



Transitions in individuality through symbiosis

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When a more complex, functionally integrated entity emerges from the association of simpler, initially independent entities, a major evolutionary transition has occurred. Transitions that result from the association of different species include the evolution of the eukaryotic cell and some obligate mutualisms. Recent studies are revolutionizing our understanding of how these intimate interspecific associations come to be, revealing how and to what extent each partner contributes to the relationship, and how partners mediate conflict. Here, we review work on the evolution of mutualistic symbioses in the context of transitions in individuality and highlight how a better mechanistic understanding of the ecological drivers of host-symbiont interdependencies can help elucidate the evolutionary path to symbiotic organismality.

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Introduction: Major transitions in evolution and individuality

Throughout the tree of life, from prokaryotes to eukaryotes, from unicellular to multicellular growth forms, all organisms share something in common. They are all made of smaller entities that came together to function as a larger, cohesive, and functionally integrated whole. Why do these simpler forms of life associate in the first place, and why do they renounce their individual autonomy to stay together? In other words, why is life organized the way it is?

In the mid-90s, Maynard-Smith and Szathmari introduced a new way to think about these questions by proposing that increasing biological complexity can be explained by a series of major transitions in evolution [1]. In each major transition, smaller, previously autonomous

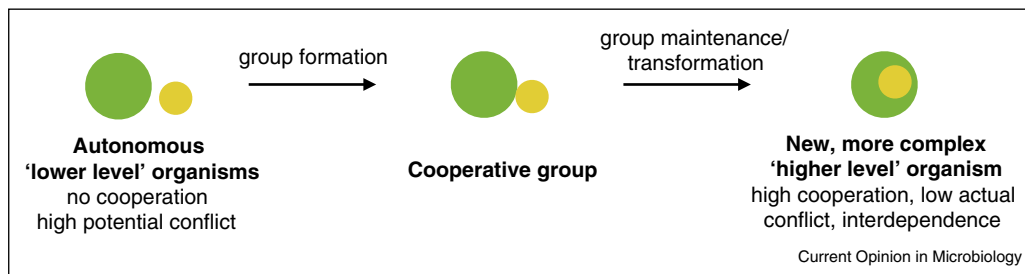
(lower-level) biological entities come together to form a new, more complex (higher-level) entity within which the smaller entities show high cooperation and low conflict (Figure 1). Central to this conceptual framework is the idea of a link between the evolution of biological complexity and the evolution of cooperation. Expanding on this idea, Queller distinguished two kinds of major transitions: ‘fraternal’ transitions involving similar, closely related units, and ‘egalitarian’ transitions involving different, more distantly related units [2,3]. Following these definitions, one can think of a multicellular organism — formed by the union of individuals from a single unicellular species — as a ‘fraternal organism’, and of the eukaryotic cell — formed by the union of individuals from two or more unrelated unicellular species — as an ‘egalitarian organism’.

Since the idea of major transitions in evolution was first introduced, great theoretical and empirical progress has been made towards understanding within-species transitions (see [4], and for recent reviews see e.g. [5,6]), in particular the evolution of multicellularity [7,8,9,10,11,12,13,14] and of eusociality [15,16]. Egalitarian transitions, however, have received less attention, partly due to the empirical difficulty of identifying and studying biological associations of functionally integrated, interdependent species — or even agreeing on what constitutes such an association [17,18]. This is, however, starting to change due to the development of novel approaches that are revealing some of the mechanisms behind the formation and maintenance of such intimate partnerships [19,20]. In this review, we focus on between-species transitions and discuss recent work on mutualistic symbioses in the light of major transitions in individuality.

Coming together: The origins of between-species associations

Mutualisms are prevalent in nature and found in all kingdoms of life, usually arising from the exchange of nutrients and/or services [21] (Table 1). In many of these associations the partners are able to live independently, but in others, one or more partners are so dependent on the association that they can no longer live outside of it. Because conflict inevitably arises when multiple species share a common environment, either due to passive competition over shared limiting resources such as nutrients or space (exploitative competition) or else because one or more species harms the others (interference competition), any theory of the origin of mutualism must answer two questions. First, how do species mediate conflict and maintain cooperation instead of pursuing

Figure 1



Common features and steps to a major egalitarian (between-species) transition. Here we illustrate a transition between a larger partner (e.g. host) and a smaller partner (e.g. symbiont). While initially autonomous entities with high potential conflict and low cooperation, the two species ultimately form a symbiotic organism with high cooperation and low conflict, as well as a high degree of mutual dependency.

their selfish interests (i.e. cheat)? Second, what favours the evolution of between-species dependencies?

