck the contents of an alga *Vaucheria*, the chloroplasts are incorporated into their reticulate gut beneath the translucent epithelium where they multiply[12,13], turning the host animals dark green. The mouths become superfluous as the green animals never eat throughout their life of about nine months; they just sunbathe because they have acquired the ability to photosynthesize! Southern blot analysis using heterologous probes indicated the presence of genes for molecules of photosystems that generate energy (ATP) and the major electron donor in biosynthesis (NADPH), and the small subunit of Rubisco – the carbon dioxide fixing enzyme in all photoautotrophic organisms[14]. The green, solar-powered organism grows and crawls along the walls of a laboratory aquarium containing artificial sea water, with overhead lighting and CO_2. Formerly a heterotroph, the slug had become transformed into a photoautotroph. Photoautotrophic slugs cultured in salt water aquaria have the same lifespan as the feeding slugs in their native marsh environment[12]. Other such examples are known (http://www.rzuser.uni-heidelberg.de/~bu6/Introduction04.html).

Although there is no consensus on the definition of a species, according to Margulis and Sagan[10], if organisms A and B are composed of 'the same set of integrated genomes, both qualitatively and quantitatively', they belong to the same species. Is the green 'plant or animal' a new species? And if so, what would be a proper scientific name for the slug – now turned green as a result of acquiring chloroplasts (with their distinctive DNA) from an alga? And what of the bacteria that, unlike the eukaryotes, are not formed by integration of genomes? According to the authors, 'Since bacteria are not formed by integration of symbionts', i.e. they possess only one genome, therefore, 'they lack species'.

Fungus symbiosis with alga (lichen)

Some twenty-five thousand species of lichens are known. These are commonly observed growing on the barks of trees and rocks. Varied opinions were, and still are, being held with regard to their nature and position in the classification of living organisms[15]. Lichen is an evolutionary novelty

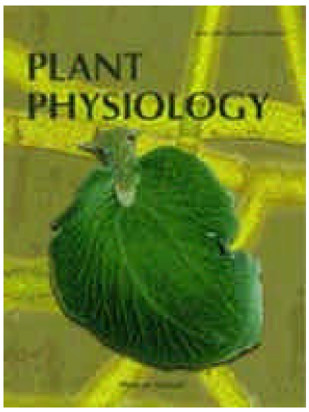

Figure 2. A plant or an animal formed by acquisition of chloroplasts from a green alga by a sea slug having the ability of locomotion as well as photosynthesis. (Reproduced with permission from American Society of Plant Biologists.)

created by the merger of cyanobacteria (some call them photosynthetic blue-green alga, a name that Margulis does not like) and non-photosynthetic, heterotrophic fungi. The credit of establishing a combination of two dissimilar organisms living together as a single entity is due to a Swiss botanist, S. Schwendener who said, 'As the result of my researches, the lichens are not simple plants, not individuals in the ordinary sense of the word; they are, rather, colonies, which consist of hundreds of thousands of individuals, of which, however one alone plays the master, while the rest in perpetual captivity prepare the nutriment for themselves and the master. This master is a fungus of the class *Ascomycetes*, a parasite which is accustomed to live upon other's work. Its slaves are green algae, which it has sought out, or indeed caught hold of, and compelled into its service. It surrounds them, as a spider its prey, with a fibrous net of narrow meshes, which is gradually converted into an impenetrable covering; but while the spider sucks its prey and leaves it dead, the fungus incites the algae found in its net to more rapid activity, even to more vigorous increase…'. So successful has this evolutionary novelty been that lichens are found in the harshest of environments where neither the alga nor the fungus can exist alone. A bushy kind called the 'reindeer moss', forms ankle-deep carpets and provides the main food for reindeer during winter. Lichens can also tolerate heat that would desiccate and kill most plants; and are therefore found growing even on rocks exposed to sun. In the last century, experimental proof of the origin of a new form was provided by dissociating the dual members of lichen, and synthesizing a lichen thallus by bringing together phylogenetically distinct organisms[16]. The example of the lichen helps appreciate the meaning of symbiogenesis and its evolutionary implications. Symbiogenesis is merger, long-term biological fusion of organisms that are different from each other to make an entirely new morphological product – new tissues, new organs, or new physiologies. It might be speculated that the merger is made permanent by gene transfer between the alga and the fungus – the alga providing nutrients and the fungus with water and minerals and protection from intense light and tolerance to desiccation.

