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A hypothesis: CRISPR-Cas as a minimal cognitive system

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Abstract

Concerning what signifies the minimal requirements for a process to be designated cognitive, various criteria have been proposed, but the problem has not been settled. The important thing to consider in establishing the criteria is which criterion has stronger explanatory power. Recent developments in immunology demonstrate that the immune system is omnipresent in the realm of living beings, including bacteria and archaea. Although the structural characteristics of immune systems are significantly different among species, the fundamental functional components, namely, recognition, information integration, reaction, and memory, are well conserved. Interestingly, these adaptive features are superimposed on those of the central nervous system. Given that adaptive cognitive ability is a prerequisite for the existence and the survival of living organisms, these results may be compatible with the idea that in bacteria without an apparent nervous system, the immune system performs neural-like functions. The presence of the clustered regularly interspaced palindromic repeats (CRISPR)-CRISPR associated protein (Cas) systems as a cognitive system in the earliest living organisms suggests that one of the fundamental functions is conserved throughout evolution. Furthermore, this interpretation can evade the critiques against the current biological paradigm that demand that cognitive mechanisms be preceded by organisms in the earlier stages of evolution, thus providing better and stronger explanatory power. I thus propose that genetic and biochemical machinery represented by the bacterial immune system serve as a minimal cognitive system.

1. Introduction

The nature of cognition has been discussed, especially the minimal requirements for a process to be recognized as cognitive (Baluška & Levin, 2016; Barandiaran & Moreno, 2006; Calvo & Baluška, 2015; Garzón & Keijzer, 2011; Godfrey-Smith, 2016; Keijzer, 2003; Lyon, 2015; McGregor, 2018; van Duijn, Keijzer, & Franken, 2006). There is a wide spectrum of views concerning the definition of cognition. The perspective called “brain-centrism” or “neurocentrism” represents one view. This traditional view claims that the cognitive process is strictly related to the presence of the brain, brain-related structures such as neurons, or neuron-associated electrophysiological functions. According to this view, the activities of organisms without brain or neuron-associated structures and functions cannot be classified as cognitive. Recent severe critiques on ‘plant neurobiology’ revealed that this view is still prevalent in biology (Alpi et al., 2007; Struik, Yin, & Meinke, 2008). At the other end of the spectrum, there are perspectives in which sensorimotor coordination elicited by the reception of external signals constitutes a sufficient condition for cognition (van Duijn et al., 2006). According to this perspective, motor elements in the output are a prerequisite for minimal cognition, and a typical example is chemotaxis in bacteria. These authors think that biochemical reactions without motor components, such as the *lac operon* system in *E. coli* are not cognitive (van Duijn et al., 2006). Recently, a more inclusive view on cognition has been developed (Baluška & Levin, 2016; Lyon, 2015). Information processing and decision making are implemented by various biological organisms and systems, and cognition should include a set of mechanisms in which information acquisition, storage, processing and use occur at any level of organization. These definitions also imply that the nervous system is not required for cognition, stressing the importance of a physiological or functional criterion rather than anatomical or

structural composition.

It seems that under any given criterion for minimal cognition, the difference between the organisms with cognitive capacity and those without it can be relatively well demarcated. In other words, each definition is equally valid within the framework of the definition selected and one can choose whichever definition one prefers and describe the phenomena by that definition. The problem is that there is no definite consensus as to which criterion should be adopted for the general discussion on cognition and that there has been little discussion on memory as an indispensable factor of cognition with a few exceptions (Baluška & Levin, 2016; Lyon, 2015; van Duijn et al., 2006). The definition that has broader explanatory power with respect to the structural, functional and evolutionary viewpoints is more important.

