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Current Opinion in Microbiology

Parasitic nematodes manipulate plant development to establish feeding sites

Shahid Siddique and Florian MW Grundler

Cyst and root-knot nematodes, the two economically most important groups of plant parasitic nematodes, induce neoplastic feeding sites in the roots of their host plants. The formation of feeding sites is accompanied by large-scale transcriptomic, metabolomic, and structural changes in host plants. However, the mechanisms that lead to such remarkable changes have remained poorly understood until recently. Now, genomic and genetic analyses have greatly enhanced our understanding of all aspects of plant–nematode interaction. Here, we review some of the recent advances in understanding cyst and root-knot nematode parasitism. In particular, we highlight new findings on the role of plant hormones and small RNAs in nematode feeding site formation and function. Finally, we touch on our emerging understanding of the function of nematode-associated secretions.

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Current Opinion in Microbiology 2018, **46**:102–108

This review comes from a themed issue on **Host microbe interactions: parasitology**

Edited by **Pascal Mäser**

For a complete overview see the Issue and the Editorial

Available online 13th October 2018

https://doi.org/10.1016/j.mib.2018.09.004

Introduction

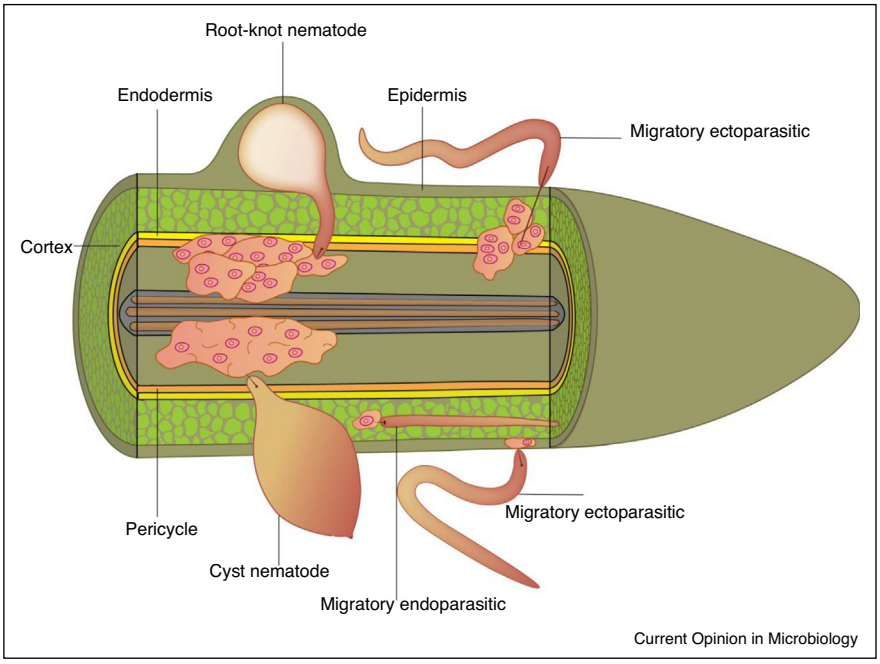
Plant-parasitic nematodes (PPNs) affect almost all major crops. The presently more than 4100 described PPN species are estimated to cause over 80 billion USD in agricultural loss per year [1]. The full extent of worldwide nematode damage is likely underestimated, particularly in developing countries, since growers are often unaware of the presence of these small, soil-borne pathogens. Additionally, the symptoms caused by PPNs are often non-specific, making it difficult to attribute crop losses to nematode damage. The small size, biotrophic life style, non-synchronized infection, and lack of a reliable transformation method make PPNs difficult experimental organisms. Studies on the molecular aspects of plant–nematode interactions have therefore lagged behind those in other pathosystems.

PPNs use a hollow protrusible stylet to break into the plant cells, withdraw nutrients, and release both proteinaceous (effectors) and non-proteinaceous molecules. The hollow stylet is connected to three enlarged, specialized esophageal gland cells, which produce the effector molecules that are secreted into the host tissues to facilitate parasitism. Each of the three esophageal glands consists of a single cell that contains an unusually long cytoplasmic extension ending in an ampulla. The effector proteins are synthesized in the gland cell and transported to the ampulla in membrane-bound granules. The ampulla in turn is connected to the lumen of the oesophagus by a valve. Some of the genes encoding oesophageal secretions are likely to have been acquired from prokaryotic microbes via horizontal gene transfer [2]. The development of stylet and esophageal gland cells producing effector molecules are among the most striking adaptations that enable PPNs to maintain a unique long-term parasitic relationship with their hosts.