Plant symbiosis with fungi (mycorrhiza)

The main body of mushrooms, the agarics, of the woody bracket fungi is mycelium which is subterranean and hence hidden. The mycelium is entwined with plant roots like a sleeve or penetrates into the cortical cell and this association was the causative factor in the colonization of land by plants[17]. The oldest plant fossils have spores similar to present-day mycorrhizal fungi, showing that symbiosis between plant roots and fungi contributed to their colonization of land, by providing plants the strength to tolerate desiccation, heat and most of all the ability to obtain nutrients through their connection to the hyphae which extends to large areas, scavenging phosphorus and nitrogen and supplying these to the plant roots to which they are connected. There are non-green higher plants such as the Indian pipe, scientifically called *Monotropa* (http://images.google.co.in/images?q=monotropa&hl=en&btnG=Search+Images) which grow in deep shade and have evolved only because their underground part is connected to the photosynthetic trees through symbiotic fungal hyphae which serve as conduits for transfer of photosynthate to non-photosynthetic plants. The mycorrhizal fungal symbionts themselves harbour obligately intracellular bacteria-like organisms[18].

Animal symbiosis with bacteria (tube worms)

Tubeworms thrive some 3–5 km deep in the sea, where mineral-enriched water with temperature of 350°C or more exits from chimney-like structures from the ocean floor and mixes with cold water (Figure 3). The animals lack a digestive system and their nutritional needs are met by their bacterial chemoautotrophic symbionts contained in a morphologically complex symbiont-housing organ called the trophosome. The current hypothesis suggests that symbionts enter through the larval mouth and digestive system and that the subsequent proliferation of endodermal midgut cells leads to the development of the trophosome – a tissue formed by acquisition of sulphur-oxidizing bacteria.

Animal symbiosis with protists (herbivores)

Cows, sheeps and deer ingest grass, but they are incapable of cellulose breakdown. Digestion in cows, for example, is by microbial symbionts in the rumen (a 70 l fermentation tank on four legs) comprising swimming cells, ciliate protists and many bacteria, which are acquired soon after birth[19]. No herbivore that lacks rumen, and is consequently deprived of microbial symbionts, will be grass-eating: hence such animals do not exist, and their evolution must be considered intimately tied to symbiosis. By the same argument, human beings too evolved and exist because of symbiotic association of enteric bacteria which synthesize and provide essential B vitamins.

Insect symbiosis with protists (aphids and termites)

Symbiotic relationships are particularly abundant in invertebrates. All species of plant phloem sap-feeding aphids harbour endosymbiotic bacteria. These endosymbionts have among the smallest genomes discovered[4]. DNA sequencing revealed total loss of genes for cell envelope, nucleotides and lipids. Some of the genes may have been transferred to the nuclear genome of the host (aphid) cell, cementing endosymbiotic relationship and species evolution. Wood-