Here we approach these questions by focusing on interactions involving a larger partner (a host) and a smaller symbiont. We classify host-symbiont associations on two axes (Figure 2): the symbiont's mode of transmission (from horizontal to vertical) and the degree of interdependency

for nutrients and/or services (from facultative to mutually obligate). By using these two interconnected continuum axes, we aim to distinguish between the symbiont's dependency for dispersal to a new host (H-V continuum) and interdependency for reproduction, growth, and survival (F-O continuum). As an association moves up and to the right in Figure 2, it becomes more like a single organism — i.e. its degree of 'symbiotic organismality' increases — until

Table 1

Examples of host-bacterial symbiont associations illustrating the diversity of modes of transmission and dependencies

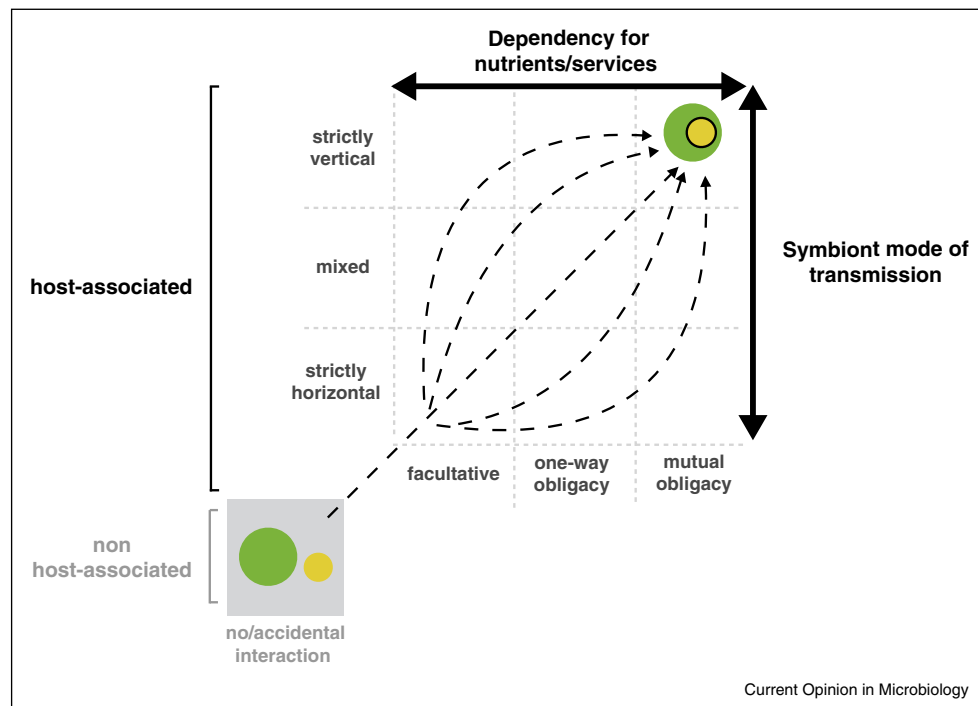
Host-symbiont	Mechanism of interaction (i.e. resources/services provided)	Symbiont transmission mode ^a	Symbiont localization	Degree of dependency ^b	References
Aphid- <i>Buchnera aphidicola</i>	Nutritional (Buchnera provides essential amino acids to the aphid in return for nutrients)	Vertical	Bacteriocytes (obligate intracellular)	Mutually dependent	[42*,82,83]
Pea aphid- <i>Regiella insecticola</i>	Defensive mutualism (symbiont protects its host against fungal pathogens or parasitoids)	Vertical (but occasionally horizontal)	Hemolymph, sheath cells and secondary bacteriocytes	Symbiont obligate to host (facultative secondary symbiont)	[76,84,85,86]
<i>Dictyostelium discoideum</i> - <i>Burkholderia</i>	Defensive mutualism	Mixed	Spores (intracellular) or sori (extracellular)	Both autonomous (facultative)	[22*,87,88]
Tubeworm <i>Riftia pachyptila</i> -endosymbiont Endoriftia	Nutritional (host provides H ₂ S and O ₂ to symbiont in return for fixed organic carbon)	Horizontal with evidence for release of symbionts upon host death	Bacteriocytes	Host obligate to symbiont	[89]
Legume- <i>Rhizobium</i>	Nutritional (legume provides carbon to rhizobia in exchange for nitrogen)	Horizontal	Nodules	Both autonomous (facultative)	[32,69]
Bobtail squid <i>Euprymna scolopes</i> - bacterium <i>Vibrio fischeri</i>	Protection- indirect via camouflage (vibrio emits light that protects the squid from potential predators at night in return for a nutrient-rich host environment)	Horizontal	Crypts of the light organ	Both autonomous (facultative)	[40]

