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Effector proteins that modulate plant–insect interactions

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Insect herbivores have highly diverse life cycles and feeding behaviors. They establish close interactions with their plant hosts and suppress plant defenses. Chewing herbivores evoke characteristic defense responses distinguishable from general mechanical damage. In addition, piercing-sucking hemipteran insects display typical feeding behavior that suggests active suppression of plant defense responses. Effectors that modulate plant defenses have been identified in the saliva of these insects. Tools for high-throughput effector identification and functional characterization have been developed. In addition, in some insect species it is possible to silence gene expression by RNAi. Together, this technological progress has enabled the identification of insect herbivore effectors and their targets that will lead to the development of novel strategies for pest resistances in plants.

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Introduction

Half of the nearly one million insect species known to date feed on plants [1•]. In more than 350 million years of plant–insect coevolution, insects have developed diverse feeding styles and behaviors (Figure 1). Plants have evolved strategies to defend themselves against insect herbivory that are based on physical barriers, constitutive chemical defenses, and the direct and indirect inducible defenses (recently reviewed in [1•]). These defense mechanisms appear to be quite effective as most plant species are resistant to most insect species. Indeed, approximately 90% of the plant-inhabiting insect herbivores are restricted to plants of a single taxonomic family or few closely related plant species, while only a minority are highly polyphagous [2]. In this context, relevant questions are: Why are most insect herbivores specialists? And how do approximately 10% of insect species establish compatible interactions with multiple plant species in different plant families?

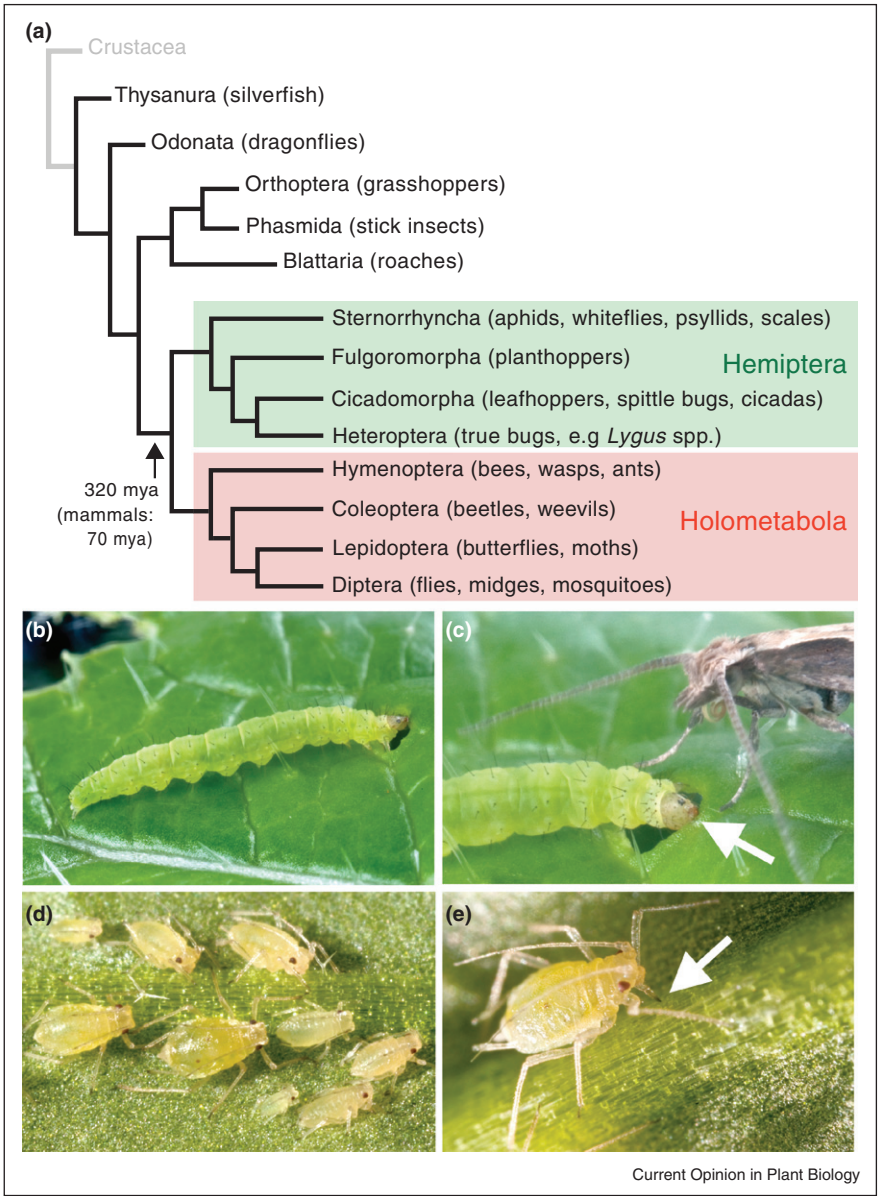
Over the years evidence has accumulated that the plant primary and secondary metabolism profiles influence insect herbivore colonization abilities. Some plants may not produce sufficient nutrients that are required for insect survival and reproduction of certain insect species [3]. In addition, secondary metabolites may deter insects from feeding or egg laying [4]. Nonetheless, although the plant primary and secondary metabolism influences host selection, there are other factors involved. Recent evidence suggests that insects produce effectors that modulate plant defense responses [1•,5]. It would be beneficial to understand the role of insect herbivore effectors in modulating plant defenses and how this may render the plant more susceptible to herbivore colonization.