Different species of PPNs feed on a range of plant tissues, including flowers, stems, leaves, and roots; however, most species feed on roots. Based on their feeding habits, PPNs can be broadly categorized as either ectoparasitic or endoparasitic (Figure 1). In this review, we focus on a complex and economically devastating group of sedentary endoparasitic PPNs including cyst nematodes (CNs; *Heterodera* spp. and *Globodera* spp.) and root-knot nematodes (RKNs; *Meloidogyne* spp.).

Infective-stage CN and RKN juveniles (J2) invade the plant root near the tip and move through different tissue layers to reach the vascular cylinder, where CNs induce the formation of a syncytium (a multinucleate fusion of cells resulting from partial cell wall dissolution) and RKNs induce the formation of 5–7 giant cells (Figure 2). In the case of RKN, proliferation of the tissue surrounding the nematode and the giant cells leads to the formation of a typical gall, which is observed as a primary symptom of infection. The establishment of feeding sites (syncytia and giant cells) enables CNs and RKNs for taking large amounts of nutrients from the plant, facilitates nematode growth, and induces a pathologically disturbed allocation of photosynthetic products that reduces plant growth and yield.

Figure 1



Overview of feeding habits of plant-parasitic nematodes. Plant-parasitic nematodes display a variety of feeding habits and can be broadly categorized as either ectoparasites or endoparasites. Migratory ectoparasitic nematodes stay vermiform throughout their life cycle and all stages are capable of feeding on roots of a broad range of host plants. Examples of migratory ectoparasitic nematodes include awl nematodes (*Dolichodorus* spp.), sting nematodes (*Belonaloaimus* spp.), needle nematodes (*Longidorus* spp.), and dagger nematodes (*Xiphinema* spp.). Members of the latter two genera extend periods of feeding at their feeding sites and are able to induce the formation of nurse cell in the root tip. They also act as vectors of specific plant viruses. Migratory endoparasitic nematodes can cause high yield losses in a variety of field crops. In addition to the direct damage they inflict on the host, these nematodes promote secondary bacterial and fungal infections. Examples of migratory endoparasitic nematodes include lesion nematodes (*Pratylenchus* spp.), burrowing nematodes (*Radopholus* spp.), and rice root nematodes (*Hirschmanniella* spp.). Sedentary endoparasitic plant-parasitic nematodes include cyst nematodes (*Heterodera* spp. and *Globodera* spp.) and root-knot nematodes (*Meloidogyne* spp.). Both cyst and root-knot nematodes induce hypermetabolic feeding sites in roots, which are the only source of nutrients for nematodes throughout their life cycle. While the host range of most *Meloidogyne* species tends to be broad, it remains rather narrow for most cyst nematode species.