In immunology, similar problems have been discussed in terms of what constitutes the immune system. As in the case of brain-centrism, the traditional view puts emphasis on the requirement for structural elements, such as lymphocytes and antibodies, which first appear in the jawed fish. However, it has recently been proposed that the immune system exists in organisms without lymphocytes and antibodies. In fact, the system is present in almost all living organisms starting from bacteria and archaea (Rimer, Cohen, & Friedman, 2014). The clustered regularly interspaced palindromic repeats (CRISPR)-CRISPR associated protein (Cas) systems are considered to be the immune systems of bacteria and archaea (Bolotin, Quinquis, Sorokin, & Ehrlich, 2005; Brouns et al., 2008; Karginov & Hannon, 2010; Lillestöl, Redder, Garrett, & Brugger, 2006; Marraffini & Sontheimer, 2008, 2010a; Mojica, Diez-Villasenor, Garcia-Martinez, & Soria, 2005; Pourcel, Salvignol, & Vergnaud, 2005; Sorek, Koonin, & Hugenholtz, 2008; Sorek, Lawrence, & Wiedenheft, 2013; Westra et al., 2012). Thus, the CRISPR-Cas systems are instrumental to search for the minimal requirements for the immune system. CRISPR-Cas

systems exhibit four functional processes: the recognition of invading DNA sequences, integration of the information received, response by destroying foreign DNA according to the integrated information, and memory of these experiences. These functional components are present in the immune systems of all living organisms; however, the structural constitutions are considerably diverse (Rimer et al., 2014). In this article, I will discuss why the bacterial immune system provides broader and inclusive criteria for minimal cognition.

2. Recent findings in immunology

2.1. The immune systems of bacteria and archaea: CRISPR-Cas

A genetic mechanism called the CRISPR-Cas system serves as a defense mechanism for approximately 40% of bacteria and approximately 90% of archaea to address an attack of bacteriophages and plasmids (Horvath & Barrangou, 2010; Marraffini & Sontheimer, 2010a). Since the original discovery of a CRISPR repeat (Ishino, Shinagawa, Makino, Amemura, & Nakata, 1987), many reports of similar genes have accumulated. The CRISPR acronym was coined in 2002 (Jansen, Embden, Gaastra, & Schouls, 2002), but the functional attributes of CRISPRs were not clear at that stage. A CRISPR locus is now defined as an array of short direct repeats interspersed with spacer sequences flanked on one side by a leader sequence that serves as a promoter for the transcription of the repeated elements (Figure 1). Whereas the repeats are identical in length and sequence (typically, 21-48 nucleotides), the spacers are highly variable in sequence. The number of spacers within a particular locus varies and reaches in some cases more than 500. Chromosomes may have multiple CRISPR loci, as evidenced in databases (Grissa, Vergnaud, & Pourcel, 2007; Rousseau, Gonnet, Le Romancer, & Nicolas, 2009). Spacer sequences most often match those of phages and other extrachromosomal elements (Bolotin et al.,

2005; Mojica et al., 2005; Pourcel et al., 2005). *Cas* genes are found in close proximity to the CRISPR loci. Cas proteins have sequence characteristics of functional domains, such as endonucleases (Haft, Selengut, Mongodin, & Nelson, 2005; Makarova, Aravind, Wolf, & Koonin, 2011; Makarova, Grishin, Shabalina, Wolf, & Koonin, 2006; Makarova, Haft, et al., 2011).

Interestingly, bacteria containing the defined spacer sequences are resistant to the corresponding phages, whereas bacteria without them are susceptible to infection. Further evidence suggests that in response to each phage infection, bacteria integrate new spacers derived from phage genomic sequences into CRISPR arrays, which confer resistance to subsequent phage infection. Foreign DNA elements are sequentially integrated in a polarized fashion next to the leader sequence (Barrangou, 2007; Lillestöl et al., 2006; Pourcel et al., 2005). Thus, the spacer content reflects different phages and plasmids that the host has encountered in a chronological order, suggesting that the CRISPR genetic system is equipped with a form of immunological memory that ensures the rejection of invading DNA molecules. All these findings point to the possibility that the CRISPR-Cas system is an adaptive and heritable immune system in bacteria and archaea. The fact that an environmental element is incorporated into the host genome as a form of memory and is handed down to daughter generations suggests that the CRISPR-Cas systems represent Lamarckian inheritance (Koonin & Wolf, 2009).