Notes:

^a Horizontal transmission: symbionts are environmentally acquired. Vertical transmission: symbionts are maternally transmitted. Mixed transmission mode: combination of both vertical and horizontal transmission.

^b We define 'obligate' as not being able to survive independently.

Figure 2



Potential paths to a major transition between species. The x axis represents the degree of dependency for nutrients and services across the facultative — obligate continuum, and is thus mainly concerned with dependence for reproduction, growth, and survival. The y axis represents the mode of symbiont transmission ranging from strict horizontal transmission to strict vertical transmission, and thus represents the degree of symbiont dependency for dispersal to a new host. When both modes of transmission are present, we use the term 'mixed'. The light grey box shows the free-living state (i.e. before association with host) characterized by a lack of, or a purely accidental interaction. The dashed arrows represent potential trajectories to reach a symbiotic organismal state when starting from a purely free-living state. As an association moves up and to the right, its degree of 'symbiotic organismality' increases, and a major evolutionary transition, characterized by strict vertical transmission and mutual dependency, occurs. Here, we consider that one species is larger (host) and the other species is smaller (symbiont).

ultimately a major evolutionary transition, characterized by strict vertical transmission and mutual dependency, occurs (e.g. the eukaryotic cell). Prior to such a transition, whether the interaction is parasitic, commensal or mutualistic is a function of the balance between the net costs and benefits of association, which is contingent on the environment [22,23,24], and associations may freely shift between these states as the partners adapt to each other's presence. Guided by these two main axes, we next discuss some of the opportunities and challenges faced by associations on the road towards symbiotic organismality, with particular emphasis on the mechanisms mediating conflict between partners and driving host-symbiont interdependencies.

From warfare to welfare: mechanisms mediating conflict and promoting cooperation

Key to maintaining a stable relationship is keeping conflict low and cooperation high [25]. Mechanisms of conflict mediation are particularly important for associations involving different species because, unlike in fraternal associations, partners cannot rely on high relatedness to minimize the evolutionary impact of within-individual

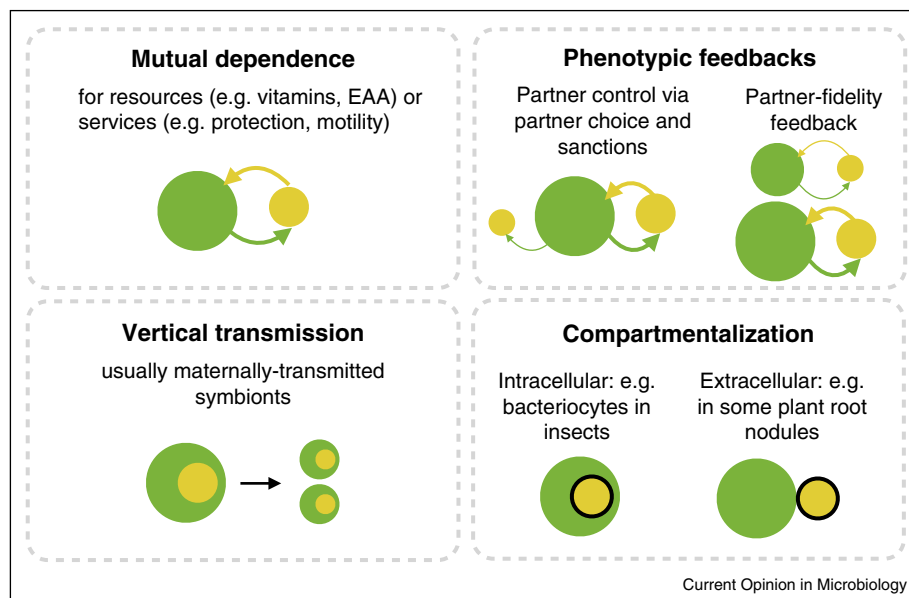
conflict and align their reproductive interests (e.g. by undergoing a single-cell bottleneck during reproduction [9]). Here we discuss strategies that can mitigate conflict during egalitarian transitions (Figure 3).