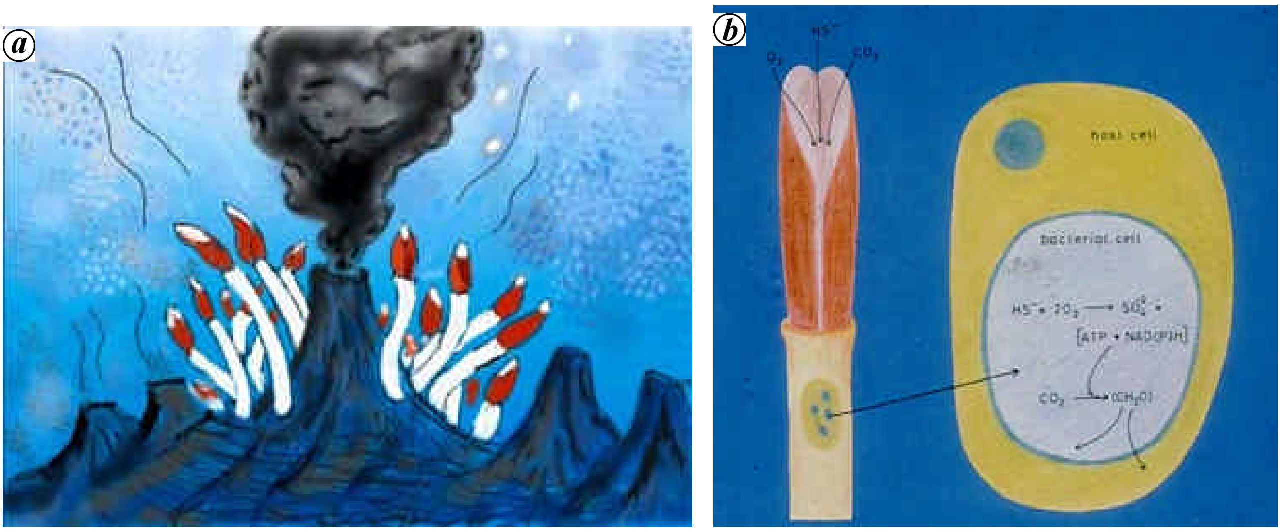

Figure 3. ***a***, Deep-sea vent tube worms, *Riftia pachyptila*, growing in the vicinity of deep-sea hydrothermal vent. ***b***, Self-supporting intracellular metabolic reaction in tube-worms under sulphur-rich, oxygen deficient conditions by endosymbiotic sulphur-oxidizing bacteria.

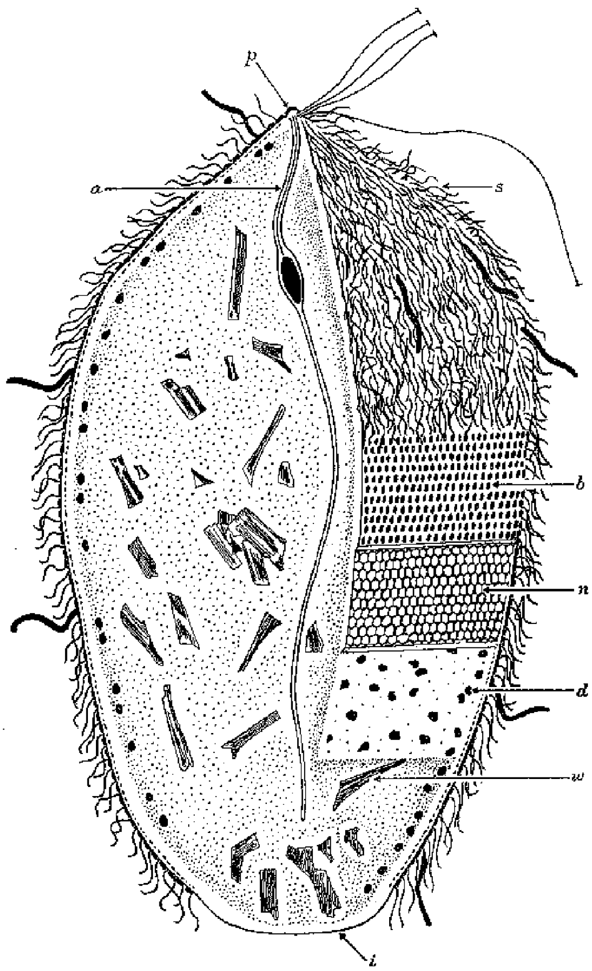

Figure 4. *Mixotricha paradoxa*, a large 500 μm × 250 μm single-celled protozoan found in the gut of termites. It is propelled not by its own cilia but by the undulations of flagella of spirochaetes that are attached to it. a, Axostyle; b, Bacterium; d, Dictyosome; i, Ingestive zone; n, Fibrous network; p, Papilla; s, Spirochaete; w, Injested wood. (Reproduced from Cleveland and Grimstone, 1964 with permission from Royal Society, London.)