The CRISPR-Cas action is generally divided into three functional steps: (1) adaptation or spacer acquisition into the CRISPR array, (2) transcription of CRISPR loci and processing of the resulting long primary CRISPR RNA transcripts (pre-crRNA) into small RNAs (crRNAs), and (3) interference or silencing of either RNA or DNA (Figure 1). During the acquisition step, the Cas proteins generate short pieces of the bacteriophage or plasmid DNA, named protospacers, which are then integrated as new spacers at the leader side of the CRISPR array. The 3- to

4-nucleotide long sequences located immediately downstream of the protospacer called protospacer adjacent motifs (PAMs) are key to the selection and the integration of the protospacer. The memory in this case is based on an acquisition of a new genetic trait by horizontal gene transfer or a modification of a genome influenced by the environment. Given that recognition of PAMs by Cas9 is required for spacer selection during spacer acquisition, the Cas protein actively participates in the formation of immunological memory in bacteria (Heler et al., 2015). In the second expression and processing step, the CRISPR repeat-spacer array is transcribed into a pre-crRNA, which is further processed into a set of small crRNAs by endonucleases encoded by the *cas* gene. The crRNAs contain variable spacers complementary to the invading nucleic acids and a partial conserved repeat. In the final interference step, the crRNAs, which form a ribonucleoprotein effector complex with Cas proteins, recognize the target sequence in the invading nucleic acid (protospacer) by base-pairing to the complementary strand of DNA. This process induces sequence-specific cleavage, thereby preventing proliferation and propagation of foreign genetic elements. Thus, in contrast to other bacteriophage-resistance mechanisms, such as the restriction-modification (R-M) system, which provides invader-nonspecific innate immunity, CRISPR-Cas functions as invader-specific and heritable microbial immunity that confers acquired resistance against viruses and plasmids.

2.2. Similarities between the CRISPR-Cas and human immune systems

Of note, many similarities exist between bacterial and human immunity. First, the acquisition step of CRISPR-Cas is analogous to recognition of or immunization with an antigen and to memorization of that experience in mammalian immunity. Without further encounters with the same bacteriophage, the CRISPR spacer is rapidly degraded. In other words, this memory is

short-lived such that continuous selective pressure is required for maintaining memory (Andersson & Banfield, 2008; Makarova et al., 2006). Controversies exist regarding the requirement for antigen for maintaining T cell memory. Classic studies demonstrated that memory T cells display low requirements for antigen. T cells proliferate in response to lower amounts of antigen than naïve T cells. However, recent studies demonstrate an increased antigen requirement for memory T cells (Mehlhop-Williams & Bevan, 2014), underlining the similarity in memory maintenance between bacteria and highly evolved species.

Second, the expression and interference steps of CRISPR-Cas are also analogous to the mammalian immune response of an immunized or a vaccinated subject against previously encountered antigens. A recent study demonstrated that bacteriophages defective in replication can also induce acquisition of spacers at a rate proportional to the quantity of replication-defective phages, further highlighting the similarity between bacterial and human immunity in vaccination with inactivated pathogenic agents (Hynes, Villion, & Moineau, 2014).

Third, in addition to CRISPR-Cas systems, the R-M system is involved in the inactivation of invading phages or plasmid DNAs. In this system, the restriction enzyme cleaves specific sites of invading DNA because it is not methylated, whereas the DNA of the host methylated by methyltransferase is protected. These two systems do not represent perfect defense mechanisms by themselves but can act collaboratively to augment the resistance of bacteria to foreign DNA (Dupuis, Villion, Magadán, & Moineau, 2013). The collaboration between the R-M and the CRISPR systems is reminiscent of the collaboration between innate and adaptive systems in mammalian immunity. These results altogether suggest that organisms situated at two extreme ends of evolution have structurally different but functionally remarkably similar elements of the immune system.