In this opinion paper, we will review progress towards the identification of herbivorous insect effectors and discuss to what extent concepts in plant–microbe interactions apply to plant–insect interactions. Following a review on effector biology [6] we adopt a broad inclusive definition of effectors including all pathogen/pest proteins and small molecules that alter host-cell structure and function. These alterations may trigger defense responses induced by avirulence factors, elicitors, microbial/pathogen/herbivore-associated molecular patterns (MAMPs, PAMPs of HAMPs) or promote infection (mediated by virulence factors or toxins) or both. We propose that specific effectors are required for establishing compatible insect–plant interactions. Dissecting the plant host factors and pathways these insect effectors may target will enable the characterization of plant defense pathways that are required for the induction of effective resistances against plant herbivores.

Herbivore feeding styles and behavior

Plant perception of insect herbivores has been particularly well investigated for lepidopterans [1•] and the Hessian fly (*Mayetiola destuctor*) [7]. Caterpillars are chewing herbivores (Figure 1b,c) and release a repertoire of elicitors that induce characteristic defense responses distinguishable from general mechanical damage (discussed below). Hessian fly larvae wound a few cells and inject saliva into these wounds while initiating interactions with the plant. In a compatible interaction, this results in the formation of gall-like nutritive tissue, whereas in an incompatible interaction the larval salivary gland components trigger cell collapse (resembling the hypersensitive response (HR)) and larval death [7].