Partner-fidelity feedback, partner choice and sanctions

Organisms that do not contribute (or contribute less) to a costly cooperative phenotype, but nevertheless reap the same benefits, should be favoured by natural selection. Given selection for cheating, how are mutualisms maintained? A number of phenotypic feedback mechanisms (Figure 3) have been proposed to explain how cheating can be prevented or limited in multispecies associations [26,27,28], including partner choice [29,30,31], partner sanctions [30,31,32,33,34] and partner-fidelity feedback [35,36].

Evidence for partner choice and post-infection partner sanctions has been found, for instance, in the facultative symbiosis between legumes and the horizontally transmitted rhizobia bacteria [30,31,32]. Rhizobia commonly live in the soil, but often colonize the root nodules of

Figure 3



Mechanisms mediating conflict between species and stabilizing mutualisms and that may be key to the formation and maintenance of a symbiotic organism. Mutual dependence, phenotypic feedbacks, and compartmentalization ensure benefits from association and keep conflict low. Vertical transmission ensures that partners have a shared reproductive fate. EAA: essential amino acids. The green and yellow arrows show the benefits provided by a species to another species.

legumes. Within the host nodules, symbiotic rhizobia fix nitrogen and provide ammonia back to their host in exchange for carbon sources and oxygen. But nitrogen fixation is costly and so rhizobia strains that fix less nitrogen can invest more energy into their own growth and reproduction. To prevent rhizobial cheats from taking over and disrupting the mutualism, the plant has evolved mechanisms to reduce infection by less beneficial symbionts (partner choice) and selectively direct more resources to root nodules colonized by beneficial rhizobia and fewer resources to less efficient nodules (partner sanctions) (Table 1) [32]. While partner choice and sanctions commonly rely on the idea that a partner's response is conditional on the behaviour of its partner [27], partner-fidelity feedback does not rely on such conditional response but instead occurs when there is a positive correlation between partners' fitness so that the benefits provided to a partner feed back as returned benefits to the actor, as occurs for instance in the association between eukaryotes and their mitochondria [26].

Partner choice and partner sanctions are more important when hosts need to re-acquire beneficial symbionts each generation from the environment, a process which requires the ability to either distinguish beneficial symbionts from a diverse pool of organisms or else sanction less beneficial or harmful symbionts. In contrast, partner-fidelity feedback is expected to play a major role in stabilizing mutualisms where symbionts are transmitted

vertically [5*,19,26]. This occurs because host and symbiont fitness are intimately intertwined, and so there is a direct negative feedback from harming the other partner [37,38]. As a consequence, partner-fidelity feedback is commonly characteristic of older, obligate partnerships with a deep history of co-evolution [5*,19,26].

Privatizing beneficial partners via compartmentalization

The ability to enhance host control and ensure partner fidelity is greatly increased in cases where symbionts reside in specialized host compartments, and more so when they are housed intracellularly [39] (Figure 3, Table 1). When symbionts are transmitted horizontally, hosts almost inevitably acquire genetically different partners, resulting in a greater potential for conflict. The partitioning of symbionts into multiple compartments (e.g. nitrogen-fixing rhizobia within nodules) results in the physical separation of symbionts, and this type of compartmentalization improves the host ability to reward beneficial symbionts or sanction harmful intruders. Such spatial separation can also be achieved, for instance, with structured crypts as seen in the light organ of the bobtail squid [40] and in the midgut of many insects [41]. Another, perhaps more extreme, example of symbiont privatization occurs when symbionts live in specialized host cells. While in some cases they can be transferred horizontally, there are also cases of vertical transmission. For instance, the obligate endosymbiont (*Buchnera*) of aphids resides inside specialized cells known as bacteriocytes (Table 1). With this intracellular

compartmentalization, the host is able to control the density of its symbiont, minimizing within-host conflict over resources. Because bacteriocytes are maternally transmitted, the host not only controls its symbiont growth, but also its symbiont's reproductive fate [42*]. Such symbiont privatization enforced by physical boundaries that are retained across generations of the host maximizes partner fidelity, as partners do not need to associate *de novo* each generation, and hence promotes the return of fitness benefits via partner-fidelity feedback.