eating termites are known as white ants which build tall mounds of mud housing their colonies (http://people.ee.ethz.ch/~kunzy/termites.htm). All termites eat cellulose as plant fibre, but lack cellulase to digest this energy source, depending on symbiotic protozoa and other microbes in their gut to digest the cellulose and absorb the end-products for their own use. The gut protozoa in turn rely on symbiotic bacteria that produce the necessary digestive enzymes. A protozoan *Mixotricha paradoxa* (Figure 4) found in the gut of termites is propelled not by its cilia but by the undulations of its attached spirochaetes. Microscopic (light and electron microscopy) or molecular techniques (polymerase chain reaction-amplified 16S rRNA gene sequences) or combinations thereof allow estimation of the number of intracellular genomes present in separate bacteria. *M. paradoxa* in termite gut has at least three other distinct secondary endosymbionts. According to Margulis[1,11,20], one integrated genome was sufficient in the merger of archaea and eubacterium, two in the proctista (a term used by the authors for classifying all eukaryotic organisms with the exception of animals, plants and fungi; for example, all algae, slime moulds, amoebas, water moulds and foraminifera), three in fungi, at least four in animals and five or perhaps seven in the plant kingdom – 'the epitome of symbiogenesis'. According to some researchers, because of large-scale gene transfer or gene loss, the future of endosymbiotic relationship is gloomy[2].

Importance of symbiosis with bacteria

Evolutionary diversity, according to Margulis, has come mainly from acquisition of different bacteria. The entire animal kingdom employs essentially one mode of metabolism: heterotrophy (dependent on an organic carbon source). Plants employ two: heterotrophy via oxygen just as in animals, and photoautotrophy – carbon dioxide-fixing, oxygen-producing photosynthesis by use of the sun's radiation. Bacteria, in addition to heterotrophy and photoautotrophy, have alternate nutritional modes: chemoautotrophy (using a chemical energy source and CO_2 as carbon) and chemoheterotrophy (using a chemical energy source and an organic substance as carbon source) that are not possessed (except via symbiotic acquisition) by either the animal or the plant. Invertebrates near deep-sea hydrothermal vents exist because of symbiosis with chemosynthetic bacteria. Cattle do not make cellulases required for digesting grass. They have evolved ectosymbiosis with microorganisms and are 'fermentation tanks on four legs'. The enteric bacteria in humans provide certain essential amino acids and vitamins. Symbiotic interactions between microorganisms and hosts are of a metabolic nature.

Why alliances are forged?

Stable physical association of dissimilar microorganisms, or consortium, is imperative for the transfer of genes. The reason for swimming organisms to come physically together and enter into a stable alliance must have been metabolic complimentarity. For example, in a recent version of SET theory it is envisaged that in the Prototerozoic Eon (2500 – 541 million years ago), when the environment was anoxic, and hot and sulphurous, an alliance was forged between one partner that reduced sulphur to toxic hydrogen sulphide and the other partner oxidized it to elemental sulphur or sulphate, which disappeared in water. Indeed, this partnership can force two different types of bacteria into a tight association or consortium. A case in point is two totally different types of bacteria forming a stable association that its original discoverer in Russia, S. A. Perfiliev had named a single marine organism, *Thiodendron* that forms white masses smelling of sulphur, until his student, G. A. Dubinina, surprised microbiologists that this organism was in fact a tight consortium, based on symbiotic reciprocity of two different types of bacteria, *Desulfobacter* (a flagellated sulphate-reducing bacterium producing H_2S) and *Spirochaeta* (a corkscrew-shaped sulphide-oxidizer) that had formed an alliance to protect itself from oxygen[21].

Test of SET

Can the path of evolutionary complexity be retraced and some extant primitive protists be merged in the laboratory to synthesize the last common eukaryotic ancestor (LECA) possessing nucleus and cytoskeleton elements from which complex forms evolved[20,22].