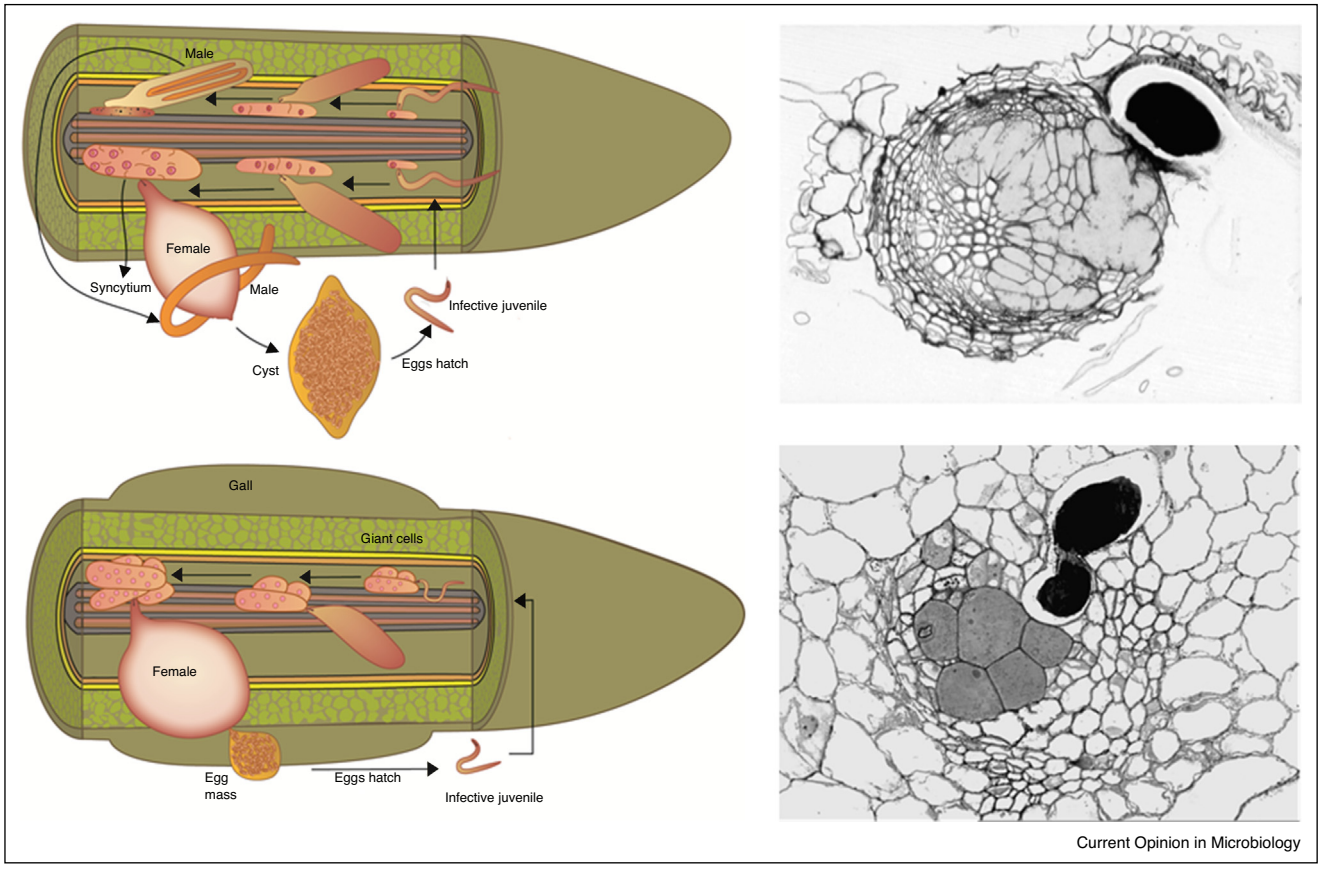
As obligate biotrophs, CN and RKN are entirely-dependent on plant-derived nutrients and solutes to fulfil their energy requirements throughout their weeks-long life cycles. Thus, both the syncytium and giant cells have evolved into a sink tissue that caters to the needs of the rapidly developing nematode. The cytoplasm of these feeding sites is dense and contains numerous organelles, including mitochondria, plastids, ribosomes, the Golgi apparatus, and the smooth endoplasmic reticulum. Furthermore, the central vacuole in these cells is replaced by several small vacuoles, and numerous ingrowths are formed at the cell wall interface with xylem cells, which are thought to increase the surface area for translocation of nutrients.

A series of transcriptomic, metabolomic, and proteomic analyses performed over the last decade showed that the genes and pathways involved in primary metabolism are specifically upregulated in both syncytia and giant cells [3–5]. As previous excellent reviews describe the metabolism and functioning of feeding sites [6–8] and discuss the modulation of plant immunity in response to nematode infection [9,10], we will focus on recent progress in understanding the formation and functioning of both types of feeding sites. The first section of our review explores homeostasis of two crucial plant hormones (cytokinin and gibberellin) that facilitate the formation and functioning of nematode feeding sites. The second section reviews current progress in understanding the role of small RNAs in syncytium and giant cell formation. The last section highlights our nascent understanding of nematode-associated secretions that are released into hosts to facilitate various aspects of parasitism.

