Antiviral Research 94 (2012) 242–257

Contents lists available at SciVerse ScienceDirect

Antiviral Research

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

Review

# Alphaviruses: Population genetics and determinants of emergence

Scott C. Weaver [a,*], Richard Winegar [b,1], Ian D. Manger [b,2], Naomi L. Forrester [a]

[a] Institute for Human Infections and Immunity and Department of Pathology, University of Texas Medical Branch, Galveston, TX, United States
[b] SRI International, Menlo Park, CA, United States

ARTICLE INFO

*Article history:*
Received 25 November 2011
Revised 5 April 2012
Accepted 7 April 2012
Available online 19 April 2012

*Keywords:*
Alphavirus
Arbovirus
Genetics
Ecology
Epidemiology
Biodefense

ABSTRACT

Alphaviruses are responsible for several medically important emerging diseases and are also significant veterinary pathogens. Due to the aerosol infectivity of some alphaviruses and their ability to cause severe, sometimes fatal neurologic diseases, they are also of biodefense importance. This review discusses the ecology, epidemiology and molecular virology of the alphaviruses, then focuses on three of the most important members of the genus: Venezuelan and eastern equine encephalitis and chikungunya viruses, with emphasis on their genetics and emergence mechanisms, and how current knowledge as well as gaps influence our ability to detect and determine the source of both natural outbreaks and potential use for bioterrorism. This article is one of a series in Antiviral Research on the genetic diversity of emerging viruses.



**Contents**



* Corresponding author. Fax: +1 409 266 6810.
*E-mail address:* sweaver@utmb.edu (S.C. Weaver).
[1] Current address: MRIGlobal, 1470 Treeland Blvd., Palm Bay, FL 32909, United States.
[2] Current address: Synbody Biotechnology, 400 Farmington Ave., Farmington, CT 06032, United States.

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http://dx.doi.org/10.1016/j.antiviral.2012.04.002

## 1. Family overview

### 1.1. Systematics, genera and species in the family

The family *Togaviridae* includes the genera *Alphavirus* and *Rubivirus* (the latter includes only rubella virus, which differs in many ways from the alphaviruses, and is not discussed further here) (Weaver et al., 2005). The alphaviruses include 29 different species of positive-strand RNA viruses that cause a wide variety of diseases in humans, domesticated and wild terrestrial vertebrates, as well as in fish (Fig. 1, Table 1). Alphaviruses of greatest immediate importance in the US as naturally emerging pathogens and/or potential biological weapons include eastern (EEEV), western (WEEV), and Venezuelan equine encephalitis (VEEV) viruses (Smith et al., 2009). Chikungunya virus (CHIKV) is an emerging alphavirus that has caused recent outbreaks in Asia, the Indian Ocean and Europe and has the potential for spread to the Continental US and other parts of the Americas (Tsetsarkin et al., 2011b).

The taxonomic structure of the alphavirus genus was originally organized according to antigenic relationships determined in serologic assays (Calisher and Karabatsos, 1988). This originally resulted in the identification of 7 antigenic complexes of mosquito-borne alphaviruses based on levels of cross-reactivity: EEE, VEE, WEE, Semliki Forest, Barmah Forest, and Middelburg. More recently, antigenic analyses have been supplanted to a large extent by genomic sequence comparisons, so some recently described alphaviruses such as Trocara have been assigned to complexes based on genetic data (Powers et al., 2001). Therefore, the current organization of the family, which includes 7 complexes, is a composite of antigenic and genetic complexes.

### 1.2. Viral structure

Fig. 2 shows the structure of VEEV, which is nearly identical to that of other alphaviruses examined, including the icosahedral nucleocapsid and envelope glycoprotein shell that is embedded in the plasma membrane-derived envelope (Kuhn, 2007). The nucleocapsid is composed of 240 capsid protein monomers and one genomic RNA molecule. The envelope glycoproteins form 80 trimer spikes, each spike consisting of 3 glycoprotein E1/E2 heterodimers. The virus attaches to host cell receptors though the E2 glycoprotein and the E1 protein includes a fusion peptide that mediates entry of nucleocapsids into the cytoplasm from endosomes.

### 1.3. Genome organization and viral replication

The genome of alphaviruses (Fig. 3) is a single stranded positive, sense RNA typically 11.4–11.8 kB in length (Kuhn, 2007). It includes a 5′ cap, and 3′ poly-A tail, and encodes 2 open reading frames (ORFs) for the non-structural and structural polyproteins, respectively. The nonstructural ORF encodes proteins for transcription and replication of viral RNA, polyprotein cleavage, and RNA capping; the structural ORF encodes the capsid protein, envelope glycoproteins E2 and E1. The expression of these proteins and replication of the viral genome all take place in the cytoplasm of the host cells, although the nsP2 and/or capsid proteins of some alphaviruses enter the nucleus where they interfere with host cell gene transcription (Aguilar et al., 2007b; Garmashova et al., 2007).

Alphaviruses replicate in the cytoplasm of cells after entry via receptor-mediated endocytosis. Several receptors have been identified for alphaviruses but they remain poorly defined for both vertebrates and mosquito vectors because most are ubiquitous and cannot explain the specificity of alphavirus–host interactions. Recently, a divalent metal ion transporter natural resistance-associated macrophage protein (NRAMP) was shown to be required for Sindbis virus (SINV) binding and entry into Drosophila cells (Rose et al., 2011). The NRAMP2 variant, a ubiquitously expressed vertebrate homolog, also appears to function as a receptor for infection of mammalian cells. Alphavirus glycoprotein chimeras demonstrated that the requirement for NRAMP2 is at the level of Sindbis virus entry. Given the conserved structure of alphavirus glycoproteins, and the widespread use of transporters for viral entry, other alphaviruses may use conserved multipass membrane proteins for infection. Fusing of the virion envelope with the endosomal membrane occurs after low pH-induced conformational changes in the envelope glycoproteins that expose a hydrophobic peptide in the E1 protein for insertion into the membrane. This results in release of the nucleocapsid into the cytoplasm and initiation of replication following their binding to ribosomes, which triggers the release of the genomic RNA for cap-dependent translation (Kuhn, 2007). Most alphaviruses contain a stop codon near the end of the nsP3 gene that is read-through at low frequency, resulting in larger amounts of nsP1–3 than nsP1–4 polyproteins. In concert with host factors, the nsPs mediate replication of a complementary, negative strand genomic RNA, followed by the production of positive strand genomic and subgenomic RNAs. Minus strand replication is favored early during infection when the nsPs are mostly uncleaved. Later, more complete cleavage of the nsPs favor plus strand synthesis, leading to higher production levels of viral proteins, particularly the structural proteins. Capsid proteins are cleaved cotranslationally in the cytoplasm from the structural polyprotein, while the remainder of the polyprotein enters the endoplasmic reticulum. Following additional cleavages mediated by cellular proteases and glycosylation, E2/E1 dimers are embedded into the plasma membrane. Ultimately, the capsid proteins within nucleocapsids interact with cytoplasmic tails of the E2 protein to initiate budding to produce enveloped virions.