Regulation of Equine Infectious Anemia Virus Expression

Equine infectious anemia virus (EIAV) is a retrovirus within the subfamily of Lentiviridae. The Lentiviridae also include the primate lentiviruses, human immunodeficiency virus (HIV-1 and HIV-2) and simian immunodeficiency virus, as well as feline immunodeficiency virus and the ungulate viruses, bovine immunodeficiency virus, visna and caprine arthritis encephalitis virus. As the causative agent of equine infectious anemia (EIA), EIAV outbreaks can have important economic impacts throughout the world [24, 49]. In the United States, spread of EIAV is controlled through surveillance programs that include identification of seropositive horses by an immunoagar diffusion test (Coggins test) and quarantine of seropositive animals. While this program has controlled the spread of EIAV in the US, the virus remains a worldwide problem. Recent vaccine attempts to control EIAV have been unsuccessful [27, 50, 118].

Recent studies with HIV [76, 103] have confirmed previous studies with EIAV [53, 55] demonstrating that during lentiviral infections virus load correlates with virus pathogenesis. With higher virus loads, the disease is more severe. Thus, it is important to understand how the level of viremia is regulated. In the past 10 years, much progress has been made in understanding the regulation of lentivirus expression. Most of this understanding comes from studies of the primate lentiviruses and good reviews of these findings are available [38, 41, 45]. Some generalities drawn from primate lentiviral studies also apply to other lentiviruses such as EIAV; however, many do not. As an infectious agent, EIAV has a number of unique and novel aspects. Both the similarities to the primate lentiviruses as well as the differences make EIAV an interesting and worthwhile lentivirus to study. These differences and similarities in the regulation of expression will be the focus of this review. Several previous reviews on EIAV pathogenesis [13, 24, 49, 105] and transcriptional regulation [13, 28] are excellent sources on earlier work in the field.

One difference between EIAV and other lentiviruses is that EIAV causes an acute disease rather than a slow, pro-
Fig. 1. A schematic diagram of the EIAV genome and the most common transcripts synthesized in infected cells. The proteins encoded by each transcript are noted.

progressive one. Two to 6 weeks following infection, an initial episode of EIA can occur which is characterized by frank viremia accompanied by fever and thrombocytopenia [24, 29, 105]. The episode usually resolves over a several-week period and seroconversion and initial T cell responses take place as the viremia is controlled [16, 43, 74, 85, 95]. Viremia can recrudesce during subsequent days to weeks causing subsequent episodes as well as chronic anemia, ventral edema and general wasting. The viremia present during each of these subsequent episodes has been shown to contain antigenic variants [54, 90, 91]. It has been postulated that viral recrudescence may be due to this variation that allows the virus to escape immune surveillance.

In most horses, the viremic episodes and accompanying clinical disease are eventually controlled resulting in a clinically quiescent, healthy, carrier status for the remainder of the horse's life. During the carrier phase of the infection, circulating infectious virions are difficult to detect [51, 58, 73, 86]. In part, the reduction of free infectious virions must result from immune control since immunosuppression of carrier horses usually causes viral recrudescence [54, 117]. In addition to the immune control of free virions, a reduction of viral RNA synthesis within infected cells also occurs [86]. Whether this reduction is due to immune clearance of those infected cells producing large amounts of RNA or due to other viral or cellular mechanisms that reduce the transcriptional or posttranscriptional activity of the virus is not currently clear. Regardless of the mechanism, the suppression of expression results in not only a clinically quiescent, but also a virally quiescent state. Thus, while HIV does not appear to have a transcriptionally latent or restricted state, EIAV does. This viral quiescence does not result in eventual viral clearance from the host. EIAV persistence during the quiescent state has been demonstrated by continued seropositivity [53], passage of blood from a seropositive carrier horse into a naive recipient [48], recrudescence of infectious virus upon immunosuppression of the infected horse [54, 117] and by detection of proviral sequences within tissues [51, 58, 73, 86] and low level viremia as detected by RT-PCR [58, 86].

Like other lentiviruses, EIAV contains a complex genome (fig. 1). The 5' and 3' long terminal repeats (LTRs) composed of the U3, R and U5 sequences flank the genome and are responsible for transcriptional initiation and polyadenylation of the viral RNA, respectively. Three different size classes of messages are made: small, medium and large. The small messages are composed of a number of differentially spliced transcripts and encode the regulatory proteins, Tat and Rev [17, 83, 101, 113] and a 27-kDa transmembrane protein, Ttm [6]. Ttm is found in EIAV-infected cells, but the role of Ttm is not known. Mid-sized messages encode the viral receptor protein, Env, and a small protein of unknown function, $2$. The mid-sized message can also potentially code for Tat [104]; however, whether Tat is synthesized from this message during an infection is not known. The envelope polypeptide is cleaved into the glycosylated surface protein (SU), gp90, and the transmembrane protein (TM), gp45. The TM protein can be processed further into a glycosylated gp32 and a carboxy terminus, nonglycosylated protein, p20 [97]. The role of this further processing in EIAV pathogenesis is not known. Gp90 and gp45 (or the gp32 and p20 cleavage products) are noncovalently linked at the surface of the virus and serve as the viral receptor [4, 24]. The large message is responsible for the synthesis of the Gag and Pol polyproteins that are processed by the virally encoded protease to produce the structural and enzymatic proteins of the virus, respectively. In addition, full length transcripts are packaged into virions to produce infectious particles.