Changes in hormone homeostasis during the formation of feeding sites

The involvement of various plant hormones in plant–nematode interaction is well-documented, and we refer readers to previous excellent reviews on the roles of auxin, salicylic acid, jasmonic acid, and ethylene [6,11]. Research during the last few years has established cytokinins and gibberellins as important players in nematode

Figure 2



Life cycle of cyst and root-knot nematodes.
(a and **b)** Second stage infective juveniles of **cyst nematodes** hatch from the eggs and enter host roots near the tip. They pass the different tissue layers and invade the vascular cylinder, where they select a single root cell to induce the formation of a syncytium. The juveniles commance feeding, lose their ability to move and increase in size. Within about two weeks they undergo three moults to reach adulthood. Females become lemon-shaped and continue feeding for about another two weeks. Female associated syncytia are large and include several hundred root cells. Male juveniles stop feeding after the third stage; the syncytia remain small. During their consecutive moults they regain a vermiform body shape and mobility. The adult males hatch from the juvenile cuticle and mate with females. After mating, females produce several hundred eggs which mostly remain within in the female body. The females die and their body wall turns into a robust cyst harbouring several hundred eggs that may stay viable in the soil for more than a decade.
(c and **d)** Second stage infective juveniles of **root-knot nematodes** hatch and penetrate roots behind the tip. They migrate towards the root tip meristem where they turn round and enter the vascular cylinder. They cease to migrate in the differentiation zone and select five to seven cells to induce the formation of giant cells. As soon as they commance feeding, they start to develop, lose their ability of locomotion and increase in size. Plant tissue surrounding the growing juvenile proliferates and forms a gall – the root-knot. The most important root-knot nematode species reproduce via parthenogenesis. After moulting three times, the adult female starts to lay eggs into a gelatinous matrix that is secreted on the root surface and embeds and protects the eggs until the juvenile hatch. N = female nematode, X = xylem, asterisks = syncytial feeding cells (2 A) and giant cells (2B), arrow heads = remnants of dissolved plant cell walls, scale bar = 20 μm.

feeding site formation, and we will therefore review recent advances in understanding the role of these two hormones in CN and RKN parasitism.

One of the first events induced by cyst and root-knot nematodes upon feeding site development is activation of the host cell cycle [12]. It is generally assumed that nematodes manipulate the production of the phytohormone cytokinin and modulate downstream signalling events to activate cell division [13]. Nematodes have been shown to produce cytokinin *in vitro* [14]; however, whether the hormone is secreted into host plants to facilitate parasitism was unknown. In three recent publications, it has been shown that both CNs and RKNs induce cytokinin signalling at their feeding sites and also in the neighbouring cells destined to be incorporated into the feeding sites. Moreover, Arabidopsis plants impaired in cytokinin biosynthesis or cytokinin signalling are significantly less susceptible to infection with both CNs and RKNs. Cytokinin signalling was also shown to be required for cell cycle activation at the site of infection, thus playing a key role in the expansion of both syncytia

and giant cells [15••,16•,17]. Notably, CNs not only produce cytokinin but also release it into infected tissues to activate the host cell cycle at and around the syncytium. These results clearly showed that cytokinins are central to feeding site formation and expansion. Intriguingly, however, mutant plant lines with increased sensitivity to cytokinin are also less susceptible to CNs, due to heightened immune responses in these plants [17]. Based on these recent studies, we suggest that a tightly controlled activation of cytokinin is required for optimal development of both CNs and RKNs.

Although cytokinin seems to play a similar role in ontogeny of both syncytia and giant cells, differences have been identified in the regulation of cytokinin biosynthesis, catabolism, and signalling genes in response to infection with CNs and RKNs, suggesting that these two types of nematode manipulate the cytokinin signalling pathway differently [16•]. For example, a cytokinin-synthesizing gene has been identified in CNs and characterised for its role in parasitism [15••], whereas identification of similar genes from RKNs has remained elusive. Considering that much higher levels of cytokinins have been previously detected in RKNs as compared to CNs [14], however, it is reasonable to assume that cytokinin-synthesizing genes are also present in RKN.