Sink or swim together: ecological and mechanistic drivers of interdependencies

Gene loss drives functional dependency

An egalitarian transition only occurs when partners are interdependent as it is key to limit conflict between partners [2]. Evidence for a link between genome reduction and symbiont lifestyle is accumulating, indicating that the smallest genomes usually belong to vertically transmitted, intracellular obligate symbionts that have evolved from bacteria that were once free-living [43]. But why and how, in the first place, would an organism give up its autonomy and become dependent on other organisms?

The loss of essential genes — driven by natural selection and/or genetic drift — is common in nature but often goes unnoticed because the functions that are lost are compensated for by ecological partners [44,45,46,47]. Many genes yield functions whose products are 'leaky', or difficult to privatize, allowing some organisms to lose these genes as long as they are in a community where other organisms have retained them. In diverse, free-living communities, this adaptive gene loss via a 'Black Queen' process can yield a variety of novel mutualistic interactions, which could potentially seed the development of more specific and intimate mutualisms [46,47].

Symbionts restricted to living and reproducing inside their host, such as vertically transmitted endosymbionts, also experience positive selection to lose genes whose products are provided by their host or other symbionts [48,49,50,51,52,53*,54], but these organisms can also evolve smaller genomes through random genetic drift. Because of their significantly reduced effective population size, the ability of natural selection to prevent the accumulation of neutral and deleterious mutations is greatly weakened, and this fact coupled with isolation from horizontal gene transfer (HGT) from other species leads to steady genome reduction [55]. This process, called Muller's ratchet [56], can lead to extreme dependency on the part of the symbiont even in the absence of a selective advantage for becoming dependent. Because Muller's ratchet is a nearly inevitable side effect of vertical transmission with a tight bottleneck, it is a major engine for pushing associations towards obligate interaction. As a consequence of such physical and functional integration, harm to the host generates a negative feedback on the symbiont's fitness

(partner-fidelity feedback), and so vertically-transmitted obligate symbionts are typically expected to be beneficial to their host [19,37]. There are exceptions, however, as illustrated by many examples of parasitic *Wolbachia* strains that are vertically transmitted in insect populations [57,58].

Interplay between gene transfer and gene loss in the evolution of interdependencies

The importance of the interplay between gene transfer and gene loss for driving egalitarian transitions is evident in the evolution of the eukaryotic cell [59]. Genes that initially belonged to the mitochondrial endosymbiont were transferred to the host's nucleus [60,61,62]. Because the products of those genes were coming back to the mitochondria, the mitochondrial genes became redundant and, as a result of such redundancy and isolation from HGT in a very small population, they were ultimately lost from the mitochondrial genome [63]. Such gene transfer and loss shifted genetic control — from the mitochondria to the host — which further aligned the interests of the two partners, and is likely to have been crucial to the evolutionary success of the eukaryotic cell.

Similar processes are ongoing within insect-microbe symbioses in the modern world, with evidence for horizontal transfer of functional genes from non-symbiotic bacteria to the host genome [53*,64*], from symbionts to other symbionts [65], and also, though rare, the direct transfer of symbiotic genes to the genome of the host [64*], which is one of the signatures of the evolution of organelles [42*]. For instance, the endosymbiont *wCle Wolbachia* provides its insect host with B vitamins, a nutritional capability that was made possible by the acquisition of a nutritional gene from a coinfecting endosymbiont [65].

While the studies discussed above focus on the role of gene loss and transfer in driving associations towards obligacy, genome expansion via gene duplication and HGT can also play an important role in the evolution of symbioses, in particular in the transition from environmental to a symbiotic lifestyle [66]. A notable example is the evolution of symbiotic nitrogen-fixing rhizobia from non-symbiotic strains by the acquisition of chromosomal symbiotic genes involved in nodulation and nitrogen fixation [67].