Furthermore, the CRISPR-Cas system has a mechanism to distinguish self from nonself because there is always a chance for the immune system to attack self, as evidenced in organisms at later stages of evolution. The question to be answered is how the crRNA identifies real targets without attacking the CRISPR locus in the host chromosome. From an analysis of CRISPRs from 330 organisms, one in every 250 spacers (0.4%) targeted 'self' genes and 59 out of 330 CRISPR-bearing organisms (17.9%) had at least one self-targeting spacer (Stern, Keren, Wurtzel, Amitai, & Sorek, 2010). Furthermore, 37% of all self-targeting spacers are found at the first or second positions in the CRISPR arrays (Stern et al., 2010). Given that the acquisition of spacers occurs first at the proximal side of the leader sequence, self-targeting spacers seem to be recent spacers and survive for a short period of time. The mechanism for self-nonself discrimination has been assessed in *Staphylococcus epidermidis* (Marraffini & Sontheimer, 2010b). According to their study, mismatches between crRNAs and a target at specific positions outside of the spacer sequence guarantee interference of invading DNA, whereas extended pairing between crRNA and CRISPR DNA repeats prevents autoimmunity. Furthermore, not only 'self' DNA but also relationship between the recognized 'self' DNA sequence and recognizing crRNA are important in bacterial systems. These two features are closely related to the situation of human immunity in which a repertoire of effector lymphocytes is modified in such a manner that self-attacking components are physically or functionally eliminated. In humans and mice, some hold the view that autoimmunity is not a cost of evolving immunity but plays a necessary, physiological function in the maintenance of the organism (Avrameas, 1991; Cohen & Cooke, 1986; Grabar, 1983). However, it is not currently clear whether autoimmunity exerted by CRISPR-Cas has a beneficial, teleonomic effect on bacteria and archaea; however, one example suggests that autoimmunity might play such a role (Vercoe et al., 2013).

Finally, recent accumulating evidence demonstrates that not all CRISPR-Cas systems are exclusively involved in the established role of DNA-encoded, RNA-mediated adaptive immunity. Rather, these systems have additional functions beyond immunity, suggesting further similarities with the human immune system. The most notable nonimmunological functions of a CRISPR-Cas system is endogenous transcriptional regulation (Barrangou, 2015; Westra, Buckling, & Fineran, 2014). Evidence of the involvement of *cas* genes in fruiting body development and sporulation of *Myxococcus xanthus* has been reported (Boysen, Ellehauge, Julien, & Søgaard-Andersen, 2002; Thöny-Meyer & Kaiser, 1993). The mechanism by which CRISPR-Cas systems regulate nonimmunological functions, including bacterial social behaviors, consists of sensing and integrating exterior information and reacting to original environmental cues by changing behavioral patterns. This three-phase mechanism is reminiscent of not only mammalian immune responses but also the functioning of the central nervous system in humans.

2.3. Essential features of the immune system

Based on the analysis above, several characteristics of the immune system have emerged. First, although the structure and the resulting mechanisms greatly differ, almost all living organisms, including plants and bacteria, are considered to be equipped with an immune system (Rimer et al., 2014). Given that a defect in the components of an immune system leads to the reduced survival or even death of the living organisms, immunity is indispensable for the existence and the survival of an organism without exception and constitutes one of the conditions for life. This notion also suggests that the only factor that counts for the definition of a given system is the functional attribute, not the structural composition.

Second, although immunological memory was thought to be a hallmark of adaptive

immunity found in organisms equipped with T and B lymphocytes, all living organisms with an immune system have a memory function; however, its structural and mechanistic representations significantly differ from species to species.

Third, the functional elements of immunity are generally summarized as follows: sensing elements in the environment, integrating the information received, reacting accordingly and efficiently, and memorizing each experience. If the term ‘essence’ is defined as the minimum characteristics that are shared by all members within a group, it is probable that these four functional characteristics, namely, recognition, information integration, reaction, and memory, constitute the essence of immunity. Significantly, these characteristics are almost completely superimposed on the functional attributes of the central nervous system.

3. CRISPR-Cas as minimal cognitive machinery

3.1. What is the problem of minimal cognition?

Cognitive capability has long been considered to uniquely belong to organisms with brain or brain-related structures and functions. This traditional and anthropocentric view, which is called neurocentrism or brain-centrism, remains dominant, as evidenced by recent severe critiques on the establishment of ‘plant neurobiology’ as a branch of biology (Alpi et al., 2007; Struik et al., 2008). On the other hand, discussions regarding minimal requirements for cognition suggest that cognitive capability may be decided independently of the presence or absence of brain, and that the boundary between organisms with cognitive capability and those without it becomes blurred (Baluška & Levin, 2016; Barandiaran & Moreno, 2006; Calvo & Baluška, 2015; Garzón & Keijzer, 2011; Godfrey-Smith, 2016; Keijzer, 2003; Lyon, 2015; McGregor, 2018; van Duijn et al., 2006). The most inclusive view of all is that necessary and sufficient conditions for cognition