Gibberellic acids (GA, or gibberellins) are a large family of tetracyclic diterpenoids that regulate a variety of growth and developmental processes in plants. Previous studies indicated the possible involvement of GA in response to infection with CNs and RKNs [18–20]. In a recent study, Yimer and colleagues performed an in-depth characterization of the role of GA in rice upon infection with a RKN. They found that RKN infection leads to accumulation of a specific GA, GA12, at the infection site. Moreover, rice mutants impaired in GA biosynthesis or signalling were less susceptible to infection with RKN. Notably, their detailed functional characterization showed that GA mediates susceptibility to nematodes by suppressing defences regulated by jasmonic acid (JA) during RKN infection [21••]. Interestingly, GA-induced susceptibility to nematodes also depends on auxin transport, as treatment of rice plants with NPA (*N-1-naphthylphthalamic acid*), a polar auxin transport inhibitor, reduced rice susceptibility to RKN. Jasmonic acid also functions in plant defence against CNs [22], and it is therefore plausible that GA might similarly suppress JA-related defences during CN infection.

The role of microRNAs (miRNAs) in plant–nematode interactions

The formation of nematode feeding sites is accompanied by massive transcriptomic changes [4,5]. Until recently, the details of the mechanisms that lead to such a global transcriptional shift remained mostly unknown, but emerging evidence points to the importance of microRNAs (miRNAs). These small non-coding RNAs regulate gene expression by binding to their target messenger RNA (mRNA), leading to mRNA degradation, translational repression, or altered transcriptional activity [23]. A number of studies have documented changes in miRNA expression in response to infection by CNs and RKNs in Arabidopsis [24–26], tomato [27], soybean [28,29], and cotton [30]. Notably, plant mutants impaired in miRNA processing have reduced susceptibility to both CNs and RKNs, indicating a role for miRNAs in feeding site formation [24,31].

Recent studies have focused on the mechanisms by which individual miRNA families contribute to transcriptome reprogramming during the formation of syncytia and giant cells (Table 1). For example, *miR858* has been shown to play a role in syncytium formation by regulating the expression of its target transcription factor *MYB83*. Constitutive overexpression of *miR858* leads to decreased susceptibility to CNs, while reduced abundance of *miR858* leads to increased susceptibility. Similarly, overexpression of a modified version of *MYB83* that cannot be cleaved by *miR858* leads to increase susceptibility to CNs. Notably, transcriptome analysis of *MYB83* overexpression lines showed that 16.6% of the syncytial transcriptome is regulated by *MYB83*, indicating a role for a *miR858-MYB83* regulatory system in gene expression modulation during CN parasitism [32••]. Together, these different studies make it clear that host miRNA pathways are powerful targets for nematodes to modulate large-scale changes in gene expression inside their feeding site. However, the mechanisms by which nematodes are able to manipulate the host miRNA expression remain unexplored. We speculate that nematodes may release effectors that interfere with the host miRNA biogenesis pathways, thus regulating the expression of specific classes of miRNAs.

Plant use a defence mechanism called host-induced gene silencing (HIGS) in which small RNAs produced within the host silence the expression of targeted pathogen or parasite mRNAs in *trans*. Plant-based HIGS is effective

Table 1

MicroRNAs (miR) involved in cyst nematode and root-knot nematode feeding site formation

miRNA	Target mRNA	Host plant	Nematode	Ref
miR858	*MYB83*	Arabidopsis	*H. schachtii*	[32••]
miR827	*NLA*	Arabidopsis	*H. schachtii*	[37]
miR396	*GRF1/GRF3*	Arabidopsis	*H. schachtii*	[38]
miR390	*ARFs*	Arabidopsis	*M. javanica*	[25]
miR159	*MYB33*	Arabidopsis	*M. incognita*	[31]
miR319	*TCP4*	Tomato	*M. incognita*	[27]

NLA, Nitrogen Limitation Adaptation; GRF, Growth-Regulating Factor; ARF, Auxin Response Factors; TCP, Teosinte branched Cycloidea Proliferating cell nuclear antigen factor.

against fungi, insects, nematodes, and parasitic plants [33]. These findings suggest that the exchange of small RNAs between plants and pathogens/parasites might be a common feature of plant–pathogen/parasite interactions. Based on these observations, we speculate that miRNAs from nematodes may similarly act *trans*-species to regulate large-scale changes in host gene expression.