In the first step (Figure 5), a swimming bacterium merged with a wall-less (capable of locomotion and engulfing) fermenting Archaea (step 1), such as *Thermoplasma acidophilum* that is capable of growth in warm and sulphurous conditions. What descended from this merger is postulated to be an anaerobe which lived in organic-rich mud in puddles or pools where oxygen was scarce. This was followed (step 2) by merger with sulphur-oxidizing marine bacterium, such as the motile, corkscrew-shaped Spirochaeta or eubacteria that were able to swim, enabling them to survive increasing oxygen concentration. Many appendages crucial to moving chromosomes at cell division in nucleated cells arose to form LECA. In step 3, the swimming LECA with low oxygen tolerance merged with an aerobic bacterium, purple bacteria or proteobacteria, which could access organic-rich habitats, and acquired aerobic Krebs cycle-oxidative phosphorylation-mitochondrial metabolism to fully cope with free oxygen in the air. From this merger evolved all fungi and animals. A third merger with free-living photosynthetic cyanobacteria that produce oxygen gave rise to ancient swimming green algae, ancestors of today's land plants. Since mammalian red blood cells lack both nuclei and mitochondria, Margulis and Sagan[10] write that a colleague, Dennis Searcy, has chosen his own red blood cell as the starting ancestral cell for forging alliances with different bacteria to form eukaryotic cell. The SET contradicts traditional theories that hold evolution by divergence and not by merging – the reason for Margulis and Sagan to regard the family trees depicting forking branches as wrong. And since bacteria have single genomes, for their theory to be consistent, it is asserted that bacteria lack species.

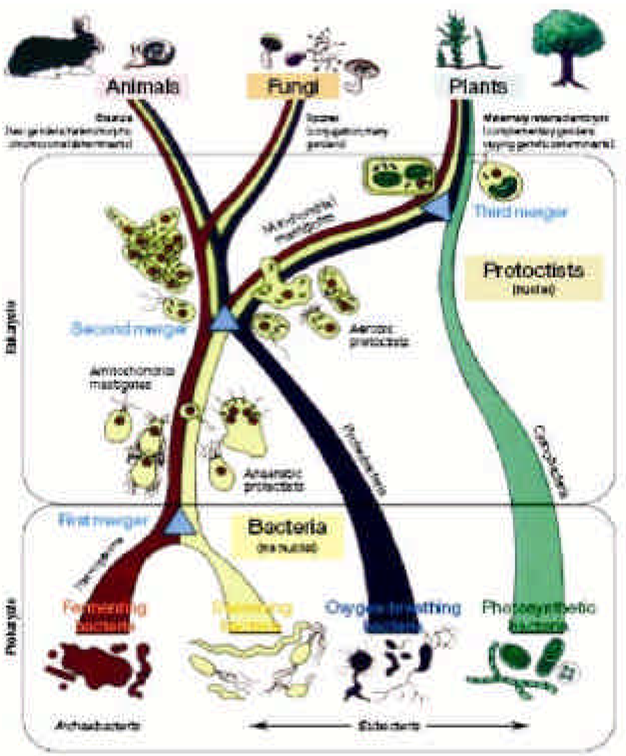

Figure 5. Margulis' depiction of 'tree of life' showing that branches of the evolutionary tree do not just diverge but also fuse. From Margulis[20].

Conclusion

A consensus now exists for Margulis' vision of evolution of large forms of life by associations, mergers and acquisition of foreign genomes. The greatest support has come from genomics, which traces life from DNA sequences. To Margulis and Sagan, sudden acquisition and merger of genomes explain 'punctuated equilibrium' – the gaps in fossil records providing an alternative to Darwin's gradualism. They challenge Darwin for emphasizing competition and selection as the sole forces shaping the origin of species. Instead, they argue that all speciation events are caused by symbioses, cooperation and the reticulation of genomes. One of the foreseeable goals of evolutionary biology is to recreate in the laboratory, the earliest eukaryotic cell by acquisition and integration of specific bacterial ancestors. 'There is more to biology than rats, *Drosophila*, *Caenorhabditis* and *E. coli*' (Foreword by E. Mayr in Margulis and Sagan[10]).

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