include a set of mechanisms, namely, information acquisition, storage, processing, and use (Baluška & Levin, 2016; Lyon, 2015). According to this perspective, biochemical and genetic reactions without motility may be classified as cognition, such as the *lac* operon system in *Escherichia coli*. There are various perspectives between the two extremes. For example, one view claims that sensorimotor coordination induced by the perception of external signals is required for cognitive activities, and a typical example is chemotaxis in bacteria (van Duijn et al., 2006). There are also discussions regarding the requirement of cognitive elements, for example, decision making, for minimal cognition (Keijzer, 2003) or the setting of separate conditions for prokaryotes and eukaryotes (Calvo & Baluška, 2015). In general, these criteria function relatively well in differentiating cognitive from noncognitive capabilities within that particular framework. However, there is no consensus as to which criterion is the most pertinent for the definition of minimal cognition and the general discussion on this topic.

3.2. Why is the CRISPR-Cas system better qualified as a model of minimal cognition?

To resolve these problems, recent findings in the field of immunology provide a hint regarding the selection of criteria for minimal cognition. One of the lessons drawn from immunology is that an anthropocentric view should be abandoned to better understand the nature and essence of immunity. Based on the classical perspectives on immunity, it is unimaginable to refer to a system without lymphocytes and antibodies as an immune system. The traditional view heavily relies on the structural constitution. According to such a view, the CRISPR-Cas system cannot constitute the immune system. However, the community of immunologists accepts the definition of immunity based on the functional constitution, which broadens and deepens the discussion concerning immunity.

The criteria for the immune system, which is summarized as signal recognition, information integration, reaction, and memory, are consistent with the minimalist definition of cognition, namely, information acquisition, processing, use, and storage (Baluška & Levin, 2016; Lyon, 2015). Among these four functional elements, the requirement of memory often escapes attention and should be more emphasized. Thus, molecular processes, such as the *lac* operon system in bacteria, may be excluded from cognition. Chemotaxis in bacteria is considered a typical example of minimal cognition given sensorimotor coordination induced by signal perception (van Duijn et al., 2006). In this case, bacteria compare their past environment with their present environment by some type of 'memory' mechanism mediated by methylation and demethylation of receptors. However, this memory is characterized by a short duration, i.e., typically seconds (Macnab & Koshland, 1972; Vladimirov & Sourjik, 2009). Whether this type of posttranslational modification should be included in memory needs to be readdressed, because that definition will extremely expand the phenomena of memory.

In humans, there are two separate memories: immunological memory for maintaining the corporeal self and mental memory for maintaining the psychological integrity of self. In bacteria and archaea, immunological memory is maintained by CRISPR-Cas systems, but mental memory (or a nervous system) is apparently absent. This fact is thought provoking. If one accepts the proposition that the presence of adaptive and cognitive functions is indispensable for organisms' existence and survival, there must be some neural-like mechanisms even in bacteria and archaea. Given the evolutionarily conserved functional, but not structural, similarity between the central nervous system and the immune system, it may not be irrational to assume that signal-initiated cognitive mechanisms and control mechanisms of behavior exerted by the CRISPR-Cas system serve as neural-like function in bacteria and archaea. If this is the case, the

immune system serves as a more universal and fundamental cognitive system in living beings, and its range of existence is far wider than the nervous system defined by anthropocentric or neurocentric views.

This proposal is also effective in evading certain critiques against a current biological paradigm. One of the critiques claims that the following conditions should be met for cognitive mechanisms to be preserved in the paradigmatic framework of physicalism (Nagel, 2012). Among others, at least in later stages of evolution, cognitive mechanisms should play an essential role in the survival and reproduction of organisms. More importantly, these mechanisms should be preceded by organisms in the earlier stages of evolution. These critiques are easily refuted by defining the CRISPR-Cas system as a cognitive system in the earliest living organisms, but not by currently proposed criteria. Thus, it is obligatory to adopt a biochemical and genetic definition of cognitive capacity, which makes us change our perspectives on cognition and may be beneficial for the general discussion on this topic.