Nematode effectors at the heart of CN and RKN parasitism

The formation of syncytia and giant cells is facilitated by the release of a cocktail of proteinaceous (effectors) and non-proteinaceous secretions inside the host cell. CN and RKN effectors can be separated into two classes based on their functions: firstly, suppression of host immune responses, secondly, formation and functioning of feeding site. An increasing number of effectors belonging to either of these two classes have been characterized over the past few years [34,35]. However, the mechanistic details by which effectors manipulate host genes and pathways have been addressed only in a few cases. For example, a number of nematode effectors have been shown to suppress host immune responses, but the exact mechanism by which these effectors achieve such suppression remains unknown. We have summarized the CN and RKN effectors that have been described during the last three years in Table 2.

Efforts to predict effectors (proteinaceous secretions) generally begin with *in silico* analysis for firstly, the presence of an N-terminal signal peptide that directs the protein into the secretory pathway, secondly, the absence of a transmembrane domain, and thirdly, similarity to available data from other nematode species. Further characterization of a putative effector often includes transcript localization by *in situ* hybridization and expression profiling during nematode development. Although these steps may help unravel the putative function of the effector, the actual localization of effectors inside the host tissues is rarely confirmed (e.g. through immunolabelling), thus making it difficult to exclude the possibility that these effectors are never secreted into host tissues.

Considering that effectors constitute only a minority of nematode-secreted proteins, additional tools are needed to establish that a secretory protein is a bona fide effector. Excitingly, a recent study identified a 6-bp (ATGCCA) dorsal gland promoter element (DOG box) that is highly-enriched in the promoter regions of several established dorsal gland effectors in cyst nematodes [36••]. Furthermore, genes with more DOG boxes in their promoter regions were more likely to encode a signal peptide for secretion. The discovery of the DOG box has opened the door to the development of tools to distinguish effectors from other secreted proteins. In the future, more such tools are needed to identify the effectors with a role in parasitism.

Future perspectives

The recent progress in understanding plant–nematode interactions underscores the necessity to elucidate the integrated molecular framework that explains how nematodes are able to form and maintain their unique feeding sites inside the plants. The research during the last several years has identified nematode secretions as key to feeding site formation and maintenance. It is becoming increasingly clear that nematodes release not only proteinaceous but also non-proteinaceous molecules to manipulate the host cell machinery. A challenge for the future will be to establish assays and tools that can better identify these secretions. It will also be crucial to develop transformation protocols for PPNs, so that we can specifically interfere with various aspects of parasitism and study the consequence of such manipulations on the infection process.

Table 2

Cyst nematode and root-knot nematode effectors involved in facilitating parasitism

Effector	Nematodes	Putative function	Ref.
MjTTL5	*M. javanica*	Immune suppression via activation of ROS scavenging	[39]
MeTCTP	*M. enterolobii*	Immune suppression via unknown mechanism	[40]
MgGPP	*M. graminicola*	Immune suppression via unknown mechanism	[41]
MiMsp40	*M. incognita*	Immune suppression via unknown mechanism	[42]
HgGLAND18	*H. glycines*	Immune suppression via unknown mechanism	[43]
HsTyr	*H. schachtii*	Feeding site formation via unknown mechanism	[44]
10A07	*H. schachtii*	Feeding site formation by manipulating post-translational machinery	[45]
GS	*G. pallida*	Feeding site formation via modulation of redox homeostasis	[46]
MiSGCR1	*M. incognita*	Facilitates early stages of infection via unknown mechanism	[47]
MiPFN3	*M. incognita*	Feeding site formation via manipulation of actin filaments	[48]
HsPDI	*H. schachtii*	Feeding site formation via modulation of redox homeostasis	[49]
CLE41/44	*H. schachtii*	Feeding site formation via vascular stem cell pathway manipulation	[50]
Hs30D08	*H. schachtii*	Feeding site formation via interaction with host SMU2 protein	[51]
HsGLAND4	*H. schachtii*	Feeding site formation via binding to promoter of *LTP* genes	[52•]

Conflict of interest statement

Nothing declared.

Acknowledgements

We apologize to all authors whose work could not be cited due to space limitations. We gratefully acknowledge Miroslaw Sobczak for providing microscopic pictures. Shahid Siddique was supported by grants from German Research Foundation (SI 1739/3-1 and SI 1739/5-1).

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- •• of outstanding interest

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