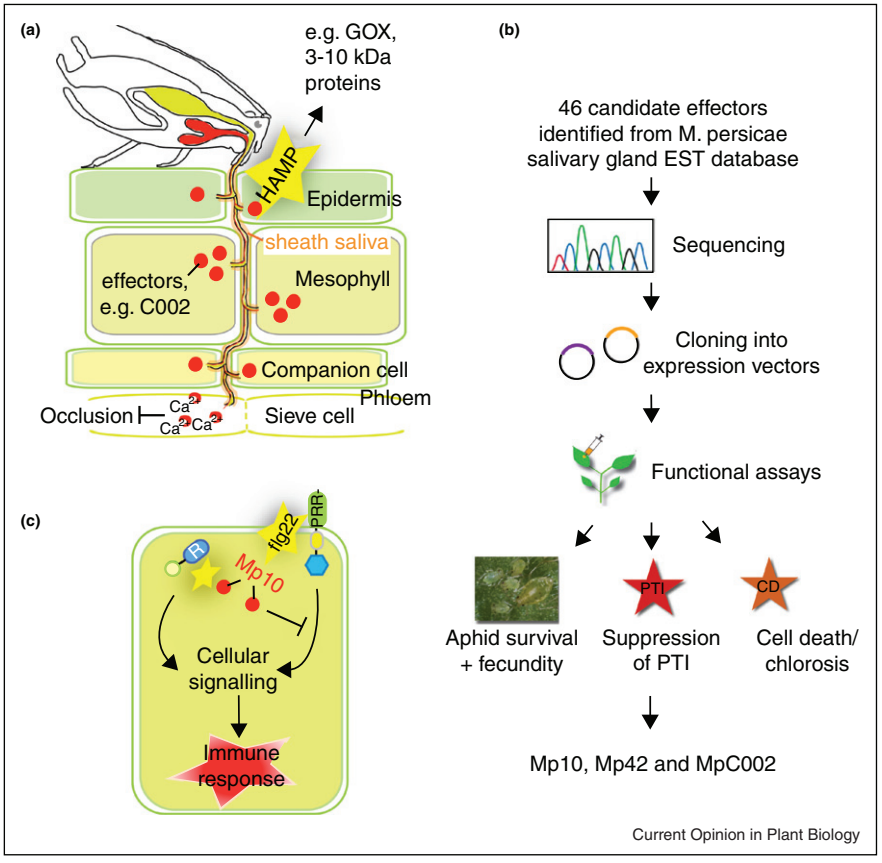
Figure 1



Insect herbivores are diverse and display a range of feeding habits. **(a)** Schematic overview of insect phylogeny. **(b)** Diamondback moth (*Plutella xylostella*) caterpillar chewing from a leaf. **(c)** Adult diamondback moth (upper right) and caterpillar (lower left). The chewing caterpillar releases oral secretions (arrow) containing elicitors or herbivore-associated molecular patterns (HAMPs), including fatty acid conjugates (FACs), inceptins, β-glucosidae and glucose oxidase (GOX), which induce the activation of plant mitogen-activated protein kinases (MAPKs), jasmonic acid (JA), ethylene (ET), salicylic acid (SA) and reactive oxygen species (ROS) [1•]. **(d)** Green peach aphid (*Myzus persicae*) nymphs and adults feeding from a leaf. (e) Aphid stylets pierce plant tissue (arrow) and navigate between the plant cells to the phloem. The stylets puncture various plant cells for acquiring plant sap and releasing saliva that contains effectors, which induce and suppress plant defense responses [14•,15]. See Figure 2a for graphic illustration.

The Hemiptera (Hemimetabola) diverged about 320 million years ago from the Holometabola, which includes lepidopteran insects (Figure 1a). Insects in the order Hemiptera have stylets for piercing and sucking (Figure 1d,e) and display typical feeding behavior [8,9] that suggests active suppression of plant defense responses. For example, aphids often initiate probing of the leaf epidermis cells immediately upon landing [10]. Each probe takes less than one minute and involves stylet penetration of the epidermis cell wall and membrane, injection of saliva and ingestion of the cell contents [11–13,14•]. In a compatible interaction, the aphid stylet will progress to the phloem (and xylem) to establish a longer term feeding [13]. While feeding, aphids produce gelling saliva that covers the stylets with a protective sheath as well as watery saliva that is secreted into plant cells and phloem

Figure 2



Characterization of aphid effectors. **(a)** Aphid stylets navigating to the phloem. The stylets puncture various cells on their path to the phloem and acquire plant sap and release watery saliva that contains effectors, which induce and suppress plant defense responses [14•,15,16,17•,18,19]. The protein-rich sheath saliva that surrounds the stylets is thought to have a protective function and may contain effectors as well. Aphids produce glucose oxidase (GOX) and 3–10 kDa proteins that are potential herbivore-associated molecular patterns (HAMPs) as they elicit defense responses in plants [20•,37,38,46]. The pea aphid *Acyrthosiphon pisum* produces C002 in its salivary glands and releases this protein into the plant; silencing of the C002 gene by RNAi (injection of dsRNA corresponding to C002 into the aphid) increases aphid lethality on plants [24,25••]. Aphids also release Ca^{2+}-binding proteins in the phloem sieve cells preventing occlusion of these cells upon mechanical damage by the aphid stylets [22••,51]. **(b)** To identify effectors from the green peach aphid *Myzus persicae*, a functional genomics pipeline was developed that resulted in the identification of additional candidate effectors, including Mp10, Mp42 and the *M. persicae* homolog of C002 (MpC002) [5]. **(c)** Mp10 suppresses the flg22-mediated PAMP-triggered immunity (PTI) and also induced chlorosis in *Nicotiana benthamiana*; the chlorosis response was dependent on the co-chaperone SGT1, which is required for the activation of NBS–LRR resistance proteins [5,47,48].

during salivation [14•,15,16] (Figure 2a). The sheath and watery saliva both contain proteins [16,17•,18,19] with diverse activities; they function as effectors and induce or suppress plant defense responses [5,20•], have enzymatic and chelating activities [16,21,22••,23] and improve aphid performance [24,25••] (Figure 2a). Other piercing-sucking insects of the order Hemiptera also introduce saliva into their plant hosts resulting in virus and pathogen transmission and induction of feeding damage and galls.