Conclusions and future directions

Most intimate, obligate symbioses are the result of an evolutionary journey where partners — once autonomous organisms — forwent the pursuit of their own selfish interests and individual autonomy to become a single symbiotic organism. Here we suggest that separating a symbiont's dependency for dispersal into new hosts (symbiont mode of transmission) and interdependency for reproduction, growth and survival (dependency for nutrients/services) into two continuum axes can help us better understand and predict the evolutionary path to symbiotic organismality (dashed arrows in Figure 2). In particular,

this approach highlights three major questions. First, does strict vertical transmission evolve before or after mutual dependency for nutrients/services? Second, are all symbiotic relationships on the path towards obligacy? Third, once a symbiont's dependency for dispersal or dependency for nutrients/services has occurred, how likely is reversal to an autonomous state? For instance, phylogenetic analyses suggest that the reversal of bacterial mutualisms to a parasitic or free-living state is rare [19,68*] and legume-rhizobia mutualisms have remained facultative despite some of these partnerships being very ancient [69]. Future work might address whether and how mechanistic or ecological constraints can be preventing some of these transitions from occurring.

A valuable tool to examine transitions in symbioses is phylogenetic analysis, which provides information about when the transitions might have occurred and the rates at which symbionts are gained and/or lost [19,41,53**,61,68*,69,70,71,72]. Experimental evolution has also proven useful in testing the conditions that favour shifts along the conflict-mutualism and horizontal-vertical continua, and there is now a diverse range of symbiosis model systems available to test further these ideas (e.g. the *Dictyostelium* farming symbiosis [22*], algal photosynthetic symbioses [23**,73], plant-bacteria symbioses [74], and insect-bacterial nutritional [65,75] and defensive [76] symbioses). Coupled together, these two approaches offer powerful complementary frameworks for exploring the origins, evolution, and breakdown of symbioses.

Another question is which partner controls vertical transmission? Do hosts enslave their symbionts by enforcing strict vertical transmission, or do symbionts force their way into their hosts' reproductive system to ensure their transmission to the next generation? There is growing evidence that both hosts and symbionts have specialized mechanisms that enable them to drive vertical transmission [77,78]. Symbiont-driven vertical transmission has been suggested, for instance, in the mutualistic endosymbiont *S. glossinidius* which invades its host's bacteriocytes using a type III secretion system, a strategy commonly used by intracellular pathogens to invade host cells [79]. In contrast, evidence for host-driven vertical transmission has been found in the pea aphid *A. pisum* which is able to selectively transfer its obligate symbiont *Buchnera* via a mechanism of exo-/endocytotic transport [80]. Which strategy is more prevalent is an open question, and more work is needed to unravel the molecular mechanisms underpinning both host-driven and symbiont-driven transmission modes.

A major challenge with studying obligate symbioses is to determine who depends on whom and how, as well as to measure the net costs and benefits of association. This becomes even more challenging when studying symbioses that involve more than two species, as indirect obligacies can arise (e.g. symbiont A depends on symbiont B that

depends on the host, and thus symbiont A depends indirectly on the host). Although our discussion in this review has focused on interactions between a host and a single symbiont, our two-axis approach can also be used in some cases to explore the route to symbiotic organismality when multiple symbionts are involved. When dependencies are hierarchical (i.e., A lives within B that lives within C), such as in the tripartite, three-way nested mealybug mutualism [53**], our approach works by separately focusing on the different host-symbiont levels. But when dependencies are non-hierarchical (e.g. symbionts A and B both living within C), symbionts may directly help or harm each other, which brings in an additional layer of complexity [81]. In such cases, our approach can still be used in the same way as long as there is a temporal separation between major transition events. This occurs, for instance, when the host acquires symbionts sequentially, and illustrates a case where several major transitions within a single host may have occurred, ultimately leading to a symbiotic organism composed of more than two former organisms. One can also envision a more complex case, where a consortium of symbionts progresses towards symbiotic organismality as a cohort, and it is this possibility that is the focus of ongoing research on the 'hologenome theory of evolution' [17]. Developing a unified theory of mutualism that integrates the mechanistic, ecological and evolutionary drivers of host-symbiont interdependencies is an important next step to obtain a more complete and general picture of how symbiotic organismality can be achieved.

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