Virology 434 (2012) 202–209

Contents lists available at SciVerse ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Review

Memory of viral infections by CRISPR-Cas adaptive immune systems: Acquisition of new information

Peter C. Fineran [a,*], Emmanuelle Charpentier [b,**]

[a] Department of Microbiology and Immunology, University of Otago, PO Box 56, Dunedin 9054, New Zealand
[b] The Laboratory for Molecular Infection Medicine Sweden (MIMS), Umeå Centre for Microbial Research (UCMR), Department of Molecular Biology, Umeå University, Umeå S-90187, Sweden

ARTICLE INFO

Available online 2 November 2012

Keywords:
CRISPR-Cas
Cas1
Cas2
Bacteria
Archaea
Viruses
Mobile genetic elements
Horizontal gene transfer
Adaptive immunity
Spacer acquisition

ABSTRACT

Multiple organisms face the threat of viral infections. To combat phage invasion, bacteria and archaea have evolved an adaptive mechanism of protection against exogenic mobile genetic elements, called CRISPR-Cas. In this defense strategy, phage infection is memorized via acquisition of a short invader sequence, called a spacer, into the CRISPR locus of the host genome. Upon repeated infection, the 'vaccinated' host expresses the spacer as a precursor RNA, which is processed into a mature CRISPR RNA (crRNA) that guides an endonuclease to the matching invader for its ultimate destruction. Recent efforts have uncovered molecular details underlying the crRNA biogenesis and interference steps. However, until recently the step of adaptation had remained largely uninvestigated. In this minireview, we focus on recent publications that have begun to reveal molecular insights into the adaptive step of CRISPR-Cas immunity, which is required for the development of the heritable memory of the host against viruses.

Contents

Introduction

During their lifetime, bacteria and archaea face the constant threat of invading foreign DNA, mainly mobile genetic elements such as phages, plasmids, transposons and genomic islands. A gain of novel genetic traits can have a beneficial or detrimental consequence on the host. For example, the horizontal transfer of genetic elements contributes largely to the acquisition of antibiotic resistance by environmental and clinical bacteria. In addition, virulence determinants can be acquired, leading to toxigenic conversion of bacterial strains. A particular threat to bacteria and archaea are their viral predators. The global phage population is genetically diverse, their abundance exceeds bacterial numbers by an order of magnitude and an estimated 10^{25} infections occur every second (Hendrix, 2003; Weinbauer, 2004; Wommack and Colwell, 2000). Therefore, an arms race is said to exist between prokaryotes and their viruses and to survive phage infection, and control the flow of genetic information, bacteria and archaea have evolved diverse defense strategies (Labrie et al., 2010).

To counteract viral infections, eukaryotic organisms launch an immune response consisting of innate (or non-specific) and adaptive (or specific) mechanisms. Most viral infections are halted by the first line of innate immune defenses that are continuously

* Corresponding author.
** Corresponding author.
E-mail addresses: peter.fineran@otago.ac.nz (P.C. Fineran), emmanuelle.charpentier@mims.umu.se (E. Charpentier).

0042-6822/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.virol.2012.10.003