R gene-mediated plant resistance to pathogens and insect herbivores

The inducible plant defense response to microbial pathogens is a multilayered process consisting of at least two phases [26]. Phase one is initiated with the recognition of PAMPs by the plant pattern recognition receptors (PRRs) resulting in PAMP-triggered immunity (PTI). A successful pathogen deploys effectors that can suppress PTI resulting in a compatible interaction unless phase two of the plant defense response is activated. Phase two is triggered upon recognition of the pathogen effectors or their activities by plant disease resistance (R) proteins resulting in effector-triggered immunity (ETI). The *R* genes often encode proteins with nucleotide binding site (NBS) and leucine rich repeat (LRR) domains (NBS–LRR proteins). The pathogen may avoid ETI by diversifying or shedding the effector gene or by suppressing ETI with other effectors.

R genes that encode proteins of the NBS–LRR family also confer resistance to insect herbivores. Three *R* genes *Mi-1.2*, *Vat* and *Bph14* have been cloned. *Mi-1.2* confers

resistance in tomato to certain clones of *Macrosiphum euphorbiae* (potato aphid), two whitefly biotypes, a psyllid, and three nematode species [27–29], *Vat* confers resistance to one biotype of the melon-cotton aphid *Aphis gossypii* [30•], while *Bph14* confers resistance to the rice brown planthopper *Nilaparvata lugens* [31]. *R*-gene mediated resistance is often limited to one clone of an insect species. Thus, specific aphid biotypes may evade and/or suppress plant defenses agreeing with the gene-for-gene model in plant–pathogen interactions [32].

Other evidence supporting the gene-for-gene model in plant–insect interactions is provided by the Hessian fly-wheat interactions, where *R* genes have been used for decades to control infestations [33,34]. Interestingly, known Hessian fly avirulence genes map to a chromosome region where several salivary genes that encode predicted secreted proteins have been found [35]. These secreted proteins could be effectors that are recognised by plant R proteins.

Effectors that elicit plant defenses in plant–insect interactors

Detectable elicitor activities are thought to be disadvantageous to insect herbivores and are generally triggered upon recognition of elicitors or HAMPs. HAMPs are invariant key components of insects and considered to exhibit similar activities as MAMPs and PAMPs in evoking the first line of plant defenses. Elicitors/HAMPs include modified lipids such as fatty acid-amino acid conjugates (FACs) [36], glucose oxidase [37,38], β-glucosidase [39] and inceptins [40] from lepidopterans, and sulfur-containing fatty acid caeliferins from a grasshopper [41] (Figure 1). The insect-derived elicitors identified to date are secreted in insect regurgitant or saliva and thereby directly delivered into the host–insect interface. The elicitors induce Ca^{2+} ion fluxes, the activation of plant mitogen-activated protein kinases (MAPKs), jasmonic acid (JA), ethylene (ET) and salicylic acid (SA) biosynthesis and signaling, production of reactive oxygen species (ROS) and enhanced volatile emission in plants (reviewed in [1•]). It is thought that the first step in the recognition of FACs by plants is the binding of these molecules to a plant receptor [42]. Interestingly, FACs induce plant species-specific responses, which fits with a receptor–ligand model for recognition. However, ion-channel forming activity has been reported for FACs [43] suggesting FACs can trigger defenses in a non-specific manner. It should be noted that plants are not only able to recognize elicitors in insect oral secretions, but also elicitors released during egg deposition [44,45].

