ANALYSIS OF THE LATENCY ASSOCIATED TRANSCRIPTS OF HERPES SIMPLEX VIRUS TYPE 1

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CONTENTS

ABSTRACT

This thesis reports a novel approach to the study of HSV-1 transcripts during latency, based on accumulating evidence that macromolecular complexes involved in the synthesis and processing of cellular and viral RNAs occupy discrete domains in cell nuclei. A method for high resolution non-isotopic *in situ* hybridization (ISH) was developed to study the intracellular location of HSV-1 latency associated transcripts (LATs) in primary sensory neurons of latently infected mice and humans. Minor LATs were localized to sharply defined intranuclear foci of 1-3 μm diameter. On average, there were 2.6 to 2.8 foci per LAT^+ neuronal profile (5 μm), representing about 10 to 11 foci per cell. In contrast, major LAT sequences were diffusely distributed throughout the nucleoplasm of latently infected neurons, with prominent sparing of nucleolar regions. Similar staining patterns were observed in latently infected human trigeminal ganglia. Thus, high and low abundance transcripts could be readily differentiated according to their spatial distribution.

Using a mixture of major LAT- and minor LAT-specific probes the characteristic staining patterns could be superimposed, demonstrating for the first time that these RNAs co-exist in latently infected neurons. In these experiments foci were readily distinguished from a background of diffuse staining however, focal staining was not a feature of probes complementary to sequences wholly within the major LAT region. The molecular basis for the segregation of major and minor LATs shown in these experiments is not clear. The data is consistent with generation of major LATs through splicing of a large primary LAT species but does not exclude the possibility that the 2 kb major LAT is a primary transcript initiated 0.7 kb downstream of the LAT promoter LAP1.

The ability to delineate the subcellular location of major and minor LATs by ISH establishes a foundation for studying the generation and transport of these molecules *in vivo*. To initiate these studies, a method for detection of nuclear antigens in sections of frozen murine sensory ganglia was developed and the distribution of cellular antigens associated with mRNA splicing in the nuclei of neurons *in vivo* was characterized.