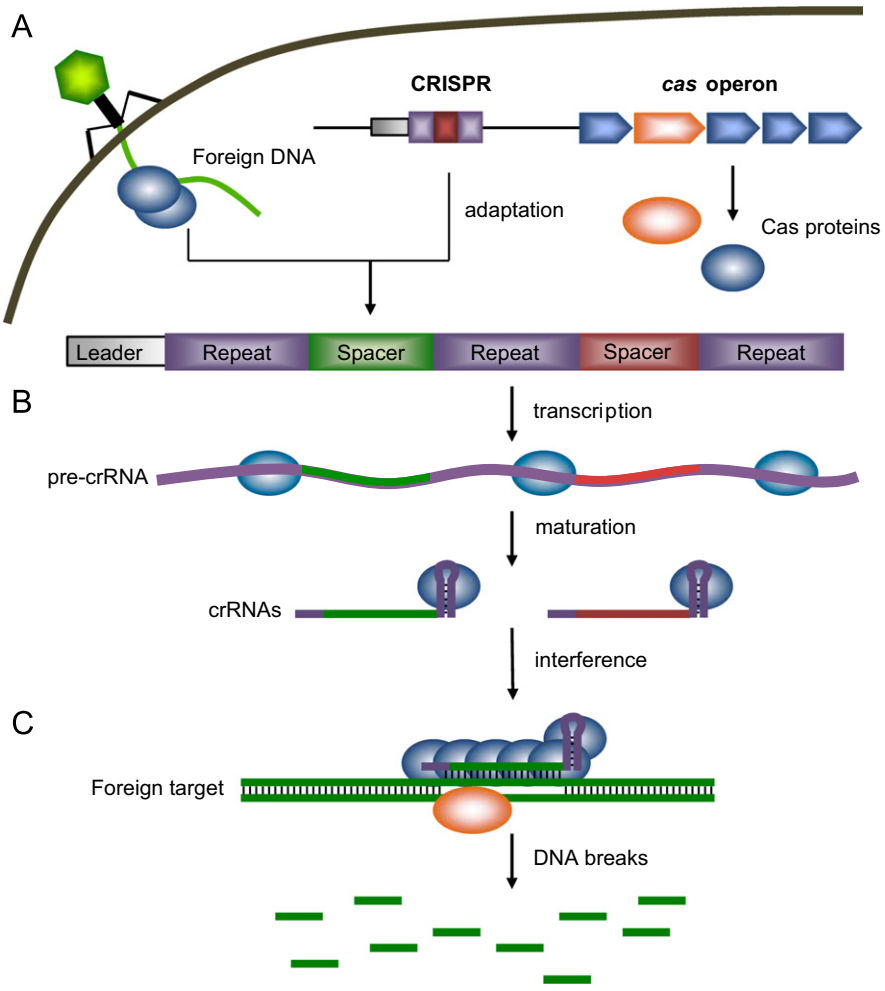

Fig. 1. Overview of CRISPR-Cas immunity to viruses of bacteria and archaea. (A) In adaptation, phage infection is recognized by Cas proteins (presumably the core Cas1 and Cas2) and a short sequence of the phage DNA (termed a protospacer, proposed to be now termed precursor-spacer or pre-spacer (Westra and Brouns, 2012)) is added to the leader end of the CRISPR array, resulting in a new spacer sequence and a duplicated repeat. Represented are the *cas* operon encoding the Cas proteins and the closely associated CRISPR array, composed of the leader sequence followed by a series of repeats-spacer units. (B) Transcription of the CRISPR array from a promoter within the leader sequence results in a precursor CRISPR RNA (pre-crRNA) transcript. The pre-crRNA is matured into individual crRNAs by a process involving Cas proteins. (C) The mature crRNAs form a ribonucleoprotein complex, which targets nucleic acids that are complementary to the spacer sequence in the crRNAs. In some cases a separate Cas nuclease (orange) is recruited, resulting in interference and destruction of the nucleic acid target. The general schematic is based on the type I system, and differences exist between the type I, II and III. For details, see the text.

active in the host without exposure to any virus. In cases when viral replication outpaces innate defenses, the host then mounts the adaptive response. Similar defense strategies against viral infection apply to microorganisms like bacteria and archaea (Bikard and Marraffini, 2012). Innate immunity against phages can be considered to involve the mechanisms of abortive infection, mutation of host receptors or restriction/modification of the incoming foreign DNA. However, in most cases these systems are not truly innate since they also display a degree of specificity. Recently, CRISPR (clustered regularly interspaced short palindromic repeats)-Cas (CRISPR-associated) has been discovered as an adaptive defense mechanism against phages (reviewed recently by Bhaya et al. (2011); Deveau et al. (2010); Horvath and Barrangou (2010); Marraffini and Sontheimer (2010); Terns and Terns (2011); van der Oost et al. (2009); Wiedenheft et al. (2012)). The system is heritable, widespread among bacteria and archaea and active in immunity against various mobile genetic elements.

CRISPR-Cas immunity is mediated by RNA and protein components that function together in ribonucleoprotein complexes. The CRISPR-Cas immune strategy consists of an adaptive phase with acquisition of memory, a biogenesis phase to generate the guide RNA components and a phase of interference of the invading cognate nucleic acids by ribonucleoprotein complexes consisting of Cas proteins and the guide RNAs (Fig. 1) (Bhaya et al., 2011; Deveau et al., 2010; Horvath and Barrangou, 2010; Marraffini and Sontheimer, 2010; Terns and Terns, 2011; van der Oost et al., 2009; Wiedenheft et al., 2012). "Adaptive" refers here to the specificity of the immune response that is customized to a particular foreign invader. A key feature in the adaptation phase of CRISPR-Cas is memory, whereby a repeated infection by the same phage is stopped immediately by the specific response. The loci are commonly composed of an array of repeat-spacer sequences encoding the RNA components and an operon of *cas* genes encoding the protein components. The array consists of a leader sequence followed by a succession of short identical repeats regularly interspaced by short spacer sequences. The spacer sequences originate from previous encounters with foreign genetic material and thus function as a memory bank that will recognize the same genetic encounter upon a repeated infection. Briefly, CRISPR-Cas immunity operates as follows. Upon infection with the genetic intruder, a short sequence of the invading DNA (termed a protospacer, proposed to be now termed precursor-spacer