4. Conclusion

I have discussed the problem of minimal cognition from the perspectives of recent developments in immunology. A thorough analysis of the immune system of bacteria and archaea, the CRISPR-Cas system, led us to better understand the fundamental inner workings of the immune system and provided not only a functional but also an ontological link between the immune system and the nervous system. Furthermore, four minimal conditions for a cognitive process, namely, the recognition of signals in the milieu, integration of that information, subsequent efficient reaction based on the integrated information, and memorization of the experience, are better met by the CRISPR-Cas system. Thus, I propose that genetic and biochemical mechanisms

represented by the CRISPR-Cas system constitute a minimal cognitive system because this proposal provides broader explanatory power than what is currently available.

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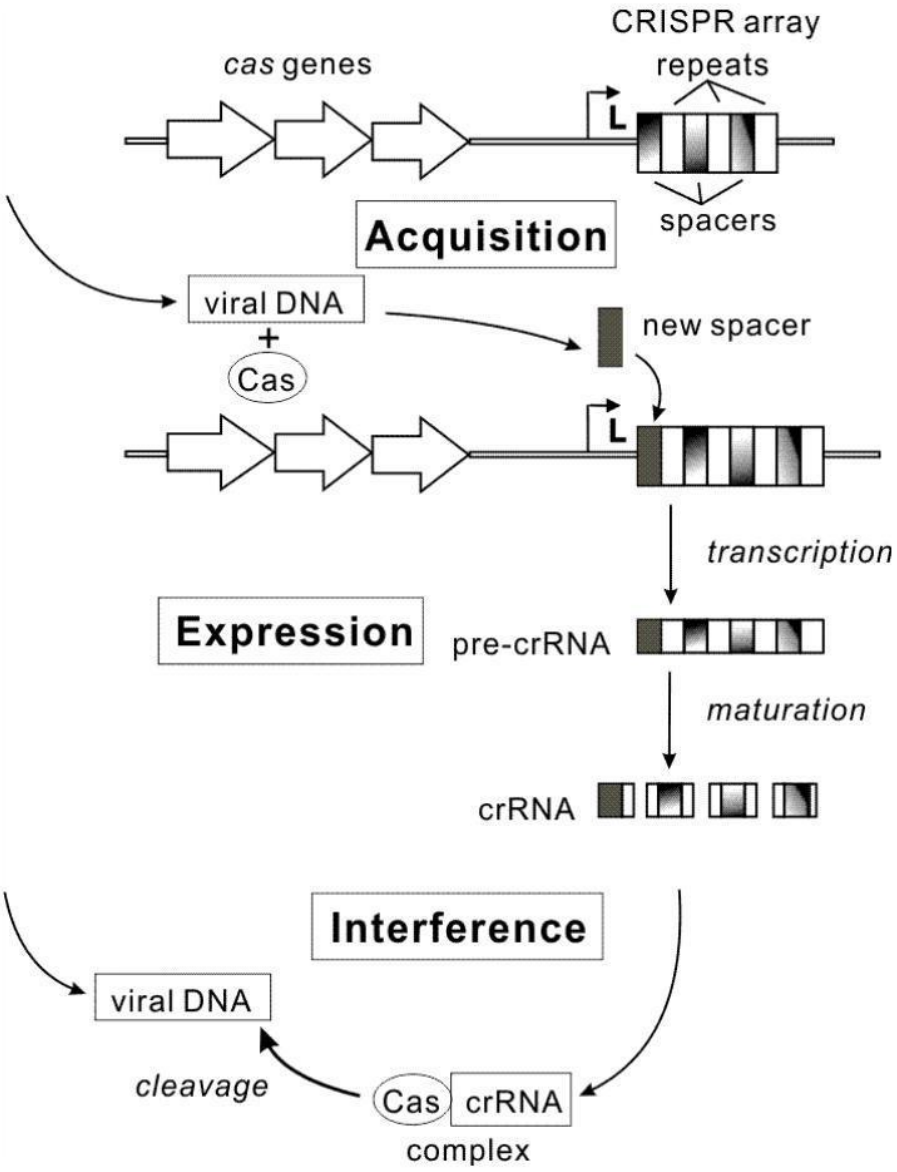

Figure 1 Simplified schematic representation of the CRISPR-Cas system. For details, please refer to the text. L: leader sequence.

About the Author

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