In aphids, pectinase in the watery saliva of the English green aphid *Sitobion avenae* triggers the release of volatiles from wheat that attract the parasitoid wasp *Aphidius avenae* [46]. Furthermore, a recent study by de Vos and Jander shows that saliva of the green-peach aphid *M. persicae* triggers defenses in *A. thaliana* thereby reducing aphid performance [20•] (Figure 2a). Although the saliva component responsible for this phenotype was not identified, characterization of aphid saliva fractions suggested the active elicitor is a peptide of between 3 and 10 kDa.

Recently, a functional genomics approach (Figure 2b) identified two candidate effectors, Mp10 and Mp42, from the aphid species *M. persicae* with possible functions in defense elicitation. Both candidates reduced aphid fecundity upon over-expression in *Nicotiana benthamiana* [5]. In addition to reducing fecundity, Mp10 also induced a chlorosis in *N. benthamiana*, which was dependent on the co-chaperone SGT1 that is required for the activation of NBS–LRR resistance proteins and plant defense responses [47,48] (Figure 2c).

Effectors that promote plant–insect interactions

The first evidence that herbivores secrete proteins in their saliva to suppress host defenses was provided by a study by Musser *et al.* [49]. This work showed that the caterpillar *Helicoverpa zea* secretes the enzyme glucose oxidase (GOX) into its host to suppress the production of nicotine and resistance in *N. tabacum*. As GOX is an enzyme found in many caterpillar species [50], as well as in phloem feeding insects, such as aphids [17•], the suppression of defenses through secretion of this enzyme could be a common strategy for different plant-feeding insects.

Aphid saliva can suppress host defenses, such as clogging of the sieve elements, to enable feeding [22••]. This occlusion of the sieve elements likely depends on an influx of Ca^{2+} into the sieve element lumen [51] (Figure 2a). However, aphid saliva contains calcium-binding proteins that are predicted to prevent calcium from binding to sieve element proteins, and thereby suppress this defense mechanism [22••] (Figure 2a). Potentially, the salivary calcium-binding proteins prevent calcium-dependent signaling cascades in the sieve elements.

A recent study by Mutti *et al.* identified the salivary gland gene *C002* from the aphid species *Acyrthosiphon pisum*, which encodes a secreted protein that is delivered inside host plant tissue during feeding (Figure 2a). Silencing of the *C002* gene in aphids resulted in altered aphid feeding behavior [24,25••]. More recently, Bos *et al.* showed that overexpression of *M. persicae* C002 (MpC002) in *N. benthamiana* enhances aphid performance, consistent with a role of C002 in aphid virulence [5]. Phylogenetic analysis of *C002* showed that this gene is fast-evolving in aphids but not present in other insects, reflecting an unknown biological adaptation [52]. This adaptation may be the result of a co-evolutionary arms race with host plants.

In addition, the Mp10 candidate effector from *M. persicae*, previously identified by a functional genomics approach, suppresses the oxidative burst induced by the bacterial PAMP flg22 but not by the fungal PAMP chitin (Figure 2c) [5]. It is therefore possible that Mp10 is a genuine suppressor of PTI. To date, the role of perception of PAMP-like molecules in plant–insect interactions remains elusive. Therefore, further identification and characterization of aphid effectors like Mp10 is needed to provide a better insight into the different layers of plant defenses that are suppressed by phloem feeding insects.

Identification of insect effectors using functional genomics

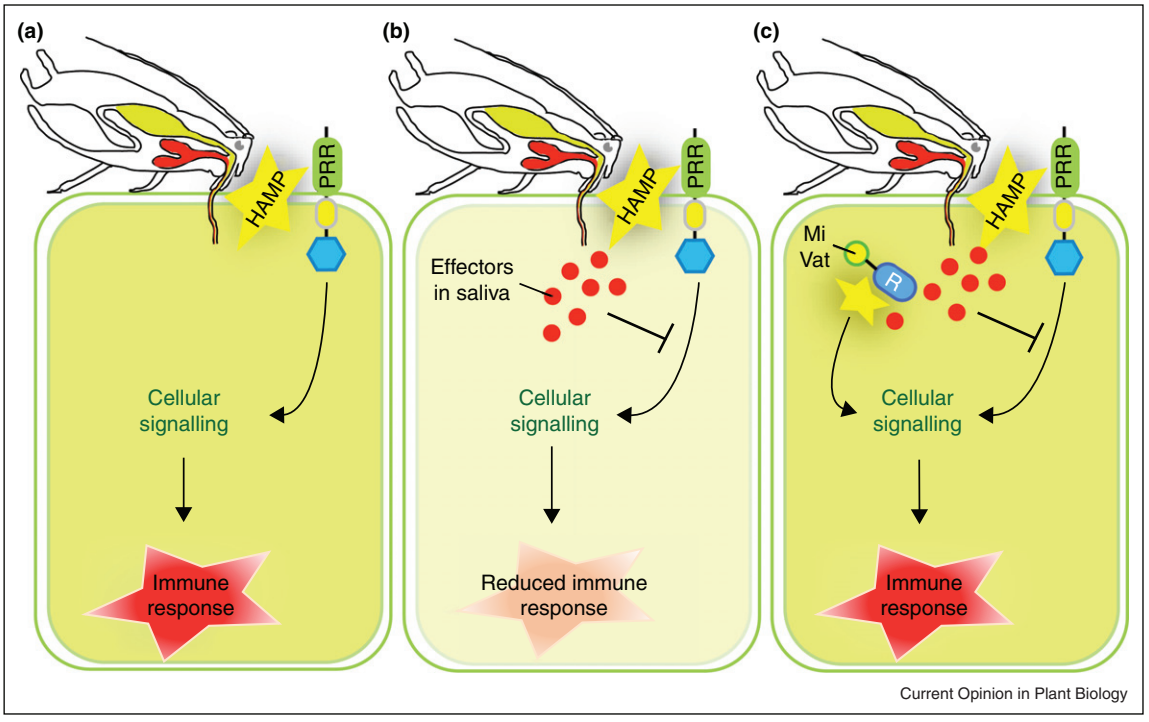
Affordable next-generation sequencing technologies have recently become available, allowing sequencing of multiple insect species and comparative genomics between and within insect species [53••,54••]. Technologies to amplify RNA from small quantities of tissue are also advancing [55,56] enabling transcriptome analyses of insect salivary glands that are predicted to produce the majority of effectors [19]. The availability of the *A. pisum* genome [57] and *M. persicae* transcriptome data [58] allowed the development of a functional genomics pipeline that identified the candidate effectors Mp10, Mp42 and MpC002 (Figure 2b) [5]. A similar approach could be applied to identify effectors in other insect herbivores providing that host plant species that allow for *in planta* assays are available.

Upon identification of effectors in a functional genomics pipeline as described above, additional functional analysis tools are needed to provide insight into the contribution of specific effectors to insect virulence. In several plant herbivores gene expression can be knocked down by feeding the insects on dsRNA-producing plants (plant-mediated RNAi) [59••,60••,61]. It is likely that plant-mediated RNAi is also possible for *M. persicae* allowing further study of the identified effectors.

Conclusions

We propose that, based on current evidence, a multi-layered plant defense response involving HAMP-triggered immunity (HTI) and ETI is also applicable to plant–insect interactions (Figure 3). Identification of plant targets of insect herbivore effectors will reveal the underlying

Figure 3



Model of the multi-layered plant defense response to aphid herbivory. **(a)** Plant cells perceive aphid herbivore-associated molecular patterns (HAMPs) leading to HAMP-triggered immunity (HTI). When the aphid is unable to secrete effectors that suppress HTI, this effective defense response deters the aphid from further feeding. For example, recognition of HAMPs may be involved in resistance of non-host plants to aphids, for example, in *Arabidopsis thaliana* resistance to the pea aphid *Acyrthosiphon pisum*. **(b)** Although plants perceive the aphid HAMPs, the defense response is effectively suppressed by aphid effectors leading to aphid colonization. We propose this model applies to compatible interactions between, for example, *Myzus persicae* and *A. thaliana*/*Nicotiana benthamiana*. **(c)** The aphid species produces effectors that effectively suppress HAMP-triggered immunity responses, but in certain clones of this aphid species one or more effectors are being recognized by *R* genes leading to a plant effective immune response and plant resistance to the aphid clone. For example, *Mi* or *Vat* confers resistance to specific clones of potato and melon aphids, respectively [27,30•].

molecular mechanisms of host manipulation that are to the benefit of the insect. Most importantly, the identification of insect herbivore effectors and their targets may generate opportunities to develop novel pest resistance strategies.

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- •• of outstanding interest

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